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Meat in nutrition: XV. Certain characteristics of gestational performance in albino rats fed a diet containing dried autoclaved pork muscle

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HEAT IN NUTRITION. XV. CERTAIN CHARACTERISTICS OF GESTATIONAL PERFORMANCE IN ALBINO RATS FED A DIET CONTAINING DRIED AUTOCLAVED PORK MUSCLE

by

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INTRODUCTION

As the study of nutrition has developed into a science, investigators in the field have established criteria to use in judging the adequacy of a dietary regime. These criteria are based on the realization that the diet must furnish the food nutrients needed by the body for the maintenance of normal physiological functions. The nutritionist, therefore, conceives the adequate diet as one that will promote growth and development in the young animal, preserve the "characteristics of youth" in the adult, and support life, reproduction, and lactation over a period of time normal for the species. If the processes are not sustained from one generation to another the species is threatened with extinction.

Many of the important discoveries in the field of nutrition followed attempts of investigators to formulate, for experimental animals, diets that met the acid test of adequacy. Success did not come easily or immediately. First, the nutritionist was forced to solve the problem of the dietary factors involved in the maintenance of life itself. Although this represented the easiest task of all, early trials met with failure. Not until the vitamins were discovered and information was developed as to their

distribution in food materials, did it become possible to prepare rations of purified food nutrients which were adequate for both growth and maintenance. Although the story of the food nutrients essential for optimal performance of these functions is still incomplete, today experimental animals fed certain synthetic diets may be brought to the reproductive period in a vigorous state of health. While this has made it possible to study the dietary factors involved in reproduction and lactation, the character of the food nutrients necessary for normal performance of these functions is still obscure. It is evident, however, that the needs of the body for reproduction and lactation are more complex than those for growth and maintenance. No doubt, further studies will lead to a clearer picture of the role which the now recognized factors play in the maintenance of good nutrition and possibly to the discovery of other important dietary essentials.

It is no wonder that our knowledge of the nutrients needed for reproduction is inadequate when we consider the many and varied physiological processes involved in the reproductive cycle, each of which may be influenced by dietary procedure. Events in the normal course of reproduction that may be affected relate to the production of

germ cells, maintenance of sex interest, fertilization of the ovum, implantation of the zygote with establishment of placental function, development of mammary glands, development of the fetus, and termination of gestation.

In the present investigation, we have been primarily interested in studying the relation of nutrition to the normal sequence of events in pregnancy. A gestation can be termed successful only if parturition is uneventful and results in the birth of vigorous live young. Many degrees of partial gestational failure lie between a successful performance and complete disaster. For example, weaklings may be born which survive for only a short period; or there may be birth of both living and dead young, or birth of dead young only, or finally, as sometimes occurs, birth of no young at all. We have designated such gestations as partial failures, and one in which death of both mother and fetus occurs as a complete failure.

Several factors may cause partial or complete gestational failure. Gynecologists recognize that endocrine dysfunction, infectious agents, inability of the female to adjust her metabolism to meet the demands of the fetus, and improper diet may all affect the character of gestation. The dietary aspects of the problem are especially interest-

ing. It is known that diet affects ovulation and copulation. Information is limited, however, in regard to the relation of diet to fertilization and implantation. If the ovum is not fertilized or the zygote not implanted in the uterine wall, partial gestational failure will result. Experimental evidence indicates that no serious consequences on the general health of the female follow. On the other hand, failure of the implanted ovum to develop normally may jeopardize the life of both mother and young.

Abnormal development of the fetus can be traced directly or indirectly to malnutrition of the mother. The maternal diet may be deficient in some food nutrient, so that the development of the fetus is handicapped by the lack of a necessary building material. For example, iodine serves as an actual building stone for the synthesis of thyroxine in the fetal thyroid gland. Vitamin E also controls the formation of new tissue, but in another way. In certain species it is known to be essential for rapid proliferation of cells in the fetus. Or again, the diet of the mother may be slightly deficient in some substance like vitamin A, which is essential for the maintenance of a normal endometrium. Degenerative changes in the maternal portion of the placenta or of the

endometrium, in many instances, lead to the death of the fetus following starvation or absorption of products of degeneration. These substances, finally, cause the death of the female.

The quality of the diet also controls fetal development indirectly. If the maternal dietary is lacking in some substence which is necessary for the normal metabolism of the mother, a breakdown in maternal metabolism may occur which prevents the fetus from receiving the food nutrients that it needs. For example, the ingestion of excessive amounts of fat, if followed by the deposition of excess fat in the liver, causes a derangement of metabolism. The presence of fat in the liver cells then limits the storage of glycogen, causing partial starvation of the fetus. A liver loaded with fat is also incapable of detoxifying metabolic products in the normal manner. Under such circumstances, the fetus receives metabolites which are actually injurious to a rapidly developing organism. In either case, the fetus dies before birth, or, if it does survive parturition, succumbs within a few days.

Our knowledge of the reasons for the spontaneous termination of gestation which normally occurs is unfortunately too slight to permit anything more than speculation on the subject, but we have reason to believe that the

physiological processes controlling the birth mechanism may be affected, also by dietary factors. For example, experimental evidence indicates that if the diet is deficient in the essential fatty acids, normal termination of pregnancy does not take place. Is it easier for the female to give birth to live young than to dead feti? If it is, then the factors already described which result in abnormal development of the feti lead indirectly to failure in parturition.

In the preceding paragraphs, ideas have been advanced which indicate that the quality of the diet of the maternal organism may profoundly affect the course pregnancy will follow. The manner in which a dietary regime exerts this influence is open to interesting question and theory. The solution of the many problems suggested offers a wide avenue of approach to the study of the food nutrients necessary for reproduction and lactation.

REVIEW OF LITERATURE

GESTATIONAL FAILURES OCCURRING IN ANIMALS FED INADEQUATE DIETS

As the knowledge of the factors essential for reproduction and lactation has increased, certain types of gestational failures have become associated with definite dietary deficiencies. Disorders of pregnancy traceable to this cause can be prevented by the addition of the missing foodstuff. In many cases, the quantity of nutrient present in the diet was adequate for the maintenance of the adult organism until the strain of pregnancy was added. Examples of instances where diet has played a specific part in the regulation of reproduction are described below.

A Partial Deficiency of Vitamin A

Mason, in 1935, described typical gestational failures in rats maintained on a diet low in vitamin A. Gestations extending as long as 26 days and resulting in the birth of both living and dead young were observed. The female at parturition was listless and showed considerable distress. Due to a loss of tone in the muscles, she had difficulty in delivering the feti. The placentae were

usually not expelled. Often the females died during or after parturition. Autopsy of animals killed when death seemed imminent showed a variable number of dead and living young in the uterus. The fetal membranes had collapsed and resorption of the feti was well advanced. Uterine infection, often accompanied by local or general peritonitis, was a constant finding. Microscopic examination of sections prepared from the uterus, feti, and placentae showed that the fetus starved to death as the result of a decreased supply of food nutrients following the infection in the uterine wall and the injury to the placenta. When vitamin A was added to the diet, the gestational performance was normal.

Lack of Vitamin B-complex

In the human being, the polyneuritis of pregnancy has long been considered a toxemia which should be treated by immediate evacuation of the uterus (Whitfield, '89; and Berkwitz and Lufkin, '32). However, observations made by Strauss and McDonald ('33) and Lukkart ('33) suggest that the condition may be due to a dietary deficiency. The polyneuritis was invariably preceded and accompanied by pernicious vomiting, which made the absorption of adequate amounts of the food nutrients impossible. The neurological symptoms of general

weakness, numbness, and muscular pain in the legs appeared about the third or fourth month of pregnancy. As the condition progressed, the muscles of the legs, arms, thorax, and abdomen became paralyzed. Often a slight anemia was present. The microscopic changes in the nerves were similar to those found in beri-beri and alcoholic neuritis. Strauss and McDonald ('33) concluded from their clinical experience with the administration of vitamin B compounds that the polyneuritis of pregnancy is similar to beri-beri and may be classed as a dietary deficiency disease.

Ross ('35) states that in North Carolina the incidence of pellagra among women and the eclampsia ratio are parallel. The patients who had eclamptic convulsions frequently subsisted on a diet deficient in vitamin G, vitamin A, vitamin C, and vitamin D.

Lack of Vitamin E

Another typical picture of gestational failure observed in rats is one associated with a deficiency of the vitamin E content of the diet. Evans and Burr, in 1927, described gestations which apparently progressed normally until the sixteenth or seventeenth day after coitus. At this time the pregnant female began to lose weight gradually and consistently

until she returned to approximately her normal non-gravid weight. Autopsy revealed that the feti were all dead and in varying stages of resorption. Microscopic examination of feti at the sixteenth or seventeenth day of the pregnancy revealed abnormalities of the yolk sac and allantois and incomplete development of the hemopoietic tissues. The retarded development of the fetus resulted eventually in death and resorption. The addition of vitamin E to the diet prevented the loss of the young.

Lack of Essential Unsaturated Fatty Acids

Evans and his co-workers ('34) state that "normal reproduction without the essential unsaturated fatty acids is impossible" (p. 433). The most common derangement of gestation noted by these authors, in rats fed a diet deficient in linoleic and linolenic acids, was a long gestation period of 23 to 25 days. Labor often lasted for 24 hours, with the delivery of both living and dead young. Eighty per cent of the young were born dead. The young born alive were weak and small (average weight, 4.0 gm.) and died soon after birth. The females were greatly weakened by prolonged labor at parturition, and 6 per cent died during labor. About 20 per cent of the females

failed to give birth to a litter, although they had shown evidence of implantation. Autopsy of two animals on the nineteenth and twenty-third days of gestation revealed dead and macerated feti. The corpora lutea were white and chalky. The addition of increased amounts of cod liver oil, carotene, and vitamin E had no apparent influence on the disorder. However, the addition of the unsaturated fatty acids alleviated the condition. In an extension of the investigation, Haeder ('37) showed that the intra-uterine death of the feti was due to starvation following degeneration of the maternal decidua.

Lack of Calcium

It has long been recognized that the maternal organism will, if necessary, draw calcium from her own skeletal tissues to furnish the fetus with an adequate supply of this element. The physiological adjustment thus made between the mother and fetus makes it difficult to assay the effect of a low-calcium diet upon gestation alone. However, in 1911, Hart, Steenbock, McCollum, and Humphrey found that if the ration of pregnant cows was limited entirely to wheat-hay and wheat-grain products, the calves were born prematurely and invariably were dead. On the other hand, cows fed a ration derived entirely from the

corn plant were able to produce vigorous live young. If calcium in the form of inorganic salts was added to the diet of wheat products, the cows were able to produce living calves.

Hecomber ('27) has further studied the effect of low calcium intake during pregnancy. He found that if pregnant female rats were fed a diet low in calcium, the intra-uterine mortality of the young was increased as compared with that in the control group. Although the number of corpora lutea per pregnancy was nearly identical for the two groups, the females on the low-calcium diet produced fewer live young than did the rats fed the adequate diet.

Lack of Iodine

As early as 1870, farmers in Michigan realized that they could prevent losses in domestic animals due to endemic goiter by the feeding of a certain crude salt. In some areas in Canada, enormous numbers of young animals were lost each year due to thyroid disease. The young were born without hair and in many cases with a marked enlargement of the thyroid. The majority of the young were born dead. The young born alive died very shortly. Smith (17) as well as Hart and Steenbock ('18) demonstrated that the condition was

directly related to lack of iodine in the diet of the mother.

Lack of Iron

If hemoglobin values may be taken as a measure of physiological well-being, the quantity of iron in the diet of the mother directly affects the vigor of the young at birth. Alt ('38), for example, showed that while the total body iron stores of the first litter produced by females on a low-iron diet were only slightly below normal, the young of the second litter had only one-fourth of the normal iron reserves. The concentration of hemoglobin in the blood of the young of this litter was also decreased. As milk has a very low iron content, suckling animals must depend upon their own reserves of iron in building hemoglobin. If the iron reserves of the liver are low at birth, the young may not be able to synthesize hemoglobin rapidly enough to develop in a normal manner.

Lack of Protein

Very few experiments have been reported in the literature dealing with the effects of either a deficient quantity

of protein or a poor quality of protein in the diet upon pregnancy. One of the earliest experiments of this nature was made by Hart, Nelson, and Pitz ('18). These investigators fed pregnant rats a diet which they believed deficient in lysine. The young when born appeared normal. However, the diet used contained 0.5 to 3.0 per cent yeast. In the view of recent work on the requirements of lysine for maintenance (Alcock, '36) and the lysine content of yeast (Csonka, '35), it seems probable that the diet was not deficient in this amino acid.

Seegers ('37) found that if pregnant rats were fed either a nitrogen-free diet or a diet containing 10 per cent gelatin as the sole source of protein, no young were born. However, if these diets were not fed until after implantation had occurred, the females were able to produce litters. The young in the litters, however, weighed less at birth, and contained less total nitrogen as well as less nitrogen per gram of body weight than did the young of control rats fed an adequate diet.

GESTATIONAL FAILURES OCCURRING IN ANIMALS FED A SUPPOSEDLY ADEQUATE DIET

In 1933, the American Committee of Maternal Welfare reported that 30 per cent of the maternal deaths in this country were due to the so-called toxemias of pregnancy. McIlroy ('33) found that about 20 per cent of the maternal deaths in England could be traced to eclampsia. Although eclampsia and the associated toxemias of pregnancy are closely related to gestational failure in human beings, the etiology of the condition is still obscure.

In a study of causes of disturbances of pregnancy, it seems pertinent to trace the similarities in pathological findings associated with gestational disturbances in man, domestic animals, and experimental animals fed supposedly adequate diets. In the case of man and the domestic animals, it is necessary to assume that a diet which prevents gross evidence of the deficiency diseases in the non-pregnant state is adequate in the known dietary essentials. However, in the case of experimental animals the adequacy of the diet in the various food nutrients can be accurately checked.

Pathological Findings

From reports in the literature dealing with the pathological changes that occur in the disturbances of pregnancy noted in the various species, data may be obtained on the symptomatology, the variations in the composition of the blood and urine, and the microscopic lesions observed in the various organs. Some typical pathological findings are described below.

In man

A wide range of symptoms has been reported for the toxemias of pregnancy, but the most common are hypertension, vomiting, albuminuria, and edema (Cruickshank, Hewitt, and Couper, '27; McIlroy, '36; DeLee, '13; and Rowe, '32.) Individual investigators believe that the appearance of one or more of these symptoms may be an important warning of an ensuing toxemic condition. Siddall and Mack ('33) considered edema alone to be an unimportant symptom, as moderate edema occurs frequently in normal pregnant women. However, those authors considered that the simultaneous presence of edema, hypertension, and albuminuria indicated a toxemic condition. On the other hand, Dieckmann ('33) believed that the appearance of albuminuria and hypertension, with or without edema,

was indicative of a toxemia.

Many subjective symptoms have been reported by different investigators. These symptoms include such ocular disturbances as loss of perspective and loss of visual acuity (Basden, '37; DeLee, '13, and Rowe, '32). In addition, the literature contains many references to a general feeling of malaise in the toxic woman. Some patients were reported as feeling lethargic (Wood and Nix, '38); others, irritable (Theobald, '35). None of these subjective symptoms appeared to be definitely associated with the toxic condition, and were probably indicative of a general metabolic disturbance.

A change in the energy metabolism may constitute a symptom of developing toxemia. Colvin and Bartholomew ('39) found that patients in early pregnancy with a basal metabolic rate in the lower limits of the normal range should be carefully watched for evidence of toxemia. In their experience, women who developed eclampsia exhibited a low metabolic rate early in pregnancy. The metabolic rate then rose sharply to above the normal level when the symptoms of toxemia appeared.

Metabolic disturbances are reflected in the composition of the blood and urine. A certain dilution of the blood is normally associated with pregnancy (Dieckmann and Wegner, '33).

Hartmann in 1933 reported that the dilution of the blood was increased in pre-eclamptic cases. The dilution was reflected in a lower concentration of hemoglobin, a decreased quantity of serum protein, and a lower cell volume per cent. Stander and Cadden ('34) believed that the high uric acid content of the blood in pre-eclamptic and eclamptic cases was the most important change in the composition of the blood. The same authors (Cadden and Stander '39) reported that the high level of uric acid in the blood was maintained in eclamptic patients notwithstanding normal excretion of the acid. Hartmann ('33) felt that a lowered carbon-dioxide combining power of the blood was an important factor in the eclamptic cases. Another fairly constant finding in the convulsive patients was a low calcium-phosphorus ratio due to a marked increase in inorganic phosphorus in the blood.

Siegel and Wylie ('33) reported that blood sugar findings are of interest in pre-eclamptic and eclamptic patients. In the patients that they studied, the pre-eclamptic cases showed hypoglycemia while the eclamptic ones exhibited hyperglycemia. The authors believed the hyperglycemia was probably caused in part by the convulsions. These authorities also felt that a day by day study

of the concentration of blood sugar in pre-eclamptics showed that these patients were suffering from an unstable carbohydrate metabolism. Mays and McCord ('35), however, found that neither hypoglycemia nor hyperglycemia was a constant factor in eclampsia.

In cases of eclampsia, Boyd ('35) found a high ratio of phospholipid to total cholesterol in the blood serum. Her observations are especially interesting when it is considered that a similar ratio has been found in the blood serum of epileptics. Bartholomew and Kracke ('36) reported a level of total cholesterol considerably above the normal value in the blood of eclamptic and pre-eclamptic patients.

A rise in blood guanidine which lasted as long as the toxic state existed has been observed in patients with severe eclampsia (Andes, Anles, and Myers '37).

The most constant change that has been noted in the urine by many investigators is an increase in the albumin content. As to the other constituents, the reports in the literature are conflicting, due probably to differences in the classification of the toxemias and in the biochemical methods used in studying the disorder. Crutchshank, Hewitt, and Couper ('27) concluded that in pre-eclamptic

cases the total nitrogen content of the urine was decreased due to a low excretion of urea. The significance of the presence of sugar in the urine has been much debated. A slight galactosuria follows the initiation of mammary development in normal patients. McIlroy ('36), however, is of the opinion that patients showing glycosuria should be examined for toxemia because the appearance of sugar in the urine might indicate liver damage. In a paper published in 1937, Savage and Wylie reported a markedly lower excretion of estrin in cases of pre-eclampsia. The available data on the composition of the urine in the toxemias of pregnancy are unfortunately limited to pre-eclamptic cases. In eclampsia, oliguria and anuria make it impossible to secure enough urine for chemical analyses.

Many obstetricians have studied the histo-pathology of the liver, kidney, and placenta in the hope of establishing the primary lesion in eclampsia. While there apparently is always some injury/to hepatic tissue in such cases, the types of lesions described vary widely. Irving ('36) reported necrosis, hemorrhage, and fatty degeneration in all portions of the lobules. DeLee in 1913 noted similar changes in livers obtained at necropsy. However, after the examination of livers from ten cases of eclampsia

Bell ('26) came to the conclusion that while a variety of lesions were present, none could be considered typical of the condition.

A study of the lesions present in the kidneys of eclamptic patients has been complicated by the variable amount of infection which may be present. Bell ('36) believed that a characteristic lesion of the glomeruli occurred in eclampsia. The glomeruli appeared enlarged and the basement membrane of the capillaries seemed thickened. Schwarz and Dorsett, as quoted by Murrey and Hrent ('38), reported similar lesions of the glomerular capillaries. Kellogg ('31) states that Hertig found lesions similar to those described above in the liver and kidneys in a case of pre-eclamptic toxemia.

Lesions in the heart have not been widely studied. However, DeLee ('13) reported that he found degenerative changes of a fatty nature in the cardiac muscle accompanied by necrosis and hemorrhage.

Young, according to Bartholomew and Colvin ('38), first associated the acute type of placental infarct with the toxemias of pregnancy in 1914. Bartholomew and Colvin in 1938 related rupture of the placental vessels and thrombosis of the villous capillaries to eclampsia. In 1936 Bartholomew and Kracke also described the presence

of large fat cells in the endothelium, which distorted and narrowed the lumen of the small placental arteries.

Details of the histo-pathology of the various organs reported in the literature will be discussed later.

In animals other than man

During the last fifteen years, reports have appeared in the literature dealing with a disturbance of pregnancy which occurs spontaneously in sheep fed a supposedly adequate diet. The condition has been widely studied both in various sections of this country and in England. By combining the reports of various investigators (M'Fadyean, '24; Elder and Uren, '35; Roderick and Harshfield, '32; and Dimock, Healy, and Bullard, '38), it is possible to obtain a fairly complete picture of the condition. The majority of the cases occurred in the winter and spring months in ewes carrying more than one lamb. The onset of the disease was sudden; the affected animals seemed normal to within a few hours before the first appearance of symptoms. Frequently, poor muscular coördination and either extreme lethargy or extreme irritability were the first symptoms noted. There appeared to be a progressive loss of visual acuity leading eventually to total blindness. The afflicted animals

either because of blindness or of some mental aberration tended to walk in circles.

The animals had little or no appetite but seemed to crave water. In the early stages of the disease, the animals ground their teeth together. As the condition progressed, respiration became very labored and rapid. At the first onset of the symptoms the animals tended to lean against any available support. The head was either retracted or drooping. In the terminal stages of the disease the animals became prostrated and passed into a coma. Convulsions occurred at irregular intervals. Often the odor of acetone could be detected on the breath.

In the sick ewes there was seldom any spontaneous attempt at parturition. However, if parturition, either spontaneous or induced, occurred, the sick animals improved rapidly. Even so, approximately 90 per cent of the sick animals died (Bergman, '35).

Complete gestational failures of unexplained origin have been described in guinea pig, rabbit, and rat colonies maintained in various experimental laboratories. Such colonies were fed diets believed to be adequate. The syndrome accompanying complete gestational failure in these experimental animals was very similar to that described in sheep and in human beings. Smith, in 1913, described a

disturbance of pregnancy in female guinea pigs which resulted in death just before or after parturition. This condition was observed only in the winter months. The chief symptom noted was extreme lethargy. When the animals that died before parturition were autopsied, fully developed young were found in the uterus.

Greene ('37) studied very carefully a similar disturbance which occurred in pregnant rabbits. He believed that syndromes comparable to those in pre-eclamptic and eclamptic cases in human beings occurred in pregnant does. He also felt that desertion of a litter by the mother might be the result of a mild asymptomatic attack of toxemia. The picture presented by the toxic does was amazingly similar to that described for the sick ewes. In a typical fatal attack, the onset of the symptoms was sudden. On some occasions an animal was in apparently excellent health not more than one-half hour before death. An extreme lethargy and poor muscular coordination were usually the first symptoms noted. The hair became rough, the eyes lustreless, and the ears cold, as the condition progressed. In every case dyspnea, cyanosis, and acetone breath were observed. Although anuria was the rule, the animals showed evidence of extreme thirst. The rabbits frequently passed into a comatose state before death,

either with or without convulsions.

Hartwell in 1927 described the death of pregnant rats following the ingestion of a synthetic diet which was thought to be adequate. The gravid females became comatose toward the end of the gestation period. Convulsions occurred frequently before death. Hartwell in describing the condition of the animals wrote, "The mothers apparently died from internal hemorrhage; their paws and tongue were quite white, their muscles very anaemic and the liver appeared bloodless. The young seemed normal in all respects." (p.1080)

In the Nutrition Laboratory of the Foods and Nutrition Department at the Iowa State College, both partial and complete gestational failures have been observed in animals fed a supposedly adequate diet. The diet used was synthetic except for the source of protein, which was dried, canned autoclaved pork muscle, incorporated in the diet at a level equivalent to 15 per cent of protein. Dyar ('35) found that it was impossible to rear three generations of rats on the pork-containing diet, because no young were born to second-generation females. In the first generation, only 45.0 per cent of the young born alive survived until seven days after birth; in the second generation, only 23.4 per cent survived. A compa-

rable percentage for animals in the stock colony as reported by Earhart ('35) was 71.4. The high mortality of the young of females fed the pork diet probably was due to a disturbance in gestation, which resulted in the birth of weaklings. Similar results in animals fed the pork-containing diet were noted later in successive years by Wellman ('36), Wilcox ('37), and Walliker ('38).

Dyar, in 1935, also reported complete gestational failure in 33 per cent of the pregnant animals fed the pork-containing diet. In 1936, King reported a 10 per cent mortality of pregnant females fed the basal pork diet and one of 70 per cent when 0.5 gm. of a liver extract was added as a supplement. The following year none of the rats fed the basal pork ration died, but 30 per cent of the animals given the basal diet supplemented with 0.5 gm. of lecithin daily were lost (Wilcox, (37). For the year 1937-1938, Walliker reported that 10 per cent of the female rats fed the basal pork ration and 20 per cent of those fed the same pork diet supplemented by 10 mg. of lipocaic[1] died at parturition.

Irrespective of the diet fed, the symptoms presented by animals that died at parturition were essentially uniform. The onset of the condition was sudden. The

1. Eli Lilly preparation

animals appeared to be in perfect health until the twenty-first or twenty-second day of gestation. Often animals which were apparently normal at 10 p.m. were found dead at 8 a.m., and those in good condition at 8 a.m. were sometimes dead at noon. The presence of blood in the urine was a warning of an approaching toxemic condition. Although the majority of the animals that died exhibited hematuria, not all the rats with bloody urine became "toxic." The first symptom noted was that the animal was cold to the touch. As the condition progressed the hair stood erect, the ears and feet became cold and pale. The animal by this time was very limp. Frequently the sick female shivered violently and ground its teeth together. In the majority of cases the head was retracted. The sick animals made no effort to eat, but tried to drink water. A sick animal was unable to support her head and rested it on a food cup. Death was usually preceded by convulsions. The majority of the deaths occurred during convulsions.

The condition of toxic sheep, rabbits, and rats studied at autopsy presents certain similarities. Roderick and Harshfield in 1932 reported that the pregnancy disease in ewes was accompanied by abnormalities in the

liver. The organ often appeared enlarged, friable, and pale yellow in color. Upon microscopic examination, such livers showed varying degrees of fatty degeneration and fatty filtration. These findings were confirmed by Elder and Uren in 1935. A similar condition of the liver has been reported in guinea pigs by Smith ('13), in rabbits by Greene ('37), and in rats by King ('36).

In the sheep the lungs appeared normal, in contrast to the marked edema and congestion of the lungs accompanied by hydropericardium and hydrothorax observed in rats and rabbits.

If the sick ewes were autopsied soon after death, the lambs were found alive (Roderick and Harshfield, '32). On the other hand, the fetal rats or rabbits were usually found dead, irrespective of whether the female died or was killed just before death.

Judged from the symptoms exhibited by animals suffering from pregnancy disease, the condition is remarkably similar in sheep, guinea pigs, rabbits, and rats. Also, the disturbance seems to be closely related to the toxemias of pregnancy which have been noted in human beings.

Theories of Etiology

Many theories have been advanced as to the cause of the toxemias of pregnancy, and numerous investigators have attempted to reconstruct the sequence of physiological events which culminates in a toxic condition. Of the hypotheses suggested, no one explains all the known phases of the disease. In general, it may be said that all the symptoms noted appear to rise from some fundamental biochemical disturbance. It is convenient, even though many of the ideas overlap, to consider the various theories regarding the etiology of toxic states in two large classes; i.e., those dealing with defects in maternal metabolism, and those dealing with defects in the placental metabolism and structure.

If the outcome of pregnancy is to be successful, the mother, as gestation progresses, must adjust her metabolism to meet the increasing demands of the fetus. Some workers feel that an inability of the maternal organism to live in symbiosis with the fetus gives rise to general metabolic disturbances, and that these are responsible primarily for an ensuing toxic condition. Others, however, do not concede this premise. In their minds, the metabolic upsets represent secondary lesions following

infection, endocrine dysfunction, or dietary deficiency. Unfortunately, it is impossible to separate completely the influence which these factors exert in disturbances of gestation, but an attempt will be made to evaluate the significance of each.

Maternal metabolic defects

Metabolic upset as a primary cause-- With the onset of pregnancy, an adaptation of physiological functions must occur. If this adjustment is made with difficulty, derangements in the maternal metabolism result. They may be reflected principally in disturbances of protein, water, and carbohydrate metabolism.

The theory that altered protein metabolism is etiological agent in producing toxemia was advanced by Strauss in 1935. He believed that the edema noted in eclamptic patients was due to a hypoproteinemia which resulted in a lowering of the colloid osmotic pressure of the plasma proteins. The bases for considering hypoproteinemia as the chief cause of water retention in pregnancy may be summarized as follows: (1) the water retention of pregnant women during the administration of sodium salts is inversely proportional to the colloid osmotic pressure exerted by the plasma proteins; (2) pregnant women who do not exhibit edema but have a low

concentration of proteins in the plasma lose weight readily when given a diet containing 260 gm. of protein and an adequate amount of calories daily; and (3) the amount of weight lost is inversely proportional to the level of the osmotic pressure of the plasma.

Others have propounded the theory that if a deranged metabolism is a causal factor in toxemias, the blood and urine should reflect the condition. However, in a study of the partition of nitrogen, Rowe, McManus, and Riley ('34) found that the only marked deviation from normal in the blood of eclamptic patients was a rise in the uric acid content.

The metabolism of carbohydrate by toxic patients has also been studied in several laboratories. When the relation between the level of blood sugar and diabetic coma and convulsions is considered, the research into the carbohydrate metabolism of toxemic patients should yield important data. However, results have been rather disappointing. In 1936, Rowe, McManus, and Plummer concluded from studying clinical cases of toxemia that the changes in quantitative sugar content of blood or urine were relatively unimportant. However, they felt that when sugar tolerance tests were used as the criteria, marked changes in the metabolism of the toxic patients

could be noted. These changes were apparent in a decreased tolerance for galactose and levulose.

Titus and Dodds ('28) also suggested that the primary factor in eclampsia might be a disturbed carbohydrate metabolism. There was, he believed, in cases of toxemia, a decreased intake or absorption of carbohydrate with a consequent depletion of the hepatic glycogen stores. The liver thus deprived of the protective action of glycogen was injured and failed to function normally. These authorities believed that the convulsions noted in eclampsia might be controlled by the administration of glucose.

Infection as a primary cause-- The theory that renal and vascular infections are the underlying causes of the toxemias of pregnancy has been advanced by Peters and his co-workers ('36, '37, and '38). Peters believes that a counterpart might be found for nearly every syndrome described as a toxemia of pregnancy in cases of chronic pyelitis, hydronephrosis, and pyelonephritis uncomplicated by pregnancy. He quoted ('36), for example, the case histories of three patients with chronic pyelitis who developed convulsive seizures at a time when they were neither pregnant nor in the terminal stages of the disease. In an analysis of the data available at the New Haven

Hospital from 1922 to 1935 inclusive, Peters found that 13 per cent of the patients suffering from toxemia also had pyelitis. At autopsy the presence of renal infection was confirmed in 11 cases out of the 25 studied. Peters ('36, p. 916) suggests "... the role of pregnancy in the etiology of toxemias may be merely to accelerate or exaggerate the progress of a pathological condition which is of itself capable of producing a similar picture in the non-pregnant subject, evoking an acute explosion or exacerbation of the disease." In papers published in 1937 and 1938, Peters reported that 40 per cent of the 68 cases of eclampsia studied presented a history of previous renal or vascular disease. He reiterated in these papers his belief that the symptoms of toxemia were those of arterial and renal infection and were not due to hepatic and metabolic disturbances. This view, however, has not been widely accepted. Most investigators seem to feel that while infection is often present in the kidneys, it is either coincident with or subsequent to the initiation of the toxemic condition.

Endocrine dysfunction as a primary cause-- Patterson, Hunt, and Nicodemus have advanced the interesting theory that hypothyroidism is the primary etiological agent in

the toxemias of pregnancy. These investigators argued as follows: There is known to be a marked hypercholesteremia in pregnancy which is exaggerated in the women who develop eclampsia. When desiccated thyroid is administered during pregnancy, the level of cholesterol in the blood falls within the normal range for non-pregnant women. However, if the administration of thyroid is discontinued, the cholesterol content of the blood rises. Therefore, they suggest that the hypocholesteremia of pregnancy is due to sub-clinical hypothyroidism. During pregnancy, any hypothyroidism present becomes accentuated due to an increased metabolism. If the maternal organism develops hypothyroidism and subsequently hypercholesteremia, the same conditions will appear in the fetus. In this case, the excess cholesterol in the fetal blood is deposited in the walls of the placental arteries, causing an endarteritis. The condition may become so severe that the arterial lumen is occluded and placental infarction results. Toxins then liberated by the degenerating placental tissues may produce a toxic state in the mother.

Simultaneously, the hypercholesteremia in the mother may injure the renal epithelium. If the combination of renal injury and absorbed toxins from the placenta

becomes severe enough to cause anuria, the sequence of events known as eclampsia will follow. If, after parturition, the thyroid is able to maintain normal metabolism, the level of blood cholesterol will fall, the cholesterol deposits will be absorbed and kidney function will improve. However, if the maternal hypothyroidism has been severe enough to cause an absorption of a large amount of fetal thyroxin, the maternal thyroid may not be able to maintain metabolism following parturition. Then the blood cholesterol rises, accentuating the kidney damage. If injury to the kidney is great enough, eclampsia may result.

In support of this theory, Patterson and his colleagues reported that pregnant rabbits subjected to complete thyroidectomy invariably died with convulsions a few days before term. The cholesterol content of the fetal blood taken at autopsy was 200 mg. per 100 cc. of blood, as compared with a value of 80 mg. for the blood of feti from normal does. The thyroid glands of the feti taken from the thyroidectomized does showed hyperplasia and evidence of hyperactivity. The maternal organism under these circumstances was apparently using large amounts of fetal thyroxin. The drain on the fetal thyroid

in turn resulted in fetal hypothyroidism and hypercholesteremia. Sections of the placentae obtained in such cases showed severe endarteritis of the placental arteries.

Vorzimer and his colleagues ('37) have taken the view that in their experience the majority of women who develop a toxemic condition during pregnancy have had a constitutional habitus which in itself was indicative of some endocrine dysfunction. Among the characteristics which they considered important as presaging a toxemia were obesity, abnormal distribution of hair, "acromegloid" changes in the facies, a male and primitive type of pelvis, and a low basal metabolic rate. One or more of the above characteristics occurred in 98 per cent of the 120 toxic patients studied by Vorzimer and his coworkers, as compared with 15 per cent in the 100 normal control cases. Moreover, 50 per cent of the toxic patients exhibited two or more of the characteristics.

A great deal of attention has been paid to the role of the posterior lobe of the pituitary in producing the toxemias of pregnancy. Fauvet ('33) has shown that edema and increased blood pressure could be induced in nonpregnant animals by the injection of extracts of the posterior lobe of the hypophysis. From these observations

he argued that the eclamptic syndrome might be produced by a pathological hyperfunction of the pars nervoxa of this gland. Anselmino, Hoffman, and Kennedy in 1932 reported that the intravenous injection of preparations of the post-pituitary to unanesthetized dogs resulted in hyperglycemia, a lowered CO_2-combining power of the blood, and an increase in the inorganic phosphorus in the blood. Similar changes in the blood have been reported for eclamptic women.

In addition, Anselmino, Hoffman, and Kennedy ('32) found excessive amounts of antidiuretic and pressor principals in the blood of women suffering from eclampsia. These investigators agreed with Fauvet that there was probably a hyperfunction of the posterior lobe of the hypophysis, but added that hyperfunction was accompanied by some disturbance of the normal balance between the pituitary and thyroid glands. It is of interest to note at this point that other investigators, such as Byrom and Wilson ('34) and Hurwitz and Bullock ('35), have been unable to demonstrate unusual amounts of pressor and diuretic principles in the blood of eclamptic patients.

Greene ('37) observed extensive histological changes in the endocrine glands especially in pars intermedia of the pituitary. He feels that these changes are closely

connected in some way with the disorder of pregnancy in rabbits.

Dietary deficiency as a primary cause-- In discussing the relation of dietary deficiencies to the toxemias of pregnancy, Theobald ('35) goes so far as to say: "There is no such thing as a toxemia of pregnancy if by this term a toxin or toxins peculiar to pregnancy be postulated, and the manifestations enumerated . . . are expressions of dietetic deficiency or deficiency disease," p. 1031. He believed calcium to be the most essential substance in the diet, his premise being that a calcium-poor diet results in hepatic dysfunction, so that the liver can no longer detoxify substances absorbed from the bowel. The presence of toxic substances in the blood stream then injures the whole body including the kidneys. Theobald based his contention on extensive clinical experience in treating patients with a toxemia of pregnancy by means of an adequate diet well-fortified by calcium and the vitamins.

Strauss ('35) believes that an adequate protein intake is an important factor in the prevention or cure of the toxemias of pregnancy. He treated 15 women suffering from pre-eclampsia with a diet rich in protein and the vitamin B complex. Each of the women showed a loss in

weight readily attributable to a decrease in edema together with a gradual decline in the toxic symptoms. Five other women in a similar toxic condition were given a diet of approximately equal caloric value but low in protein. No loss in weight occurred and two of the women became worse during a period of two weeks. He, therefore, concluded that the improvement in the first group of women was due to an increased intake of protein. The protein intake has a marked influence upon the level of protein in the blood. It is possible that if the body is losing protein because of renal injury, an excess amount of this food nutrient must be ingested if the blood proteins are to be maintained at the normal level. Harden, McEllroy, and Huggins ('35) also have suggested that eclampsia occurs if the concentration of the blood proteins is decreased.

Several investigations have pointed to the possibility that the disturbance of pregnancy noted in rats may have a dietary basis. The work of Hartwell ('27) suggested that the quality of fat in the diet might be an important factor. Pregnant rats fed a diet composed of caseinogen, potato starch, marmite, salt mixture, butter, and cod liver oil frequently died in convulsions near term. However, rats fed a similar ration containing

slightly more caseinogen, less butter fat, and no cod liver oil were able to produce five or six litters.

In the laboratory at the Iowa State College, the feeling has grown that the disorder of pregnancy noted in rats and believed to be analogous to eclampsia in women is due to a dietary deficiency. The evidence upon which this hypothesis is based is described below. The rats developing a toxic condition were maintained on a basal diet containing 25 per cent of dried autoclaved pork muscle. If the pork content of the diet was increased to 50 per cent, the number of fatalities was decreased. Then it was found that a diet identical to the basal pork ration except that beef was substituted for the pork at the 50 per cent level was capable of supporting reproduction into the sixth generation.[1]

Attempts have been made to identify the missing factor by adding various supplements to the basal pork ration. Of some 25 different materials used as supplements, only two besides the large amount of beef caused an improvement in gestational performance. Rogosheski in 1936 reported that when the basal pork ration was fortified with 2 gm. of fresh liver daily, the reproductive performance of the females fed this diet was equal

1. Unpublished data in the files of the Nutrition Laboratory at Iowa State College

to that of the animals in the stock colony.

The second substance which proved to be effective was lipocaic, a fat-metabolizing hormone isolated from the pancreas by Dragstedt and his co-workers ('36). Only 4 mg. daily of this substance was required as a supplement to the basal pork ration. Pregnant females on this diet gave a reproductive performance equal to that of the animals of the stock colony (Wilcox, '37).

In this connection it is also interesting to note that certain substances such as choline chloride, lecithin, liver extract, and riboflavin actually seemed to increase the incidence of the disorder.

Formation of toxic substances in the placenta

Although occasional cases of toxemia occur postpartum, the majority improve rapidly following delivery. It is natural, therefore, that many theories of the etiology of the toxemias of pregnancy have centered around the belief that some toxic substance is elaborated in the placenta. Young and Miller in 1921 were the first to present such a theory. They found acute infarcts upon histological examination of the placentae of patients suffering from eclampsia. They attributed the formation of these infarcts to a blockage of the maternal blood

stream. Bartholomew and Kracke ('32) described similar acute placental infarcts in patients suffering from eclampsia. The work of these last investigators, however, indicated that thrombosis or even rupture of the exposed fetal arteries was the cause of the infarcts. Bartholomew and his co-workers believed that the typical syndrome of eclampsia was due to absorption of toxic substances such as guanadine or histamine from the infarcted areas. This theory was supported by the fact that non-pregnant guinea pigs injected with an autolysate of human placental tissue developed the clinical syndrome of eclampsia together with the typical hepatic and renal lesions. Bartholomew and Colvin ('38) agree with Patterson, Hunt, and Nicodemus ('38) that hypercholesteremia resulting in cholesterol deposits in the arterial walls is a factor in the formation of the infarcts. The view that the toxic substance absorbed by the maternal organism is guanidine has been supported by the observation of Andes, Andes, and Meyers ('37) that the concentration of guanidine in the blood of toxic patients is above the normal level.

Smith and Smith ('35) have found excessive amounts of prolan accompanied by low estrin values in the sera of women with eclampsia. Analyses of extracts of various organs have led these authors to believe that the placenta

elaborates these hormones. Smith and Smith, therefore, suggest that the changes in the normal balance between estrin and prolan may cause the changes noted in eclampsia.

An interesting new hypothesis has been offered by Page ('39). He argues that if for any reason the placenta fails to receive a sufficient blood supply, it might be able to increase the systemic blood pressure and so increase its blood supply. The placenta has no nerve connections by which this could be accomplished, so it is necessary to consider that the placenta elaborates a pressor substance. Such a pressor substance, especially if produced in large amounts, might be toxic to the maternal organism.

PURPOSE OF THE EXPERIMENT

In recent studies conducted in the Nutrition Laboratory of the Foods and Nutrition Department of the Iowa State College, it has been observed that a supposedly adequate diet containing dried autoclaved pork muscle as the sole source of protein is incapable of supporting normal gestation when fed to the female albino rat. Dyar ('35) observed that 33 per cent of the rats fed the basal pork ration designated as Pork I died at parturition with symptoms characteristic of eclampsia. The observations first reported by Dyar have been confirmed again and again (King, '36; Wilcox, '37, and Walliker, '38). Factors responsible for the appearance of the syndrome have been studied in the laboratory.

The quality of the diet as a possible etiological agent in the production of the disorder was an especially interesting basis for speculation. Upon first analysis, it seemed that the ration as formulated might be low in vitamin E, vitamin A, or some factor of the vitamin B-complex. However, experimental data collected in the laboratory supported the belief that the diet contained an adequate supply of the known vitamins. The possibility that the proteins of the dried autoclaved pork

muscle were not well utilized in the animal economy was also eliminated (Stevenson, '38). We believe, therefore, that the disease is not related to a deficiency of any of the recognized dietary essentials. Instead, evidence points to the hypothesis that the diet as formulated is deficient in some unknown dietary factor. For example, Rogosheski ('36) reported greatly improved gestational performance in rats fed the basal pork diet fortified by 2 gm. of fresh liver daily. Similar results were reported by Wilcox ('37) when 4 mg. of lipoeaic were used as a dietary supplement. The findings were so clean-cut and decisive that we could only conclude that these substances contain some dietary factor or factors essential for normal gestational performance in the albino rat.

Relatively few reports in the literature suggest that a toxemic-like condition similar to the one described by Dyar can be produced experimentally by dietary manipulation. The importance of her findings and their possible significance in clinical situations is evident. Dyar and her successors described only the gross external symptoms of the disorder. It was important, therefore, to establish in detail the nature of the abnormal reproductive performance noted in experimental animals and to determine

whether any histologic and metabolic disturbances occurred that resembled the pathologic changes reported as characteristic of toxemic pregnancies in human beings. The first objective of the research herein reported was, therefore, a study of the general pathological changes associated with gestational failure in female albino rats fed a diet containing dried, autoclaved pork muscle. The deviations from normal were analyzed in terms of the physical state of the animals, and the biochemical and histological changes in the tissues. Although the potency of certain dietary supplements (liver and lipocaic) in preventing nutritive disaster had been demonstrated in the laboratory, the possibility existed that the findings were merely fortuitous. For this reason the second objective was to determine whether feeding certain dietary addenda prevented the appearance of the symptoms studied in the sick rats. This we believed would constitute a true test of the efficacy of the supplements added to the Pork I ration.

GENERAL PLAN OF THE EXPERIMENT

The experiment herein described was planned primarily to establish certain characteristics of the typical disorder that occurs in pregnant rats receiving a supposedly adequate diet containing dried, autoclaved pork muscle as the source of protein. To achieve this purpose the gestational performance of rats fed the pork-containing diet known as Pork I was compared with that of normal animals reared on the standard diet routinely fed the stock colony. Inasmuch as it seemed possible that lesions occurring in the gravid animals might be due in part to the strain of pregnancy, both virgin and mated females were maintained on each experimental diet for the sake of comparison. The value of liver, lipocaic, and liver extract as dietary supplements was tested. The groups receiving these materials were treated exactly as were the rats in the control and experimental series. The animals used in this experiment may, therefore, be divided into three main groups as follows:

1. Control group (Steenbock V)
2. Experimental group fed the basal pork ration (Pork I)

3. Experimental groups fed the basal pork ration fortified with a supplement

   a. Fresh liver (Pork 7)

   b. Lipocaic (Pork 39)

   c. Liver extract (Pork I)

Further information concerning each experimental group is given below. The control group consisted of 30 females fed the stock ration, Steenbock V. One-half of the 30 animals were mated, the other half were kept as virgins.

The first experimental group contained 30 females. They were fed the basal pork ration (Pork I). Fifteen females were mated with males from the stock colony. However, data collected in the laboratory showed an apparent increase in both fetal and maternal mortality when males fed the basal pork ration were used for mating purposes instead of males fed the stock diet. In view of these observations, an additional group of 15 females grown on the Pork I diet were mated with males also fed the Pork I diet.

Fresh liver was chosen as a dietary supplement because Rogosheski ('36) found that the daily addition of 2 gm. of fresh liver to the basal pork ration resulted in a slightly better reproductive performance than was

observed even in the stock colony. The Pork I ration supplemented with fresh liver was known as Pork 7. Only two groups of five animals each, i.e., virgin rats, and females mated with males from the stock colony, were maintained on this diet.

The effect of lipocaic was tested because Wilcox in 1937 showed that the reproductive performance of female rats receiving as little as 4 mg. of lipocaic as a daily supplement to the basal pork ration was equal to that of the females in the stock colony. The Pork I ration fortified by lipocaic was called Pork 39. The sample of lipocaic used in the present study (1937-38) was obtained from the Eli Lilly Company.[1] This firm had prepared the lipocaic as a by-product in the manufacture of insulin. Dr. Dragstedt found that this lipocaic was approximately one-half as potent as that fed by Wilcox in 1936-37[2] in preventing fatty livers in depancreatized dogs given insulin. Therefore, the 1937-38 lipocaic was fed at the 10 mg. level in order to insure the presence of an adequate amount of the protective substance. Forty-five animals distributed in the same way as the females fed the Pork I ration were maintained on the diet.

Dragstedt ('37) has indicated that lipocaic is a

1. The material was obtained through the courtesy of Dr. L. Dragstedt.
2. Personal communication from Dr. L. Dragstedt to Dr. P. P. Swanson

pancreatic hormone. Therefore, it is possible that serious metabolic consequences may follow the feeding of an excessive dose of lipocaic.[1] This hypothesis was tested by giving one group of animals in the lipocaic series 500 mg. of the substance daily as a supplement to the basal pork ration. This dietary group contained 30 animals. It was not possible to obtain enough lipocaic to offer this quantity of the supplement to as large a group of animals as was used in the other series.

It seemed necessary to add a third group to the lipocaic series because as the study progressed it became evident that the reproductive performance of the animals fed 10 mg. of lipocaic would not equal that reported by Wilcox ('37). For this reason, the daily dosage was increased to 40 mg. so as to supply an adequate amount of the active substance. The group of 30 animals fed this modification of the diet were similar to the group fed the Pork I diet, with the exception that no pregnant females mated with males fed the same test were included.

The effect of supplementing the Pork I ration with liver extract was studied because King ('36) showed that the incidence of the reproductive failures observed by

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1. Personal communication from Dr. L. Dragstedt to Dr. P.P. Swanson

Dyar ('35) was increased when 0.5 gm. of a water-soluble, alcohol-soluble extract of liver was added to the basal diet. The symptoms exhibited by the animals before death seemed identical with those shown by the females which died on the Pork I ration (Dyar '35). It seemed advisable, therefore, to compare the lesions produced on the Pork I diet supplemented with the liver extract with those present in the organs of animals fed the Pork I ration. A group of 45 animals was fed this diet. The Pork I diet supplemented with liver extract was designated as Pork 31.

To determine the effect of pregnancy, per se, it was necessary to obtain comparable observations on pregnant and virgin animals; therefore, each gravid female was paired with a virgin female. The pair was fed the same diet. The virgin animal was killed when she had been fed the experimental diet the same number of days as the pregnant member of the pair.

Previous investigations in the laboratory of the Foods and Nutrition Department at the Iowa State College showed that the average length of gestation in rats fed the Pork I diet was 22.3 days (Wilcox, '37; King, '36). In the present study, the pregnant females were killed

21.5 days after the initiation of pregnancy in order to obtain data concerning the condition of the animal just prior to the birth of the litter. As the experiment called for the destruction of the animal, the second gestation period was chosen for certain of the observations made. This choice was influenced by our belief that the effect of diet upon various organs would be more pronounced at this time than at the end of the first pregnancy. The plan also afforded opportunity to enlarge the study by observations on the progress of the first pregnancy.

In order to study the reproductive performance in the various experimental groups, data were obtained from three sources. Some observations were made while the animal was alive, others were obtained at autopsy, and still others were from analyses made of tissues removed at autopsy. These data furnished information that could be used in the evaluation of gestational behavior in pregnancies I and II. Gestation I was studied in regard to (1) the manner in which pregnancy progressed from the time of ovulation to parturition, (2) the condition of the litter at birth, and (3) the vitality of the litter.

The same indices could not be used in studying Gestation

II since the mother was killed. However, autopsy records added new and pertinent information. Gestation II was assessed as follows: (1) the manner in which pregnancy progressed from the time of ovulation to parturition, (2) intra-uterine development of feti, and (3) size and condition of feti and placentae.

Other data were collected to secure information regarding the pathological changes associated with gestational failure. The items studied were (1) physical vigor and general condition of the animals, (2) appearance of the visceral organs, (3) water consumption during pregnancy, (4) changes in body weight during pregnancy, (5) weights and moisture content of certain internal organs, (6) fat content of the liver, (7) histological appearance of certain internal organs, and (8) histological appearance of feti and placentae.

In some instances the amount of tissue obtained from one animal was too small to be used for all the analyses desired. Therefore, the females, both virgin and mated, were arbitrarily divided into two lots. The organs from one lot of animals were used to determine the weights and moisture content of the organs and the fat content of the liver. The weights of individual feti and placentae were also obtained from this lot of animals. The organs, feti,

and placentae of the second lot of females were used in the preparation of histological sections. Whenever possible, the organs of the females exhibiting symptoms of toxemia were so divided that they could be used for the entire series of analyses.

The general design of the experiment is summarized in Table I.

TABLE I. DISTRIBUTION OF ANIMALS IN VARIOUS EXPERIMENTAL GROUPS AND USE MADE OF ORGANS REMOVED AT AUTOPSY

Diet of females	Reproductive status	Diet of males used in mating	Number of animals used	
			For preparation of histological sections	For determinations other than histological
Steenbock V (Control ration)	Mated	Steenbock V	5	10
	Virgin		5	10
Pork I (Basal ration)	Mated	Steenbock V	5	10
		Pork I	5	10
	Virgin		5	10
Pork 7 (Pork I plus fresh liver)	Mated	Steenbock V	5	-
	Virgin		5	-
Pork 39A (Pork I plus 10 mg. lipocaic)	Mated	Steenbock V	5	10
		Pork 39A	5	10
	Virgin		5	10
Pork 39B (Pork I plus 40 mg. lipocaic)	Mated	Steenbock V	7	8
	Virgin		7	8
Pork 39C (Pork I plus 500 mg. lipocaic)	Mated	Steenbock V	5	5
		Pork 39C	5	5
	Virgin		5	5
Pork 31 (Pork I plus liver extract)	Mated	Steenbock V	5	10
		Pork 31	5	10
	Virgin		5	10

EXPERIMENTAL PROCEDURE

ANIMALS USED

General

The animals used in the investigation herein described were albino rats (Mus Norvegicus Albinus) of Wistar stock, strain A, belonging to the stock colony maintained by the Foods and Nutrition Department of the Iowa State College. These animals had been inbred by brother and sister matings for 71 generations. The group from which the experimental animals were taken represented the twenty-first generation bred in the Iowa State College laboratory. In this interval they had been reared on several modifications of a stock diet composed of mixed grains, that was originally described by Steenbock in 1923. In 1932 the formula of the diet, known as Steenbock V, was permanently established, and thereafter the components of the diet were kept as uniform as possible from season to season, and from year to year (Swanson, Stevenson, and Nelson '38).

The number of young in the litters produced by the stock colony females was reduced to eight on the fourth day after birth. The young were weaned when 28 days old. Three or four young females were caged together from the

time of weaning until they reached sexual maturity, as judged by the opening of the vaginal orifice. At this time they were divided into the experimental groups making up the experiment.

The uniformity of the stock animals in regard to growth has been discussed by Timson ('32). She found that average increments in weight made during specific intervals by animals representing successive generations over a period of four years were nearly identical.

However, at the present time no information is available concerning the uniformity of successive generations of animals in the colony in regard to reproduction. Therefore, inasmuch as the study herein described deals especially with gestational performance, it seemed wise to investigate the uniformity of the colony in regard to factors that are a measure of the progression of normal pregnancy. The variation in the implantation per cent, placental index, and fertility per cent of animals of the stock colony representing the five generations reared from 1935 to 1938 was determined. (See page 103 for definition of terms.) The mean values and standard variations of the means for each generation are presented in table II. An analysis of variance of the data (table 1, in the appendix) showed no significant difference between

generations in regard to either implantation per cent, placental index, or fertility per cent.

TABLE II. GESTATIONAL PERFORMANCE OF RATS COMPOSING FIVE GENERATIONS OF THE STOCK COLONY

Generation	Implantation per cent		Placental index		Fertility per cent	
	Mean	s	Mean	s	Mean	s
18th	88.6	21.4	100.0	0.0	88.6	21.4
19th	81.7	24.9	97.6	10.8	79.4	55.4
20th	83.3	24.1	97.9	10.2	81.2	24.7
21st	80.4	24.9	96.4	13.1	76.8	25.4
22nd	90.4	19.9	100.0	0.0	90.4	19.9

Distribution of Animals Used in Experiment

The rats used in the present experiment were taken from the first, second, and third litters produced by females in the stock colony. Observations made in the laboratory have led to the belief that the young of the second litter were the most vigorous. Recent data collected by Graham ('39) show that if the hemoglobin content of the blood is taken as an index of physiological well-being, the young representing the second litters are superior to individuals making up later litters. For the study herein described, an attempt was made to distribute

the rats representing different litters as evenly as possible among the experimental groups. The data presented in table III show that with the exception of the group fed the Pork 39 B diet, approximately two-thirds of the animals in each group were derived from the first litter. The other one-third were for the most part representatives of the second litter. Of the 235 rats used, only 7 were derived from the third litter. In the group of rats fed the Pork 39 B diet, the relations between the number of animals derived from the first and second litters were just reversed, i.e., one-third from the first litter and two-thirds from the second litter. The deviation in the source of animals in this experimental group was due to the fact that the group was not started until it became evident that the reproductive performance of the animals fed the Pork 39 A diet would not equal that reported by Wilcox ('37) for animals fed the Pork I diet supplemented by 4 mg. of lipocaic.

TABLE III. SOURCE OF EXPERIMENTAL ANIMALS IN RESPECT TO PARITY OF MATERNAL RAT

	Number of rats derived from litter 1	Number of rats derived from litter 2	Number of rats derived from litter 3	Total number of rats used
Steenbock V	20	10	0	30
Pork I (Basal ration)	31	14	0	45
Pork 7 (Pork I plus fresh liver)	7	3	0	10
Pork 39A (Pork I plus 10 mg. lipocaic)	26	14	5	45
Pork 39B (Pork I plus 40 mg. lipocaic)	7	23	0	30
Pork 39C (Pork I plus 500 mg. lipocaic)	18	10	2	30
Pork 31 (Pork I plus liver extract)	30	15	0	45
TOTAL	139	89	7	235

Uniformity of Animals Used in Experiment

If the results obtained by the feeding of various diets to experimental animals are to be compared, the animals used must be as uniform as possible at the beginning of the experiment. Also, it is important that the animals used be vigorous and in good condition. Mason

and Ellison ('35), in regard to this point, wrote "Utilization of inferior young has invariably led to atypical results, especially in studies concerned with the male or female reproductive system." (p. 10). The following characteristics were used to check the uniformity of the animals used in the experiment herein described: (1) body weight at weaning, (2) age at sexual maturity, (3) body weight at sexual maturity, (4) body weight when study of vaginal smears was initiated, (5) age at initiation of first pregnancy, (6) body weight at initiation of first pregnancy.

As can be seen from table IV, the average values for the characteristics described above were very uniform for the animals composing the various experimental groups, with the exception that the animals fed the Pork 39 B diet were older and heavier at sexual maturity than the animals in any other group. However, all the groups were uniform in regard to age and body weight at initiation of the first pregnancy. The variation in age and body weight at sexual maturity shown by the group fed the Pork 39 B diet was probably due to the fact that these animals were for the most part derived from the second litter while most of the animals in the other groups were taken from the first litters.

TABLE IV. AVERAGE UNIFORMITY OF ANIMALS USED IN THE EXPERIMENT[1]

Experimental groups	No. of animals	Body weight at time of weaning	Age at sexual maturity	Body weight at sexual maturity	Body weight when study of vaginal smears was initiated[2]	Age at initiation of first pregnancy	Body weight at initiation of first pregnancy
		gm.	days	gm.	gm.	days	gm.
Steenbock V (Control ration)	30	48.4	43.1	82.9	117.8	70.8	144.1
Pork I (Basal ration)	45	47.0	43.2	86.7	113.0	68.1	140.3
Pork 7 (Pork I plus fresh liver)	10	48.5	44.0	84.4	114.2	70.0	135.0
Pork 39A (Pork I plus 10 mg. lipocaic)	45	47.7	46.9	85.1	111.0	76.6	141.8
Pork 39B (Pork I plus 40 mg. lipocaic)	30	50.1	52.2	110.8	118.0	72.3	138.4
Pork 39C (Pork I plus 500 mg. lipocaic)	30	48.9	45.1	87.8	110.2	74.7	139.4
Pork 31 (Pork I plus liver extract)	45	46.4	44.2	84.5	113.4	74.0	137.2

1 For data pertaining to the uniformity of the sub-groups used to obtain the average values for the groups listed, see table 2 in the appendix
2 Study initiated when rats were 2 months old

CARE OF ANIMALS

At sexual maturity the female rats were housed separately in round wire-mesh cages with raised bottoms. Each cage was set in an enamel pan lined with a paper towel. The raised floor of the cage and the size of the wire mesh helped to prevent the animals eating their excreta. The paper towels were changed daily. Food and water were presented to the rats ad libitum in glass jars wired to the sides of the cage. The food dishes and water cups were washed and sterilized three times each week. The cages and pans were washed and sterilized weekly. The temperature of the laboratory was recorded once daily. During the period from September, 1937, to May, 1938, when the experiment was in progress, the temperature was fairly uniform, ranging from 75 to 80 degrees Fahrenheit. An attempt was made to reduce the incidence of lung infections in the animals by keeping the air as free from dust as possible (Moise and Smith, '30). As air is pulled into the room from the hall by two large exhaust fans, dust from the hall finds its way into the laboratory. To prevent this infiltration of dust, a filter made of two layers of cheese cloth was tacked to the ventilator in the door opening into the

hall. The cloth filter was changed weekly. The use of a compound of paraffin oil and sawdust in sweeping the floor also helped to keep the dust out of the air.

The food of the rats receiving the various pork containing diets was weighed daily except Sunday, and the amount of food consumed was recorded. A double portion of food was offered to the rats on Saturday. A surplus of food, i.e., 2 to 5 gm. more than the animal was expected to eat, was offered daily. The uneaten food was discarded when the food cups were changed. By this careful regulation of the amount of food offered, the development of rancidity in the food and excessive waste were avoided. No record was kept of the food consumed by the animals fed the mixed grain diet (Steenbock V).

If accurate analyses of the fat and moisture content of the liver are to be made, the animal must be in the post-absorptive state at the time of death. Also a uniform metabolic state is desirable if histological sections of organs from different animals are to be compared. The animals were therefore starved for 10 hours before they were killed.

The amount of water consumed daily by the pregnant females was recorded from the twelfth day of pregnancy.

until parturition or death. Each pregnant animal was offered 100 cc. of distilled water in a crystal water fountain twice daily. The water remaining in the fountain was measured at approximately 8 A. M. and 6 P. M. every day, and the amount consumed calculated "by difference."

Each animal was weighed weekly. The pregnant females were also weighed daily from the twelfth day of gestation until seven days after the birth of the litter. In addition, the gravid females were weighed daily at 8 A. M., 12 noon, 4 P. M., 6 P. M., and 10 P. M. from the eighteenth day of pregnancy until parturition. The plan of frequent weighings was used for two reasons. First, several investigators in the field of human medicine have discussed the relation between fluctuations of body weight in late pregnancy and eclampsia (Siddall and Mack, '33, '38; Strauss, '37). Second, with the frequent handling early symptoms of a toxemic condition were detected. The eighteenth day was chosen for the initiation of the study of the fluctuations in body weight, as the rat fetus makes its maximum increase in weight from the eighteenth to the twenty-first day of the gestation period (Huber, '15).

The young were weighed separately as soon as possible

after birth. The lungs of the young found dead at this time were removed and placed in water. If the lungs floated, it was believed that the fetus had drawn air into its lungs and therefore had been born alive. Conversely, if the lungs failed to float, the fetus was judged not to have breathed after birth, and was recorded as having been born dead (Webster'30).

As a routine procedure the litters were reduced to six on the fourth day after birth. We felt that the ability of a female to rear six young was an adequate and not unjust measure of lactation performance. Therefore, if six or more young were born, the mother rat was expected to rear six rats. If less than six young were born, the female rat was given an opportunity to rear the entire litter. If possible, three males and three females were retained from each litter. Those animals whose weight most nearly approached the average weight of the litter were saved. The entire litter was weighed daily as a group until the young were weaned. In addition, the males and females were weighed separately when four, seven, 14, 17, 19, 21, and 28 days old.

BREEDING TECHNIQUE

Vaginal Smears

The rhythmical changes occurring in the oestrous cycle of the female rat may be followed by histological examinations of the vaginal epithelium (Long and Evans, '22). Samples for the study of the cellular changes were obtained from the females by lightly touching the surface of the vagina with a glass rod. The specimen was removed daily at the same hour. When the smear was taken the rat was supported on its back in the left hand of the technician, the thumb and index finger holding the head. The tail was curled around the fifth finger of the right hand, and at the same time a sterile glass rod was gently inserted a short way into the vagina. As soon as the rod was withdrawn from the vagina, the tip was touched to a drop of distilled water on a clean glass slide. The smear thus formed was examined under the 16 mm. objective of a microscope. A substage light equipped with a blue ground glass filter was used for illumination. The glass rods were made of tubing 2 mm. in diameter, cut a convenient length and carefully fire-polished on both ends. To facilitate the analysis of the data obtained on oestrual rhythm, the rats were weighed daily at the time the

vaginal smear was taken.

Several precautions were taken to prevent infection as a result of vaginal examination. Immediately after use the rods were placed in a strong soap solution. They were then washed and inserted into thick-walled glass test tubes containing about 5 cc. of distilled water. The tubes, plugged with cotton, were sterilized for 20 minutes in a pressure cooker at 15 pounds pressure.

The classification given by Long and Evans ('22) was used as a basis for the determination of the stages of the oestrous cycle. These authors have divided the cycle into five distinct stages, i.e.:

Stage 1. Epithelial cells, the pro-oestrous period

Stage 2. Epithelial and cornified cells, the oestrous period

Stage 3. Many cornified cells

Stage 4. Cornified cells and many leucocytes, the metaoestrous period

Stage 5. Leucocytes, epithelial and cornified cells, the dioestrous period

No vaginal smears were examined before the females were eight weeks old, because matings among the rats fed the Pork I diet are not fertile until the animals are 65

days old (Wilcox '37, King '36). After the onset of pregnancy, the smears were examined carefully for the presence of red blood cells. In the normal rat the implantation of the embryo in the uterine wall between the twelfth and fourteenth days of pregnancy results in the appearance of free blood in the vagina (Long and Evans '22). The occurrence of free blood in the smear or on the rod before the twelfth day (Mason '37) indicates some abnormality in the establishment of the fetal-maternal syncytium. The study of the vaginal smears was discontinued during lactation. Samples of the vaginal contents were not removed from the virgin females.

Mating of Animals

After the study of the vaginal smears was initiated, one complete oestrous cycle was allowed to pass before the females were mated. The rat will mate late in the pro-oestrous period or in the oestrous period. Long and Evans ('22) have shown that the average length of the pro-oestrous period is 12 hours, and that of the oestrous period is 12 hours. Therefore, to be certain that the male would be present as the female came into heat, the animals were, whenever possible, mated when the females were in the pro-oestrous period. If, however, this period

was missed, the female was mated in stage 2, or the oestrous period. If no vaginal plug or sperm was found by the time the female had passed into stage 3, the male was removed. The vaginal smears were studied in the morning. The vaginal contents of the females that were mated were checked at 12 noon, 4 P.M., 6 P.M., and 10 P.M. for the presence of sperm or plugs.

Brother males were used for the first mating. In cases where it was impossible to use a brother rat, a male of the same age (± three days) as the female was used. Males of proven fertility between three and six months of age were used for the later matings.

COMPOSITION AND PREPARATION OF DIETS

In the study herein reported, five diets were fed. They were called Steenbock V, Pork I, Pork 7, Pork 39, and Pork 31. The composition, character, and preparation of each diet are described below.

Steenbock V Ration

The ration fed to the stock colony of rats grown in the laboratory as the source of experimental animals was given to one group of rats. This diet, Steenbock V, was a mixed whole grain diet based on one originally formulated by Steenbock in 1923. The ration as fed in the laboratory consisted of two parts, a basal portion and a supplementary portion.

The formula of the basal portion of the ration is shown below:

Yellow cornmeal ----------	64.0 gm.
Crude casein ----------	5.0 "
Linseed meal ----------	16.0 "
Ground alfalfa ----------	2.0 "
Sodium chloride ----------	0.5 "
Calcium carbonate --------	0.5 "
Yeast ----------	1.5 "
Irradiated yeast ---------	0.5 "
Wheat germ ----------	10.0 "
	100.0 "

The basal portion of the diet was fortified with milk,

lettuce, and meat. These supplements were prepared in the following manner:

1. Dried whole milk[1] was used as the source of the milk given to the rats. The dry preparation was liquified by mixing 130 gm. of powder with 1 qt. of water for five minutes in a Hobart mixer at high speed. To each quart of milk was added 1 tsp. of cod liver oil[2], and 2 cc. of a solution[3] containing small amounts of iodine, manganese, aluminum, and copper. The milk was fed daily according to the following schedule:

a. Each lactating female received 50 cc. per day;

b. Each pregnant female received 25 cc. per day;

c. Each male received 12.5 cc. per day;

d. Each resting female received 12.5 cc. per day.

2. The lettuce used was ordinarily obtained from the Memorial Union Cafeteria or the Institutional Tea Room. It consisted mainly of discarded outside leaves from head lettuce. The leaves were washed in cold water and the

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1. Klim is powdered whole milk distributed by the Borden Co., New York. Sufficient dry Klim for one year was purchased once each year. This milk represented winter milk from one day's run in the factory.
2. Refined Norwegian vitamin tested Cod Liver Oil, U.S.P., imported by The Pearson-Ferguson Co., Kansas City, Mo.
3. The solution contained 0.08 gm. of potassium iodide, 0.316 gm. manganese sulfate, 0.098 gm. potassium aluminum sulfate, and 0.875 gm. of anhydrous copper sulfate in 100 cc. of distilled water.

crushed or decayed portions carefully removed. 10 gm. of lettuce weighed on a Chatillion scales were given to each rat three times each week.

3. Raw beef round which had been freshly ground through a clean meat grinder was obtained from a local market. 5 gm. of the meat measured with a calibrated aluminum spoon were given to each rat three times every week. The lettuce and meat were fed on alternate days.

Pork I Ration

General formula

The basal pork ration known as Pork I was fed to one group of experimental rats. The formula of the diet was as follows:

Canned pork muscle (dried to one-half its original weight)	25 gm.
Cornstarch[1]	53 "
Yeast[2]	5 "
Agar Agar[3]	2 "
NaCl[4]	1 "
Salt mixture[5]	4 "
Butterfat	8 "
Cod liver oil[6]	2 "
	100 gm.

1. Purchased in the local market in 280 lb. lots.
2. Yeast foam tablet powder purchased from Northwestern Yeast Co., Chicago, Ill.
3. Bacto-Agar purchased from the Difco Laboratories, Inc., Detroit, Mich.
4. Purchased in local market.
5. Osborne, T. B., and Mendel, L. B., J. Biol. Chem., 37, 223-299, 1919.
6. Refined Norwegian vitamin tested cod liver oil, U.S.P., purchased from the Pearson-Ferguson Co., Kansas City, Mo.

The diet was synthetic except for the source of protein. To keep the protein in the diet as uniform as possible, lots of green skinned hams, varying in size from 300 to 1000 pounds, were purchased at one time. The hams were boned, stripped of excess fat, and ground once through the medium plate of the meat grinder. The ground pork was packed into no. 2 enameled tin cans, one pound of meat being weighed into each container. The cans were then sealed and processed at 15 lb. pressure for 65 minutes. At the end of this time, the cans were carefully examined for leaks and cooled rapidly in cold running water. They were stored at room temperature until needed.

When the cans were opened for use, all the visible fat was removed from the surfaces. The meat was dried on metal trays covered with a piece of cheese cloth so that the meat did not come directly in contact with the metal. One thousand grams of meat were spread on each tray and dried to one-half the original weight in a current of warm air (85 to 95 degrees Centigrade). Usually one to two hours were required for the drying process.

The butterfat used in the diet was prepared from butter purchased at the Iowa State College Dairy. Four

pounds of butter were melted over a water bath and held at the melting point for three hours without stirring. At the end of this time the coagulated protein was skimmed from the top of the melted butter and the liquid fat decanted into a funnel equipped with a copper jacket to hold hot water. A plug of cotton was used as a filter. The butterfat was cooled and stored in the icebox until needed.

The diet was mixed twice weekly in a Hobart electric mixer at low speed (92 r.p.m.) The diet was then packed into enameled tin cans and stored in the ice-box.

In view of the observations made by Whipple ('34) relating to the reproductive behavior of rats fed a diet containing rancid fat, several precautions were taken to prevent the development of rancidity in the diet. For instance, the meat was protected from direct contact with metal during the drying process. Also, the diet was mixed frequently, in order that it might be kept fresh. As a check on rancidity, samples of canned meat dried to one-half the original weight, and of unopened cans of meat were sent to the National Live Stock and Meat Board for analyses on the rancidity of the fat.[1]

The determinations of the peroxide values were made on three samples of fat obtained from the cans of meat;

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1. The peroxide determinations reported in the table were made through the courtesy of Dr. R. C. Newton, of Swift and Company, Chicago, Illinois.

i.e., the fat extracted from the dried meat, the fat scraped from the surface of the meat in the unopened cans, and the fat extracted from the canned meat after the visible fat had been removed (table V). Of the three kinds of fat tested, only that scraped from the top of the cans of meat had a peroxide value sufficiently high (4.7 - 9.0) to indicate that considerable oxidation had occurred[1] (Lease and Steenbock, '39). However, if moderately rancid lard has a peroxide value of 30, even the visible fat in the cans of meat was only slightly rancid. It should be recalled also that the visible fat was routinely removed before the meat was dried.

1. The determinations of peroxide values were made by the Institute of American Meat Packers through the courtesy of W. Lee Lewis, Secretary of the Committee on Nutrition.

TABLE V. PEROXIDE VALUES OF FAT OBTAINED FROM CANNED PORK[1]

Sample	Fat extracted from sample as received	Fat taken from top of cans of cooked meat	Fat extracted after drying in vacuum oven to 50% loss in weight	Fat extracted after drying in air oven with a current of air at 180° F.[2]
A_1 Pork (dried at Ames)	2.2	--	--	--
B_1 Pork (not dried)	--	6.3, 4.7, 5.6	0.4, 0.8, 1.4	0.4, 1.4
C_2 Pork (not dried[3])	--	8.4	3.6	3.6
C_3 Pork (not dried)	--	9.0	3.0	3.0

[1]Peroxide values expressed as milli-equivalents of peroxide per 1000 gm. of fat.
[2]This procedure was equivalent to that used in the laboratory for drying the meat.
[3]Canned 14 months before tests were made.

Adequacy of the Pork I diet

Judged by present day standards, the diet as formulated should provide sufficient amounts of all known dietary essentials. This view is supported by considerable experimental evidence.

Gestational disturbances similar in some respects to those reported by Dyar ('35) have been observed in animals maintained on a diet low in vitamin A (Mason '35). However, Dyar ('35) showed that 1 gm. of the Pork I diet fed daily as a supplement to the basal A-free diet supplied enough vitamin A to support normal growth in rats previously depleted of their stores of the vitamin in question. Dyar's data are reproduced in figure I. Neither did the female rats fed the Pork I ration show a persistent cornification of the vaginal epithelium. Such a change in the character of the vaginal epithelium.has been reported by Mason and Ellison ('35) as the first evidence of an inadequate intake of vitamin A.

Evans and Burr ('28) have shown that the vitamin B complex is necessary for normal reproduction in the rat. Each rat receiving the Pork I diet consumed approximately 0.6 gm. of yeast daily as calculated from the amount of food eaten. Husseman and Hetler ('31) as well as Evans

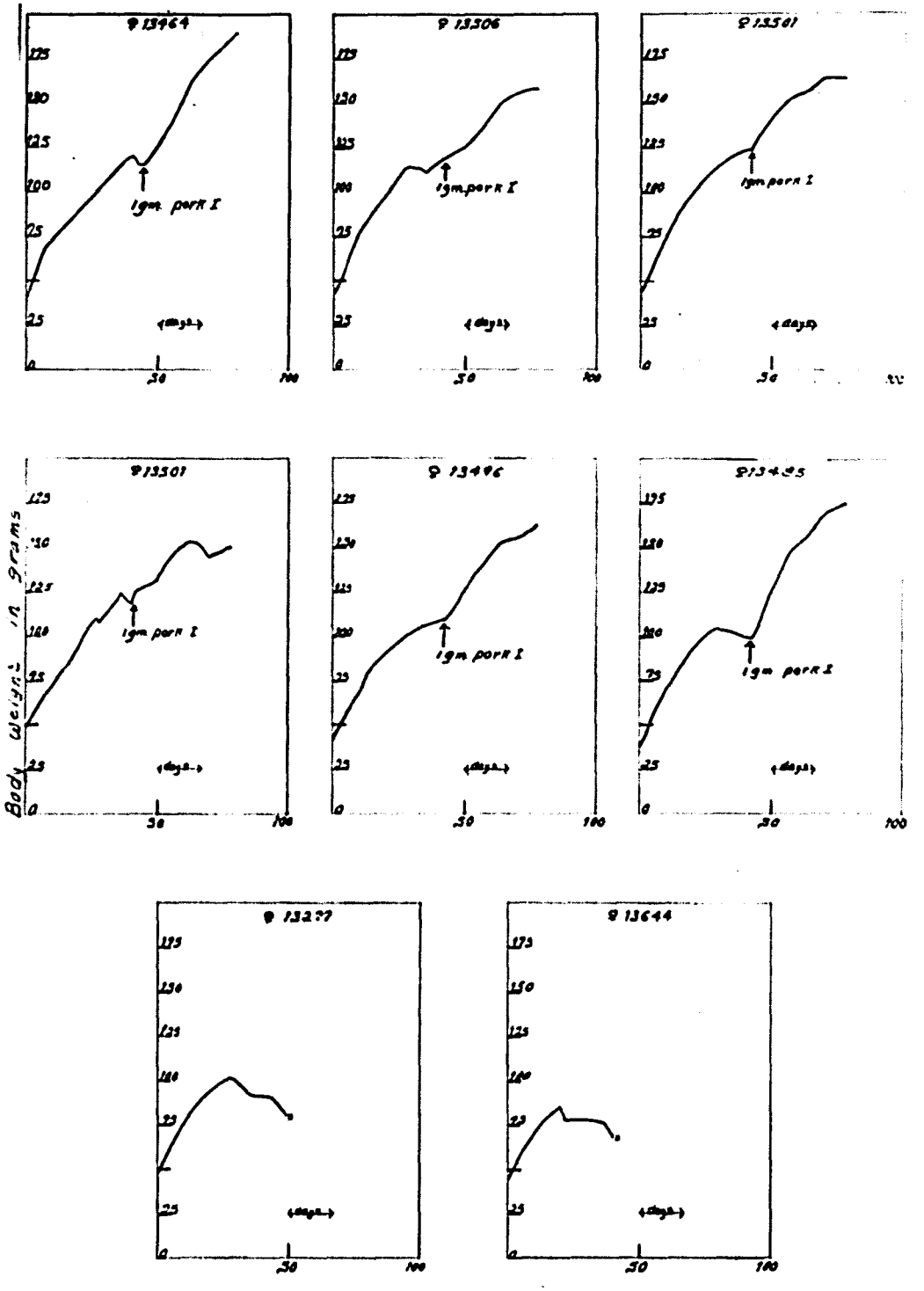

FIG. I. GROWTH INDUCED BY FEEDING 1 GRAM OF PORK I DIET TO RATS DEPLETED OF BODY STORES OF VITAMIN A. (GRAPHS REPRODUCED FROM DYAR, '35)

and Burr ('28) have shown that 0.6 gm. of yeast furnish sufficient vitamin B for growth and reproduction. The possibility of a deficiency was tested, however. When a diet similar to Pork I but containing 15 per cent yeast was fed to a group of 10 females, no improvement occurred in the reproductive performance. In fact, one female died in pregnancy with convulsions and other symptoms of toxemia.[1]

It was not considered necessary to include a source of vitamin C as such in the diet. Suden and Alley ('35) report that the teeth of rats reared for three generations on a scorbutic diet were histologically normal. Höjer ('26) has shown that a disturbance in the normal histology of the teeth is the first indication of vitamin C deficiency in guinea pigs. For this reason rats are believed not to require vitamin C in the diet.

The diet contained 2 per cent cod liver oil, which had a vitamin D potency of at least 85 international units per gm. of oil.[2] Therefore, the rats received approximately 17 international units of vitamin D per day as judged by the food consumed. As 2.7 international units have been shown to be adequate for the prevention of rickets in rats, the animals receiving the Pork I diet

1. Unpublished data in the files of the Nutrition Laboratory of the Foods and Nutrition Department.
2. Cod liver oil contains at least 85 U.S.P. units of vitamin D, which is equivalent to one international unit of vitamin D as defined and adopted by the Conference on Vitamin Standards of the Permanent Commission on Biological Standardization of the League of Nations in June, 1931. (U.S.P. XI, 261, 1936)

probably received an ample supply of vitamin D.

The Pork I ration as formulated contained no specific source of vitamin E. While the meat, cod liver oil, and butter fat probably provided an ample supply of this factor, it was possible that oxidation had destroyed the vitamin. However, in checking this point Dyar ('35) found that the addition of 10 drops of wheat germ oil daily to the Pork I diet failed to improve the reproductive performance of the females. When 10 drops of the same wheat germ oil were fed to females maintained on a mixed grain diet treated with ferric chloride and ether to destroy the vitamin E (Waddell and Steenbock, '32), normally developed litters were born. Moreover, when females maintained on a ration so treated were transferred to the Pork I diet following positive mating, they were able to produce normal young.[1] (See figure 2.) Therefore, the Pork I ration is believed to contain an adequate amount of vitamin E.

Gestational failures similar to those observed in females fed the Pork I ration have been reported by Evans, Lepkovsky, and Murphy ('34), in rats fed a diet deficient in the unsaturated fatty acids. However, the Pork I diet was thought to be adequate in the essential fatty acids, as lard, cod liver oil, and butter fat were all excellent

1. Unpublished data in the files of the Nutrition Laboratory of the Foods and Nutrition Department.

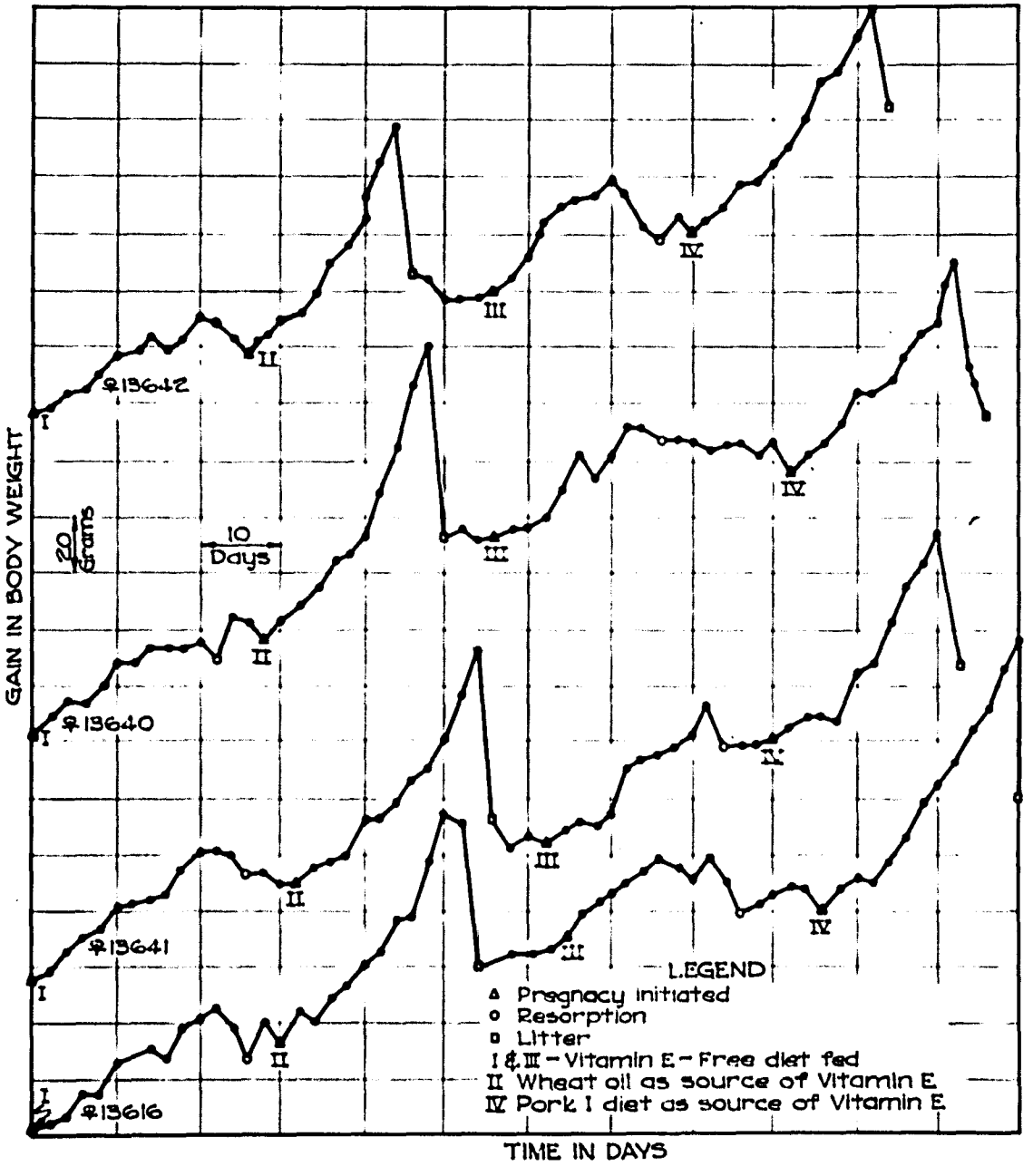

FIG. II. NORMAL PREGNANCIES INDUCED IN RATS DEPLETED OF BODY STORES OF VITAMIN E BY FEEDING EITHER WHEAT GERM OIL OR THE PORK I DIET AS A SOURCE OF VITAMIN E

sources of unsaturated acids. Jones ('31) showed that the Pork I diet contained 24 per cent of fat.

Whipple ('34) found that slightly rancid fats in the diet produced certain abnormalities in reproduction in rats. The fats she used had a peroxide value of 15 to 20. The fat extracted from the pork dried to one-half the original weight had a peroxide value of 1.2 to 2.2.[1] Therefore, the adequacy of the Pork I diet was probably not affected by the rancidity of the fat.

In evaluating the diet, the possibility that the protein was inadequate in quality for reproduction could not be overlooked. However, Rogosheski ('36) found that the addition of 1 gm. each of cystine, glycine, and glutamic acid to the Pork I ration failed to improve the reproductive performance of the females. Then in 1938, Stevenson demonstrated that the proteins of the Pork I ration were well utilized for maintenance by the adult male rat. She found that the biological value of the dried, canned, autoclaved muscle was as high as that reported in the literature for raw pork. Of course the fact that the proteins of the diet were adequate for maintenance does not mean that they were also adequate for

1. This range was recorded in a personal communication to Dr. P. Mabel Nelson and Dr. Pearl P. Swanson from W. Lee Lewis, Secretary of the Committee of the American Institute of Meat Packers but not in analytical table submitted.

reproduction. In this connection, Wilcox reported normal reproductive performance in rats fed 4 mg. of lipocaic daily as a supplement to the Pork I diet. It does not seem possible that 4 mg. of lipocaic contained enough protein or amino acids as such to materially influence the quality or quantity of protein in the diet.

Pork 7 Ration (Pork I plus Fresh Liver)

The diet known as Pork 7 was fed to one group of rats and consisted of Pork I supplemented with 2 gm. of fresh liver daily. The liver was purchased at a local market twice each week. The 2 gm.-portions were weighed to within 25 mg. on a torsion balance. The weighed pieces were stored on a watch glass in an electric refrigerator at a temperature of -18 to -20 degrees Centigrade. The liver was fed while still frozen and was usually consumed by the animals before it had a chance to thaw.

Pork 39 Ration (Pork I plus Lipocaic)

The basal pork ration, Pork I, supplemented with lipocaic was known as Pork 39. The material was added at three different levels, i.e., 10 mg., 40 mg., and 500 mg. The three diets containing lipocaic were fed to three different groups of rats. To facilitate the accurate

measurement of small quantities like 10 mg. and 40 mg., the lipocaic was mixed with corn starch in such proportions that 0.5 gm. of the mixture was equivalent to either 10 mg. or 40 mg. of lipocaic. The doses of lipocaic were measured with a calibrated aluminum spoon and fed in separate dishes. See table 3 in the appendix for the accuracy of successive portions of the two mixtures of corn starch and lipocaic measured with a calibrated spoon.

The rats refused to eat 500 mg. of lipocaic daily. Therefore, the daily dose of the supplement was mixed with the Pork I diet. The spoon used to deliver the 500 mg. of lipocaic was checked for accuracy in the measurement (table 3 in the appendix). The lipocaic used in 1938-1939 was obtained in two lots from the Eli Lilly Co. through the courtesy of Dr. Lester Dragstedt. It was prepared as a by-product in the manufacture of insulin. The material was a fine, dry, gray powder with a characteristic sour odor and bitter taste. It did not appear to be at all hydroscopic. Portions of lipocaic large enough to supply the supplement needed during one week were stored in glass jars with paraffined corks. These jars were kept in the electric refrigerator until needed.

Pork 51 Ration (Pork I plus a Liver Extract)

The Pork I diet supplemented by 500 mg. of a water-soluble, alcohol-soluble extract of liver was fed to one experimental group of animals. The dry powder was measured with a calibrated aluminum spoon and mixed directly with the diet. The average results obtained from ten weighings of the amount delivered by the spoon at different times are shown in table 3 in the appendix. Samples from two lots of liver extract were fed.

The liver extract[1] was prepared at the Wilson Laboratories by a method similar to that described for the preparation of Lilly Liver Extract No. 343.[2] As the preparation was very hydroscopic, it was stored in air-tight tin cans in an electric refrigerator. Amounts large enough to supply the supplement needed during one week were stored in the ice-box in covered half-pint fruit jars placed in a desiccator containing anhydrous calcium chloride.

1. Supplied through the courtesy of Dr. David Klein, of the Wilson Laboratories, Chicago, Illinois.

2. The detailed preparation of this material is described on page 273 of the New and Non-official Remedies, published by the American Medical Association, Chicago, 1935.

LENGTH OF EXPERIMENT

The mated females were killed 21.5 days after the initiation of the second pregnancy. In every instance, the animals mated between 6 P.M. and 8 A.M. The calculations for the time of death were as follows: If sperm or a vaginal plug was found at 10 P.M. the time was calculated from that hour. A four-hour error was possible in this calculation as mating might have occurred any time after 6 P.M. However, if sperm or a vaginal plug was found at 8 A.M., a more complicated problem was presented. In order to have as accurate a basis as possible for the calculation, a time half-way between 10 P.M. and 8 A.M. was chosen; i.e., 3 A.M. The use of this hour resulted in a possible error of five hours in calculating the time of coitus. A leeway of three hours in the time of death was allowed before or after the hour that represented the exact lapsing of the 21.5 days. For example, females which mated by 10 P.M. could be killed between 7 A.M. and 1 P.M.; while the others could be killed between 12 noon and 6 P.M. In actual practice the majority of the animals were killed at either 1 P.M. or 4 P.M.

The females that showed symptoms of a toxemic

pregnancy were killed just as death seemed imminent. The animals that died during a gestation period between the intervals of observation were examined as soon as possible after death.

Each virgin female was killed when it had been on the diet the same number of days as the pregnant female for which it served as a control.

AUTOPSY TECHNIQUE

Gross Observations

Just before the termination of the experiment, the general physical condition of each animal was described according to the outline shown in form 1 in the appendix. In the autopsy procedure, a definite routine was followed. The color and consistency of each organwere recorded as it was removed. The lungs, the base of the tongue, and the inner and middle chambers of the ears were studied for evidence of infection. The stomach and intestines were examined for ulcers and signs of hemorrhage. The relative amounts of fat in the subcutaneous, abdominal, perirenal, pericardial, and intermuscular depots were recorded. Any abnormal condition in the uterus such as hemorrhage or dead feti was noted. The outline used for recording the autopsy data is shown in form 2 in the appendix.

Technique Used in Removing Organs

Organs used for weight, moisture, and fat analyses

The animal was first stunned by a blow on the head and an incision extending from the anus to the diaphragm was made on the ventral median line. The abdominal wall

was then cut transversely from the edges of the incision exposing the viscera. This cut was made in such a way that a minimum loss of blood occurred.

The uterus of a pregnant animal was ligated at the oviducts and the cervix. Different colored threads were used for the ligatures of the left and right oviducts, so that the position of the feti in each horn could be recorded. The uterus was cut just posterior to the cervical ligature and removed together with the ovaries and some fat. The removal was performed with care so that there was a minimum loss of blood.

The liver was next removed by cutting the mesenteric attachments and the blood vessels. After the organ was excised it was blotted to remove any free blood present on the surface of the lobes, and freed of adhering fat. The color and consistency of the organ were recorded at this time.

The kidneys were removed by cutting the renal blood vessels and the perirenal fat. They were freed of all adhering fat and split longitudinally. The cut surface was blotted; the color and consistency of the cortex, medulla, and pelvis were noted. The spleen was removed by cutting the mesenteric attachments and the blood vessels at the hilum. After excision, it was cleaned

and blotted dry. The color and consistency of the organ were observed.

After these organs had been removed, the medial abdominal incision was extended anteriorly to the left of the sternum through the diaphragm and the ribs. This procedure exposed the heart which in the majority of cases was still beating. The vessels attached to the heart were cut near the atria. The atria and the attached blood vessels were removed carefully, leaving the atrioventricular valves intact. The ventricles were then split longitudinally to remove any clots of blood present. The cut surface was blotted well.

At this point in the procedure the pancreas was examined and any marked deviation from a normal appearance was noted. Next the stomach and intestines were removed and placed in a jar of cold water. Later these organs were examined for ulcers and signs of hemorrhage.

The mammary glands on the left side of the rat were then removed and freed of muscle and fat. A small muscle between the layers of glandular tissue was so difficult to remove that it was routinely left in place, and weighed with the mammary tissue.

The uterus after weighing was split from the cervix to the oviducts. A diagram was made of the relative

position of the feti in each horn of the uterus. Each fetus was freed of the amniochorion and the umbilical cord and dried on a paper towel. The cord was cut about 1/8 of an inch from the umbilicus in order to prevent excessive bleeding from the cut vessels. The activity of the fetus and relative development were recorded at this time. Each placenta was freed from the cord and membranes and dried.

The same general procedure was followed for the virgin animals except for the mammary glands, which were so poorly developed that they could not be removed.

Organs used for histological examination

In general the routine autopsy procedure described in the preceding section was followed in the removal of organs and tissues used for histological observation. However, none of the organs were freed of fat. Except in the case of the liver, the entire organ, just as it was removed from the animal was placed in the fixative. The pancreas and a portion of the duodenum were removed with the spleen and "fixed". The uterus was tied off as described above and immersed in the fixative.

Since the entire liver was too large to use for the preparation of sections, a wedge-shaped slice extending

from the free to the attached edge of the lobe was cut from the center of the large left lobe. The entire caudal lobe was also taken for a sample. Each of the samples was cut into thirds before it was placed in the fixative.

DETERMINATION OF WEIGHTS AND MOISTURE CONTENT OF ORGANS

Immediately after excision, each organ was placed in a tared weighing bottle. The weights of the heart, kidney, spleen and mammary glands were obtained to approximately the fourth decimal place on an analytical balance. The organs were dried in a Weber electric air oven at 105 degrees Centigrade until they reached constant weight. The per cent of water in the fresh organs was then calculated.

In the case of the liver, both fat and moisture determinations were made on the same organ. Approximately 1 to 2 gm. of liver were used for the determination of moisture. The remainder of the organ was used for fat analysis.[1]

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1. The moisture and fat analyses of the liver were made by Miss Ethelwyn Wilcox.

The weights of both the intact and the stripped uterus were obtained to the first decimal place on a trip balance. The feti and placentae were placed in tared weighing bottles immediately after they were removed from the uterus. Each placenta was placed in a weighing bottle with the fetus to which it had been attached. The feti and placentae were weighed to approximately the fourth decimal place on an analytical balance. The bottle containing both the fetus and the placenta was weighed first, then the placenta was removed from the weighing bottle with forceps and the bottle plus the fetus re-weighed. The weight of the placenta was therefore obtained "by difference." This procedure minimized the error in the weighing of the small placenta.

HISTOLOGICAL TECHNIQUE

Preparation of Sections[1]

The excised organs to be used for histological analysis were always placed immediately in the fixative. All of the organs used in this study were fixed in Zenker's solution[2]. This fixative consisted of two

1. Becker, E. R., and Roudabush, R. L. 1935. Brief directions in histological technique, Collegiate Press Inc., Ames, Iowa
2. The use of Zenker's solution as a fixative was recommended by Dr. L. S. Stone of the Yale University School of Medicine.

parts, A and B, which were combined just before use. Solution A contained 5 gm. of potassium dichromate, 10 gm. of mercuric chloride, and 1 gm. of sodium sulfate in 100 cc. of solution. 95 cc. of this solution were mixed thoroughly with 5 cc. of solution B, glacial acetic acid.

To prevent the dilution of the fixative by the amniotic fluid a concentrated Zenker's solution[1] was used to fix the feti and placentae. 3 cc. of the concentrated Zenker's solution were injected into the amniotic cavity of each fetus. The intact uterus was then submerged in a large volume of Zenker's solution of the usual strength.

The spleen, liver, pancreas, and pieces of liver were fixed for 12 hours. Kidneys and heart were removed from the solution after 12 hours, split longitudinally and replaced in fresh fixative for another 12 hours. The fixative surrounding the uterus was replaced by a fresh solution every 12 hours for two days (48 hours). The uterus was then cut between each fetus and returned to the fixative for another 12 hour period.

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[1] The concentrated Zenker's solution contained 10 gm. of potassium dichromate, 20 gm. of mercuric chloride, and 2 gm. of sodium sulfate in 100 cc. of solution. 95 cc. of this solution were combined with 10 cc. glacial acetic acid.

At the end of the fixation period the organs were washed in water and dehydrated in ethyl alcohol by passing the tissue through solutions of increasing strength[1]. The uterus and accompanying feti were kept in each alcohol about three times as long as the other organs. Cedar oil was used as the clearing agent.

The first organs studied were imbedded in Altman's imbedding mixture.[2] However, for the majority of the organs Tissuemat purchased from the Fisher Scientific Company was used. This product which exhibited physical properties which are very similar to those of Altman's imbedding mixture was found to be very satisfactory. The paraffin blocks were stored in the icebox until they could be cut. The sections were all cut eight microns thick with a Spencer rotary-type microtome.

Three to five serial sections of each organ were mounted on a slide. Five such slides were made of each organ. The sections on each slide were taken from five different portions or the organ. The sections were floated with distilled water onto slides coated with

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[1] The detailed procedure is given in the appendix.

[2] Altman's imbedding mixture contains 850 gm. of paraffin, 100 gm. of stearin, and 50 gm. of beeswax. The ingredients are melted, thoroughly mixed and filtered.

thin film of Meyer's albumin[1] and were flattened by warming either on a hot plate or above the flame of an alcohol lamp. The slides were allowed to dry not more than 18 to 36 hours before they were stained.

The sections were de-Zenkerized in 70 per cent iodinized alcohol before they were stained. All the slides were stained in undiluted Delafield's hematoxylin, destained in 0.33% hydrochloric acid and then "blued" in tap water. The sections were then counterstained in ethyl eosin. Gum damar was used as the mounting medium.

[1] Purchased from the Arthur H. Thomas Company.

Microscopic Analysis of Histological Sections

In analyzing the histological sections from any one organ, a composite description was made of all the sections mounted on the five slides prepared from each organ. Component parts of the organ were always examined in the same order. For example, in studying sections taken from a kidney, the cortex was examined first, then the medulla, and lastly the pelvis. In so far as it was practical, all sections from one organ, i.e., liver, from every rat in all the experimental groups were described before a different organ was studied.

The following terms were used to describe conditions noted in the microscopic study of the sections:

Hyperemia, an increased amount of blood within the blood vessels;

Hemorrhage, the presence of free blood outside of the blood vessels;

Thrombosis, the clotting of blood within the blood vessels during life;

Infarct, an area of necrosis resulting from the sudden stoppage of blood in a blood vessel;

Edema, the presence of an excessive amount of fluid in the tissues;

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1. Runnells, R. A.
1938. Animal pathology, Collegiate Press Inc., Ames, Ia.

Hyaline degeneration, the formation of hyaline in a cell or group of cells;

Hyaline casts, collections of hyaline material in the renal tubules or in the urine;

Cloudy swelling, a cellular degeneration characterized by swollen cells with granular cytoplasm and indistinct nuclei;

Fatty infiltration, the deposition of fat in one or, at the most, a few large globules within the cells of the epithelium of the liver and in the connective tissue of other organs;

Fatty degeneration, a disturbance of fat metabolism of the cells of the liver or kidney epithelium resulting in the appearance of many small droplets of fat normally held firmly by the cell protoplasm;

Fat necrosis, the splitting of fat into fatty acids and glycerol by the action of pancreatic lipase;

Necrosis, the sudden local death of cells in the living body.

DATA COLLECTED FOR EVALUATION OF GESTATIONAL PERFORMANCE

Gestation I

In studying gestation, data were collected that served as indices of the success of the function. They fell into three main classes, i.e., those that described the course of the gestation itself, those that described the condition of the litter born, and those that described the vitality of the litter and thereby reflected the success of the gestation. The data collected for the study of the first gestation follow. Their significance in the evaluation of reproduction is discussed in "Results."

A. For the study of the average progression of Gestation I
   a. Fertility index[1]
   b. Implantation per cent[1]
   c. Placental index[1]
   d. Implantation (Appearance of erythrocyte sign)
      1. Time of occurrence

1. The implantation per cent is the ratio of the $\frac{\text{number of implantations}}{\text{number of positive matings}}$,
   the placental index is the ratio of the $\frac{\text{number of successful parturitions}}{\text{number of implantations}}$, and
   the fertility index is the ratio of the $\frac{\text{number of successful parturitions}}{\text{number of positive matings}}$.

2. Persistence in days

B. For the study of the condition of litter I at birth

a. Total weight of live young in litter

b. Individual weight of young

c. Number of young in litter

d. Per cent of young born dead

C. For the study of the average vitality of litter I

a. Per cent of young dying one to four days after birth

b. Individual weight of young four days after birth

c. Rearing performance[1]

d. Individual weight of young at weaning

Gestation II

Only a part of the data used to study gestation I could be obtained for the second pregnancy, as the females were sacrificed before parturition. However, the autopsy records furnished additional information concerning the condition of the feti, placenta, the number of resorptions, and the number of corpora lutea, that was especially useful in analyzing the success of the gestation. The following data were collected.

1. The rearing performance is the ratio of $\frac{\text{number of young reared}}{\text{number of young that should have been reared.}}$

A. For the study of the average progression of Gestation II
   a. Implantation per cent
   b. Implantation (Appearance of erythrocyte sign)
      1. Time of occurrence
      2. Persistence in days

B. For the study of the average development of feti[1] in uterus
   a. Total number of feti
   b. Total weight of feti
   c. Fertilization per cent[2]
   d. Per cent of feti resorbed per litter
   e. Per cent of total litters resorbed

C. For the study of the average condition of feti and placentae
   a. Individual weight of feti
   b. Individual weight of placentae
   c. Per cent of normally developed feti dead at autopsy
   d. Fetal-placental index[3]

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1. These data were obtained at autopsy.
2. The fertilization per cent has been defined herein as the ratio of $\frac{\text{the number of feti}}{\text{the number of corpora lutea}}$ and must be distinguished from "fertility per cent", used in evaluating the success of gestation I.
3. The fetal placental index has been defined herein as the ratio of $\frac{\text{weight of placenta}}{\text{weight of fetus}}$.

DATA COLLECTED FOR STUDY OF PATHOLOGICAL CHANGES ASSOCIATED WITH GESTATIONAL FAILURE

One of the primary purposes of the investigation herein reported was to determine what pathological changes were associated with gestational failure. The relative condition of rats dying of the pregnancy disorder and those surviving parturition in all experimental groups was described. Findings were compared with similar data collected from a normal control group. In the study, the physical condition of the rats was analyzed, and the visceral organs were described in regard to external appearance, histo-pathology, size, water content, and, in the case of the liver, fat content.

The outline used in describing the physical vigor and general condition of the animals is shown in form 1, in the appendix. The females were weighed daily for the study of changes in body weight during pregnancy. The outline used to collect data concerning the appearance of the visceral organs is shown in form 2 in the appendix. The amount of water consumed by the females was recorded daily from the twelfth day of pregnancy until parturition or death.

The relative weights and water content of the liver[1], kidney, heart, spleen, and mammary glands removed from

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1. The group of analyses on the liver was made by Miss Ethelwyn Wilcox.

rats representing all dietary groups were determined.

The organs used for the study of cellular changes accompanying the pregnancy disorder were the liver, kidney, heart, spleen, pancreas, feti, and placentae.

RESULTS AND DISCUSSION

Even before the experiment was completed and the data were analyzed, certain facts pertaining to the reproductive behavior of the animals fed the various experimental diets were obvious. For instance, the females fed the Steenbock V diet, designated as the control ration, never developed the characteristic symptoms of the pregnancy disorder. Also, the animals maintained on this ration were able to produce vigorous live young and to rear these young successfully. It might be said in passing that the stock colony belonging to the Nutrition Laboratory has been maintained for seven years on the Steenbock V diet and no deaths due to pregnancy disorder have ever occurred.

The occurrence of the typical pregnancy disorder first described by Dyar ('35) in animals fed the Pork I ration was again confirmed. Among the animals reared especially for this study, 6.7 per cent and 13.3 per cent of the females in the two Pork I groups studied died with typical symptoms of the disturbance (table V). The general symptomatology described by Walliker ('38) was again noted. The onset of the symptoms was always sudden,

TABLE V. INCIDENCE OF PREGNANCY DISORDER IN FEMALES FED VARIOUS EXPERIMENTAL DIETS

Experimental groups	Per cent of females dying	
	Mated with males fed Steenbock V	Mated with males fed diet of female
Steenbook V (Control diet)	0	0
Pork I (Basal diet)	6.7	13.3
Pork 7 (Pork I plus fresh liver)	0	0
Pork 39A (Pork I plus 10 mg. lipocaic)	0[1]	0
Pork 39B (Pork I plus 40 mg. lipocaic)	0[1]	-
Pork 39C (Pork I plus 500 mg. lipocaic)	10	20
Pork 31 (Pork I plus liver extract)	20	13.3

and no reliable subjective measurement was found for predicting the appearance of the syndrome. In some cases, animals appearing perfectly normal died within fifteen minutes. The majority of the females became ill on the twenty-first or twenty-second day of gestation. The disorder occurred during either the first or the second littering, although other studies in the laboratory have noted deaths at the third pregnancy.[2] It was observed also that even the females that did not die at parturition

1. In other studies conducted in the laboratory at the same time, females died in these experimental groups.
2. Unpublished data in the files of the Nutrition Laboraotry.

were unable to rear their young successfully, many young dying within four days after birth.

Fresh liver was the only supplement tested in the present experiment which prevented the appearance of the symptoms of the pregnancy disorder. These observations confirmed the findings reported by Rogosheski upon the effect of feeding fresh liver as a supplement to the Pork I diet. The addition of lipocaic and liver extract to the Pork I diet failed to prevent the appearance of the pregnancy disease.

The results obtained with the use of lipocaic were indeed disappointing. As can be seen from table V, no rats died in the experimental groups receiving the daily addition of 10 mg. or 40 mg. of lipocaic to the diet. However, deaths were noted in groups of animals used in other experiments conducted simultaneously in the laboratory where lipocaic was fed (Walliker, '38). When the dosage of lipocaic was increased to 500 mg. daily, 20 per cent of the animals in one group died. Not only were many animals lost in the group fed the diet containing 500 mg. of lipocaic, but also animals that survived parturition exhibited poor reproductive performance in terms of number of young alive the fourth day after birth. In general, increasing the amount of lipocaic fed did not

improve the reproductive behavior of the females.

These findings are in direct contrast to results of a study reported by Wilcox ('37) in which 4 mg. of lipocaic exerted a marked beneficial effect upon the reproductive performance of the animals. Unfortunately the lipocaic used by Walliker and the present investigator differed markedly from that fed by Wilcox. The preparation used in the laboratory in the year 1936 to 1937 (Wilcox, '37) was a white crystalline material isolated from ox pancreas by Dr. Lester Dragstedt. The lipocaic fed in this study (1937 to 1938) was furnished by the Eli Lilly Company through the courtesy of Dr. Dragstedt. It was prepared as a by-product in the manufacture of insulin from fetal pancreas. This material, which was a grayish-yellow powder with a peculiar odor and flavor, was found by Dr. Dragstedt to be about one-half as effective as his original preparation in preventing fatty livers in depancreatized dogs maintained with insulin.[1] No conclusions may be drawn as to the effect of lipocaic upon reproduction until it is possible to obtain a uniform product.

The highest mortality of females (13.3 per cent and 20 per cent) occurred in the two groups of animals fed liver extract as a supplement to the Pork I diet. The high incidence of the pregnancy disorder among this group

1. Personal communication from Dr. Lester Dragstedt to Dr. Pearl Swanson.

of animals confirmed the observation made by King ('36) that the number of females dying at parturition was increased when liver extract was added to the basal pork diet. A poor reproductive behavior was also observed among the animals fed this diet whose behavior at parturition was apparently normal.

It should be noted that in every experimental group, except the lot fed liver extract, the incidence of pregnancy disorder was increased when the male used for mating received the same diet as did the female.

Although 100 per cent mortality of the females at parturition has never been noted, the animals surviving were consistently unable to rear all of their young. This failure was due to a high death-rate among the young early in the lactation period. These observations made us wonder whether or not the vitality of the young of the pork-fed females was of such low order that they were unable to survive extra-uterine existence. If this were the case, the early loss of litters might be due to an atypical or sub-acute form of the pregnancy disorder. The following section deals with an attempt to evaluate the gestational performance of the females comprising the various experimental groups that did not develop the pregnancy disorder but none the less failed to rear their young.

EVALUATION OF SUCCESS OF FIRST GESTATION

To add to the reliability of data collected on differences in gestational performance between the groups of animals studied, a set of data obtained from normal animals was necessary for comparison. The experimental group of animals fed the Steenbock V diet was believed normal. However, this group contained only fifteen animals, and variations in reproductive behavior have been observed in small groups of these rats from year to year. Therefore, it was decided to use observations made on 217 females in the stock colony as a standard of normal gestational performance. Five generations (the eighteenth through the twenty-second) of the stock colony were represented in this standard group. Observations that are indicative of reproductive behavior, such as items pertaining to the progression of gestation, the condition and vitality of the litter, were tabulated for the first pregnancy. The individual items considered were the same as those used in studying the experimental groups and are listed in table VI.

Certain of the data seemed to indicate that the gestational performance of the animals used in the present experiment was abnormal. Therefore, it was necessary to

deviated from the normal picture as represented by observations made on the standard group. The general character of the data introduced many obstacles in working out a plan of analysis and made it imperative to test the significance of differences in two ways.

In the first analysis, the chi-square test was used in evaluating items reported on a percentage basis, which did not show a normal distribution. Due to this non-normality, some of the records were divided by counting the number reported as 100 per cent and the number reported as less than 100 per cent. For the remaining records, the number reported as 0 per cent and the number reported as above 0 per cent were counted. The variables examined are given below.

A. Implantation per cent (100%:less than 100%)

B. Placental index (100%:less than 100%)

C. Fertility index (100%:less than 100%)

D. Dead young in litters (0%:more than 0%)

E. Young dying in litters before 4 days of life (0%:more than 0%)

F. Rearing performance (0%:more than 0%)

Not all variables were included in the analyses described above. The remaining variables could be compared directly with similar data from the standard series. The

standard deviation from the mean for each item was calculated for the standard group and was taken as an index of the normal range for that item. Then for the standard deviation for each variable thus obtained, several standard errors of the mean based on the size of the different experimental groups were calculated. Values were determined which give the range within which 99 per cent of the means of random samples drawn from the standard group might be expected to fall (Fisher, '36). A mean value of an item, derived from an experimental group, which fell outside this range was significantly different from the mean of the standard group, and abnormality of function in regard to the item was inferred.

Some discussion of the indices used to evaluate gestational performance may be helpful. It is difficult to obtain accurate information of the progression of gestation without killing the animals at intervals during pregnancy. However, the items listed in table VI under "average progression of gestation" do give some information on the normal sequence of events from coitus to parturition. A composite picture of the success of fertilization, implantation, and parturition is obtained by the use of the fertility index. This figure measures only the relation between number of positive matings and number of successful

parturitions. However, by using the implantation per cent and the placental index, it is possible to determine whether or not lack of fertility in any group of animals is due to untoward occurrences before or after implantation.

The implantation per cent measures the relation between number of positive matings and number of implantations. It is expected that in normal animals a certain proportion of positive matings are followed by implantation, and low values indicate an early break in reproductive processes. One of the reasons for a failure in implantation following the appearance of sperm in the vagina may be abnormal ova or spermatozoa. However, if males of proved fertility are used for mating, as was the case in about one-half of the present experiments, failure of implantation may be charged against the female alone. The day of gestation on which placentation occurs can be detected by examining the vaginal contents for the presence of free blood. An early appearance of the erythrocyte sign has been demonstrated in abnormal conditions. It is possible also that retarded implantation is indicative of a disturbance in placental development. Undue persistence of the red blood cells also may be regarded as an atypical condition.

The placental index measures the relation between number of implantations and number of parturitions. For

instance, if the feti die and are resorbed before the end of gestation, or if the birth mechanism in the female fails to function, the placental index becomes zero.

The length of the gestation period is also an index of the normality of pregnancy. Prolonged gestations have been reported in abnormal uterine conditions such as follow the feeding of diets low in vitamin A or in the essential fatty acids.

With these considerations in mind, the average gestational performance of each experimental group was analyzed in relation to the behavior of the standard group using the data presented in table VI. In this series, the females had been mated with males fed the adequate control diet.

In so far as the progression of gestation was concerned, the observations made on the experimental group fed the Steenbock V diet were not, on the whole, significantly different from those made on the standard group (table VI). They differed only in respect to the day on which implantation occurred. Gestation proceeded normally in all other experimental groups also, with the exception of the animals fed the Pork 31 diet. In this group, the reproductive mechanism seemed to be stimulated. The animals exhibited an unusually high fertility, shown by the fact that every

TABLE VI. DATA RELATING TO FIRST GESTATION OF FEMALES MATED WITH MALES FROM

Observations made during gestation and lactation	Standard group	Stee V (C Rati
Average progression of gestation I		
Proportion of fertility indices = 100	148:217	14:
Proportion of implantation per cents = 100	169:217	14:
Proportion of placental indices = 100	210:217	15:
Implantation (Appearance of erythrocyte sign)		
Day of occurrence in gestation	13.6	14.
Persistence in days	1.8	1.
Length of gestation in days	22.2	22.
Average condition of litter I at birth		
Total weight of live young in litters	43.3	38.
Individual weight of young	5.1	5.
Number of young in litter	8.6	7.
Proportion of total litters containing no dead young	186:27	14:
Average vitality of litter I		
Proportion of total litters dying 1 to 4 days after birth	29:217	4:
Individual weight of young 4 days after birth	6.2	6.
Proportion of rearing performances = 0	37:217	5:
Individual weight of young at weaning	49.5	50.

[1]The data from which the averages were taken are shown in tables 4, 5, and 6
[2]Data given by Rogosheski ('36) included.
*Significant
**Highly significant

) WITH MALES FROM THE STOCK COLONY[1]

Standard group	Experimental groups						
	Steenbock V (Control ration)	Pork I (Basal diet)	Pork 7[2] (Pork I + fresh liver)	Pork 39A (Pork I + 10 mg. lipoeaic)	Pork 39B (Pork I + 40 mg. lipoeaic)	Pork 39C (Pork I + 500 mg. lipoeaic)	Pork 31 (Pork I + liver extract)
148:217	14:15	12:14	14:17	10:15	11:15	9:0	14:0**
169:217	14:15	12:14	14:17	10:15	11:15	9:0	14:0*
210:217	15:0	14:0	17:0	15:0	15:0	9:0	14:0
13.6	14.5**	14.7**	14.0	14.4**	14.8**	14.5	13.5
1.8	1.6	1.2	1.1	1.9	1.8	2.2	1.6
22.2	22.1	22.3	22.2	22.4	22.3	22.8	22.3
43.3	38.1	39.4	35.1	31.0**	30.5**	33.4	30.4**
5.1	5.0	4.9	4.9	4.8	4.3**	4.1**	4.6
8.6	7.6	7.7	7.2	6.9	6.5**	6.7	6.3**
186:27	14:1	10:3	16:1	12:3	13:2	6:3	13:1
29:217	4:14	3:11	9:17*	11:13**	8:15**	4:8**	12:14**
6.2	6.3	5.5	6.0	5.8	5.2	6.0	3.8
37:217	5:15	6:13*	10:17*	11:15**	13:15**	5:9**	12:14*
49.5	50.0	43.9	51.1	40.0	38.9	47.1	48.5

ables 4, 5, and 6 in the appendix

positive mating resulted in implantation and in a litter. In addition, implantation in these animals occurred earlier than in the females of any other dietary group.

Apparently the dietary factors involved in the experimental diets have little effect upon the outward evidence of the normal progression of gestation. The fact that we were unable to detect any differences does not indicate, however, that real differences did not exist. Observations such as those discussed above are only crude measures of the conditions that prevail during gestation.

The data pertaining to the condition of the young at birth contributed valuable information to our knowledge of the normality of gestation. The amount of new live tissue which the female is able to produce during gestation reflects the degree of success of the function. Feti low in vigor may be able to live while in the uterus but are often unable to survive after parturition. The total live weight of the litter at birth is affected by three factors: i.e., the number of young born, the per cent of young born dead, and the individual weight of the young.

Examination of the data presented in table VI shows that in so far as the condition of the litter at birth was concerned, there were no significant differences between litters produced by animals fed the Steenbock V, the Pork I,

or the Pork 7 diets and members of the standard group.

The animals fed the supplements of lipocaic produced a smaller amount of new live tissue than did the females in the standard group. Differences were significant only in the groups fed the two lower levels of the material. In the case of the animals fed the Pork 39B diet (40 mg. lipocaic) both the number of young in the litter and the individual weight of the young were smaller than the corresponding values in the standard set of data. When the dosage of lipocaic was increased to 500 mg., the individual weight of the young was the only item that differed significantly from the standard value. However, it must be remembered that as this group is small, deviations from normal must be very large in order to be significant.

The females consuming the Pork 31 diet, in spite of their high fertility, were unable to produce litters of normal weight or size.

The inability of the young to survive during the first few days of extra-uterine life may reflect conditions during gestation. It is thought that the proportion of young dying in the first four days of life and the weights of the individual young at this time are indices of intra-uterine development. Likewise, rearing performance and weight of young at weaning also give some indication of the development of

the young during gestation, because the amount of milk secreted is affected not only by the nutritional state of the mother, but also by the vigor of the litter.

When the rearing records of the various groups of rats were compared, no significant difference was found between the animals fed the Steenbock V diet and standard set of animals. In the litters produced by the females fed the Pork I diet and the standard group of rats, the difference in the proportion of total litters dying before the fourth day of life approached significance, showing the pork ration exerted some adverse influence. The rearing performance of the Pork I group was significantly poorer than that of the standard. In addition, the weights of the individual young both at four days and at weaning were considerably lower than the corresponding weights of young in the standard group! These items were not tested statistically for significance of the differences, because of variations in litter size.

The addition of fresh liver to the Pork I diet did not improve the vitality of the young born to females fed the basal pork ration, as far as proportion of total litters dying before four days and rearing performance was concerned. However, the weights of the young at four days and 28 days after birth compared favorably with similar

data for the young of animals in the control group.

In the groups of animals fed lipocaic as a supplement to the basal pork ration, the rearing performance was poor. In addition, with the exception of the Pork 390 group, the young were below par in regard to the body weight of the individual young at four days and at weaning.

Neither was the rearing performance of the females receiving liver extract as a supplement to the basal pork diet normal. Also, the young at four days weighed only 3.8 gm. However, by the end of suckling period they were as large as the young in the standard group.

In general, it may be said that the first gestation of the experimental group of animals receiving the Steenbock V diet was as successful as that of the standard series. The rats fed the Pork I diet exhibited a better gestational performance than was expected from the results of other studies in the laboratory (Dyar, '35; King, '36; Walliker, '38). Of all the supplements tested, only the addition of fresh liver to the Pork I diet improved the rearing performance of the females and maintained the gestation near a normal level.

It is to be expected that if the diet of both parents is deficient, the young produced will be in a poorer physical condition than if the diet of only one parent is

lacking in some dietary essential. Comparison of the data presented in table VII with those in table VI shows that the nutritive state of the male affects the condition of the progeny. While matings between males and females maintained on the Pork I diet were very fertile, total weight of the litter, size of litter, and individual weight of young were all significantly lower than the values reported for the standard group. It will be recalled that no significance could be attached to differences in these items when males of the stock colony were used for mating. The differences in proportion of total litters dying before four days and of rearing performances equal to zero of females belonging to the Pork I and standard groups, respectively, were highly significant. The condition was more acute than it was when males fed the Steenbock diet were used.

The females in only two of the experimental groups given lipocaic as a supplement to the Pork I ration were mated with males fed the same diet as they themselves received. In both groups the use of males maintained on the pork diet plus lipocaic resulted in a poorer gestational performance than was noted when the females were mated with males from the stock colony. The bad effect of the diet of the males on the condition and vitality of the litter

TABLE VII. DATA RELATING TO FIRST GESTATION OF FEMALES MATED WITH MALES FED THE

Observations made during gestation and lactation	Standard group	Pork I (Basal ration)
Average progression of gestation I		
Proportion of fertility per cents = 100	148:217	13:0*
Proportion of implantation per cents = 100	159:217	10:13
Proportion of placental indices = 100	210:217	13:0
Implantation (Appearance of erythrocytes)		
Day of occurrence in gestation	13.6	13.9
Persistence in days	1.8	1.6
Length of gestation in days	22.2	22.1
Average condition of litter I at birth		
Total weight of live young in litters	43.3	29.7**
Individual weight of young	5.1	4.6*
Number in litter	8.6	6.8*
Proportion of total litters containing no dead young	186:27	13:0
Average vitality of litter I		
Proportion of total litters dying 1 to 4 days after birth	29:217	8:13**
Individual weight of young 4 days after birth	6.2	5.3
Proportion of rearing performance = 0	37:217	11:13**
Individual weight of young at weaning	49.5	45.4

*Significant
**Highly significant

STATION OF FEMALES MATED WITH MALES FED THE SAME DIET

d lactation	Standard group	Experimental groups			
		Pork I (Basal ration)	Pork 39A (Pork I + 10 mg. lipocaic)	Pork 390 (Pork I + 500 mg. lipocaic)	Pork 31 (Pork I + liver extract)
s = 100	148:217	13:0*	12:15	8:0	12:14
ents = 100	159:217	10:13	12:15	8:0	10:12
= 100	210:217	13:0	15:0	8:0	12:10
hrocytes)					
n	13.6	13.9	15.2**	14.2	13.9
	1.8	1.6	1.7	2.0	2.1
	22.2	22.1	22.2	22.4	22.4
h					
tters	43.3	29.7**	32.9**	29.1**	32.8
	5.1	4.6*	4.5**	4.4**	4.6
	8.6	6.8*	7.2	6.3**	7.1
aining no dead young	186:27	13:0	12:3	6:0	10:1
g 1 to 4 days	29:217	8:13**	13:15**	4:6**	6:11**
s after birth	6.2	5.3	5.8	6.3	6.3
s = 0	37:217	11:13**	14:15**	6:8**	8:12**
aning	49.5	45.4	40.3	38.5	34.5

was greater in the group fed the 500 mg. level of lipocaic than in the group receiving 40 mg. of the supplement daily.

It is difficult to explain the effect of using males fed the Pork 31 diet upon the gestational behavior of females fed the same diet, especially when heretofore the feeding of the supplement to the female seemed to have an adverse influence upon the success of pregnancy. It will be recalled that if males fed the stock diet were used for mating, the litters born were small in size and light in weight as compared with those of the standard group. The reverse was true in this experiment when males fed the same diet were used for mating. This phase of the experiment should be repeated.

EVALUATION OF SECOND GESTATION

The success of the first pregnancy cannot be regarded as a true measure of the effect of dietary factors upon gestational performance. It will be recalled that the animals were not given the various experimental diets until they were 40 to 50 days old, and that they were only 70 days old at the initiation of the first pregnancy (table IV). Therefore, during the first gestation period the female had an opportunity to draw upon substances stored in her body tissues during the time prior to the initiation of the experiment when she received the adequate stock diet. When pregnancy is imposed at an early age, the female is faced with the double problem of producing a litter and completing her own growth. For this reason, the behavior of the female in the first pregnancy is likely to be more erratic than in later gestation periods. Therefore, we think that data collected during the second gestation period yield more pertinent information regarding the true effect of the dietary regime upon the success of gestation than do data relating to the first gestation. The animals were killed just prior to the termination of the second gestation. The data were enriched considerably by examination of the uterine contents at autopsy.

Unfortunately no autopsy data similar to those obtained from the animals in the various experimental groups were available for the standard group of rats. It was necessary, therefore, to use information obtained at autopsy from the experimental group fed the Steenbock V diet as the standard. This group of animals was too small, however, to permit statistical comparisons of autopsy data.

As in the study of gestation I, the progress of the second gestation was analyzed (table VIII). The fertility per cent and placental index could not be calculated as they depend upon the occurrence of parturition. The gestation records of the control group of Steenbock V rats approximately duplicated those of the standard group in their second pregnancy. In general, the proportion of successful implantations and time of implantation of the fertilized ova were normal in all groups. However, in two lots, i.e., Pork I and Pork 390, blood persisted in the vagina for an unduly long time after implantation.

The average intra-uterine development of the feti in the second gestation was studied next by means of data obtained at autopsy. In the analysis the following considerations were kept in mind. The number of young born gives no indication of the number or state of feti in the uterus, as feti in various stages of resorption may be

TABLE VIII. DATA RELATING TO THE SECOND GESTATION OF FEMALES MATED WITH MALE

Observations made during gestation and at autopsy prior to parturition	Standard group	Steenb V (Con diet)
Average progression of gestation II		
Proportion of implantation per cent = 100	170:217	15:0
Implantation (Appearance of erythrocyte sign)		
Day of occurrence in gestation	13.9	14.0
Persistence in days	1.2	1.3
Average intra-uterine development of feti		
Number of normally developed feti		11.3
Total weight of feti in gm.		55.9
Fertilization per cent[2]		87.7
Per cent of feti resorbed		15.4
Per cent of total litters resorbed		0.0
Average condition of feti and placentae		
Individual weight of well-developed feti in gm.		5.0
Individual weight of placentae in gm.		0.4
Per cent of normally developed feti dead at autopsy		0.0
Fetal-placental index[3]		0.0

[1]The data from which the averages were taken is shown in tables 4, 5 and 6 in

[2]The fertilization per cent is the ratio of $\frac{\text{the total number of feti}}{\text{the total number of corpora lutea}}$

[3]The fetal-placental index is the ratio of the $\frac{\text{weight of placenta}}{\text{weight of feti}}$

*Significant
**Highly significant

ES MATED WITH MALES FROM THE STOCK COLONY[1]

Standard group	Experimental groups						
	Steenbock V (Control diet)	Pork I (Basal ration)	Pork 7 (Pork I plus fresh liver)	Pork 39A (Pork I plus 10 mg. lipoic)	Pork 39B (Pork I plus 40 mg. lipoic)	Pork 39C (Pork I plus 500 mg. lipoic)	Pork 31 (Pork I plus liver extract)
170:217	15:0	14:16	14:17	11:15	11:15	9:10	13:15
13.9 1.2	14.0 1.3	13.7 2.2**	14.0 1.0	13.6 2.0	14.8** 1.9	14.0 2.5*	13.4 1.7
	11.3	10.4	10.9	11.0	10.7	8.1	10.0
	55.93	43.48	48.99	39.76	39.67	20.74	34.79
	87.7	96.2	91.3	97.0	95.2	75.6	98.3
	15.4	15.4	12.4	43.7	47.3	66.5	17.4
	0.0	0.0	0.0	0.0	6.7	40.0	0.0
	5.08	4.11	4.79	3.28	3.89	2.27	3.48
	0.481	0.383	0.398	0.377	0.368	0.508	0.442
	0.0	0.0	0.0	0.0	1.4	2.1	0.0
	0.0946	0.0932	0.0831	0.1151	0.0946	0.224	0.107

)les 4, 5 and 6 in the appendix.
of feti
of corpora lutea
ıcenta
:1

present. However, it is possible at autopsy to determine the condition, total number, and weight of the feti. Even in a normal pregnancy in rats fed an adequate diet, a certain number of resorptions occur due to crowding of the feti or failure in the development of adequate placental circulation. An excessive mortality of the feti, however, is closely connected with the nutritional state of the female (Evans and Burr, '27). The per cent of the total feti resorbed is no more important than the per cent of total litters resorbed. If only half of the feti in a litter are resorbed, the remaining feti may survive parturition and be reared. In such cases, the gestation is not as great a failure as if the entire litter were resorbed.

The actual fertility of an animal is difficult to measure from autopsy data, because the number of embryos implanted depends on the vitality of the germ cells and the establishment and maintenance of adequate placental function. The number of corpora lutea present in the ovaries represents the number of ova extruded at ovulation. Normally a certain number of ova do not develop into feti, due either to failure in fertilization or to failure of the zygote to implant. If the embryo is implanted, even though it dies very shortly, the site of implantation can

be detected. The ratio of number of feti (every embryo implanted being counted as a fetus) to the number of corpora lutea is a measure of the success of fertilization and implantation.

The data concerning the development of the feti in the uterus are presented in table VIII. The animals fed the basal pork rations produced fewer and smaller feti than did the Steenbock V females. The fertilization per cent was increased in all but one group of animals fed the various pork rations. This was the group receiving 500 mg. of lipocaic. Fertilization was especially high in the group fed liver extract.

The group of animals fed fresh liver in addition to the Pork I diet produced nearly as many and as large feti as did the Steenbock V females. The fresh liver was the only supplement which improved the gestational performance of the females fed the basal pork ration. Indeed, the addition of lipocaic or of liver extract seemed to have a detrimental effect upon the number and weight of feti produced. It is significant that the increasing doses of lipocaic resulted in progressively poorer uterine contents. Not only were the feti produced fewer in number and lighter in weight in females fed the 500 mg.

level of the supplement than in the group receiving the 10 mg. level, but the fertilization per cent was decreased. Normally developed feti were found dead in the uterus only in the groups fed the 40 mg. and 500 mg. doses of lipocaic. In addition the relation between the weight of the fetus and the weight of the placenta was abnormal in the group fed the high level of lipocaic. The number of resorptions in the rats fed the control, Pork I, Pork 7, and Pork 31 diets were approximately the same. The addition of lipocaic to the basal pork diet increased resorptions from approximately 15 to 66 per cent. The relative number of feti resorbed increased as the dose of lipocaic increased.

It was noted in the discussion relating to the success of gestation I that in any experimental group the females mated with males fed the same diet exhibited a poorer gestational performance than did the females mated with males from the stock colony. Whether these findings held true in the second gestation was now investigated (table IX). Analysis of the data therein presented shows that the gestations in the basal control group receiving the Pork I ration were poorer when males fed this diet were used for mating than when tested fertile males from the stock colony were used. There were fewer and smaller

TABLE IX. DATA RELATING TO SECOND GESTATION OF FEMALES MATED WITH MALES FED THE

Observations made during gestation and at autopsy prior to parturition	Standard group	Steenboc V (Contr ration)
Average progression of gestation II		
Proportion of implantation per cent = 100	170:217	15:0
Implantation (Appearance of erythrocyte sign)		
Day of occurrence in gestation	13.9	14.0
Persistence in days	1.2	1.3
Average intra-uterine development of feti		
Total number of feti		11.3
Total weight of feti		55.93
Fertilization per cent[2]		87.7
Per cent of feti resorbed		15.4
Per cent of total litters resorbed		0.0
Average condition of feti and placentae		
Individual weight of well-developed feti		5.08
Individual weight of placentae		0.481
Per cent of normally developed feti dead at autopsy		0.0
Fetal-placental index[3]		0.0946

[1]The individual data from which these averages were obtained is shown in table 8

[2]Fertilization per cent is the ratio of $\frac{\text{total number of feti}}{\text{total number of corpora lutea}}$

[3]Fetal-placental index is $\frac{\text{weight of placenta}}{\text{weight of fetus}}$

*Significant **Highly significant

MALES MATED WITH MALES FED THE SAME DIET[1]

	Standard group	Experimental groups				
		Steenbock V (Control ration)	Pork I (Basal ration)	Pork 39A (Pork I + 10 mg. lipocaic)	Pork 39C (Pork I + 500 mg. lipocaic)	Pork 31 (Pork I + liver extract)
	170:217	15:0	12:14	12:15	8:9	12:14
	13.9	14.0	14.5	15.8**	15.3**	14.0
	1.2	1.3	2.4**	2.1**	2.1	3.4**
		11.3	9.7	10.1	7.8	10.7
		55.93	31.38	32.96	23.44	36.79
		87.7	98.9	92.4	74.1	96.9
		15.4	22.0	43.1	48.7	16.3
		0.0	0.0	0.0	50.0	1.6
		5.08	3.78	3.01	2.49	3.05
		0.481	0.399	0.305	0.424	0.379
		0.0	0.0	1.7	2.4	0.0
		0.0946	0.106	0.102	0.170	0.124

obtained is shown in table 8 in the appendix.

r of feti
r of corpora lutea

feti and more resorptions.

Comparison of the data obtained in the study of the two gestations confirmed our early assumption that an inadequate dietary regime does not exert as marked an influence on the course of the first pregnancy as it does on the second. On this basis, we can conclude that gestation is less successful in animals fed the Pork I diet than in similar animals maintained on the Steenbock V ration. Of the three supplements fed, only fresh liver is capable of supporting normal gestation. The addition of liver extract or lipocaic to the basal pork diet results in even poorer gestational performances than was observed in animals fed the unsupplemented pork ration. The influence of the substances is not the same. The feeding of lipocaic strangely enough caused marked resorption of the feti. The liver extract, on the other hand, retarded the development.

PATHOLOGICAL CHANGES ASSOCIATED WITH GESTATIONAL FAILURE

It was pointed out in the preceding section of the discussion that not only did a certain per cent of animals fed the basal pork ration develop the pregnancy disorder, but also that the females surviving, in the main, were

unable to rear their litters successfully. The following part of the experiment was planned to describe in detail the pathological condition of the animals that died. We were also anxious to determine whether or not incipient symptoms of the pregnancy disorder could be detected by a careful study of the animals that survived parturition. It seemed possible that the post-parturient death of the young might bear some relation physiologically to the conditions noted in the pregnancy disorder.

In studying the incidence of pathological changes, the experimental animals were divided into three groups. The first of these groups consisted of the females fed the control diet, and was used as the standard in evaluating any condition observed. The second group included all the animals fed the pork-containing diets that did not develop the pregnancy disorder. The third group contained all rats that became acutely ill and developed typical symptoms of the disturbance. As was indicated in the "general plan" of the experiment, certain indices were chosen as basis of comparison of the condition of the three groups of animals. They were: general physical condition of the animals, water consumption, changes in body weight in pregnancy, weights and moisture contents of certain organs, fat content of organs, cellular changes in

organs, and cellular changes in feti and placentae.

General Physical Condition of Animals

The condition of rats as judged by musculature, sleekness, alertness, appearance of hair, and other characteristics of like nature is an index of the physiological well-being of the animal. In analyzing the physical condition of the rats in the present experiment, an attempt was made to detect incipient symptoms of the pregnancy disorder. Differences in external appearance between healthy and obviously sick animals were easily discerned, but it was difficult to distinguish border-line cases. A scoring system developed from data recorded in form 1 (appendix) was used in judging the state of health of each rat not in a moribund state at the end of the experiment. If the condition of the rat in terms of any characteristic was that usually observed in a normal, healthy animal, it was rated + + + + (numerical score, 4). The scores obtained in this way were compared with similar ones secured from the normal control rats. Some of the items studied contributed more information than others. Therefore, the relative value of each item checked in the subjective rating was weighted. The rating scale used is

shown in table X. An animal in perfect physical condition, if rated by this scale, would receive a score of 62.

As can be seen from data presented in table XI, all groups of pregnant animals fed the pork-containing diets, with one exception, rated somewhat less than did the normal control group. This group, i.e., the animals fed the Pork I diet supplemented by fresh liver, scored nearly as high as the control group. The subjective rating also indicated that pregnancy is associated with a somewhat improved physical condition. Whether the differences are real is problematic, however.

All in all, these analyses revealed that when the pregnancy disorder did not develop, it was impossible to distinguish on the basis of physical appearance alone, between the animals maintained on the normal control diet and those fed the pork rations.

The onset of the disease, however, produced a marked change in the condition of the rat. The pictures shown in plates I, II, and III illustrate the difference in external appearance between the normal control animals and the sick females. Plates II and III show the progression of the disorder from the first appearance of the symptoms to death during a convulsion. These symptoms were never observed in the pregnant females fed either

TABLE X. NUMERICAL VALUE ASSIGNED TO EACH CHARACTERISTIC

Characteristic	Value assigned to each plus in rating scale	Highest possible score
Condition	2	8
Alertness	2	8
Fat	2	4[1]
Absence of gauntness	1	4
Muscle tone, general	1	4
abdominal	1	4
Hair, creamy	1/4	1
fine	1/4	1
smooth	1/4	1
thick	1/4	1
Tail, clean	1/2	2
smooth	1/2	2
Respiration, normal	2	8
Gait, normal	1	4
Visible mucous membranes, pink	1	2[1]
Absence of exudates, nasal	1/2	2
oral	1/2	2
anal	1/2	2
vaginal	1/2	2
Total		62

[1]No value above two plus was given any rat in the series.

TABLE XI. CERTAIN AVERAGE CHARACTERISTICS OF CONDITION OF FEMALES FED VARIOUS DIETS AT END OF EXPERIMENT[1]

Experimental	Reproductive status	Score for general physical appearance	Rectal temperature	Appearance of liver[2]				Number of stomach ulcers
				Yellow	Friable	Mottled	Spongy	
			degrees					
Steenbock V (Control ration)	Pregnant	49.1	96.9	1.6	2.3	1.2	1.2	0.0
	Virgin	46.6	--	0.9	1.6	0.7	0.8	0.0
Pork I (Basal ration)	Mated	47.6	96.5	2.8	3.0	1.8	1.8	0.1
	Virgin	43.9	--	1.5	2.1	1.0	1.3	0.6
Pork 7 (Pork I plus fresh liver)	Mated	48.8	97.1	2.2	1.8	2.8	3.0	0.6
	Virgin	49.1	--	1.7	2.1	0.8	1.0	0.0
Pork 39A (Pork I plus 10 mg. lipocaic)	Mated	46.4	96.9	2.4	3.0	1.9	1.7	0.5
	Virgin	42.6	--	1.9	3.1	1.2	1.6	0.6
Pork 39B (Pork I plus 40 mg. lipocaic)	Mated	45.4	97.1	2.7	3.3	1.4	2.0	0.7
	Virgin	43.0	--	1.9	2.6	1.4	1.2	1.4
Pork 39C (Pork I plus 500 mg. lipocaic)	Mated	47.8	97.0	2.4	3.2	1.4	2.1	0.7
	Virgin	46.7	--	1.7	2.7	1.5	1.5	0.8
Pork 31 (Pork I plus liver extract)	Mated	45.8	96.6	2.6	2.9	2.8	2.3	0.4
	Virgin	45.0	--	1.7	2.4	1.4	1.9	0.5

[1]The data for the individual animals from which the average values were obtained is shown in table 9 in the appendix

[2]Rated on 0 to ++++ scale

PLATE I. APPEARANCE IMMEDIATELY PRIOR TO PARTURITION OF CONTROL RAT FED STEENBOCK V DIET

PLATE II. APPEARANCE IMMEDIATELY PRIOR TO PARTURITION OF RAT FED PORK I RATION -- EARLY SYMPTOMS OF THE PREGNANCY DISORDER

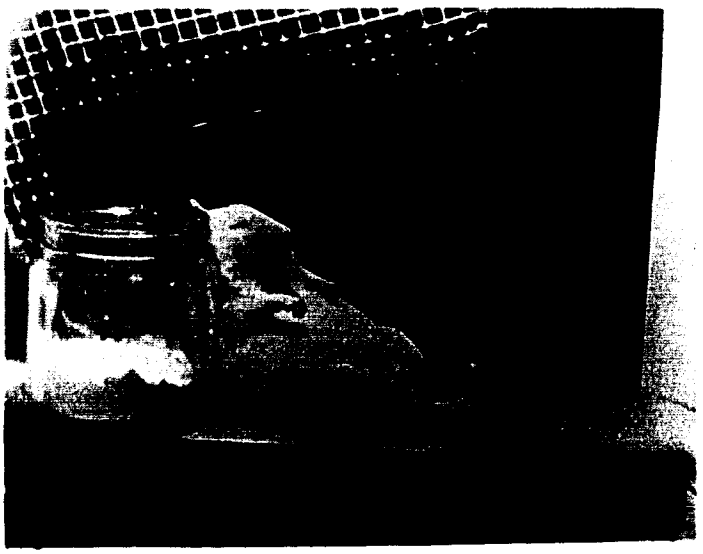


PLATE III. APPEARANCE IMMEDIATELY PRIOR TO PARTURITION OF RAT FED PORK I RATION -- LATE SYMPTOMS AND CHARACTERISTIC POSITION AT DEATH

the Steenbock V ration or the Pork I diet supplemented with fresh liver (Pork 7).

A detailed description of the sick rats is given in table XII. Erect hair, white ears and paws, lethargy, and cyanosis characterized these animals. In addition, the majority of the females showed dyspnea, hematuria, and bloody nasal and vaginal exudates. Many of the animals died during convulsive seizure. Irrespective of the diet fed, the symptoms exhibited by the sick animals were practically identical. As far as can be judged from symptomatology, the pregnancy disorder noted in these animals was the same as that reported by Dyar ('35) and her successors (King, '36; Wilcox, '37; and Walliker, '38).

King ('36) reported that animals showing symptoms of the pregnancy disorder were always cold to the touch. To obtain objective data relating to this point, the rectal temperatures of all pregnant females were taken just before they were killed. It was found that the body temperatures for the pregnant animals that exhibited no evidence of a stormy parturition showed surprisingly little variation from the normal control group. The average value for the entire series of mated animals was 96.8 degrees Fahrenheit (table XI). The average body temper-

TABLE XII. DESCRIPTION OF SICK FEMALES AT END OF EXPERIMENT

DIET OF FEMALES	DIET OF MALES	RAT NUMBER	SEVERITY OF TOXEMIA	OUTCOME OF TOXEMIA	MUSCLE TONE		CONDITION OF HAIR	RESPIRATION	DE
					GENERAL	ABDOMINAL			NASA
Pork I	Steenbock V	21706[1]	Acute	Fatal	Very Poor	Very Poor	Rough	Rapid	Bloodt
		21740[1]	Acute	Fatal	———	———	———	———	Slight, Bloodt
		21842[1]	Acute	Fatal	———	———	———	———	———
		21881	Acute	Sacrificed	Very Poor	Very Poor	Normal	Labored	Bloodt
		22300[1]	Acute	Fatal	———	———	———	———	Copiou Bloodt
	Pork I	21928	Acute	Fatal	———	———	———	———	Bloodt
		21929	Acute	Sacrificed	Very Poor	Very Poor	Normal	Labored	———
Pork 39A (Pork I plus 10 mg. lipocaic)	Steenbock V	21708[1]	Acute	Fatal	———	———	———	———	Slight, Bloody
		21741[1]	Acute	Sacrificed	Very Poor	Very Poor	Rough	Labored	Bloody
Pork 39B (Pork I plus 40 mg. lipocaic)	Steenbock V	21841[1]	Acute	Fatal	Very Poor	Very Poor	Normal	Labored	Slight, Bloodt
		21843[1]	Acute	Sacrificed	Poor	Poor	Rough	Rapid	Copious Bloody
		21908[1]	Acute	Fatal	Very Poor	Very Poor	Normal	Labored	Slight, Bloody
		21911[1]	Acute	Sacrificed	———	———	———	———	Copious Bloody
		21965[1]	Acute	Fatal	Normal	Normal	Normal	Normal	Copious Bloody
Pork 39C (Pork I plus 500 mg. lipocaic)	Steenbock V	21876	Acute	Fatal	———	———	———	———	Bloody
	Pork 39C	21621	Acute	Sacrificed	Very Poor	Very Poor	Rough	Labored	———
		21957	Acute	Sacrificed	Very Poor	Very Poor	Rough	Labored	———
Pork 31 (Pork I plus liver extract)	Steenbock V	21618	Acute	Fatal	———	———	———	———	Bloody
		22008	Acute	Sacrificed	Very Poor	Very Poor	Rough	Labored	Copious Bloody
	Pork 31	21901	Acute	Fatal	Very Poor	Very Poor	Rough	Labored	Copious Bloody
		21939	Acute	Fatal	Very Poor	Very Poor	Rough	Normal	Bloody
		21940	Acute	Fatal	Very Poor	Very Poor	Rough	Normal	Bloody
		22147	Acute	Fatal	———	———	———	———	———

[1]Animals not grown for this experiment

'ERIMENT

LE TONE	CONDITION OF HAIR	RESPIRATION	DESCRIPTION OF EXUDATES				RECTAL TEMPERATURE IN DEGREES F.	CHARACTER OF URINE	COLOR OF EARS AND FEET	COLOR OF VISIBLE MUCOUS MEMBRANE
ABDOMINAL			NASAL	ORAL	ANAL	VAGINAL				
Very Poor	Rough	Rapid	Bloody	Bloody	———	———	———	Bloody	White	Blue
———	———	———	Slight, Bloody	———	———	———	———	Bloody	White	Blue
———	———	———	———	———	———	———	———	Normal	White	Blue
Very Poor	Normal	Labored	Bloody	———	———	Copious, Bloody	92.3	Bloody	White	White
———	———	———	Copious, Bloody	———	———	———	———	Bloody	White	Blue
———	———	———	Bloody	Bloody	———	———	———	Bloody	White	Blue
Very Poor	Normal	Labored	———	———	———	Copious, Bloody	92.0	Bloody	White	White
———	———	———	Slight, Bloody	———	Bloody	———	———	Bloody	White	Blue
Very Poor	Rough	Labored	Bloody	———	Bloody	———	91.2	Bloody	White	Blue
Very Poor	Normal	Labored	Slight, Bloody	———	———	Copious, Mucous	93.6	Bloody	White	Blue
Poor	Rough	Rapid	Copious, Bloody	———	———	———	———	Bloody	White	Blue
Very Poor	Normal	Labored	Slight, Bloody	———	———	———	91.2	Bloody	White	Blue
———	———	———	Copious, Bloody	Bloody	———	———	———	Bloody	White	Blue
Normal	Normal	Normal	Copious, Bloody	———	———	———	———	Slightly, Bloody	White	White
———	———	———	Bloody	———	———	Bloody	———	Normal	White	White
Very Poor	Rough	Labored	———	———	———	Bloody	———	Bloody	White	White
Very Poor	Rough	Labored	———	———	———	Copious, Bloody	92.1	Bloody	White	Blue
———	———	———	Bloody	———	———	Bloody	———	Bloody	White	Blue
Very Poor	Rough	Labored	Copious, Bloody	———	———	———	93.1	Bloody	White	Blue
Very Poor	Rough	Labored	Copious, Bloody	———	———	Bloody	———	Bloody	White	Blue
Very Poor	Rough	Normal	Bloody	———	———	———	———	Bloody	White	White
Very Poor	Rough	Normal	Bloody	———	———	———	———	Bloody	White	White
———	———	———	———	———	———	———	———	Bloody	White	Blue

ature of the sick animals was 92.1 degrees Fahrenheit. The average normal figure was lower than that reported by Horst ('33). She noted an average rectal temperature of 99.3 degrees Fahrenheit for male rats caged at a temperature of 78.8 to 82.4 degrees Fahrenheit. The animals in the Nutrition Laboratory were maintained at a temperature of 75 to 80 degrees Fahrenheit. Donaldson ('24) has quoted data published by Sieren de Haan which showed that the body temperature of the rat increases with a rise in environmental temperature. The difference in the temperature of the two laboratories probably accounted for the lower rectal temperature noted in our rats as compared to those studied by Horst. Unfortunately, no temperatures were taken of the virgin females, so data are not available in regard to the effect of pregnancy per se on the rectal temperature of the albino rat.

The appearance of certain visceral organs removed at autopsy was also studied subjectively. The investigators (Roderick and Harshfield, '32; and Green, '37) working on the pregnancy disorder in sheep and rabbits found soft, yellow, friable livers, often very mottled, in animals that died. In view of these reports, the livers of all the animals were examined and scored on four points

relating to a pathological state: i.e., yellowness, friability, mottled condition, and sponginess. Again a rating scale from "o" to "four plus" was used. This time, however, an ascending value indicated an increase in the abnormal condition. The lower the score, the better the condition of the liver. The scores describing the appearance of the livers of animals in the experimental groups are shown in table XI.

The fact that pregnancy per se exerts a definite influence on the condition of the liver is shown by the fact that in every dietary group the livers of the pregnant animals scored higher than those of the virgins fed the same diet. Among the mated animals, the livers of those fed the Steenbock V diet received the lowest scores. In fact, the scores on the livers of the pregnant animals in the normal control group were no higher than those of virgins in the other groups. The livers of the virgin rats fed the Steenbock V diet received the lowest scores of any group studied. We think the conditions observed in the group of animals fed an adequate diet and not subjected to the physiological strain of pregnancy represent a normal picture for the organ. The scores on the livers of the virgin control rats fed the various diets containing pork were all higher than those on the livers

of the virgins in the normal control group. Thus, it seems possible that even in virgin animals the pork-containing diets are not capable of maintaining as healthy liver tissue as is found in the animals fed the Steenbock V diet. From these data we are led to the belief that the ingestion of the basal Pork I ration results in some injury to liver tissue which is accentuated by pregnancy. This idea is supported by objective evidence presented in later sections.

No differences, judged by gross observation, could be detected in the kidney, spleen, or pancreas of the normal control rats and those of the animals fed the various experimental diets whose gestation was uneventful. Other studies in the laboratory[1] have shown that stomach ulcers may be produced in male rats by the feeding of the Pork I or Pork 7 diets for 12 months. With one exception, all the groups of animals fed the diets containing pork muscle showed some evidence of stomach ulcers. The condition was not as severe as that noted in the male rats, probably because the duration of this experiment was less than seven months. But as with the male rats, not a single animal, virgin or mated, fed the Steenbock V diet developed ulcers.

Lung infection is common in rats over six months of

1. Unpublished data in the files of the Nutrition Laboratory.

age (Moise and Smith, '29). This condition affects the animals so severely that when it occurs it is difficult to distinguish between the results related to dietary deficiencies and those due to lung infection. However, the results in this experiment are not colored by complications due to respiratory infection, since only five of the 235 rats used in the study showed evidence of even mild lung infection. Pus pockets at the base of the tongue or in the middle ear are also common autopsy findings in adult rats. However, not one of the animals in this experiment had any signs of aural or lingual infection.

The appearance of the visceral organs of the individual sick animals is described in table XIII. Whether or not the pregnancy disorder develops, yellow, friable livers seems to be characteristic of the rats receiving the diets containing dried, autoclaved pork muscle. The average scores for the condition of the livers of the sick animals noted were within the range of those for animals in the experimental groups other than the control group.

The kidneys in the rats that were acutely ill were swollen, very firm, and gorged with blood. The heart,

TABLE XIII. APPEARANCE OF VISCERAL ORGANS OF SICK FEMALES

DIET OF FEMALE	DIET OF MALE	RAT NUMBER	MAXIMUM LENGTH OF TIME FROM DEATH TO AUTOPSY	APPEARANCE OF LIVER[1]				APPEARANCE OF KIDNEYS	APPEARANCE OF PANCREAS	APPEARANCE OF HEART	APPEARANCE OF LUNGS	APPEARANCE OF PLEURAL CAV
				YELLOW	FRIABLE	MOTTLED	SPONGY					
Pork I	Steenbock IV	21706	30 Min.	2	4	2	4	Swollen, gorged with blood	Normal	Contracted, coronary vessels distended	Filled with blood	Filled with serous fluid
		21740	30 Min.	3	4	3	1	Normal	"	"	"	"
		21842	4 Hr.	1	4	0	4	Swollen, gorged with blood	"	"	"	"
		21881	15 Min.	4	3	2	4	"	"	"	Normal	Slight amount of serous flu
		22300	10 Hr.	3	2	3	3	"	"	"	Filled with blood	Filled with serous fluid
	Pork I	21928	1 Hr.	2	4	4	2	"	"	"	"	"
		21929	5 Hr.	4	4	2	4	"	"	"	Normal	Slight amount of serous flu
Pork 39A (Pork I plus 10 mg. lipocaic)	Steenbock IV	21708	30 Min.	3	4	2	3	Swollen, gorged with blood	Normal	Contracted, coronary vessels distended	Filled with blood	Filled with serous fluid
		21741	15 Min.	4	3	1	3	"	Surrounded by jelly-like mucous	Normal	Normal	No excess fluid present
Pork 39B (Pork I plus 40 mg. lipocaic	Steenbock IV	21845	10 Hr.	1	4	4	3	Swollen, gorged with blood	Normal	Normal	Filled with blood	Filled with serous fluid
		21695	15 Min.	3	4	2	3	"	"	Contracted, coronary vessels distended	"	"
		21911	2 Hr.	2	4	2	4	"	"	"	"	"
		21908	15 Min.	4	4	2	2	"	Surrounded by jelly-like mucous	"	"	"
		21841	15 Min.	3	2	4	2	"	"	"	"	"
Pork 39C (Pork I plus 500 mg. lipocaic)	Steenbock IV	21876[2]	4 Hr.	1	1	1	1	Normal	Normal	Normal	Normal	No excess fluid present
	Pork 39C	21937	15 Min.	1	4	2	1	Swollen, gorged with blood	"	Contracted, coronary vessels distended	Filled with blood	Filled with serous fluid
		21821	15 Min.	4	3	2	4	"	"	"	"	"
Pork 31 (Pork I plus liver extract)	Steenbock IV	21818	1 Hr.	4	4	2	4	Swollen, gorged with blood	Normal	Contracted, coronary vessels distended	Filled with blood	Filled with serous fluid
		21901	15 Min.	3	2	1	3	"	"	Normal	"	No excess fluid present
		22008	30 Min.	0	3	2	3	"	"	Contracted, coronary vessels distended	"	Filled with serous fluid
	Pork 31	21939	30 Min.	4	3	1	4	"	"	"	"	"
		21940	15 Min.	2	4	3	3	"	"	"	"	"
		22147[2]	2 Hr.	2	3	3	4	"	"	"	"	"

[1] Rated on 0 to 4+ scale
[2] Died on the 16th day of pregnancy

VISCERAL ORGANS OF SICK FEMALES

...OF ...OM ...TO ...SY	APPEARANCE OF LIVER: YELLOW	FRIABLE	MOTTLED	SPONGY	APPEARANCE OF KIDNEYS	APPEARANCE OF PANCREAS	APPEARANCE OF HEART	APPEARANCE OF LUNGS	APPEARANCE OF PLEURAL CAVITY	APPEARANCE OF UTERUS	APPEARANCE OF FETI	CHARACTER OF BLOOD
.	2	4	2	4	Swollen, gorged with blood	Normal	Contracted, coronary vessels distended	Filled with blood	Filled with serous fluid	Very hyperemic	Dead, well-developed	Thin, did not clot, bluish
n.	3	4	3	1	Normal	"	"	"	"	Hemorrhage at base of placentae	"	bluish
	1	4	0	4	Swollen, gorged with blood	"	"	"	"	Placentae loosely attached to uterine wall	"	"
L	4	3	2	4	"	"	"	Normal	Slight amount of serous fluid	"	"	Thin, clotted slowly
	3	2	3	3	"	"	"	Filled with blood	Filled with serous fluid	"	"	Bluish
	2	4	4	2	"	"	"	"	"	Very hyperemic	"	Thin, clotted slowly, bluish
	4	4	2	4	"	"	"	Normal	Slight amount of serous fluid	"	"	"
n.	3	4	2	3	Swollen, gorged with blood	Normal	Contracted, coronary vessels distended	Filled with blood	Filled with serous fluid	Very hyperemic	Dead, well-developed	Bluish
n.	4	3	1	3	"	Surrounded by jelly-like mucous	Normal	Normal	No excess fluid present	"	"	Thin, Clotted slowly
	1	4	4	3	Swollen, gorged with blood	Normal	Normal	Filled with blood	Filled with serous fluid	Hemorrhage at base of placentae	Dead, well-developed	Bluish
n.	3	4	2	3	"	"	Contracted, coronary vessels distended	"	"	Very hyperemic	"	"
	2	4	2	4	"	"	"	"	"	Hemorrhage at base of placentae	"	"
n.	4	4	2	2	"	Surrounded by jelly-like mucous	"	"	"	"	"	Thin, Clotted slowly, bluish
n.	3	2	4	2	"	"	"	"	"	"	"	"
	1	1	1	1	Normal	Normal	Normal	Normal	No excess fluid present	Very hyperemic	Dead	bluish
n.	1	4	2	1	Swollen, gorged with blood	"	Contracted, coronary vessels distended	Filled with blood	Filled with serous fluid	"	Dead, well-developed	Thin, Clotted slowly, bluish
n.	4	3	2	4	"	"	"	"	"	"	"	"
	4	4	2	4	Swollen, gorged with blood	Normal	Contracted, coronary vessels distended	Filled with blood	Filled with serous fluid	Very hyperemic	Dead, well-developed	Bluish
n.	3	2	1	3	"	"	Normal	"	No excess fluid present	"	Soft, hyperemic	Thin, Clotted slowly, bluish
in.	0	3	2	3	"	"	Contracted, coronary vessels distended	"	Filled with serous fluid	"	"	"
in.	4	3	1	4	"	"	"	"	"	"	"	"
in.	2	4	3	3	"	"	"	"	"	"	"	"
:	2	3	3	4	"	"	"	"	"	"	Dead	bluish

especially in the animals that died, was contracted, and the coronary vessels were greatly distended. The lungs in such cases were filled with blood. Often the pleural cavity contained 3 to 5 cc. of fluid. In three cases the pancreas was completely surrounded by a mucous, semi-solid substance. The material seemed to be completely held within the peritoneal covering of the pancreas and separated the lobules of the gland so that they floated like leaves in the semi-liquid mass. The spleen appeared normal to gross observation.

The uterus in the animals that were sacrificed when moribund was usually much colder than the rest of the body. The blood vessels were always distended. Although the feti were invariably dead, in general they were so well developed and firm that it did not seem possible that they could have been dead very long. In four cases only were the feti soft and apparently disintegrating. Hemorrhage was sometimes found at the base of the placentae, and in other instances the amniotic fluid seemed tinged with blood. The placentae were very loosely attached to the uterine wall.

The blood of the animals that were destroyed as death seemed imminent had a peculiar bluish tinge, which

gradually disappeared as the autopsy progressed. This observation considered in conjunction with the dyspnea and cyanosis strengthened the belief that the animals suffered from anoxemia. The blood of normal rats clots rapidly and often before an autopsy is finished the shed blood in the body cavities is coagulated. The blood of the sick rats, however, was thin and watery and often failed to clot during the time consumed by the autopsy (15 to 35 minutes).

The stomachs of the rats that died were invariably filled with water, as if one of the last acts of the animal had been to drink. Stomach ulcers were found in only three of the animals that died. No evidence of lung infection was found.

Variations in Water Consumption

The data concerning the water consumption of the animals at two-day intervals from the twelfth to the twenty-first days of gestation are presented in table XIV. The water intake of the pregnant control rats and the non-pregnant rats fed the various pork-containing diets increased slowly but rather steadily as gestation progressed.

The sick rats, on the other hand, drank relatively

TABLE XIV. AVERAGE CONSUMPTION OF WATER DURING TWO-DAY INTERVALS IN GESTATION PERIOD BY PREGNANT RATS FED VARIOUS EXPERIMENTAL DIETS[1]

Experimental groups	Consumption of water during two-day intervals in gestation period				
	12th-13th	14th-15th	16th-17th	18th-19th	20th-21st
	cc.	cc.	cc.	cc.	cc.
Steenbock V (Control ration)	14.4	14.5	15.5	16.7	17.1
Pork I (Basal ration)	14.4	17.2	17.0	18.0	17.6
Pork 7 (Pork I plus fresh liver)	17.2	17.4	16.8	17.8	17.7
Pork 39A (Pork I plus 10 mg. lipocaic)	14.6	13.9	15.8	15.3	15.8
Pork 39B (Pork I plus 40 mg. lipocaic)	13.4	17.2	18.0	19.8	20.8
Pork 39C (Pork I plus 500 mg. lipocaic)	16.4	16.7	18.4	17.6	17.8
Pork 31 (Pork I plus liver extract)	14.7	15.4	14.2	15.8	18.4
Sick rats	18.6	18.2	19.1	24.5	15.4

[1]The data for the individual animals from which the averages were obtained is given in table 11 in the appendix.

large amounts of water as early as the twelfth or thirteenth days of gestation, their fluid intake increasing rapidly until a peak was reached at the eighteenth to nineteenth day. The average water consumption then fell markedly just before the first appearance of the symptoms of the disorder, i.e., the twentieth and twenty-first days of pregnancy. These data are difficult to explain. It is possible, however, that the water metabolism of the animals developing pregnancy disorder is disturbed as early as the twelfth day of pregnancy. Also, the fall in the water consumed at the twentieth day may coincide with the beginning of water retention.

Variations in Gain in Body Weight

Many factors may affect the actual gain in body weight made by a pregnant female during the gestation period. Of these, the more important are (1) the body weight and age of the female at initiation of pregnancy, (2) the amount of food and water consumed, (3) the number of feti, and (4) the total weight of the feti. As yet no satisfactory scheme has been found to correlate the effect of these factors in such a way that the normal gain in weight of a given female can be estimated.

In order to determine if the sick females made abnor-

mal gains in body weight during pregnancy, the question was approached from three angles. In the first case, averages were obtained relating to the body weight at the initiation of pregnancy of 22 females showing symptoms of the disorder, and to the number of feti in the uterus of these females. Then, from rats fed the Steenbock V diet a set of 22 normal females was picked in such a way that the average values for the two characteristics were nearly identical with those determined for the sick animals. The gains in weight made by the matched groups during the gestation period are shown in figure III. Rates in gain in the two groups were nearly the same until the twenty-first day of pregnancy. After that time the gain in body weight of the sick females was enormous in relation to that made by the normal group. This comparison, however, did not take into account the possibility that the feti produced by the sick animals might be larger than those of the normal rats. Therefore, two groups of 10 rats each, paired as to average total weight of feti and average number of feti, were drawn from the larger groups. The average daily increments in weight of the two groups are shown in figure IV. These curves show again that the sick rats made an excessive gain in weight between the twenty-first and twenty-second days of

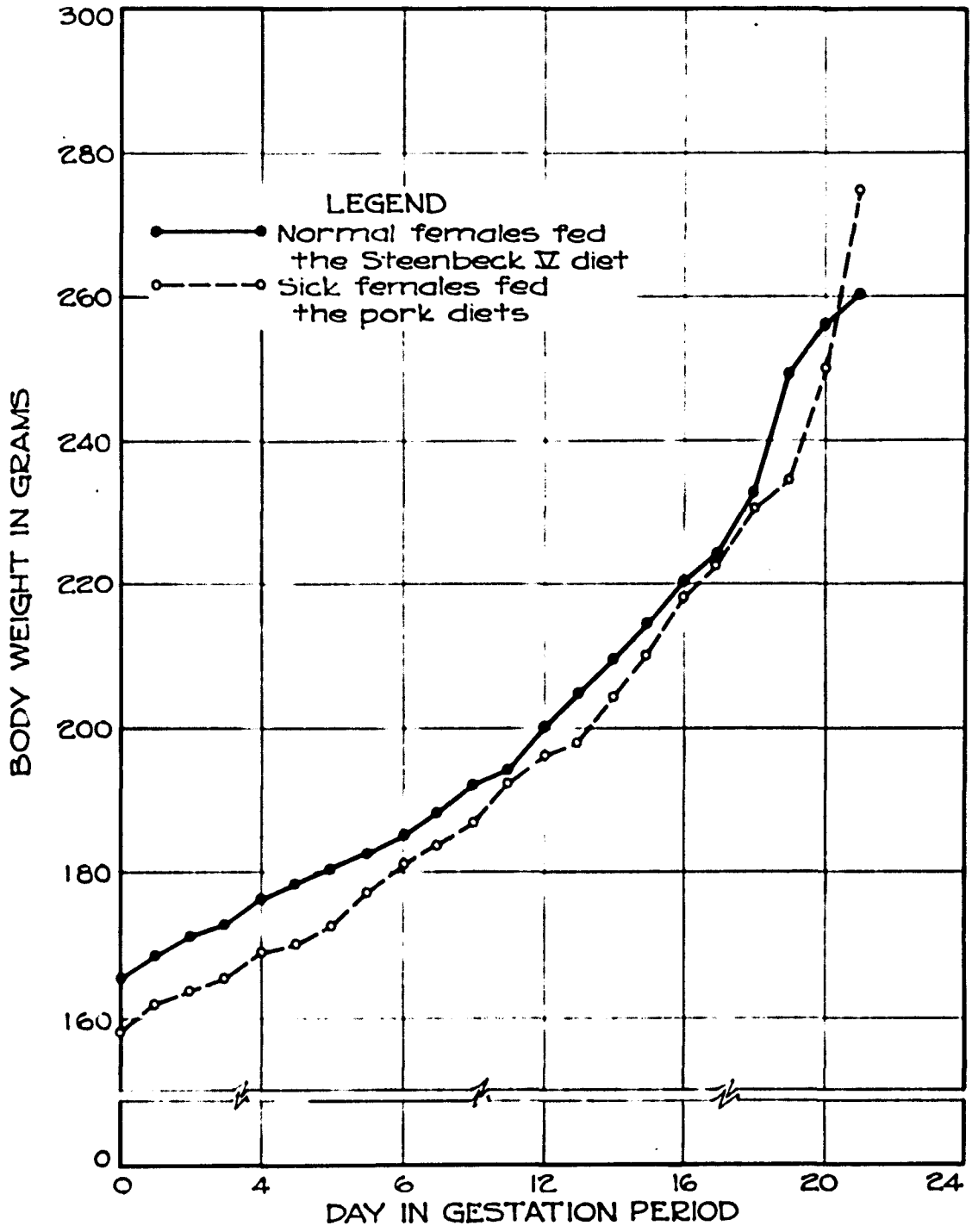


FIG. III. GAINS MADE IN WEIGHT DURING PREGNANCY BY NORMAL FEMALES AND SICK FEMALES MATCHED AS TO INITIAL WEIGHT OF MATERNAL RAT AND NUMBER OF YOUNG

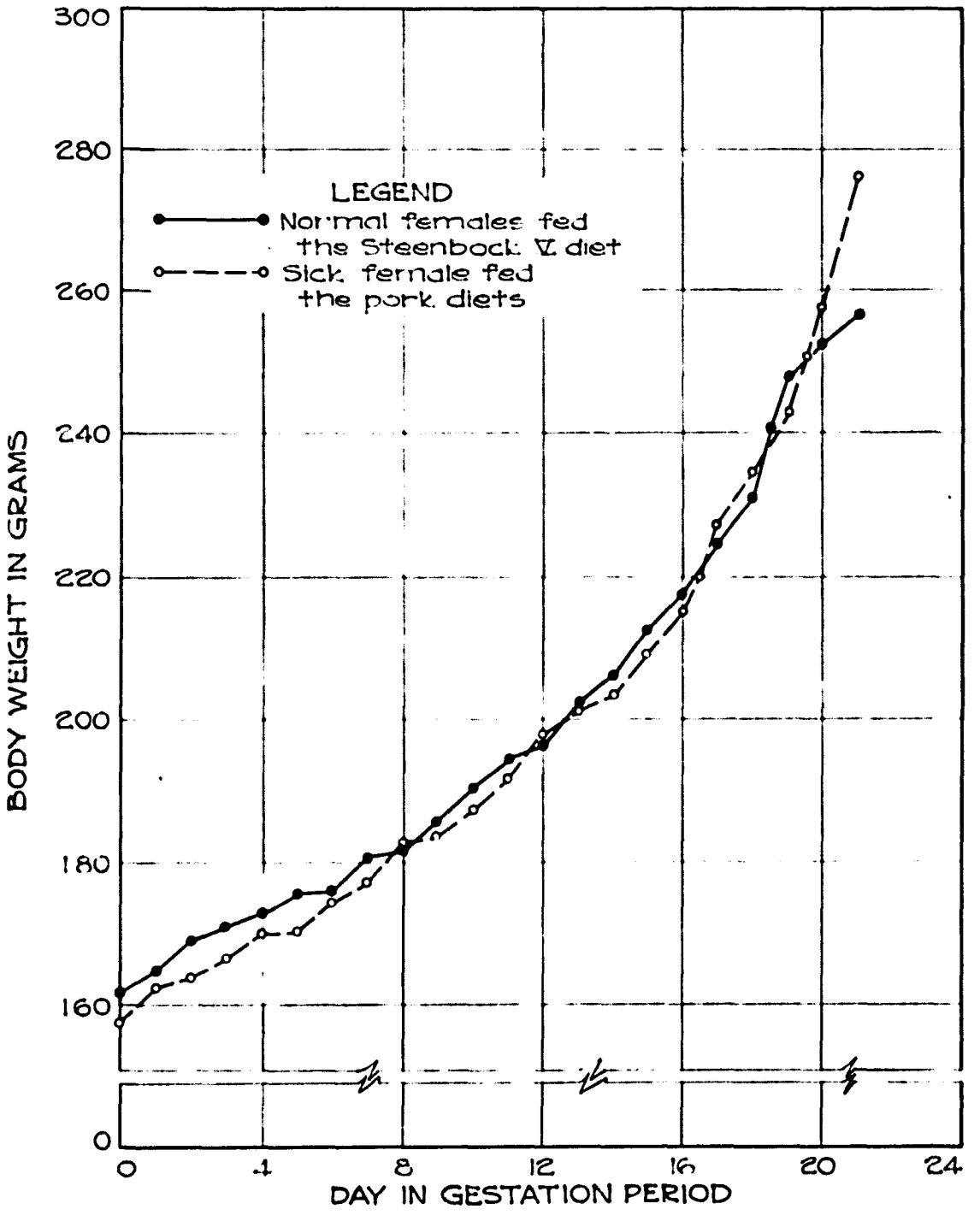


FIG. IV. GAINS MADE IN WEIGHT DURING PREGNANCY BY NORMAL FEMALES AND SICK FEMALES MATCHED AS TO NUMBER OF YOUNG IN LITTER AND TOTAL WEIGHT OF LITTER

gestation.

Still a third method was used to evaluate the significance of the gains in weight. As a basis for this method, it was argued that while the gains made by normal, pregnant rats vary widely, the proportion of the total gain made in any given interval of time should be fairly constant. Therefore, the per cent of the total gain in body weight made by each rat between the twenty-first and twenty-second days of pregnancy in the two matched groups of 22 rats each was calculated. The percentages obtained are arranged below in numerical order.

Steenbock V Females	Sick Females
1.4	5.0
2.6	6.3
2.7	6.5
3.2	7.6
3.3	8.6
6.5	8.8
6.7	9.7
6.9	10.7
7.4	11.6
7.4	12.2
7.8	12.2
8.4	13.8
9.2	19.9
10.2	14.2
10.5	14.7
10.8	15.9
11.1	16.7
11.6	16.8
12.0	17.6
12.8	20.4
13.5	19.2
13.6	32.2

The relative gain in weight made in the last 24 hours of gestation by the females derived from the stock colony was 8.1 per cent of the total increment, and by the sick rats, 12.5 per cent. Not only was the average per cent gain for the group of sick rats higher than that of females fed the Steenbock V diet, but also the total range covered by the values was greater. The lowest percentage gain of total weight in the last day by the moribund animals was 5.0, as compared with 1.4 per cent by the control females. In addition, while the greatest per cent of the total gain in weight made by a stock female was 13.6 per cent, one of the sick females gained a third of the total increase in pregnancy during the last 24 hours of the gestation period.

Judging from the results obtained by using these three methods, we feel justified in concluding that the rats developing symptoms of the pregnancy disorder make excessive gains in body weight between the twenty-first and twenty-second days of pregnancy. These abnormal gains in weight must be related to a disturbance in metabolism resulting in water retention.

Variations in the Fat Content of Liver

An excessively large quantity of fat in the liver is an index of a pathological condition and may affect the ability of the organ to function normally. If large amounts of fat are deposited in the hepatic cells, the amount of glycogen stored therein is reduced. In pregnancy, a deficiency of liver glycogen leads to malnutrition and partial starvation of the fetus and possibly to hypoglycemia in the mother. If glycogen is not stored in the hepatic cells, the gravid animal has no reserve source of energy to call upon during periods of increased metabolism like labor. An excess of fat in the liver also decreases the ability of that organ to detoxify injurious substances.

Gross observations made upon the livers of the sick rats by previous investigators in the Nutrition Laboratory led to the belief that yellow livers were a constant factor in the pregnancy disorder. Therefore, the fat content of the livers of virgin and pregnant rats fed the various experimental diets was determined quantitatively.

The amount of fat present in the livers of the virgin rats is shown in table XV. The fat in the livers of animals fed the pork diets in general was 2 to 5 per cent

TABLE XV. AVERAGE FAT CONTENT OF LIVERS OBTAINED FROM FEMALES FED VARIOUS EXPERIMENTAL DIETS[1]

Experimental groups	Number of animals used		Percent fat in liver on H_2O free basis	
	Virgin females	Pregnant females	Virgin females	Pregnant females
Steenbock V (Control diet)	9	10	20.64	22.04
Pork I (Basal ration)	9	15	25.01	39.41
Pork 7 (Pork I plus fresh liver)	0	4	--	28.38[2]
Pork 39A (Pork I plus 10 mg. lipocaic)	11	19	23.81	38.24
Pork 39B (Pork I plus 40 mg. lipocaic)	7	8	23.51	38.52
Pork 39C (Pork I plus 500 mg. lipocaic)	3	10	22.41	34.26
Pork 31 (Pork I plus liver extract)	8	17	23.82	29.59
Sick rats	--	19	--	31.64

[1]These data are reproduced through the courtesy of Miss Ethelwyn Wilcox. The individual data from which the averages were obtained are shown in table 13 in the appendix.

[2]These analyses were made by Miss Marian Edwards.

higher than that in the livers of the control group. However, the percentages reported for the livers of similar animals fed the various pork-containing diets differed very little among themselves. It seems that the ingestion of the various pork diets produces a small but consistent increase in the amount of fat deposited in the livers of the virgin rats.

While pregnancy caused an increase of only about 1.5 per cent in the quantity of fat in the livers of the normal control animals, a striking increase in this cellular constituent occurred in the rats fed the Pork I diet. The increment amounted to 14 per cent in rats that did not die. Livers of the gravid rats fed the basal pork ration contained approximately 80 per cent more fat than was found in the organs taken from comparable pregnant rats fed the Steenbock V diet. Although the addition of fresh liver or liver extract resulted in a decreased amount of fat in the hepatic cells, these supplements failed to protect the liver as completely from fat deposition as did the Steenbock V diet. The quantity of liver fat in the lipocaic-fed rats was similar to that in the livers of the animals receiving the Pork I diet.

There was less fat present in the livers from animals that exhibited the characteristic symptoms of the preg-

nancy disorder than there was in the organs removed from rats fed the Pork I diet. The livers of the sick rats contained approximately 32 per cent of fat in contrast to 39 per cent in the livers of rats that survived parturition.

We can conclude from the data presented above that the feeding of the various pork diets consistently resulted in a slight increase in liver fat of virgin rats. Pregnancy greatly increased this deposition of fat in the pork-fed animals. The average amount of fat in the livers of rats dying of the pregnancy disorder was within the range found in the animals fed the pork-containing diets that did not develop the characteristic symptoms of the disturbance. The feeding of fresh liver or liver extract reduced the fat content of the liver, while the addition of lipocaic had no apparent effect.

Variations in Weight and Moisture Content of Organs

If experimental variables are reduced to a minimum, comparative weights and moisture contents of organs removed from test animals may reflect the influence of a specific dietary regime. In the experiment herein reported, certain organs, i.e., liver, kidney, spleen, heart, and in the case of the gravid animals, the mammary gland

were studied. Unfortunately, with the exception of the liver there are so few analyses[1] represented in the various experimental groups that the results indicate only trends. For this reason, no attempt has been made to evaluate the effect of the various supplements. The findings have been discussed in three groups, i.e., those referring to (1) the normal control animals receiving the adequate diet, (2) the animals receiving the various pork diets that did not develop the disorder, (3) the animals that were acutely ill. The number of analyses of the organs made in each group are shown in table XVI.

Since organ weight is known to be a function of body weight (Donaldson '24), the ratio of organ weight to body weight is a more reliable measure of organ size than is actual weight. The data on both the actual weights and the ratios of the various organ weights to body size obtained from virgin rats are presented in table XVII. In general little difference was observed between the actual or relative weights of specific organs removed from virgin animals representing the control and pork-fed groups.

The effect of pregnancy upon the size and the relative weight of organs in normal rats was slight except in

1. A large number of these data were lost in the Margaret Hall fire.

TABLE XVI. NUMBER OF ANALYSES MADE RELATING TO WEIGHT AND PERCENTAGE WATER IN ORGANS

Group	Analyses made on liver	Analyses made on kidney	Analyses made on spleen	Analyses made on heart	Analyses made on mammary gland
VIRGIN ANIMALS					
Normal control animals	9	6	6	6	-
Animals fed the various pork-containing diets	38	28	28	28	-
PREGNANT ANIMALS					
Normal control animals	10	3	3	3	3
Animals fed the various pork-containing diets that were not sick	83	15	15	15	15
Animals fed the various pork-containing diets that were sick	19	12	12	12	12

the case of the liver (table XVII). This organ was approximately 30 per cent larger in the normal pregnant female than it was in the virgin animal. In the animals that did not develop the pregnancy disorder, the pork-containing diets produced no additional effect upon the size of the liver. However, the occurrence of the pregnancy disorder caused an enlargement of the liver. The difference in the ratio of liver weight to body weight in these rats was significant (significance ratio,[1] 5.2) when compared with the ratio for rats given the same diet that did not become sick, and approached significance when compared with the normal controls.

The kidney, spleen, and heart of normal rats consuming pork in the diet were like those of the control rats. First examination suggested that the kidney and spleen were larger in the moribund animals than in the rats that were not toxic. However, upon statistical analysis, none of the differences were found to be significant, although the differences in the organ weight/ body weight ratios referring to the kidney and spleen approached significance. The difference in the ratio of liver weight to body weight was significant when the sick rats were compared with pork-fed females whose gestation was normal (significance ratio, 7.3).

1. This ratio is d/p.e.d, where d is the difference between the means and p.e.d is the probable error of the mean difference. If the value of the ratio is equal to 3.0, the difference in the two means may be considered significant. (Sherman, '32, p. 572.)

TABLE XVII. AVERAGE WEIGHTS AND ORGAN WEIGHT TO BODY WEIGHT RATIO OF CERTAI

Group	Liver		Kidney		PREGN
	Weight of fresh organ in grams	Organ weight/ body weight	Weight of fresh organ in grams	Organ weight/ body weight	Weigh fresh in gr
Normal control animals	7.015	0.0376	1.473	0.0079	0.4
Rats fed the various pork-containing diets that were not sick	6.791	0.0365	1.489	0.0076	0.3
Rats fed the various pork-containing diets that were sick	6.637	0.0422	1.809	0.0089	0.5
					VIRG
Normal control animals	5.339	0.0284	1.472	0.0077	0.3
Rats fed the various pork-containing diets	5.216	0.0281	1.492	0.0081	0.3

[1]The data from which the averages were taken is shown in tables 19 to 23.

DY WEIGHT RATIO OF CERTAIN ORGANS REMOVED FROM EXPERIMENTAL ANIMALS[1]

Kidney		Spleen		Heart		Mammary gland	
ght of sh organ grams	Organ weight/ body weight	Weight of fresh organ in grams	Organ weight/ body weight	Weight of fresh organ in grams	Organ weight/ body weight	Weight of fresh organ in grams	Organ weight/ body weight
PREGNANT ANIMALS							
..473	0.0079	0.475	0.0019	0.672	0.0035	4.993	0.0188
..489	0.0076	0.391	0.0020	0.694	0.0039	4.013	0.0163
..809	0.0089	0.532	0.0034	0.724	0.0037	4.222	0.0172
VIRGIN ANIMALS							
..472	0.0077	0.377	0.002	0.704	0.0037	--	--
..492	0.0081	0.346	0.002	0.691	0.0036	--	--

n in tables 19 to 23.

Although the variation in actual weight of the mammary glands was great, the ratios of glandular weight to body weight were very constant in the three groups of animals (table XVII).

Pregnancy, in itself, apparently had little or no effect upon the amount of water present in the organs, with the possible exception of the spleen. The number of animals in these groups was too small to test the significance of the differences noted.

When the amount of moisture present in the various organs obtained from the normal control group and the non-moribund animals fed the pork diet were compared, the liver was the only organ in which the difference between the means was significant (significance ratio , 4.46).

Surprisingly enough, the livers of the pork-fed rats that did not die contained less water than did those of normal animals. This decrease noted in the rats representing the various pork diets is explained, perhaps, by the high fat content of their livers. The cells, overloaded with fat, may be partially dehydrated.

As was noted in the case of the unmated females, the diet fed had no effect upon the amount of water retained in the other organs of the pregnant rats. The wide variation in water content found in the mammary glands

was probably due to variations in the fat content of this organ. No fat analyses were made, so the moisture content could not be reported on a fat-free basis.

Edema is considered by many obstetricians one of the most constant symptoms of eclampsia. It is difficult to detect this condition in the pregnant rat. Therefore, it seemed that moisture determinations would yield pertinent information as to the relative hydration of the animals. The moisture contents of the same organs described above were determined for both virgin and mated animals in the various experimental groups. The data obtained were again divided into three parts, i.e., those pertaining to the normal control, and the sick and the well pork rats.

The moisture contents of the various organs removed from the virgin rats are given in table XVIII. No difference in the per cent of water present could be detected in organs obtained from the virgin animals fed the adequate diet and those receiving the diets containing pork muscle.

The liver, kidney, and spleen of the sick rats showed a marked increase in the per cent of water present when they were compared with the same organs removed from other gravid rats fed the same diet. (Significance ratios, 7.3,

TABLE XVIII. AVERAGE MOISTURE CONTENT OF ORGANS REMOVED FROM EXPERIMENTAL ANIMALS

	Moisture content of liver in per cent[1]	Moisture content of kidney in per cent	Moisture content of spleen in per cent	Moisture content of heart in per cent	Moisture content of mammary gland in per cent
VIRGIN ANIMALS					
Normal control rats	68.9	78.9	76.2	77.6	--
Rats fed the various pork-containing diets	68.3	78.4	78.1	77.6	--
PREGNANT ANIMALS					
Normal control rats	70.3	77.3	80.1	78.2	71.2
Rats fed the various pork-containing diets that were not sick	67.2	78.2	77.6	77.1	58.1
Rats fed the various pork-containing diets that were sick	70.8	82.9	82.2	77.6	67.5

[1]These data are reproduced through the courtesy of Miss Ethelwyn Wilcox.

13.05, 6.76, respectively.) It is interesting that in the moribund the water content of liver has returned to the normal value.

Cellular Changes in Organs

Reports in the literature dealing with the normal histology of the albino rat are very limited. It is difficult, also, to compare or compile published material which is available, because factors in the experimental procedure, such as the diets fed, the histological methods used, and the age, sex, and genetic history of the rats, varied widely. Therefore, for the purpose of the experiment herein described, the microscopic anatomy of the organs taken from the virgin rats in the control group fed the Steenbock V diet has been considered the normal picture for the colony. As was stated in the portion of the thesis dealing with the experimental procedure, the histological technique used in preparing the tissue sections was very carefully controlled so that comparisons made between organs obtained from different animals would be valid.

Therefore, when the virgin rats fed the Steenbock V diet are used as the standard, any deviations from normal noted in the organs obtained from animals in any other

experimental group can be considered as due to the effect either of the diet or of pregnancy; or to the influence of both factors.

Pathology of the liver

Examination made on sections of liver obtained from the virgin rats fed the Steenbock V diet showed large, octagonal-shaped hepatic cells arranged in cords or rows radiating from the intralobular vein (plate IV no. 1). The nuclei were stained dark blue. The cytoplasm was faintly granular, and the cell outlines were distinct. The erythrocytes in the hepatic sinuses were stained bright red, and showed no hemolysis. There was no evidence of any pathological changes beyond an occasional cell which seemed slightly swollen and more granular than the others. While the histological picture presented by the sections of liver taken from virgins fed the various pork-containing diets was essentially normal, slightly more evidence of cloudy swelling was noted than in the normal liver tissue. In the sections representing the livers of virgin rats in the Pork I and Pork 31 groups, a few cells were found that showed fatty infiltration (plate IV, no. 3 and 7.) These data lend support to the belief that even in virgin animals the pork-containing diets are unable to maintain

1. Section of liver from virgin control rat (21551) fed Steenbock V diet. X 160.

2. Section of liver from virgin rat (21617) fed Pork 7 diet. X 160.

3. Section of liver from virgin rat (21631) fed Pork I diet. X 160.

4. Section of liver from virgin rat (21734) fed Pork 39A diet. X 160.

5. Section of liver from virgin rat (21867) fed Pork 39B diet. X 160.

6. Section of liver from virgin rat (21622) fed Pork 39C diet. X 160.

7. Section of liver from virgin rat (21627) fed Pork 31 diet. X 160.

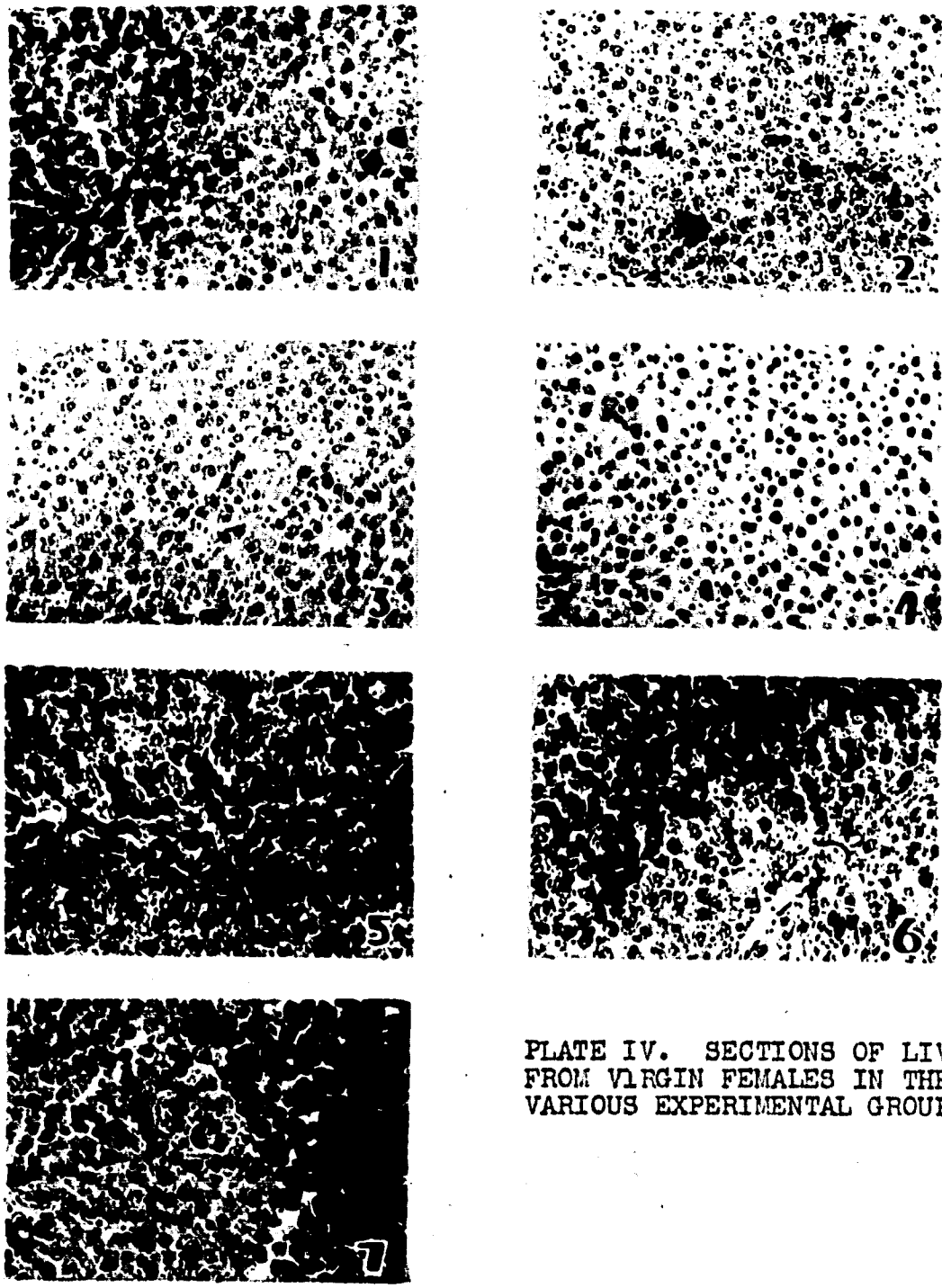


PLATE IV. SECTIONS OF LIVER FROM VIRGIN FEMALES IN THE VARIOUS EXPERIMENTAL GROUPS

quite as healthy liver tissue as the Steenbock V diet.

Pregnancy per se induced certain changes even in the livers of rats fed the Steenbock V diet (plate V, no. 1 and 2.) In three of the five livers examined some fatty infiltration was present. The degree of infiltration was so slight, however, that no more than three cells containing fat were found in any one field examined with the 16 mm. objective. In a few areas the liver cells seemed swollen and the cytoplasm was very granular. The cell outlines in such areas were indistinct and the nuclei were stained a pale blue. Throughout the entire organ the cells seemed more swollen than did those in the livers from the virgin rats fed the same diet. The relative amount of cloudy swelling observed in the livers of virgin and pregnant females was more marked in the rats fed the Pork I than in the Steenbock control animals (plate V, no. 3 and 4).

In addition, even when no evidence of the pregnancy disorder developed, other cell changes were noted in the pork-fed rats. These were apparent in the livers of rats fed the experimental diets containing pork alone or pork supplemented with lipocaic or liver extract. A histological analysis of the livers from these rats indicated that the hepatic cells were, in general, characterized by

1. Section of liver of normal control female (21551) fed Steenbock V diet. X 160.

2. Section of liver of normal pregnant control female (21504) fed Steenbock V diet. X 160.

3. Section of liver of virgin female (21631) fed the Pork I diet. X 160.

4. Section of liver of pregnant female (21458) fed the Pork I diet. X 160.

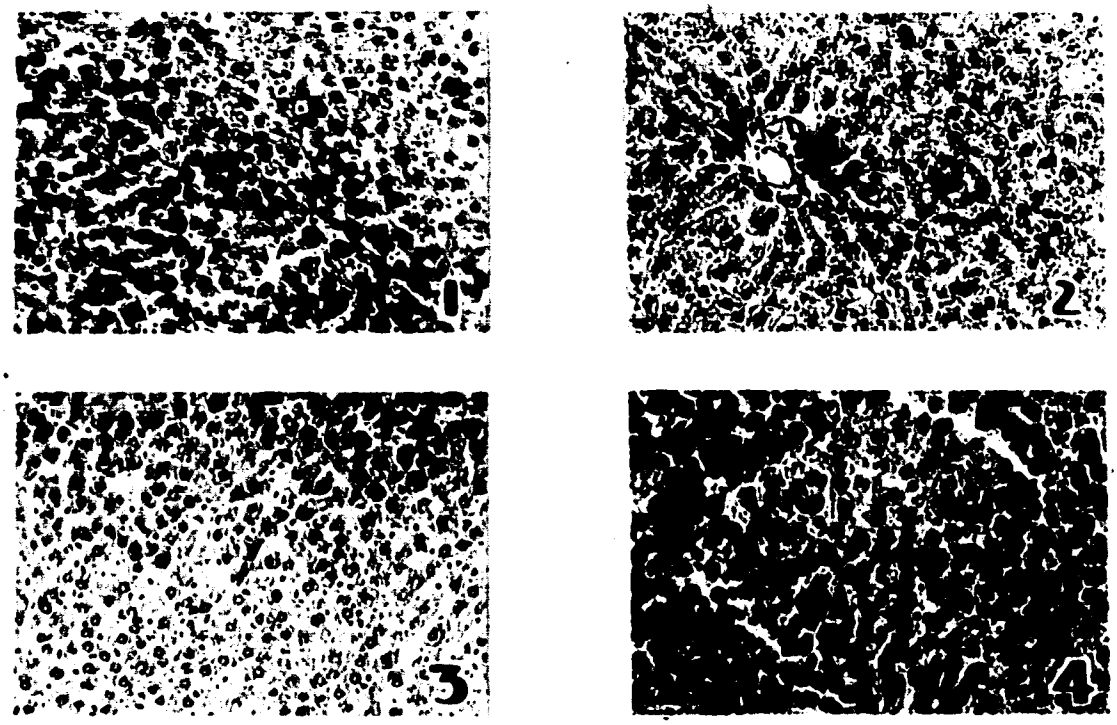


PLATE V. SECTIONS OF LIVER FROM VIRGIN AND PREGNANT FEMALES

moderate cloudy swelling, indistinct cell outlines, poorly staining nuclei, and slight fatty infiltration (plate VI, no. 4, 5, 6 and 7).

The livers of the gravid rats fed the Pork I diet fortified with an addition of fresh liver (Pork 7) presented much the same picture as did those of the virgins in the normal control group. The cell outlines were even more distinct and nuclei more deeply stained than in the livers of the normal control group of mated animals (plate VII, no. 2).

It is difficult to make a reliable distinction between the animals fed the Pork I, the Pork 39, or the Pork 31 rations (plate VII, no. 3, 4, and 7). The degree of the changes appeared to be less marked, however, in the liver tissue of animals receiving the Pork 39 diets than in the hepatic epithelium of gravid females receiving either the Pork I or the Pork 31 diet. Probably the livers of the Pork 31 group showed the greatest change in cell structure of any of the series studied. This was the only group in which fatty degeneration was observed. In six of the ten livers analyzed, about one-twentieth of the hepatic cells showed a derangement of the fat metabolism of the cell as indicated by the presence of small droplets of fat in the cytoplasm, i.e., fatty degeneration.

1. Section from liver of normal pregnant control rat (21504) fed Steenbock V diet. X 160.

2. Section from liver of pregnant rat (21747) fed Pork 7 diet. Three cells show fatty degeneration. X 160.

3. Section from liver of pregnant rat (21458) fed Pork I diet. X 160.

4. Section from liver of pregnant rat (21756) fed Pork 39A diet. X 160.

5. Section from liver of pregnant rat (21795) fed Pork 39B diet. X 160.

6. Section from liver of pregnant rat (21421) fed Pork 39C diet. X 160.

7. Section of liver from pregnant rat (21473) fed Pork 31 diet. X 160.

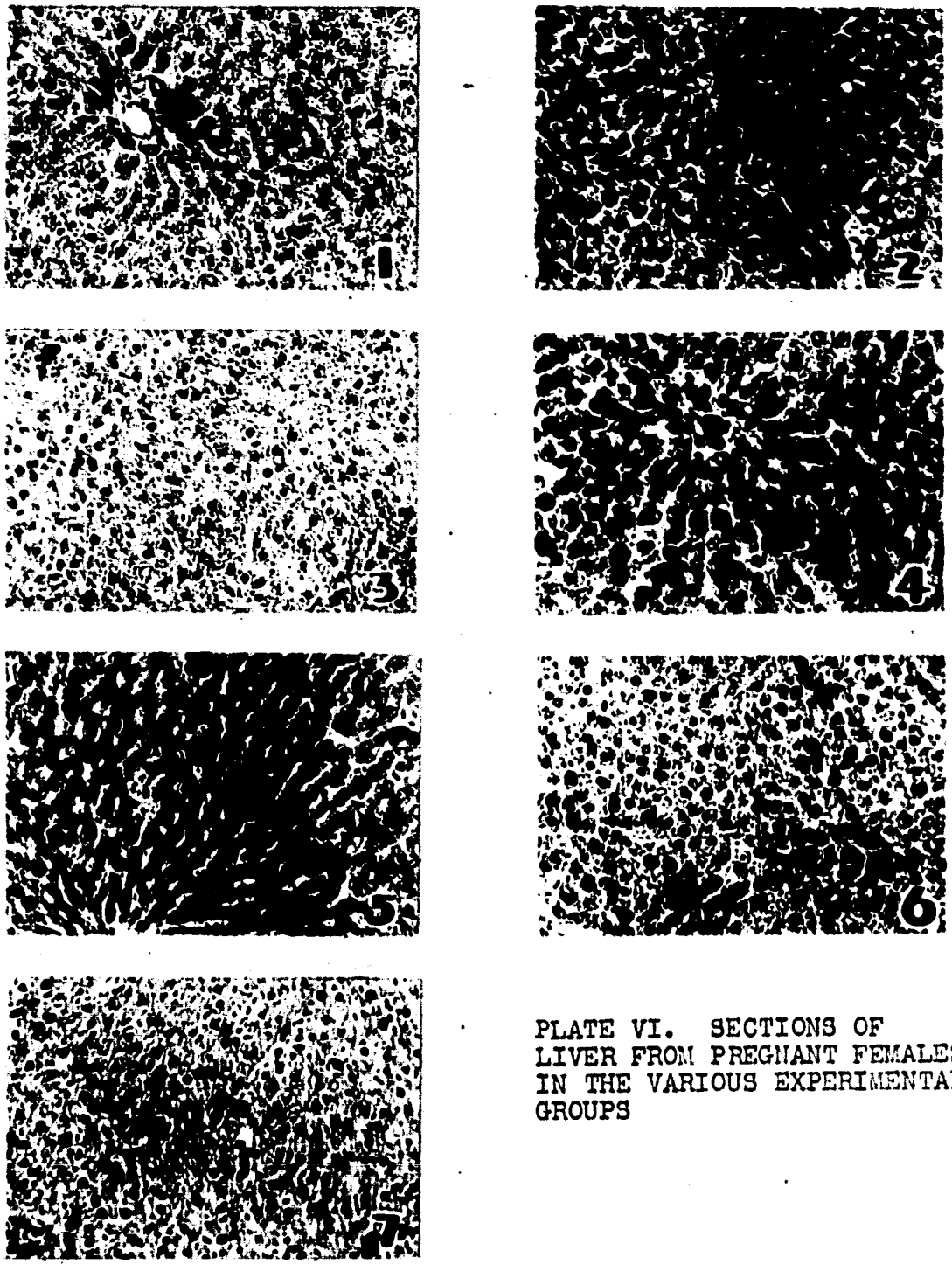


PLATE VI. SECTIONS OF LIVER FROM PREGNANT FEMALES IN THE VARIOUS EXPERIMENTAL GROUPS

1. Section from liver of normal control rat (21504) fed Steenbock V diet.


normal liver cells

2. Section from liver of sick rat (21708) fed Pork I diet.


liver cells - fatty degeneration

3. Section from liver of sick rat (21876) fed Pork 39C diet.


acute fatty degeneratio

4. Section from liver of sick rat (21618) fed Pork 31 diet.


patty infiltration

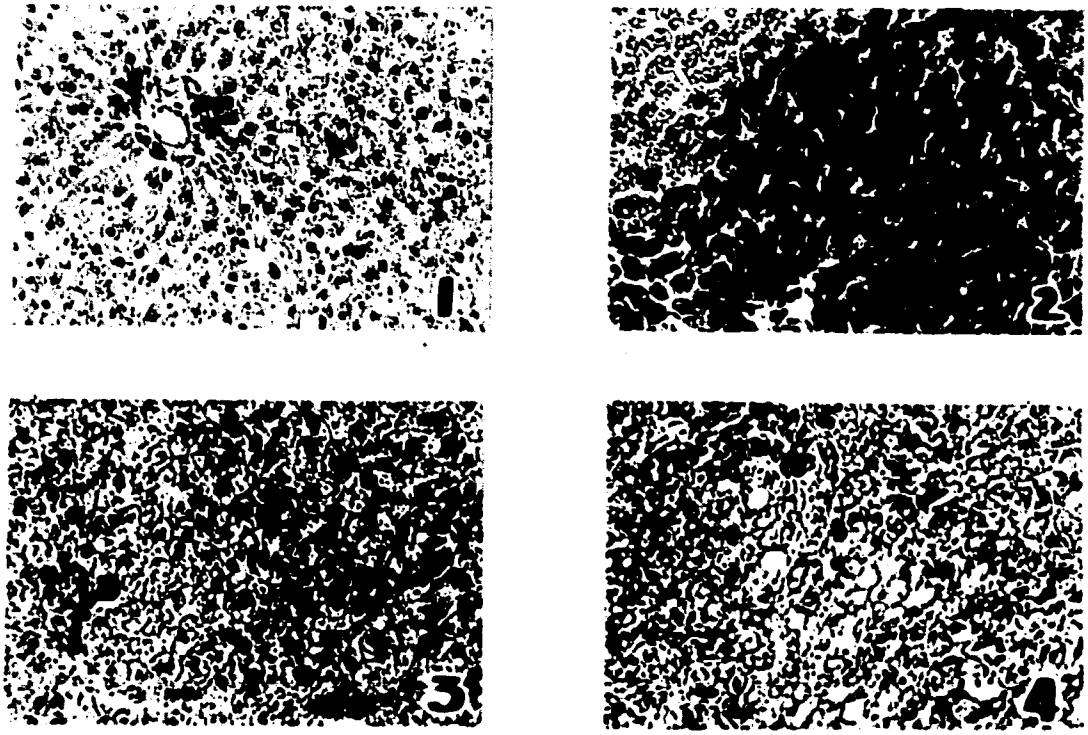


PLATE VII. SECTIONS OF LIVER FROM A PREGNANT CONTROL FEMALE AND THREE SICK RATS

The slight derangement noted in the livers of the rats fed the pork diets became an acute abnormality in the animals developing the pregnancy disorder. Marked fatty degeneration accompanied by varying degrees of fatty infiltration appeared in the livers from all the sick rats regardless of the particular pork diet on which the animals had been maintained (plate VII , no. 2, 3,and 4). Areas of focal necrosis were also present. The pathological changes resulted in an almost complete loss of the characteristic liver structure, only the vascular and biliary vessels remaining unchanged.

We can conclude from these data that the ingestion of the basal pork-containing diet results in some injury to the liver cells in virgin animals. The injury is in turn accentuated by pregnancy. The lesions present in the pregnant control group are also aggravated in the pork-fed rats. The liver supplement was the only dietary addition that definitely prevented the development of the liver condition and kept the organ in as good condition as it was in the virgin rats fed the Steenbock V diet. It is possible, therefore, that the fresh liver contains some dietary essential which is necessary directly or indirectly for the maintenance of normal hepatic epithelium. There is also evidence that the addition of

lipocaic to the basal pork ration resulted in improvement in the condition of the liver.

Complete gestational failure in rats fed the basal pork ration is accompanied by acute fatty degeneration of the liver cells and varying degrees of fatty infiltration and focal necrosis.

Pathology of the kidneys

The kidneys of even the virgin rats fed the control diet (Steenbock V) showed some deviation from cell structure generally considered normal. In the proximal tubules the renal epithelium often was striated and had a tendency to condense at the periphery of the cells. (plate VIII, no. 1). The glomeruli appeared normal, the changes occurring only in the tubular epithelium. The same conditions prevailed in the sections of kidney representing virgin rats fed the Pork I diet supplemented with fresh liver. All other diets containing pork induced greater pathological changes in kidney structure than were observed in the two groups of virgins already described. Some of the tubules in about one-half of the sections of kidney examined contained hyaline material. There seemed to be no difference in the amount of kidney damage irrespective of whether the animals were fed the unsupplemented pork

ration (Pork I) or this diet supplemented with either liver extract (Pork 31) or lipocaic (Pork 39) (plate VIII, no. 2, 7, and 4).

Changes similar to those noted in the kidneys of the virgin rats consuming the Steenbock V diet were noted in the renal tubules of gravid animals fed the same diet. The stratification of the epithelium and the condensation of the cytoplasm had increased somewhat with the pregnancy. No signs of hyaline casts were found (plate IX, no. 1 and 2). The differences noted between the virgin and gravid animals maintained on the Steenbock V diet were even more apparent in the rats fed the Pork I ration (plate IX, no. 3 and 4).

The kidney damage appeared more extensive in organs taken from pre-parturient females in the other experimental groups (rats fed Pork I, Pork 7, Pork 31) than in similar rats in the control group (plate X). The changes observed were mainly cloudy swelling, marked stratification and condensation of the cytoplasm and some hyaline degeneration. In some areas the characteristic structure of the tubular epithelium was lost and the cells had pulled away from the basement membrane.

Again it was difficult to distinguish between organs

1. Section from kidney of normal virgin rat (21551) fed Steenbock V diet. X 160.

2. Section from kidney of virgin rat (21617) fed Pork I diet. X 160.

3. Section from kidney of virgin rat (21631) fed Pork 7 diet. X 160.

4. Section from kidney of virgin rat (21734) fed Pork 39A diet. X 160.

5. Section from kidney of virgin rat (21867) fed Pork 39B diet. X 160.

6. Section from kidney of virgin rat (21622) fed Pork 39C diet. X 160.

7. Section from kidney of virgin rat (21627) fed Pork 31 diet. X 160.

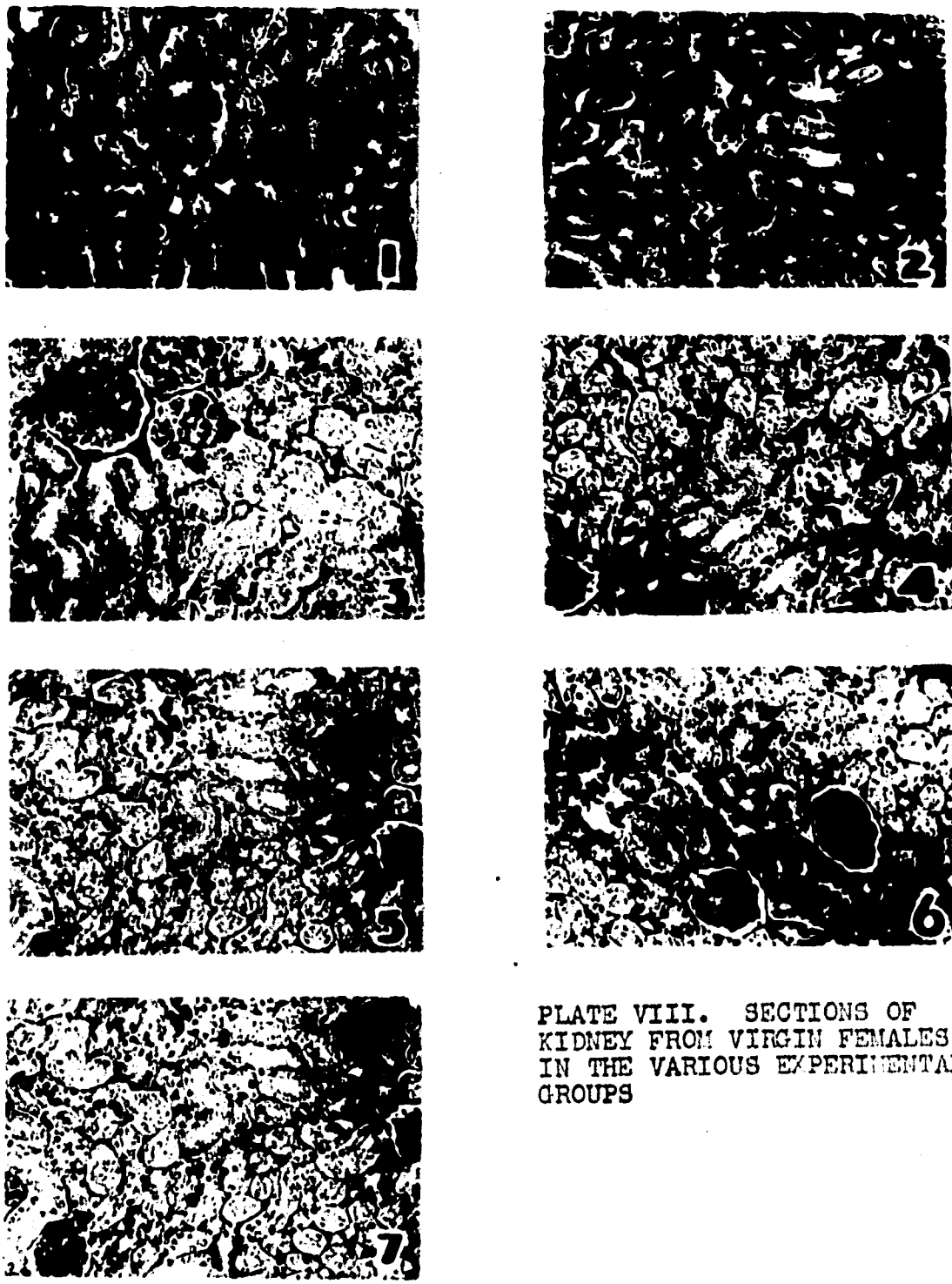


PLATE VIII. SECTIONS OF KIDNEY FROM VIRGIN FEMALES IN THE VARIOUS EXPERIMENTAL GROUPS

1. Section of kidney from normal virgin control rat (21551) fed Steenbock V diet X160.

2. Section of kidney from normal pregnant control rat (21504) fed Steenbock V diet X160.

3. Section of kidney from virgin rat (21631) fed Pork I diet X160.

4. Section of kidney from pregnant rat (21458) fed Pork I diet.

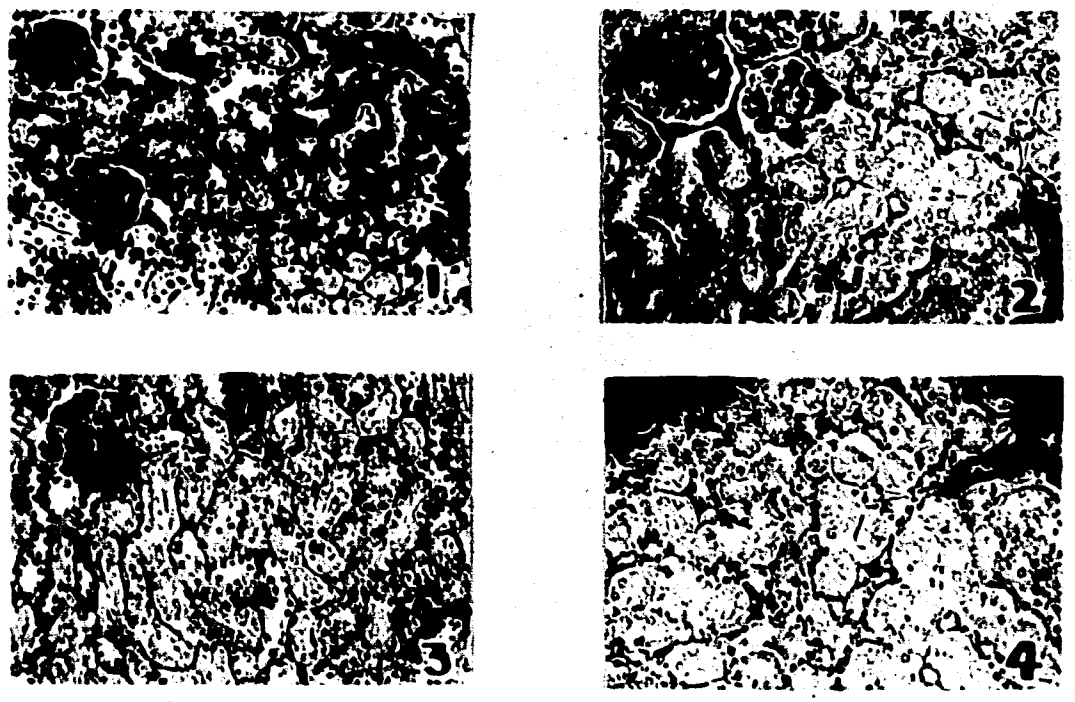


PLATE IX. SECTIONS OF KIDNEY FROM VIRGIN AND PREGNANT FEMALES

1. Section from kidney of normal pregnant control rat (21504) fed the Steenbock V diet. X 160.

2. Section from kidney of pregnant rat (21747) fed Pork 7 diet. X 160.

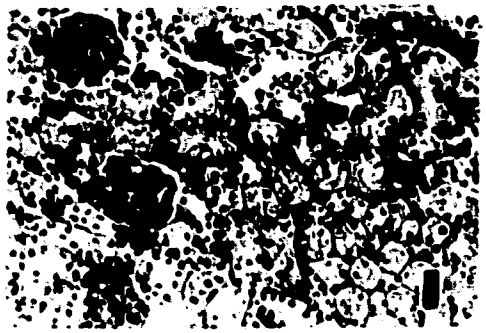
3. Section from kidney of pregnant rat (21458) fed Pork I diet. X 160.

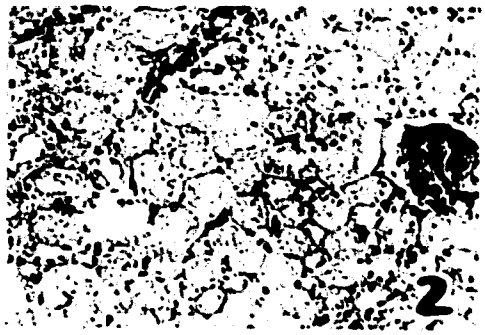
4. Section from kidney of pregnant rat (21756) fed Pork 39A diet. X 160.

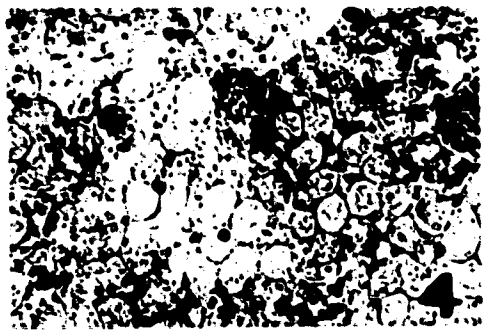
5. Section from kidney of pregnant rat (21795) fed Pork 39B diet. X 160.

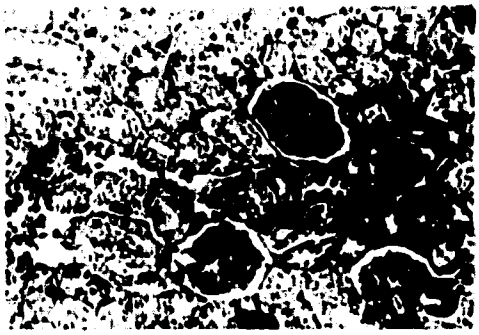
6. Section from kidney of pregnant rat (21421) fed Pork 39C diet. X 160.

7. Section from kidney of pregnant rat (21473) fed Pork 31 diet. X 160.









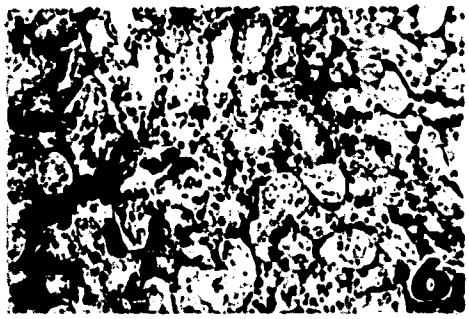


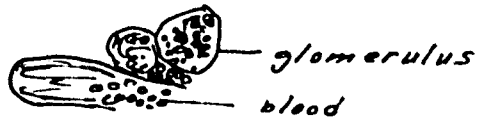
PLATE X. SECTIONS OF KIDNEY FROM PREGNANT FEMALES IN THE VARIOUS EXPERIMENTAL GROUPS

removed from animals fed the various pork-containing diets. However, fewer hyaline casts were noted in the sections from kidneys of the mated females ingesting the Pork I diet supplemented with fresh liver (Pork 7) than in any other corresponding group of animals except those fed the control diet (plate X, no. 2).

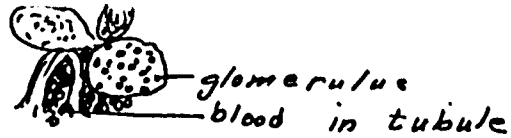
If pregnancy disease develops, the damage to the kidney is greatly increased and much renal tissue is destroyed. The first thing noted upon examination of the sections was extensive hemorrhage within the tubules. (plate XI, no. 2, 3 and 4). The renal epithelium was completely destroyed. Large deeply-stained nuclei could be found, but the cytoplasm was so fragmented that the cell outlines were completely lost. The renal blood vessels were congested. With the exception of the glomeruli the hemorrhage apparently occurred throughout the organ. The glomeruli showed marked hyperemia, but otherwise were normal. There were no signs of either recent or long standing renal infection. As far as could be determined, no fatty degeneration of the renal epithelium had occurred. The changes described were found in greater or less degree in the kidneys of every rat that showed symptoms of the pregnancy disorder. The animals that were sick for

1. Section from kidney of normal pregnant control rat (21504) fed Steenbock V diet.

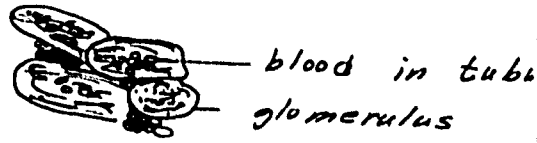
2. Section from kidney of sick rat (21708) fed Pork I diet.



3. Section from kidney of sick rat (21876) fed Pork 39A diet.



4. Section from kidney of rat (21618) fed Pork 31 diet.



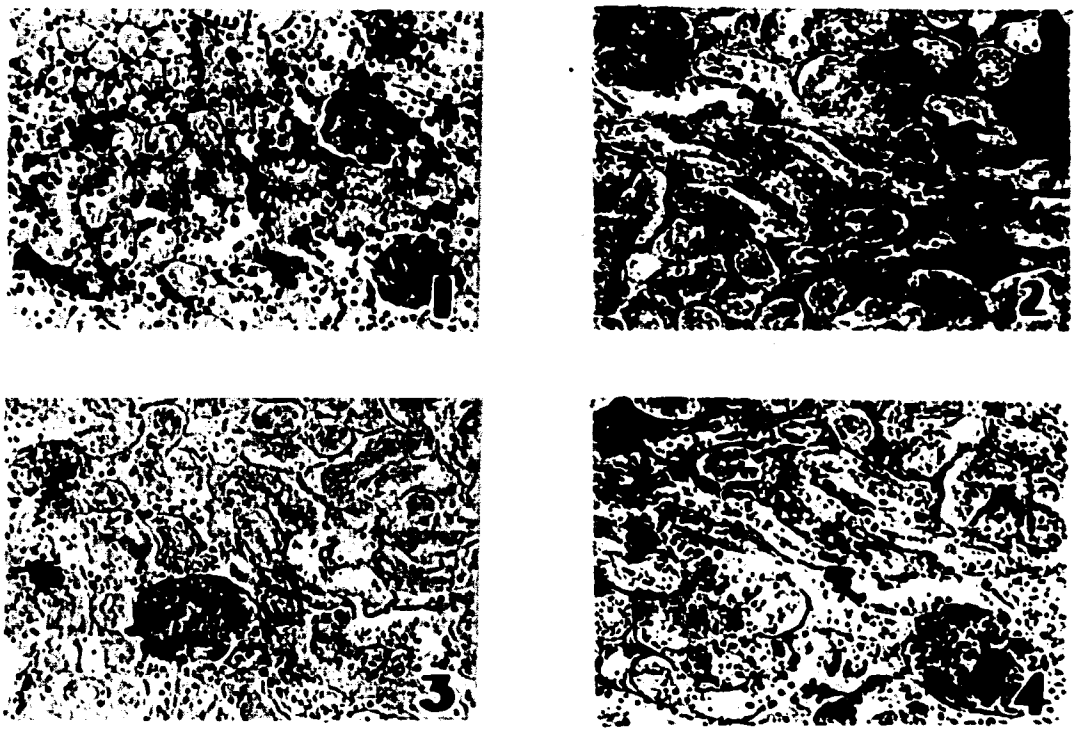


PLATE XI. SECTIONS OF KIDNEY FROM A PREGNANT CONTROL FEMALE AND THREE SICK RATS

several hours before they died showed more extensive renal damage than did the rats that died suddenly without the appearance of the characteristic symptoms.

Thus, it seems that even rats fed an adequate diet show evidence of renal injury. The condition is aggravated by pregnancy and by the diet consumed during this interval. If the pregnancy disorder develops, the kidney tissue is almost completely destroyed.

Pathology of spleen, heart, and pancreas

No differences could be detected between spleens of the virgin rats comprising any of the experimental groups and the normal control females. The sections of spleen obtained from the gravid females fed the various diets likewise showed no evidence of any pathological changes. In addition, there were no discernible deviations from the normal histology of this organ in the rats that suffered from the pregnancy disorder. In passing, it should be noted that in one of the sick rats (21937) some hemolysis in the spleen was noted (plate 2, appendix). As the spleen presented no evidence of pathological changes, photomicrographs are included of only the spleens from the virgin and pregnant females in two groups, i.e., the control group fed the Steenbock V

diet and the group receiving the Pork I ration (plate XII, no. 1, 2, 3 and 4). Typical photomicrographs of spleens obtained from animals in each experimental group are presented in plates 1 and 2 in the appendix.

Sections of heart muscle removed from animals in the various experimental groups likewise yielded no indications of pathological changes due either to diet or to pregnancy. The only abnormalities noted in the hearts of the sick animals were a marked hyperemia and occasional slight hemorrhage. As in the case of the spleen, only photomicrographs of heart muscle from the normal females and those fed the basal pork ration are presented (plate XII, nos. 5, 6, 7 and 8). Photomicrographs of cardiac tissue obtained from animals in the other experimental groups are shown in plates 3 and 4 in the appendix.

Inasmuch as the addition of a pancreatic extract to the basal pork ration had been shown capable of preventing the pregnancy disorder (Wilcox, '37), it was possible that changes in the pancreas might be responsible for the condition. The sections of the pancreas, therefore, were examined with interest. However, results of the study of this gland were disappointing. In so far as could be determined when hematoxylin and eosin were used for staining, no difference existed between the pancreas of

1. Section of spleen of virgin rat (21551) fed Steenbock V. diet. X 160.

2. Section of spleen of pregnant rat (21504) fed Steenbock V diet. X 160.

3. Section of spleen of virgin rat (21631) fed Pork I diet. X 160.

4. Section of spleen of pregnant rat (21458) fed Pork I diet. X 160.

5. Section of heart of virgin rat (21551) fed Steenbock V diet. X 160.

6. Section of heart of pregnant rat (21504) fed Steenbock V diet. X 160.

7. Section of heart of virgin rat (21631) fed Pork I diet. X 160.

8. Section of heart of pregnant rat (21458) fed Pork I diet. X 160.

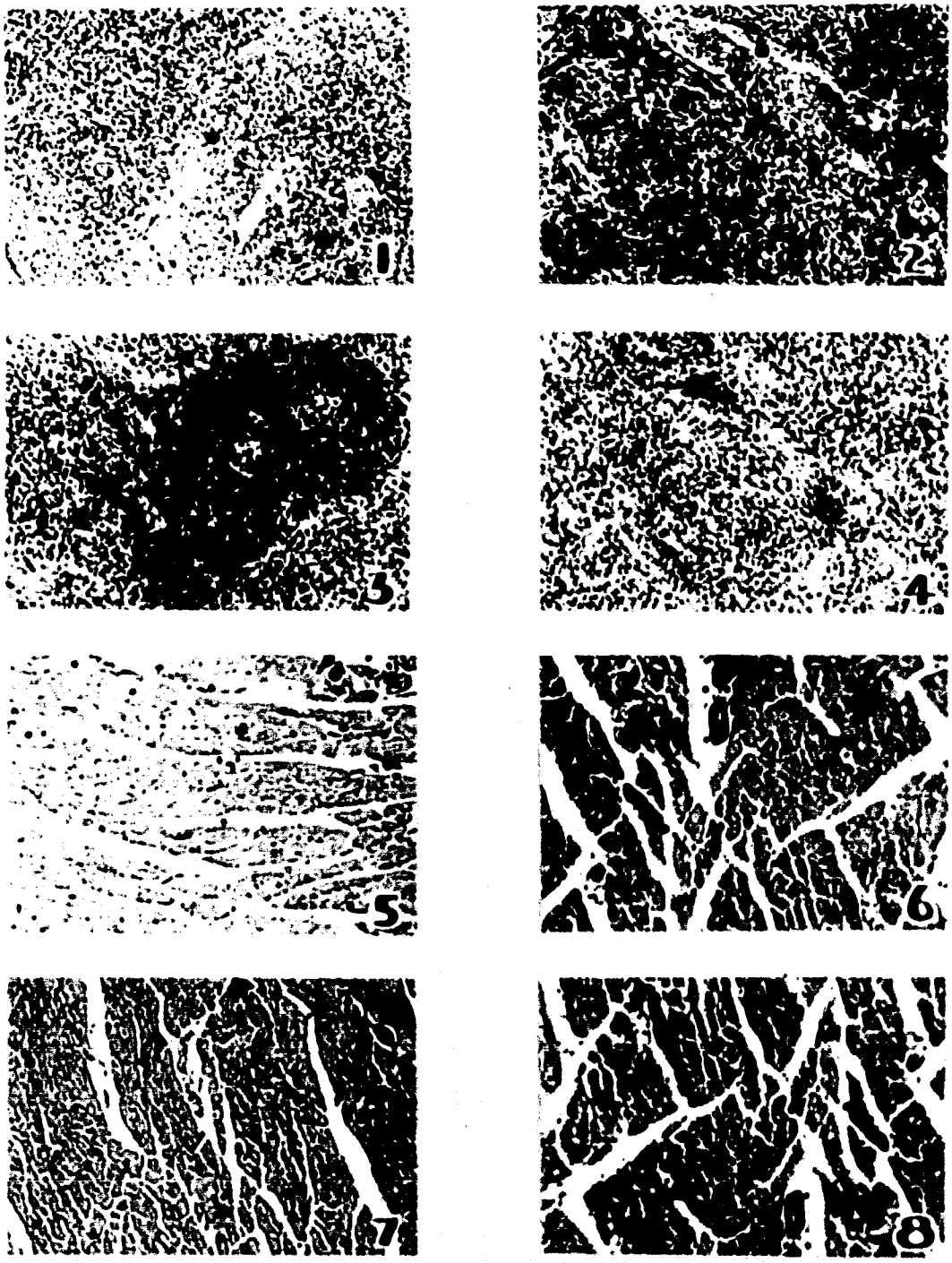


PLATE XII. SECTIONS OF SPLEEN AND HEART FROM VIRGIN AND PREGNANT FEMALES

the normal virgin rats and those of animals in the other experimental groups. Photomicrographs of sections from the pancreas of animals fed the control diet, the basal pork diet, and the Pork I ration supplemented with lipocaic are presented (plate XIII). The pancreas of the sick animals also appeared normal (plate XIII, no. 7), with the exception of those rats in which the pancreas at necropsy was found enclosed in a jelly-like substance. The acini of such a pancreas were contracted and stained very deeply. The connective tissue supporting the lobules was broken. No evidence of fat necrosis was found, however. The islets appeared normal (plate XIII, no. 8). We plan to study the islets more intensively in the future.

General summary of pathology of organs

From the data presented we can conclude that the ingestion of the basal ration containing dried, autoclaved pork muscle as the sole source of protein results in some injury to the renal and hepatic epithelium during pregnancy. The condition prevailing in the gravid animals is apparent in an incipient stage in the virgin females. The addition of fresh liver to the basal diet mitigates the effect of the pork ration upon the liver and kidneys and prevents the incidence of the pregnancy disorder. There

1. Section of pancreas of virgin female (21551) fed the Steenbock V diet. X 160.

2. Section of pancreas of normal pregnant control female (21504) fed the Steenbock V diet. X 160.

3. Section of pancreas of virgin female (21631) fed the Pork I diet. X 160.

4. Section of pancreas of pregnant female (21458) fed the Pork I diet. X 160.

5. Section of pancreas of virgin female (21651) fed the Pork 39A diet. X 160.

6. Section of pancreas of pregnant female (21475) fed the Pork 39A diet. X 160.

7. Section of pancreas of sick female (21911) fed the Pork 39B diet. X 160.

8. Section of pancreas of sick female (21841) fed the Pork 39B diet. Pancreas surrounded by jelly-like material. X 160.

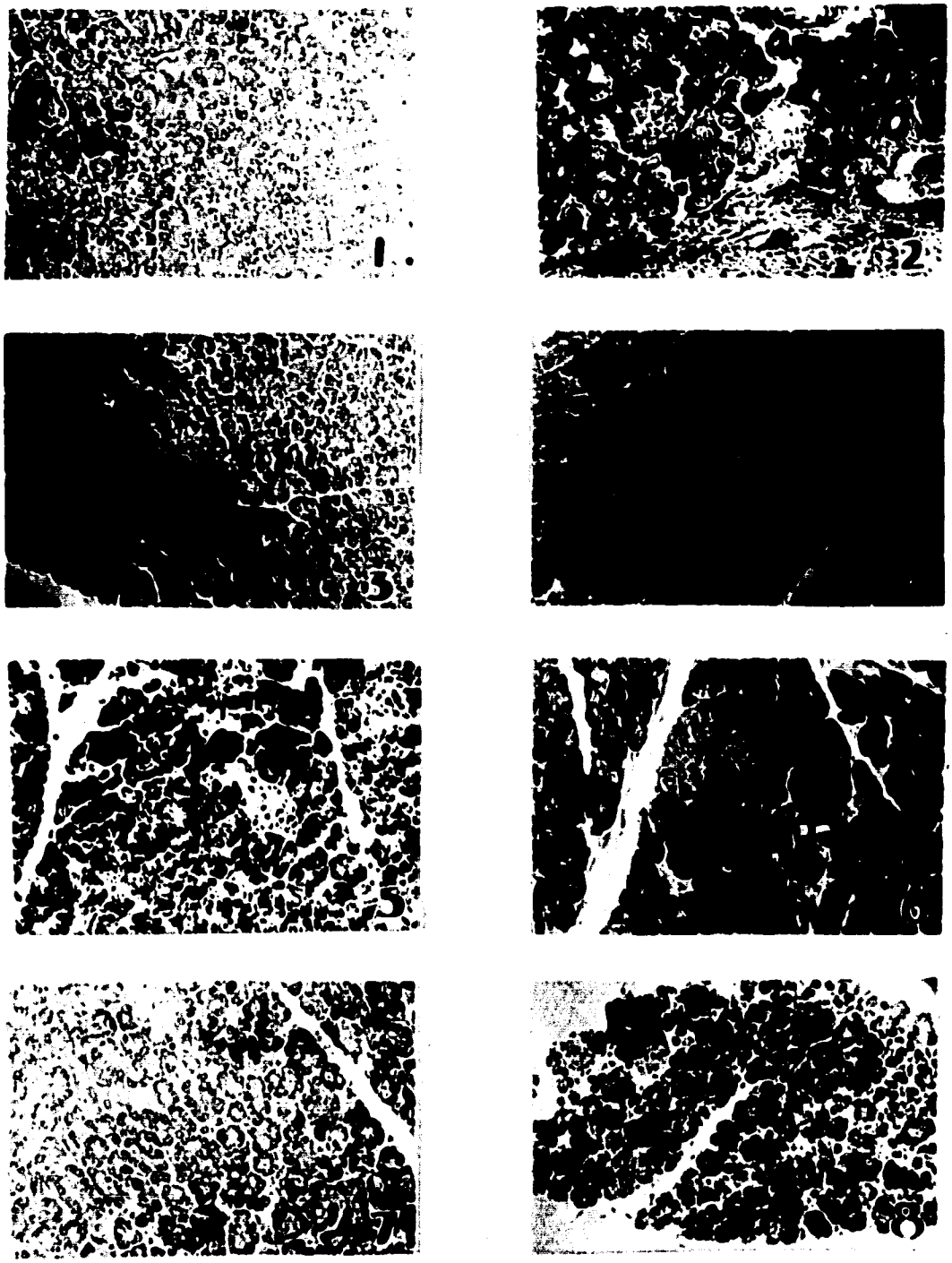


PLATE XIII. SECTIONS OF PANCREAS FROM VIRGIN AND PREGNANT FEMALES FED STEENBOCK V, PORK I, AND PORK 39B DIETS

is slight evidence that lipocaic also exerts a beneficial influence. The pregnancy disorder resulting in complete gestational failure is associated with definite lesions in the liver and kidney. In the liver the normal structure is destroyed and the hepatic cells show marked fatty degeneration accompanied by varying degrees of fatty infiltration and necrosis. The principal lesion in the kidneys is hemorrhage in the tubules and fragmentation of the cytoplasm of the tubular epithelium.

Apparently no definite pathological changes in the heart and spleen are associated with the ingestion of the pork-containing diets or with the development of the pregnancy disorder. The hyperemia and hemorrhage noted in hearts removed from the moribund animals were probably caused by the convulsions observed in these rats. Changes in the pancreas taken from the sick rats were found only in cases where the pancreas was surrounded by a jelly-like substance.

Cellular Changes in Feti and Placentae

If the symptoms heretofore described are characteristic of a deranged pregnancy, the feti and placentae of the animals acutely ill might be expected to reflect the condition. Also, examination of fetal and placental tissues

might furnish reason why so many young of the pork-fed females die in the first four days after birth. Again the histology of the feti and placentae of females fed the Steenbock V diet was taken as the standard.

The visceral organs of these feti were well-developed and could be readily identified (plates XIV and XV). Relatively speaking, the liver is by far the largest organ in the body of the fetus, occupying practically the entire abdominal cavity. The ventral portion of the fetal livers in normal rats showed a marked hyperemia. This hyperemic condition in the liver was a constant finding and probably was due partly to the effect of gravity. The livers of the normal control rats also showed moderate necrosis. However, as the intestinal loops nearest the necrotic areas in the liver showed some epithelial degeneration, we believe that the hepatic necrosis is an artifact due to slow penetration of the fixative into the liver. This view is supported by Corey's (32) observation that fetal degeneration begins in the liver and gastro-intestinal tract.

The placenta of the rat is discoidal, the maternal blood being separated from fetal blood by a layer of endothelial cells and by the chorion. The placentae removed from rats fed the adequate diet were very vascular. Practically every area

1. Heart

2. Liver

3. Intestine

4. Uterine wall

5. Amnio-chorion

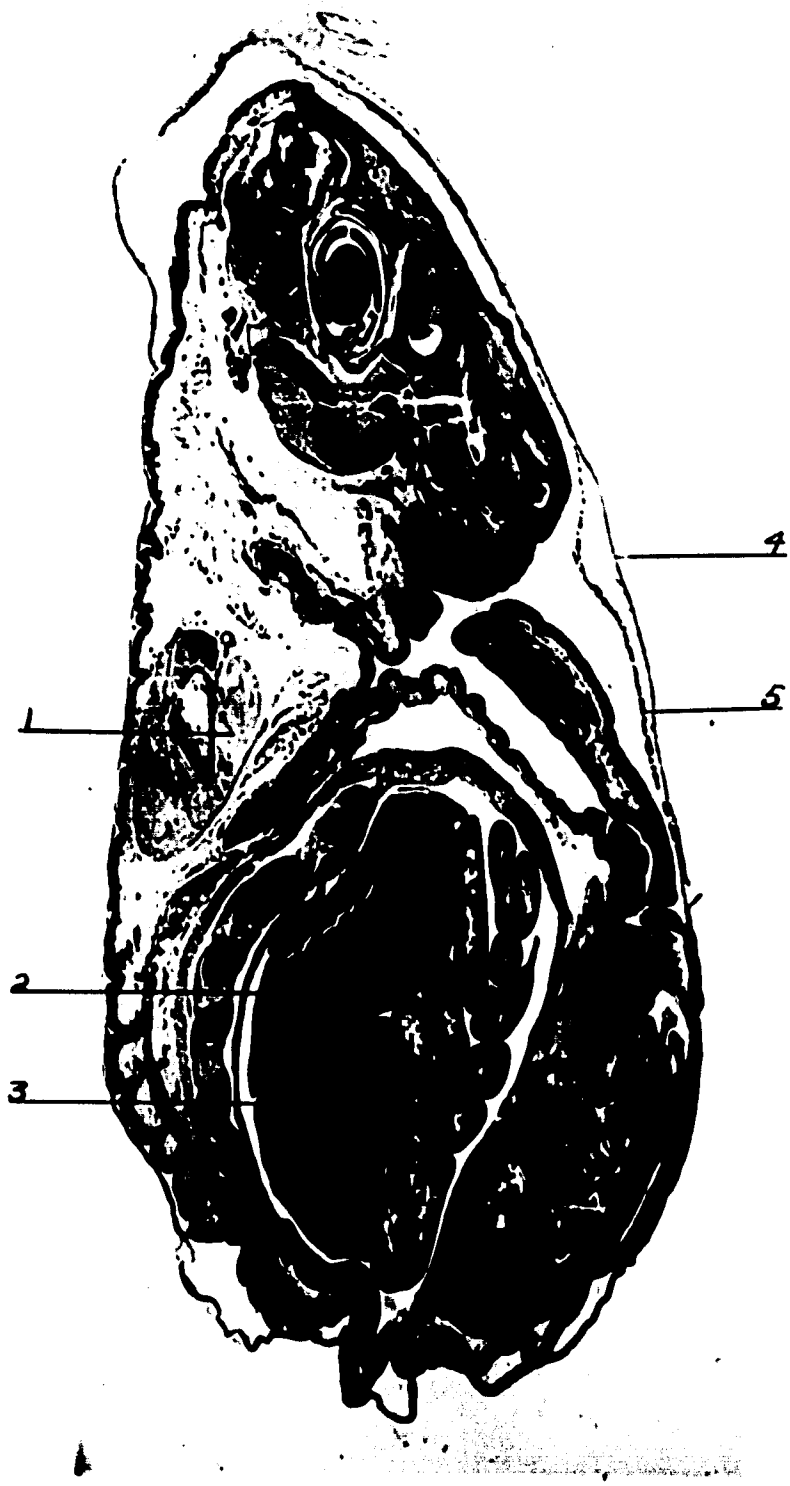


PLATE XIV. SECTION OF FETUS FROM A NORMAL PREGNANT CONTROL FEMALE

1. Heart

2. Lung

3. Placenta

4. Liver

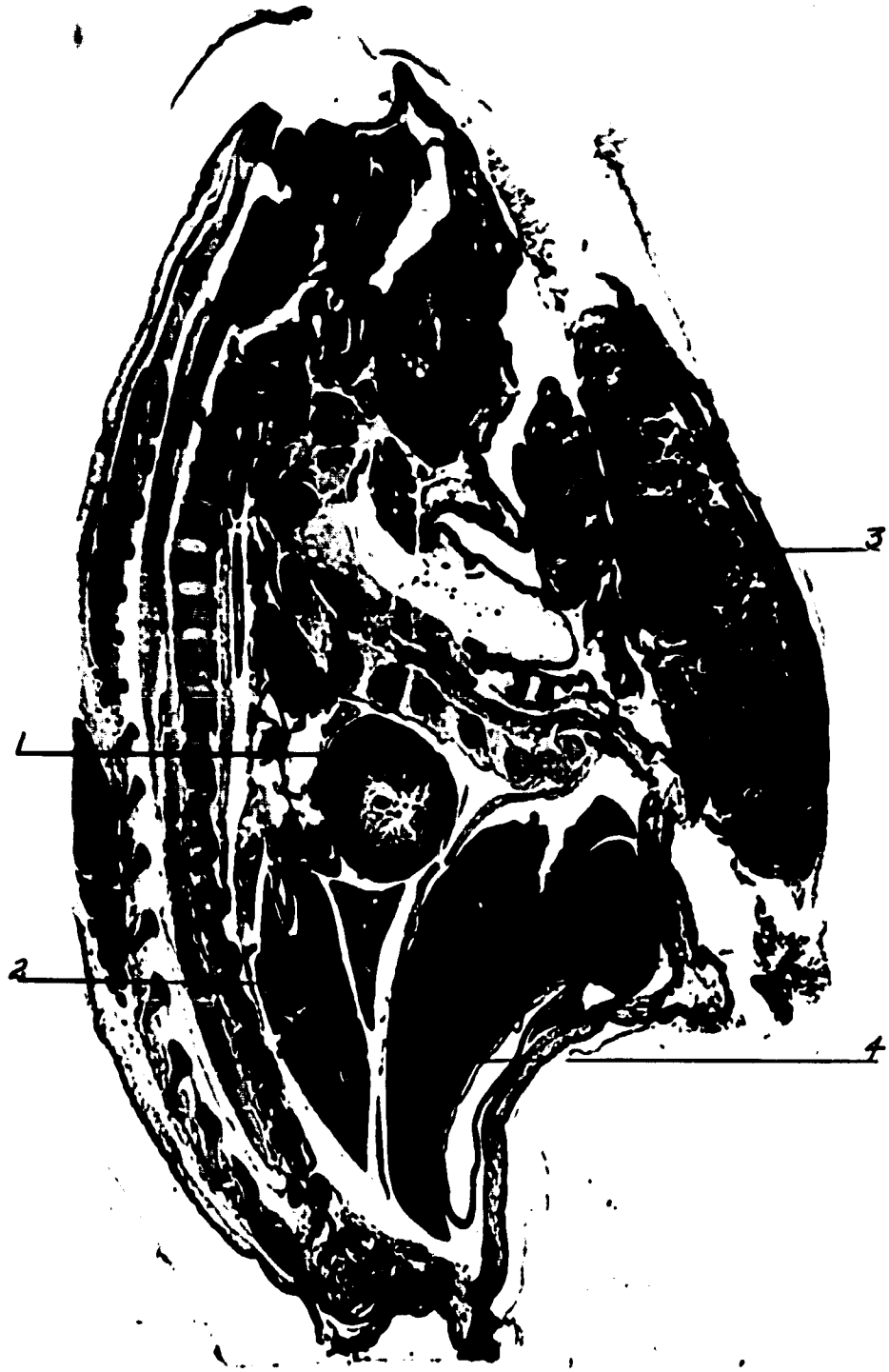


PLATE XV. SECTION OF FETUS FROM A SICK RAT

not occupied by endothelium or supporting tissue was filled with blood. The blood in both the fetus and the placentae showed no signs of hemolysis. The cell outlines of the erythrocytes were distinct and the cells themselves were stained a bright red (plate XVI, no. 1).

No deviations from the normal histology could be detected in the feti or placentae from the pork-fed rats that did not suffer from the pregnancy disorder (plate XVI). However, only one fetus and placenta from each litter was studied. It is possible that when all feti developing in a pregnancy are studied, we may find changes in some of the feti or their accompanying placentae which will explain the poor survival of the young. The changes noted in the livers of feti obtained from rats fed the pork-containing diet were essentially the same as those observed in feti of the normal animals.

However, pathological changes noted in the feti and placentae from the rats that were acutely ill formed a constant and characteristic picture regardless of the diet of the mother. Even upon gross observation after fixation the placentae in such cases were much less vascular than normal. Microscopic observation showed many of the sinuses devoid of blood. Some hemolysis of the fetal blood occurred in the placenta. Even in areas where the blood

1. Section of a placenta of a normal pregnant control rat (21504) fed Steenbock V diet. X 160.

2. Section of a placenta of pregnant rat (21747) fed Pork 7 diet. X 160.

3. Section of a placenta of pregnant rat (21458) fed Pork I diet. X 160.

4. Section of a placenta of pregnant rat (21756) fed Pork 39A diet. X 160.

5. Section of a placenta of pregnant rat (21795) fed Pork 39B diet. X 160.

6. Section of a placenta of pregnant rat (21421) fed Pork 39C diet. X 160.

7. Section of a placenta of pregnant rat (21473) fed Pork 31 diet. X 160.

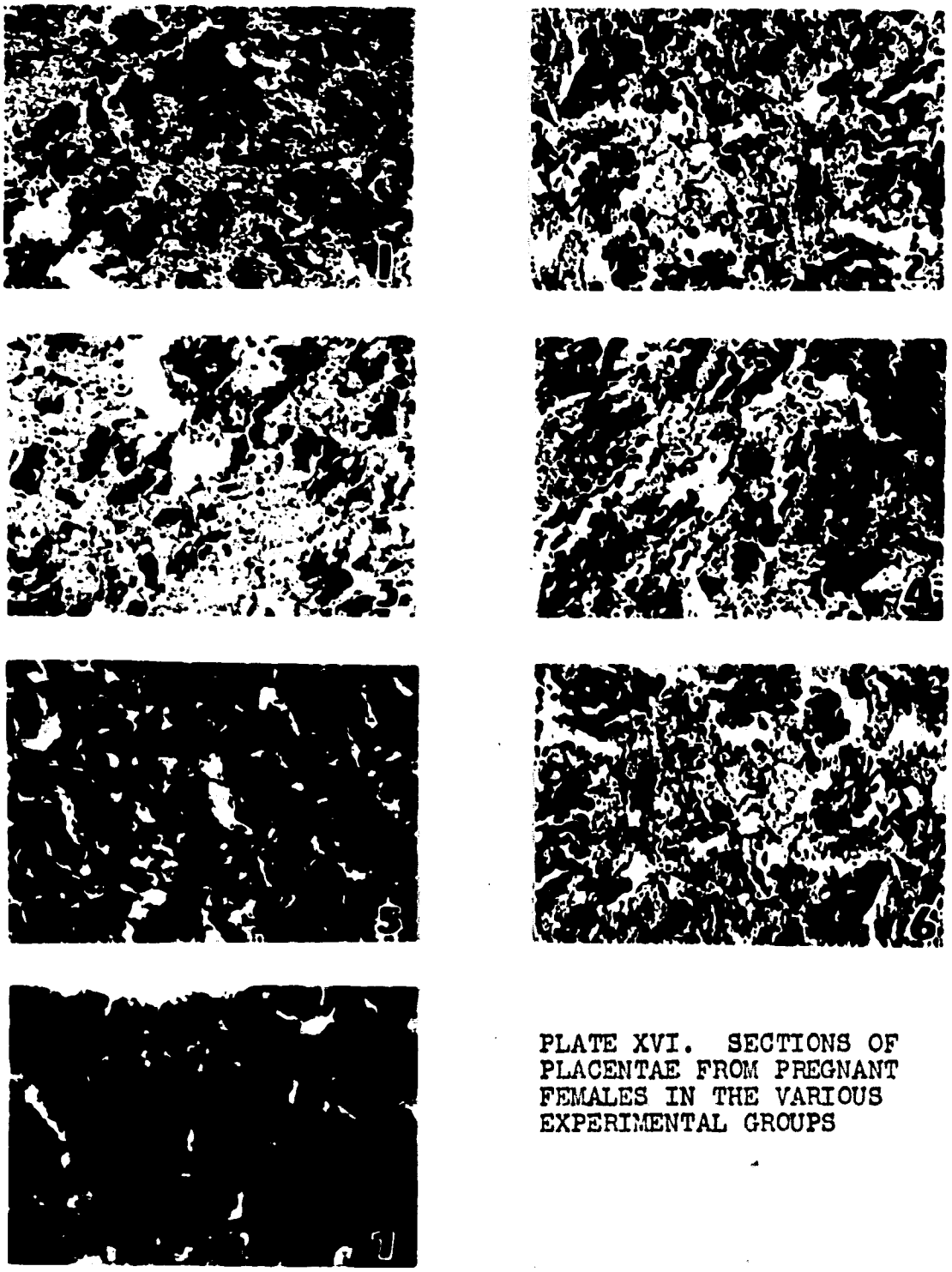


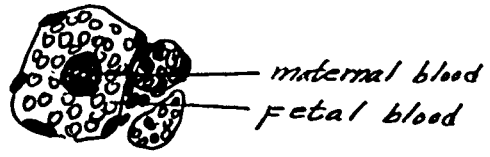
PLATE XVI. SECTIONS OF PLACENTAE FROM PREGNANT FEMALES IN THE VARIOUS EXPERIMENTAL GROUPS

cells are still intact, the erythrocytes failed to stain in a normal fashion. There was no evidence, however, of infarcts or endarteritis (plate XVII, no. 1, 2 and 3).

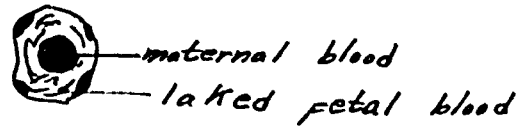
Inspection of the umbilical vein in these cases always disclosed a large thrombus in that vessel (plate XVIII, no. 1). The blood in the umbilical vein, the liver, and the heart was hemolyzed as in the fetal circulation in the placenta (plate XVIII, no. 3). It was difficult to obtain much reliable information as to the condition of the livers of feti taken from the sick rats, as we were in doubt about the cause of necrosis. However, in these feti the necrosis seemed more advanced than was to be expected from the corresponding degeneration of the intestinal epithelium. Therefore, it seemed possible that only a portion of the necrosis was due to poor penetration of the fixative. A marked leucocytosis was also noted in the liver. No signs of infection were found in the placentae, the feti, or the uterine wall. As far as could be determined, the other structures in the fetus were normal.

The general microscopic picture of the feti and placentae obtained at autopsy from females that died leads to the conclusion that some hemolytic agent is at least indirectly responsible for the death of the feti.

1. Section of a placenta from a normal rat (21504) fed the Steenbock V diet.



2. Section of a placenta from a sick rat (21708) fed the Pork I diet.



3. Section of a placenta from a sick rat (21876) fed the Pork 39A diet.

4. Section of a placenta from a sick rat (21618) fed the Pork 31 diet.

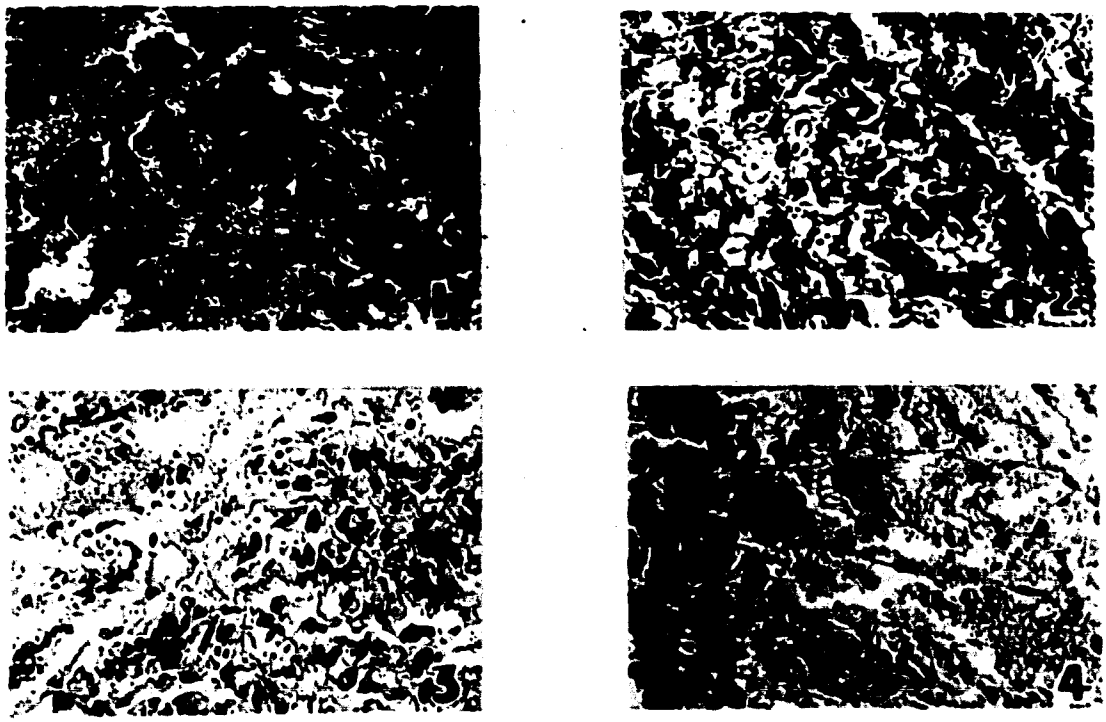
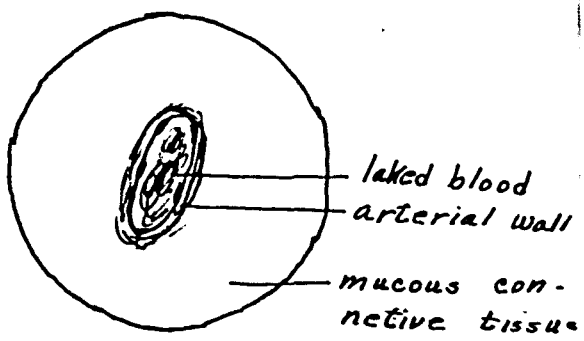
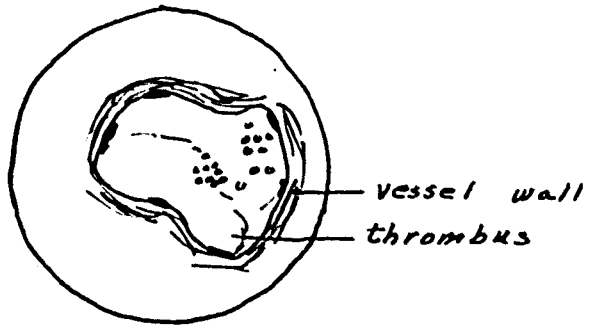


PLATE XVII. SECTIONS OF PLACENTAE FROM A PREGNANT CONTROL FEMALE AND THREE SICK RATS

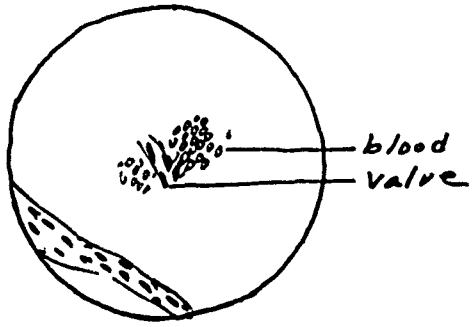
1. Section of umbilical cord showing artery from fetus of sick rat (21708) fed the Pork I diet.



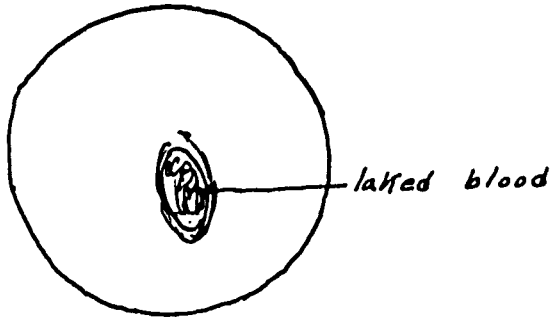
2. Section showing thrombus in umbilical vein from fetus of sick rat (21708) fed the Pork I diet.



3. Section showing blood in the umbilical vein of fetus from normal rat (21504) fed Steenbock V diet.



4. Section showing hemolysis of blood in blood vessel from fetus of sick rat (21708) fed the Pork I diet.



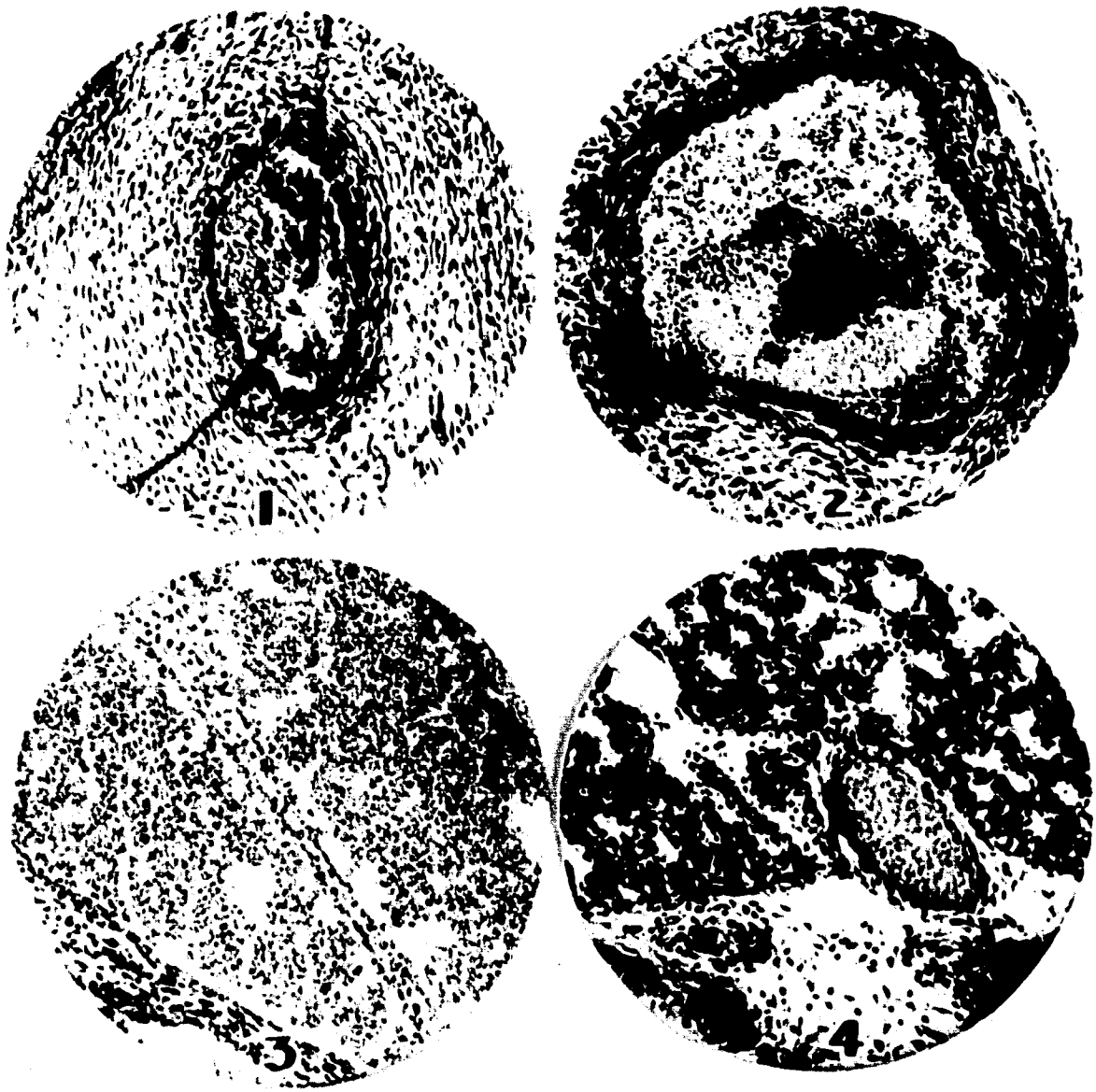


PLATE XVIII. SECTIONS OF THE UMBILICAL BLOOD VESSELS FROM A PREGNANT CONTROL RAT AND A SICK RAT

DISCUSSION

CORRELATION AND DISCUSSION OF FINDINGS

The preceding sections have described two types of gestational failure, i.e., partial and complete, occurring in animals fed the basal pork ration known as Pork I. In complete gestational failure, the most noteworthy condition is a failure of the birth mechanism; in partial, a high mortality of young in the first four days of life.

The findings of the present investigation permit a clear definition of the syndrome of acute pregnancy disorder in rats. The symptoms are enumerated in table XIX and may be summarized briefly as follows: The liver is invariably affected, being large in size, yellow in color, and friable in consistency. Chemical analysis of the organ reveals a significantly high content of fat, and histological analysis, acute fatty degeneration and infiltration. If the maternal rat is killed before death, the feti are always found dead in the uterus. Observations made on sections of the feti and placentae suggest that hemolysis of the fetal blood and thrombosis of blood in the umbilical vein are the primary factors in the death of the feti. The "toxic" animals exhibit unusually large gains in weight during the last twenty-four hours of

TABLE XIX. SUMMARY OF CHARACTERISTICS NOTED IN ANIMALS FED THE PORK I DIET (Con'd on next page)

Characteristic	Normal control animals	Animals fed the Pork I diet that were sick	Animals fed the Pork I diet that were not sick
GENERAL CONSIDERATIONS			
General physical condition	Good	Poor	Good
Percent total gain in weight made in last 24 hours of gestation	8.1	12.5	--
Water consumption per day in gestation in cc.			
From 12th-18th day	14-17	19-24	14-18
From 20th-21st day	17-17	24-15	18-18
LIVER			
Gross appearance	Dark red, not friable	Yellow, friable	Yellow, friable
Size (organ wt/ body wt.)	0.00376	0.00422**	0.00365
Per cent moisture	70.3	70.8**	67.2*
Per cent fat	22.0	31.6*	39.7*
Cellular changes	Very slight cloudy swelling	Marked fatty degeneration and infiltration	Moderate cloudy swelling

*Significantly different from the normal control animals.
**Significantly different from the "non-toxic" animals fed Pork I.

TABLE XIX. SUMMARY OF CHARACTERISTICS NOTED IN ANIMALS FED THE PORK I DIET
(Cont'd on next page)

KIDNEY			
Gross appearance	Normal	Swollen gorged with blood	Normal
Size (organ wt./ body wt.)	0.0079	0.0081	0.0076
Per cent moisture	78.2	82.9**	77.3
Cellular changes	Cloudy swelling of tubular epithelium	Hemorrhage complete destruction of tubular epithelium	Moderate cloudy swelling of tubular epithelium
SPLEEN			
Gross appearance	Normal	Normal	Normal
Size (organ wt./ body wt.)	0.0019	0.0034	0.0020
Per cent moisture	80.1	82.2**	77.6
Cellular changes	None	None	None

*Significantly different from the normal control animals.

**Significantly different from the "non-toxic" animals fed Pork I.

TABLE XIX CONT'D. SUMMARY OF CHARACTERISTICS NOTED IN ANIMALS FED THE PORK I DIET

HEART			
Gross appearance	Normal	Normal	Normal
Size (organ wt./ body wt.)	0.0035	0.0037	0.0039
Per cent moisture	78.2	77.6	77.1
Cellular changes	None	None	None
PANCREAS			
Gross appearance	Normal	Sometimes surrounded with jelly-like material	Normal
Cellular changes	None	Acini contracted, connective tissue gone	None
FETI AND PLACENTAE			
Gross appearance	Well-developed, feti alive	Well-developed, feti dead	Well-developed, feti alive
Cellular changes	None	Hemolysis of fetal blood, thrombus in umbilical vein	None

*Significantly different from the normal control animals.
**Significantly different from the "non-toxic" animals fed Pork I

gestation. Changes in the moisture content of the liver, kidney, and spleen suggest that some disturbance of water balance takes place which may account for the large gains in body weight.

It was noted earlier that, at least as far as external symptoms were concerned, the pregnancy disorder occurring in rats fed the basal pork diet was closely related to "toxic" disturbances of pregnancy in human beings, sheep and rabbits. It is interesting now, in the light of the pathological changes described in the preceding section, to again examine the similarity of the disturbances of pregnancy noted in different animals.

The onset of the disease is always sudden. Lethargy, loss of muscle tone, coma, and convulsions are the more common findings. In the rabbit, sheep, and rat, dyspnea, cyanosis, and a marked decrease in body temperature occur. In general, the present study emphasizes the close parallelism in symptomatology in the different animals.

It is considerably more difficult to evaluate the similarity of changes noted in human beings and in the rat, than to compare the changes occurring in any other two species. Although the symptoms recorded for eclampsia in man vary more widely than those given for the other vertebrates, many observations have been made on eclamptic

women that have as yet not been reported in the studies made on rats, sheep, or rabbits.

Weight changes have been carefully studied in human patients with eclampsia. Siddall and Mack ('38) reported that an excessive gain in weight was not an indication of approaching toxemia, and that such a gain was not an aid in diagnosing a toxic condition. It was observed in the present study that sick rats made greater gains in weight during the last day of gestation than did matched control rats fed an adequate diet. These findings are not necessarily in conflict with those of Siddall and Mack. It is impossible to eliminate variations in inheritance, age, body type, and previous history in studying human cases. The animals used in this study, however, were a homogeneous group in regard to these factors. The uniformity which can be obtained in properly controlled experimental animals may make it possible to solve the riddle presented by eclampsia.

The variations in water consumption noted in the sick animals are unexplainable until further data are available. It will be recalled that while the water consumption of these animals was high from the twelfth day to the nineteenth day of gestation, a sharp decline in the water consumption occurred in the last two days. When the rela-

tive hydration of the organs of these rats is considered, it seems possible that retention of water in the tissues may account for the lack of thirst. Although we have as yet no quantitative information on the volume of urine excreted by the moribund animals during the last two days of gestation, we do have a feeling that the pregnancy disorder causes marked oliguria, if not anuria.

The changes in organ weights which may occur in the so-called toxemic pregnancies have not been studied in animals other than the rat. In the experiment reported herein, it was shown that the ratio of organ weight to body weight was significantly higher for the liver of sick rats than for the same organ in animals in which no symptoms developed. Edema has been reported as a characteristic symptom in human beings and sheep. Changes in moisture content of the liver, spleen, and kidney of the sick rats suggest that these animals also suffer from edema. The increase in organ weight noted is probably due to water retention.

Sheep are the only animals in which the fat content of the liver has been studied quantitatively. Roderick, Harshfield, and Merchant ('33) reported that the relative quantity of fat in the liver of the toxic animals was

approximately 60 per cent, the normal value being near 7 per cent. Such a marked change in liver fat has not been noted consistently in the sick rats. In the present study, the average per cent of liver fat calculated on the dry basis, for the rats that died, was approximately 31 as compared with 22 in the normal animals, and 39 in non-moribund animals fed the Pork I diet. In more than one-third of the cases the quantity of liver fat in the sick rats was above 39 per cent.

Fatty degeneration and infiltration were consistent microscopic changes occurring in organs of the rats suffering from the pregnancy disorder. The one feature that invariably distinguished the livers of moribund pork-fed rats from those of rats that survived was the fatty degeneration and infiltration of the hepatic cells. Similar changes have been reported in the livers of human beings, rabbits, and sheep suffering from derangements of gestation. Indeed, so constant has been this finding that it seems to be the fundamental change in the disturbance.

Abnormalities of the kidney always accompanied the pregnancy disorder. Changes believed to be characteristic of eclampsia have been reported in the glomeruli of the

kidney by Bell ('26) and others. However, no glomerular changes were noted in the kidneys of rats used in this experiment. The marked cellular changes occurred only in the tubular epithelium. No evidence of fatty changes in the renal epithelium were observed in the sick rats.

Bartholomew and Colvin ('38) believe that placental changes in human beings suffering from eclampsia are so characteristic that the diagnosis of toxemia may be made from examinations of unknown placentae. These authors found infarcts in the placenta which had apperently been caused by an endarteritis. The cells in the vessel walls showed evidence of fatty infiltration. Patterson, Nicodemus, and Hunt ('38) described similar changes in the placentae of "toxic" rabbits. The changes noted in the placentae of the sick rats, however, were quite different from those reported for human beings and rabbits. A marked decrease in vascularity accompanied by hemolysis of the fetal blood and thrombosis of the blood in the umbilical vein were constant findings in the feti and placentae of the sick rats. No changes were ever seen in either the uterine wall or the placentae of the sick rats that corresponded to conditions described by Mason ('35) as characteristic of vitamin A deficiency. These negative findings confirm our belief that a lack of vitamin A is

not involved in the disorder.

In sheep and in human beings, the incidence of the disturbance is increased in females carrying more than one fetus. A similar condition seems to prevail in the rats. For example, the sick females maintained on the Pork I diet had an average of 11.8 feti whose average individual weight was 5.0 gm. as compared with 10.6 feti, each weighing 4.1 gm. produced by non-moribund animals fed the same diet. The incidence of the pregnancy disorder may be associated with an unusually large production of fetal tissue.

In spite of certain differences noted in the various kinds of animals, it is probable that the primary lesion in stormy pregnancies is the same. If this is true, then the production of a pregnancy disorder similar to eclampsia in easily controlled experimental animals such as the rat may aid in the solution of the complex problem of toxemic pregnancies occurring in women.

THEORETICAL CONSIDERATIONS

The preceding study of the pregnancy disorder indicates that liver injury is the common symptom in all animals, fatty degeneration and infiltration of the cells in the liver being the primary lesion observed in human

beings, sheep, rabbits, and rats. If we start with this basic fact, it can be assumed that metabolic defects are bound to follow. On the basis of observations made in the present investigation, we have developed a hypothesis that may explain the sequence of events in the progression of pregnancy disease. First, liver function is lowered by fatty changes in the hepatic cells. As a result, metabolites normally detoxified in this organ pass into the systemic circulation unchanged. The hemolysis noted in the fetal blood of rats that die suggests the possibility that some "toxic" substance is circulating in the maternal blood. In the early stages of dysfunction, the kidney is able to excrete the "toxic" substance. Eventually, injury to the kidney, however, results from the strain of attempting to free the body of the deleterious substance. As degeneration progresses in the liver, renal injury likewise advances, raising the threshold of excretion of the offending substance. The concentration of this material in the blood finally reaches a level incompatible with life. Whatever the "toxic" substance may be, it is capable of causing vascular spasm and a general redistribution of blood in the animals. The presence of a large concentration of the metabolite in the blood may cause lesions in the brain and spinal cord, these lesions in turn resulting in convul-

sions. It must be recognized, however, that other factors may be causal agents. For example, fat in the liver cells may have pushed out the glycogen, leaving the animal with only muscle glycogen to draw on for extra glucose needed in labor, thereby causing a hypoglycemia possibly severe enough to cause convulsions.

The accumulation of the "toxic" substance in the maternal blood stream finally causes the hemolysis of the fetal blood noted in the placentae from sick rats, and the formation of a thrombosis in the umbilical vein.

Now that a sequence of events has been suggested that would account in part for the symptoms noted in the sick rats, it is interesting to cogitate whether or not there is a substance produced in normal metabolism, the accumulation of which is capable of producing the syndrome described.

Andes, Andes, and Myers ('37) have reported an increase in the level of blood guanidine in eclamptic women. This substance might well be the metabolite causing the train of symptoms. Much has been written concerning the relation between guanidine in the blood and liver disease. The majority of evidence seems to indicate that a rise in blood guanidine occurs in most cases of liver damage (Andes, Andes, and Myers '37). Several conditions have been shown

to follow a rise in blood guanidine. Major ('25a, '25b, '26a, '26b) for example, suggested that guanidine is capable of causing high blood pressure by a contraction of the arterioles. He found, however, if the guanidine were injected slowly or if the injection were followed by the administration of an hepatic extract, no rise in blood pressure occurred. Apparently the liver extract destroyed the guanidine or in some way counteracted its effect. Would we, therefore, be justified in assuming that unless the liver is actively functioning guanidine is not converted into creatine?

In connection with guanidine poisoning, Blatherwick, Sayhan, and Hill ('27) have reported that the administration of guanidine compounds results in marked kidney damage. These authors quote the following report on the pathology of the kidneys: "In sections of the kidney stained with hematoxylin and eosin there has been intense damage of the cells lining the convoluted tubules. The injury varies from slight swelling of the cell bodies to complete destruction with granular debris in the lumina. The lumina of some of the convoluted tubules have been obliterated by the intense swelling. There is no noteworthy change of the glomeruli," (p. 683) It seems, therefore, that if liver damage occurs in animals fed the basal

pork ration, a rise in blood guanidine explains the kidney damage and vascular changes noted in the sick rats.

In support of our hypothesis, we tested experimentally the premise that fatty degeneration in the liver is a primary factor in setting up the train of events occurring in pregnancy disorder. To this end, two pilot experiments were planned and executed in the laboratory. Phosphorus poisoning is known to cause fatty degeneration and infiltration in the liver. Therefore, in the first experiment, pregnant females fed the Steenbock V and Pork I diets were poisoned with phosphorus on the eighteenth day of pregnancy.[1] Symptoms similar to those observed in the experimental rats developing the pregnancy disorder were observed in six of the ten rats fed the Steenbock V diet, and in eight of a similar group of ten rats receiving Pork I. On the twenty-first day of pregnancy the animals were cold to the touch and lethargic. Their hair was roughened and their nose, ears, and paws, white. They also exhibited dyspnea. However, none of the rats showed the limpness and hematuria noted so often in animals dying at parturition. These rats, poisoned with phosphorus, often died in convulsions. Upon autopsy, the livers were large, very yellow, spongy, and friable. The kidneys were swollen, but showed no signs of hemorrhage. The feti were well-developed

1. The phosphorus was injected sub-cutaneously in one dose of 0.1 cc. of a 1 per cent solution of yellow phosphorus in sweet almond oil for each 100 gm. of body weight.

and invariably dead. The placentae were soft, slightly yellow, and very friable. When calculated upon the weight of the fresh liver, the per cent of fat in the livers of rats fed the Steenbock V given phosphorus ranged from 8 to 11 per cent. These values compare favorably with the quantity of fat present in the livers of normal pregnant rats receiving the adequate diet. A section from the liver of one of the poisoned rats in this group is shown in plate XIX. Every one of the livers of rats treated with phosphorus showed fatty degeneration and infiltration. Peri-portal necrosis also occurred in the livers of the poisoned rats. The fact that this type of necrosis was not observed in the sick rats supports our belief that a toxic substance did not cause the primary liver injury observed in rats dying of the pregnancy disorder.

It seemed possible, however, that the phosphorus in itself had a detrimental effect on the animal beyond that shown by the liver injury. Therefore, an attempt was made in the second experiment to produce fatty livers by dietary means. Best ('36) and his colleagues have shown that fatty livers may be produced in rats fed a diet containing 40 per cent fat. A group of five female rats were maintained on a diet similar to the one described by Best.

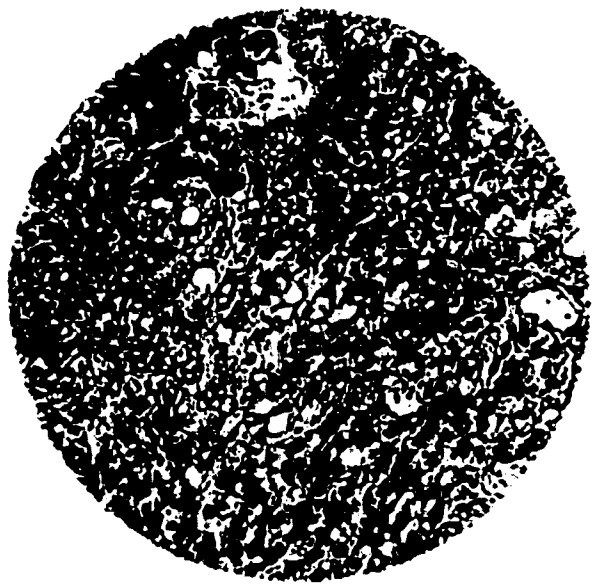


PLATE XIX. SECTION OF LIVER FROM PREGNANT FEMALE RECEIVING THE STEENBOCK V RATION PLUS PHOSPHORUS

However, the fertility of the animals fed this excessively high fat diet was so low that up to date it has been impossible to induce pregnancy. However, another attempt will be made to approach the study from this angle in the future.

The preceding hypothesis has been built upon the premise that a high content of fat in the liver is the primary change in the rats fed the Pork I diet. Why of two litter mates fed the same diet and with approximately the same fat content in the liver, one should develop fatty degeneration of the hepatic cells and die at parturition, while the other is able to give birth to a litter in a normal fashion is still a puzzle. However, it may be that when the hepatic cells are overloaded with fat, the increase in metabolism associated with preparation for parturition causes degeneration of the cells so that the cell protoplasm is no longer able to bind the lipids. Irrespective, though, of the mechanism of fatty degeneration of the cells, the high fat content of the liver, produced by feeding of the Pork I diet, is the predisposing factor.

One of the main purposes of the study was to determine whether the pathological changes associated with partial gestational failure were similar to those noted in the rats that died with typical symptoms of the pregnancy disorder.

The lack of vitality of the young was not as marked in the present experiment as in others conducted in the Nutrition Laboratory. For example, Gray ('36) reported that 65 per cent of the live young born to females fed the pork diet were dead before they were four days old, compared with 21 per cent of the young of the normal control animals. It will be recalled that it was thought that the inability of the young of the pork-fed rats to survive extra-uterine existence was caused by a derangement of the gestation process. If that were the case, then those females fed the basal pork diet that survived parturition were probably suffering from an atypical form of the pregnancy disorder. Any pathological changes found in the rats that died might be present, therefore, to a lesser degree in the other females fed the Pork I diet. This did not prove to be true. A large quantity of fat in the liver was the only constant finding in both groups of animals (table XIX).

Among the animals receiving the Pork I diet that did not die, the per cent of feti resorbed (15.4) was the same as in the animals fed the adequate control diet. The question arises as to why the young of the females fed the Pork I diet died before they were four days old, while the young of the rats given the Steenbock V ration

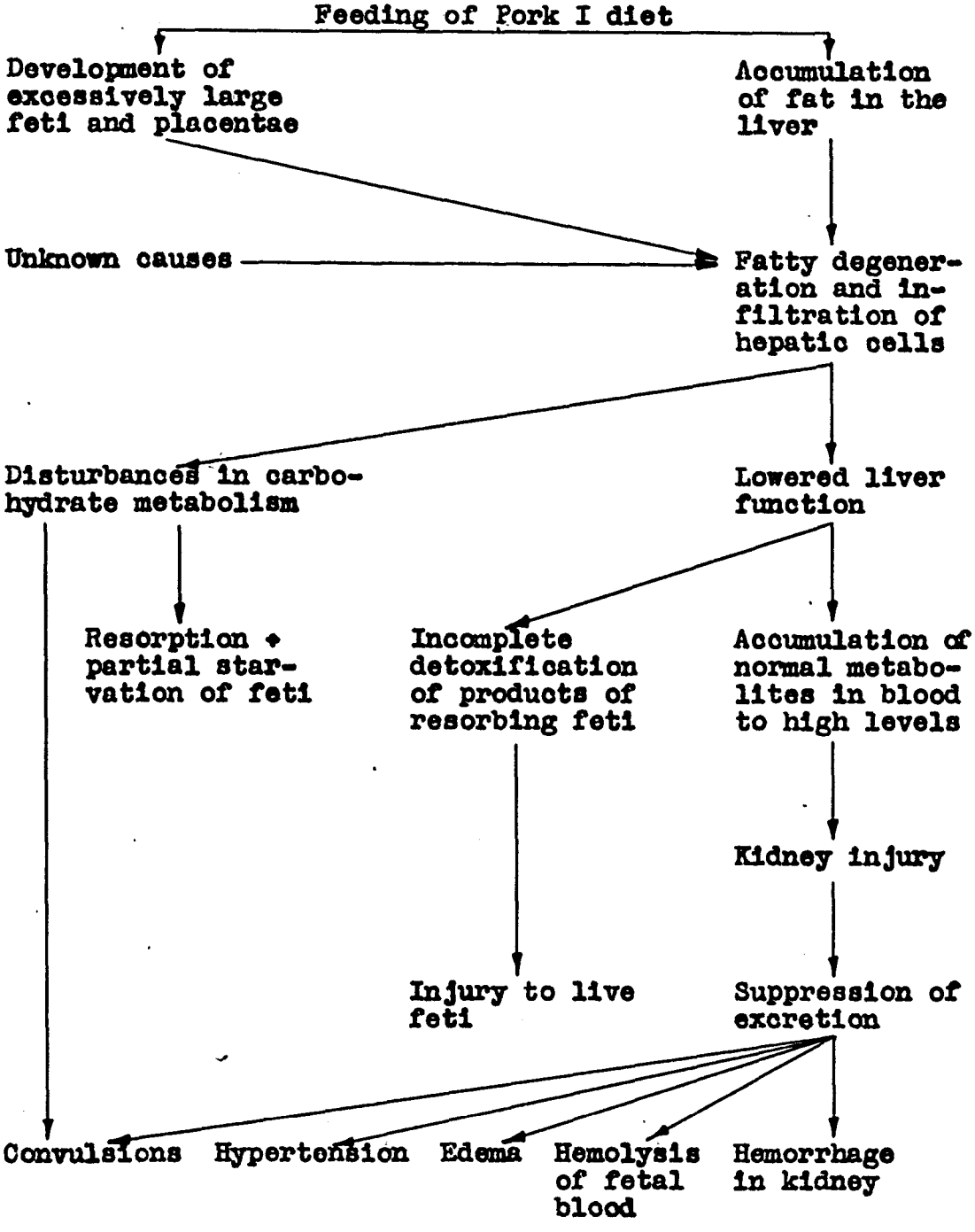
survived. It is possible that this difference in the mortality of the young is related to the difference in the fat contents of the livers of the two groups of animals. Again we postulate: The products of cellular degeneration absorbed by the maternal blood from resorbing feti are carried to the liver for detoxification. The injurious materials are completely removed from the blood by the liver in the case of the animals fed the adequate diet. However, since the livers of the animals receiving the Pork I diet are overburdened with fat, the detoxification is relatively incomplete, and injurious substances are allowed to pass into the general circulation. These "toxic" substances in turn may injure the feti, so that they are born in a moribund state and die very shortly. The characteristic symptoms of the pregnancy disorder fail to develop, in these cases, since the small quantity of live fetal tissue produced does not cause a great enough change in the metabolism of the female preparatory to parturition to produce the characteristic degeneration of the hepatic cells.

Or possibly, the accumulation in the liver affects the storage of glycogen. Partial starvation of the feti results and the young are born in a poorly nourished condition, unable to withstand the rigors of extra-uterine

existence. In another study[1] relating to the establishment of the syndrome of pregnancy disorder, data have already been collected showing that a depletion occurs in the glycogen stores in livers of pork-fed rats that are not "toxic." The changes which may occur following the feeding of the Pork I diet are summarized in diagram I.

1. Ph.D. research by Helen Farrankop, of the Foods and Nutrition Department.

DIAGRAM I. THEORETICAL REPRESENTATION OF SEQUENCE OF EVENTS RESULTING IN ACUTE PREGNANCY DISORDER



SUMMARY AND CONCLUSIONS

The reports of Dyar ('35) and of her successors (Wilcox '37, Walliker '38) dealing with the occurrence of gestational failures, both partial and complete, in rats fed the basal pork ration known as Pork I formed the basis of the experiment herein described. In partial gestational failure, the most striking abnormality was a high mortality of the young during the first four days of life; in the complete, a failure of the birth mechanism resulting in the death of both mother and feti. The Pork I diet, containing 25 per cent of dried autoclaved pork muscle, was believed to be adequate in all known dietary essentials. Except for the source of protein, the diet was synthetic. The chief purpose of the present study was to establish the syndrome connected with the partial and complete gestational failures in animals fed the Pork I ration. In addition, the effect of adding three supplements, lipocaic, fresh liver, and liver extract, to the basal pork diet was investigated.

The 235 animals used in the experiment were divided into three experimental groups, i.e., (1) the control group of rats fed the Steenbock V diet which has never been known to produce complete gestational failure, (2) the

group of rats receiving the basal pork ration, and (3) the group of animals receiving the Pork I ration supplemented by fresh liver, lipocaic, or liver extract. In order to determine whether pregnancy per se exerted an effect upon any conditions present, pregnant and virgin females were maintained in each experimental group. The experimental groups of pregnant animals were further subdivided on the basis of the diet of the males used for mating.

The data collected were used to evaluate the gestational performance of rats in the three experimental groups and also to describe the pathological changes associated with gestational failure. The females in the pregnant series were allowed to bear and rear one litter. They were then killed 21.5 days following the initiation of the second pregnancy. The general physical condition of the animals and the appearance of certain organs were described at the end of the experiment. The liver, kidney, heart, spleen, feti, placentae, and, in some cases, the pancreas were removed at autopsy. The virgin animals in each group were killed when they had received the diet the same number of days as the pregnant animals in that group.

In the first part of the present study the gestational performance of animals in the three experimental groups was

evaluated. Data collected on the progression of the first gestation period and the condition and vitality of the first litter were used in this analysis. In addition, data pertaining to the second pregnancy, obtained by observation on the progression of gestation, and the condition of the uterine contents at autopsy were studied. It was concluded that the second pregnancy was a better measure of the effect of diet upon the gestational performance of the female than was the first pregnancy.

Ten of the females fed the various pork-containing diets died at parturition with typical symptoms of the pregnancy disorder. In general, even among the animals that did not die, the feeding of the Pork I diet resulted in a poorer gestational performance than that noted in animals fed the adequate control ration. Gestational failures, both partial and complete, were more numerous in females mated with males also receiving the Pork I diet, than when males from the stock colony were used for mating. Fresh liver was the only supplement added to the Pork I diet that prevented the appearance of the pregnancy disorder. The feeding of lipoeaic markedly increased the occurrence of resorptions, as many as 66 per cent of the feti being lost in rats fed 500 mg. of the supplement daily.

The second part of the investigation consisted of a

study of the pathological changes associated with gestational failure. Changes from normal in the following items were considered: (1) general physical condition of the animals, (2) gain in body weight during pregnancy, (3) water consumption in pregnancy, (4) fat content of the liver, (5) weight and moisture content of organs, and (6) histology of organs, feti, and placentae.

The general physical condition of the animals both in regard to external appearance and condition of certain visceral organs was rated subjectively. In addition, the rectal temperature of the pregnant animals was taken. The gains made in body weight during gestation were studied, pairing experimental animals with normal females from the stock colony matched in respect to body weight at the initiation of pregnancy and number and weight of feti. The amount of water consumed by the pregnant animals was measured twice daily from the twelfth day of pregnancy until parturition. The average weight and moisture content of the liver, kidney, spleen, heart, and mammae were determined. In addition, analyses were made of the fat content of the liver.[1] Histological sections were prepared of the liver, kidney, heart, spleen, and pancreas, as well as of the feti and placentae. A standard method using Zenker's solution for the fixative and haematoxylin and ethyl eosin as stains

1. These data were included through the courtesy of Miss Ethelwyn Wilcox.

was followed in the preparation of the sections.

The feeding of the pork diet to the virgin animals increased the relative quantity of fat in the liver and induced cellular changes in the liver and the kidney. These differences were accentuated by pregnancy. In the normal control group, pregnancy did not produce these changes.

Between the "non-toxic" pregnant animals fed the various pork-containing diets and the normal control group, the only consistent differences noted were in the liver. The livers from the rats receiving pork muscle were higher in fat and lower in moisture than in the normal control animals. In addition, an increase in cloudy swelling in the hepatic cells was observed upon histological examination of the sections prepared from livers of pork-fed rats. Some degenerative changes were also noted in the kidneys of these rats.

Deviations from normal were marked in the animals developing the pregnancy disorder. In general, the symptoms were similar to those described by Walliker ('38). The sick animals make excessive gains in body weight during the last day of pregnancy. Differences between the moisture contents of the liver, kidney and spleen of these animals

and of those not "toxic" suggested that the large gains in body weight were due to a disturbance in water balance. The liver was yellow in color, large in size, and friable in consistency. The kidneys were swollen and gorged with blood. Both chemical and histological analyses of the liver revealed marked abnormalities in the organ. The quantity of fat was high and the hepatic cells showed marked fatty degeneration and infiltration. The feti were well-developed, but invariably dead. Hemolysis of fetal blood and a thrombus in the umbilical vein were constant findings.

Finally, an attempt was made to evaluate the significance of the findings. In so doing, similarities between pregnancy disorder, eclampsia in women, and disturbances of gestation reported in rabbits and sheep were indicated, a theory developed explaining the train of events observed in the pregnancy disorder, and changes found in partial gestational failure correlated with those observed in complete gestational failure.

LITERATURE CITED

Alcock, R. S.
1936. The synthesis of proteins in vivo
Physiol. Rev., 16, 1-18

Alt, H. L.
1938. Iron deficiency in pregnant rats
Amer. J. Dis. Child., 56, 975-984.

American Committee on Maternal Welfare
1935. The management of preeclamptic toxemia and eclampsia
J. Amer. Med. Ass., 104, 1703-1705.

Andes, J. E., Andes, E. J., and Meyers, V. C.
1937. Guanidine-like substances in blood. III. Blood guanidine in normal pregnancy, toxemias of pregnancy, and cirrhosis of the liver
J. Lab. Clin. Med., 23, 9-18.

Anselmino, K. J., Hoffman, Friedrick, and Kennedy, W. P.
1932. The relation of hyperfunction of the posterior lobe of the hypophysis to eclampsia and nephropathy of pregnancy
Edin. Med., J., 39, 376-388.

Bartholomew, R. A., and Kracke, R. R.
1936. The probable role of the hypercholesteremia of pregnancy in producing vascular changes in the placenta, predisposing to placental infarction and eclampsia
Amer. J. Obstet, Gynec., 31, 549-562.

Bartholomew, R. A., and Colvin, E. D.
1938. Diagnosis of the occurrence of toxemia of pregnancy by examination of the unknown placenta
Amer. J. Obstet. Gynec., 36, 909-927.

Basden, M. M.
1937. Toxaemia of pregnancy: its significance and treatment
J. State Med., 45, 474-483.

Bell, J. W.
1926. Postmortem findings in ten cases of toxemias of pregnancy
Amer. J. Obstet. Gynec., 12, 792-802.

Bergman, H. D.
1935. Nutritional diseases common to domestic animals
Iowa Veterinarian, 6, No. 1, 7, 25-32.

Berkwitz, N. J., and Lufkin, N. H.
1932. Toxic neuronitis of pregnancy: A clinicopathological report
Surg., Gynec. Obst., 54, 743-757.

Best, C. H., Mawson, M.E.H., McHenry, E. W., and Ridout, J. H.
1936. The effect of diets low in choline
J. Physiol., 86, 315-322.

Blatherwick, N. R., Sahyun, Melville, and Hill, Elsie
1927. Some effects of synthelin on metabolism
J. Biol. Chem., 75, 671-683.

Boyd, E. M.
1935. Blood lipids in eclampsia
Amer. J. Obstet. Gynec., 30, 323-332.

Byram, F. B., and Wilson, C.
1934. The alleged pituitary origin of the eclamptic and preeclamptic toxaemias of pregnancy
Quart. J. Med., 27, 361-368.

Cadden, J. F., and Stander, H. J.
1939. Uric acid metabolism in eclampsia
Amer. J. Obstet. Gynec., 37, 37-47.

Colvin, E. D., and Bartholomew, R. A.
1939. Behavior of the basal metabolism in the course of developing toxemia of pregnancy: correlation with cholesterol; placental infarcts and retinal examination
Amer. J. Obstet. Gynec., 37, 584-604.

Corey, E. L.
1932. The maceration and resorption of fetuses in the rat
Anat. Rec., 56, 195-198.

Cruickshank, J. N., Hewitt, J., and Couper, K. L.
1927. The toxaemias of pregnancy. A clinical and biochemical study
Gr. Brit. Med. Res. Council, Special Rpt. Series No. 117.

Csonka, F. A.
1935. Proteins of yeast (Saccharomyces cerevisiel)
J. Biol. Chem., 109, 703-715.

DeLee, J. B.
1913. The principles and practice of obstetrics
W. B. Saunders Company, Philadelphia.

Dieckmann, W. J.
1933. Comparative studies of the blood in the nonconvulsive toxemias of pregnancy
Amer. J. Obstet. Gynec., 26, 543-555.

Dieckmann, W. J., and Wegner, C. R.
1932. Eclampsia without convulsions or coma
Amer. J. Obstet. Gynec., 23, 657-663.

Donaldson, H. H.
1924. The rat
Wistar Institute Memoirs, 6.

Dragstedt, L. R., Van Prohaska, John
1936. Observations on a substance in pancreas (a fat metabolizing hormone) which permits survival and prevents liver changes in depancreatized dogs
Amer. J. Physiol., 117, 175-181.

Dyar, Elizabeth
1935. Meat in nutrition. VI. The relation of vitamin E to reproductive abnormalities induced in the albino rat by the feeding of a diet containing pork muscle
Unpublished Thesis, Library, Iowa State College, Ames, Iowa

Earhart, C. R.
1935. Stock colony studies. II. Reproductive performance of the albino rat (strain A, Wistar stock) under standard conditions
Unpublished Thesis, Library, Iowa State College
Ames, Iowa

Elder, Cecil, and Uren, A. W.
1935. Pregnancy disease in sheep
Mo. Agr. Exp. Sta. Bul. 345.

Evans, H. M., and Burr, G. O.
1927. The antisterility vitamine fat soluble E
Univ. Calif. Memoirs, 8.

Evans, H. M., and Burr, G. O.
1928. On the amount of vitamin B required during lactation
J. Biol. Chem., 76, 263-272.

Evans, H. M., Lepkovsky, Samuel, and Murphy, E. A.
1934. Vital need of the body for certain unsaturated fatty acids. V. Reproduction and lactation upon diets containing saturated fatty acids as their sole source of energy
J. Biol. Chem., 106, 441-444.

Fauvet, E.
1933. Eclampsia, a hypophyseal disorder
Arch. f. Gynak. 155, 100-
Abstracted in Amer. J. Obstet. Gynec., 155, 360, 1936.

Fisher, R. A.
1936. Statistical methods for research workers
Boyd and Oliver, London.

Graham, A. M.
1939. Stock colony. IV. Factors affecting concentration of hemoglobin in the blood of the albino rat, Wistar stock, strain A
Unpublished Thesis, Library, Iowa State College, Ames, Iowa.

Green, H. S. N.
1937. Toxemia of pregnancy in the rabbit. I. Clinical manifestations and pathology
J. Exper. Med., 65, 809-832.

Harden, Boyd, McEllroy, W. S., and Huggins, R. R.
1935. Protein stabilization in preeclampsia and eclampsia
Amer. J. Obstet. Gynec., 30, 524-533.

Hart, E. B., McCollum, E. V., Steenbock, H., and Humphrey, G. C.
1911. Physiological effect on growth and reproduction of rations balanced from restricted sources
Wis. Agr. Exp. Sta., Research Bull., 17.

Hart, E. B., Nelson, V. E., and Pitz, W.
1918. Synthetic capacity of the mammary gland. I. Can this gland synthesize lysine?
J. Biol. Chem., 36, 291-307.

Hart, E. B., and Steenbock, H.
1918. Thyroid hyperplasia and the relation of iodine to the hairless pig malady. I
J. Biol. Chem., 33, 313-323.

Hartwell, G. A.
1927. Growth and reproduction on synthetic diets. II
Biochem. J., 21, 1076-1086.

Hojer, A. J.
1926. A method for determining the anti-scorbutic value of foodstuffs by means of histological examination of the teeth of young guinea pigs
Brit. J. Exper. Path., 7, 356-369.

Huber, G. C.
1915. The development of the albino rat, mus norvegicus albinus. L. From the pronuclear stage to the stage of mesoderm anlage; end of first day to the end of the ninth day
J. Morph., 26, 247-358.

Hurwitz, David, and Bullock, L. T.
1935. Failure to find pressor and antidiuretic substances in patients with toxemia of pregnancy
Amer. J. Med. Sci. 189, 613-619.

Hussemann, D. L., and Hetler, R. A.
1931. The vitamin B and G requirements of lactation
J. Nutr., 4, 127-140.

Irving, F. C.
1936. The vascular aspect of eclampsia
Amer. J. Obstet. Gynec., 31, 466-476.

Jones, L. J.
1931. The effect of certain constituents of the diet on hemoglobin formation in the rat
Unpublished Thesis, Library, Iowa State College, Ames, Iowa

Kellogg, F. S.
1931. Observations on toxemic nephritic group cases with special reference to classification
Amer. J. Obstet. Gynec., 21, 275-279.

King, Helen
1936. Meat in nutrition. XI. Effect of supplementary feeding of certain liver fractions on the reproductive performance of the albino rat fed a diet containing pork muscle
Unpublished Thesis, Library, Iowa State College, Ames, Iowa

Lease, E. J., and Steenbock, H.
1939. Diet and rate of depletion of hepatic vitamin A
J. of Nutr., 17, 85-90

Long, J. A., and Evans, H. M.
1922. The oestrous cycle in the rat and its associated phenomena
Univ. Calif. Memoirs, 6.

Luikart, Rolf
1933. Avitaminosis as likely etiologic factor in polyneuronitis complicating pregnancy, with the report of a case
Amer. J. Obstet, Gynec., 25, 810-815.

Macomber, Donald
1927. Effect of a diet low in calcium on fertility, pregnancy and lactation in the rat
J. Amer. Med. Ass., 88, 6-11.

Meader, E. C.
1937. The effect of fat in simplified diets on the reproductive organs of the female albino rat during gestation
Anat. Rec., 70, 73-77.

Major, R. H.
1925a. The possible relationship between guanidine and high blood pressure
Amer. J. Med. Sc., 170, 228-232.

Major, R. H.
1925b. The excretion of guanidine bases on two cases of arterial hypertension with reduction of blood pressure
Bull. Johns Hopkins Hosp. 36, 357-360.

Major, R. H.
1926a. The effects of guanidine compounds on the blood pressure when introduced slowly into the circulation and into the gastro-intestinal tract
Bull. Johns Hopkins Hosp. 39, 215-221.

Major, R. H.
1926b. Studies on the effect of hepatic extract upon experimental hypertension produced ir by guanidine compounds
Bull. Johns Hopkins Hosp., 39, 222-231.

Mason, K. E.
1935. Foetal death, prolonged gestation, and difficult parturition in the rat as a result of vitamin A deficiency
Amer. J. Anat., 57, 303-349.

Mason, K. E., and Ellison, E. T.
1935. The demonstration of oestrus in the vitamin A-deficient rat by supravital study of the vaginal smears
J. Nutr., 10, 1-11.

Mays, C. R., and McCord, W. M.
1935. A study of blood sugar levels in eclampsia
Amer. J. Obstet. Gynec., 29, 405-414.

McIlroy, Louise
1936. The toxaemias of pregnancy
William Wood and Company, Baltimore

Moise, T. S., and Smith, A. H.
1929. Observations on the pathogenesis of pulmonary suppuration in the albino rat
Soc. Exper. Biol. Med. Proc., 26, 723-725.

Moise, T. S., and Smith, A. H.
1930. Atelectasis in the pathogenesis of abscess of lung
Arch. Vet. Med., 45, 92-101.

M'Fadyean, John
1924. Fatty infiltration of the liver as a cause of death in pregnant ewes
J. Comp. Path. Therap., 37, 287-293.

Mussey, R. D., and Hunt, A. B.
1938. The late toxemias of pregnancy (so-called nephritic toxemias of pregnancy)
Int. Abstract Surger., 66, 550-561.

Page, E. W.
1939. The relation between hydatid moles, relative ischemia of the gravid uterus, and the placental origin of eclampsia
Am. J. Obstet. Gynec., 37, 291-293.

Patterson, W. B., Hunt, H. F., and Nicodemus, R. E.
1938. The etiology of eclampsia
Amer. J. Clinical Path., 8, 120-136.

Peters, J. P.
1937a. Nature of eclampsia
Yale J. Biol. Med., 9, 233-245.

Peters, J. P.
1937b. Toxemias of pregnancy
Yale J. Biol. Med., 9, 311-326.

Peters, J. P.
1938. The nature of the toxemias of pregnancy
J. Amer. Med. Ass., 110, 329-331.

Roderick, L. M., and Harshfield, G. S.
1932. Pregnancy disease of sheep
North Dakota Agr. Exp. Sta. Tech. Bul. 261.

Roderick, L. M., Harshfield, G. S., and Merchant, W. R.
1933. Further demonstration of the functional pathology of pregnancy disease in ewes
Cornell Vet., 23, 348-353.

Rogosheski, E. R.
1936. Meat in nutrition. IX. Effect of supplementary feeding of fresh liver and of an amino acid mixture on the reproductive performance of the albino rat fed a diet containing pork muscle
Unpublished Thesis, Library, Iowa State College, Ames, Iowa.

Ross, Robert A.
1935. The toxemias of pregnancy and certain deficiency diseases
Virginia M. Monthly 62, 424-
Abstracted in Amer. J. Obstet. Gynec., 32, 363; 1936.

Rowe, A. W.
1932. The toxemias of pregnancy. I. Some observations of hepatic factor
J. Amer. Med. Ass., 99, 2083-2087.

Rowe, A. W., McManus, M. A., and Riley, G. A.
1934. The toxemias of pregnancy. II. The nitrogen metabolism
J. Lab. Clin. Med., 19, 923-929.

Rowe, A. W., McManus, M. A., and Plummer, A. J.
1936. The toxemias of pregnancy. IV. The carbohydrate metabolism
Amer. J. Obstet. Gynec., 31, 856-866.

Savage, J. E., and Wylie, H. B.
1937. A chemical Test for pregnancy applied to the determination of estrin in the uterus of normal and toxemic patients in the last trimester of pregnancy
Amer. J. Obstet. Gynec., 33, 771-777.

Seegers, W. H.
1937. The effect of protein deficiency on the course of pregnancy
Amer. J. Physiol., 119, 474-479.

Siddall, R. W., and Mack, H. C.
1938. Weight changes and toxemia of late pregnancy
Amer. J. Obstet. Gynec., 36, 380-386.

Siegel, I. A., and Wylie, H. B.
1933. Blood sugar findings in eclampsia and preeclampsia
Amer. J. Obstet. Gynec., 26, 129-37.

Sherman, H. C.
1932. Chemistry of foods and nutrition, 4th ed.
The Macmillan Company

Smith, G. E.
1917. Fetal athyrosis. A study of the iodine requirements of the pregnant sow
J. Biol. Chem., 29, 215-225.

Smith, G. V., and Smith, O. W.
1935. Evidence for the placental origin of the excessive prolan of late pregnancy toxemia and eclampsia
Surg., Gynec. Obst. 61, 175-183.

Smith, Theobald
1913. Some bacteriological and environmental factors in the pneumonia of lower animals with special reference to the guinea-pig
J. Med. Res., 29, 291-324.

Stander, H. J., and Cadden, J. F.
1934. Blood chemistry in preeclampsia and eclampsia
Amer. J. Obstet. Gynec., 28, 856-871.

Steenbock, H.
1923. A satisfactory ration for stock rats
Science, 58, 449-450.

Stevenson, G. T.
1938. Meat in nutrition. XIII. The biological value of canned autoclaved pork muscle
Unpublished Thesis, Library, Iowa State College, Ames, Iowa.

Strauss, M. B.
1935. Observations on etiology of the toxemias of pregnancy. Relationship of nutritional deficiency, hypoproteinemia, and elevated venous pressure to water retention in pregnancy
Am. J. Med. Sci., 190, 811-824.

Strauss, M. B.
1937. Observations on the etiology of the toxemias of pregnancy. II. Production of acute exacerbation of toxemia by sodium salts in pregnant women with hypoproteinemia
Amer. J. Med. Sci., 194, 772-783.

Strauss, M. B., and McDonald, W. G.
1933. Polyneuritis of pregnancy. A dietary deficiency disorder
J. Amer. Med. Ass., 100, 1320-1323.

Suden, C. T., and Alley, O. E.
1935. Further evidence of unsusceptibility of the rat to a dietary deficiency of vitamin C
Soc. Exp. Biol. Med. Proc., 32, 753-754.

Swanson, P. P., Stevenson, G. T., and Nelson, P. M.
1938. A method of increasing precision in vitamin A assay
J. Nutr. 15, 103-123.

Theobald, G. W.
1935. The dietetic deficiency hypothesis of the toxemias of pregnancy
Proc. Royal Soc. Med., 28, 1388-1399

Timson, G. M.
1932. A statistical analysis of the growth of rats in the stock colony of the foods and nutrition department of Iowa State College during years 1928-1931
Unpublished Thesis, Library, Iowa State College Ames, Iowa

Titus, Paul, and Dodds, Paul
1928. The etiologic significance of lowered blood sugar values in vomiting of pregnancy
Amer. J. Obstet. Gynec., 16, 90-96.

Vorzimer, J. J., Fishberg, A. M., Langrock, E. G., and Rappaport, E. M.
1937. The endocrine basis of toxemia of pregnancy
Amer. J. Obst. Gynec., 33, 801-813.

Waddell, J., and Steenback, H.
1931. Vitamin E in iron treated dry rations
J. Nutr., 4, 79-93.

Walliker, C. T.
1938. Meat in nutrition. XIV. Effect of supplementary feeding of two levels of lypocaic on the reproductive performance of the albino rat fed a diet containing dried autoclaved pork muscle
Unpublished Thesis, Library, Iowa State College, Ames, Iowa.

Webster, R. W.
1930. Legal medicine and toxicology
W. B. Saunders Company, Philadelphia.

Wellman, H. E.
1936. Meat in nutrition. X. Effect of supplementary feeding of oven-dried and of vacuum-dried liver in the reproductive performance of the albino rat fed diet containing pork muscle
Unpublished Thesis, Library, Iowa State College, Ames, Iowa

Whipple, D. V.
1934. Studies concerning the nutritional value of slightly rancid fat
Amer. J. Med. Sci., 187, 864-865.

Whitfield, D. W.
1889. Peripheral neuritis due to the vomiting of pregnancy
Lancet, 1, 627-628.

Wilcox, Ethelwyn, B.
1937. Meat in nutrition. XII. Effect of supplementary feeding of lecithin and lipocaic on the reproductive performance of the albino rat fed a diet containing pork muscle
Unpublished Thesis, Library, Iowa State College, Ames, Iowa.

Wood, J. E., and Nix, Harold
1938. Hypertension in the late toxemias of pregnancy
J. Amer. Med. Ass., 110, 332-336

Young, James, and Miller, D. A.
1921. The etiology of eclampsia and the preeclamptic state
Brit. Med. J., 1, 486-490.

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APPENDIX

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PROCEDURE FOR THE PREPARATION OF THE HISTOLOGICAL SECTIONS

1. Fix tissues in freshly mixed Zenker's solution for 12 to 24 hours.*

2. Wash in running tap water for 12 to 24 hours.

3. Dehydrate according to the following schedule:

   A. 35% alcohol - 1 to 2 hours

   B. 50% alcohol - 2 to 3 hours

   C. 70% alcohol - 12 to 24 hours

   D. 83% alcohol - 12 to 24 hours

   E. 95% alcohol - 1 to 2 hours

   F. Absolute alcohol, two changes - one half hours each time

   G. Equal parts of absolute alcohol and cedar oil - 2 to 3 hours

4. Clear in cedar oil for at least 24 hours.

5. Infiltrate according to the following schedule:

   A. Equal parts of cedar oil and Tissuemat - 1 to 2 hours

   B. Tissuemat - 4 hours

   C. Fresh Tissuemat - 6 hours

   D. Fresh Tissuemat - 4 hours

* The time allowed to fix the tissues and for the other operations depends in part on the size of the piece of tissue used.

6. Embed in fresh Tissuemat

7. After the sections have been cut and fixed on the slides they should be allowed to dry for at least 12 hours but not more than 36 hours.

8. Remove the Tissuemat from the sections by immersing the slides in Xylol I for 10 minutes and Xylol II for 3 minutes.

9. Hydrate the sections by passing the slides through solutions of ethyl alcohol of decreasing concentrations according to the following schedule:

A. Absolute alcohol - 3 minutes

B. 95% alcohol - 1 minute *

C. 70% alcohol - 1 minute

D. Distilled water - 2 minutes

10. Stain the slides for 5 minutes in undiluted Dilafield's hematoxylin.

11. Immerse slides in tap water for one minute.

12. Destain the sections in 0.33% hydrachloric acid for two seconds.

13. "Blue" the sections in alkaline tap water for 2 minutes.

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* At this point the slides were immersed for 5 minutes in 70% iodinized alcohol to remove any of the Zenker's solution left in the tissues.

14. Wash the slides in two changes of distilled water 2 minutes each.

15. Dehydrate the sections by immersing the slides in solutions of ethyl alcohol according to the following schedule:

A. 70% alcohol - 2 minutes

B. 95% alcohol - 2 minutes

16. Counterstain the sections in a solution of 0.5% ethyl eosin in 95% alcohol.

17. Immerse the slides in absolute alcohol for 3 minutes.

18. Transfer the slides to xylol for five minutes.

19. Place the cover slips using gum damar as the mounting media.

FORM 1. CHARACTERISTICS NOTED IN DESCRIBING CONDITION OF ANIMALS AT END OF EXPERIMENT

Rat No._____ Date_____

Diet_____ Hour_____

Age in days_____

No. of pregnancy_____

Day in gestation period_____

Weight before starving_____

Weight after starving_____

General condition[1]
condition_____ alert_____

fat_____ gaunt_____

Muscle tone
general_____ abdominal_____

Hair
creamy_____ smooth_____

fine_____ thick_____

Tail, clean_____ smooth_____

Teeth, straight_____ orange_____

Eyes, pink_____ popping_____

Respiration_____

Gait_____

Visible mucous membranes
pink_____ cyanotic_____

Exudates[2]
nasal_____ oral_____

anal_____ vaginal_____

Hematuria_____

Rectal temperature_____

Remarks_____

_____

1 In recording the degree to which any condition is present use a scale ranging from minus (-) to four plusses (++++)
2 Indicate character of exudate

FORM 2. CHARACTERISTICS NOTED IN DESCRIBING THE CONDITION OF THE ORGANS AT AUTOPSY

Rat No._____ Diet_____

AUTOPSY FINDINGS:[1]
Liver, yellow_____mottled_____

friable_____spongy_____

_____

Kidneys:
Cortex, color_____friable_____

Medulla, color_____friable_____

Pelvis, color_____friable_____

_____

Pancreas, any gross abnormalities:

_____

Corpora Lutea
No. in left ovary_____right_____

Color_____

Fetal sites, no. of_____

Live feti, no. of_____

Resorptions, no. of_____

Stomach ulcers, no. of_____

Condition of the lungs:

	Infection	Emphysema
Lobe 1.	_____	_____
2.	_____	_____
3.	_____	_____
4.	_____	_____
5.	_____	_____

Pus pockets:
Ear_____

Base of the tongue_____

Remarks:_____

_____

[1]Use a scale ranging from minus (-) to four plusses (++++) insofar as possible in recording the degree to which any condition is present.

TABLE 1. ANALYSIS OF VARIANCE OF GESTATIONAL PERFORMANCE OF RATS COMPOSING FIVE GENERATIONS OF THE STOCK COLONY

A. Implantation per cent			
Source of variation	Degrees freedom	Sum of squares	Mean square
Total	162	86 171.19	
Between means of generations	4	2 840.68	710.17
Within generations	158	83 330.51	527.41

F = 710.17 / 527.41 = 1.35. 1% point for 4 and 162 df is 3.91, 5% point is 2.67

B. Placental index			
Total	162	12117	
Between means of generations	4	316	79.00
Within generations	158	11801	74.69

F = 79.00 / 74.69 = 1.06. 1% point for 4 and 162 df is 3.91, 5% point is 2.67

C. Fertility index			
Total	162	90874	
Between means of generations	4	4990	1248
Within groups	158	85884	544

F = 1248 / 544 = 2.29. 1% point for 4 and 162 df is 3.91, 5% point is 2.67

TABLE 2. UNIFORMITY OF THE EXPERIMENTAL ANIMALS

Dietary group	Reproductive status	Diet of males	Body weight at time of weaning	Age at sexual maturity	Body weight at sexual maturity	Body weight when study of vaginal smears was initiated	Age at initiation of first pregnancy	Body weight at initiation of first pregnancy
			gm.	days	gm.	gm.	days	gm.
Steenbock V	Virgins (1)*	-	47	44	86	115	-	-
	Virgins (2)**	-	49	46	84	114	-	-
	Mated (1)	Steenbock V	48	42	80	117	70	150
	Mated (2)	Steenbock V	51	40	80	128	72	134
Pork 7	Virgins (1)		48	48	80	116	-	-
	Mated (2)	Steenbock V	49	44	87	113	70	135

* Organs from animals numbered (1) were used for determination of the weights of organs, etc.

** Organs from animals numbered (2) were used to prepare histological sections.

TABLE 2. (Cont'd) UNIFORMITY OF THE EXPERIMENTAL ANIMALS

Pork I	Virgin (1)	-	47	45	86	110	-	-
	Virgin (2)	-	41	37	74	111	-	-
	Mated (1)	Steen-bock V	47	45	89	114	64	130
	Mated (2)	Steen-bock V	51	43	95	116	62	152
	Mated (1)	Pork I	47	41	86	115	78	148
	Mated (2)	Pork I	45	44	80	112	61	132
Pork 39A	Virgin (1)	-	47	46	77	110	-	-
	Virgin (2)	-	49	49	84	111	-	-
	Mated (1)	Steen-bock V	48	46	88	110	76	134
	Mated (2)	Steen-bock V	46	45	86	111	78	156
	Mated (1)	Pork 39A	48	48	95	114	75	148
	Mated (2)	Pork 39A	48	46	72	109	84	142
Pork 39B	Virgin (1)	-	53	52	114	123	-	-
	Virgin (2)	-	52	50	107	116	-	-
	Mated (1)	Steen-bock V	45	48	101	116	69	132
	Mated (2)	Steen-bock V	50	58	118	115	76	145

TABLE 2. (Cont'd) UNIFORMITY OF THE EXPERIMENTAL ANIMALS

Pork 39C	Virgin (1)	-	57	45	88	113	-	-
	Virgin (2)	-	45	44	82	107	-	-
	Mated (1)	Steen-bock V	51	50	95	112	80	144
	Mated (2)	Steen-bock V	47	48	92	108	70	137
	Mated (1)	Pork 39C	48	41	82	110	80	145
	Mated (2)	Pork 39C	48	44	88	111	68	131
Pork 31	Virgin (1)	-	46	43	85	112	-	-
	Virgin (2)	-	48	44	85	120	-	-
	Mated (1)	Steen-bock V	44	43	83	116	69	133
	Mated (2)	Steen-bock V	49	41	73	110	73	142
	Mated (1)	Pork 31	48	46	88	114	81	143
	Mated (2)	Pork 31	46	47	89	109	70	130

TABLE 3. ACCURACY OF SPOONS CALIBRATED TO MEASURE 0.50 GM. OF VARIOUS SUPPLEMENTS

A. Spoon No. 1 used to measure supplement for Pork 39A diet

Date	Number of tests made	Average weight of supplement (gm.)
9-27-37	10	0.5026
11-4-37	10	0.5002
12-24-37	10	0.5038
2-14-37	10	0.5009
4-4-37	10	0.5043
Average		0.5024

B. Spoon No. 2 used to measure supplement for Pork 39B diet

10-19-37	10	0.5034
11-3-37	10	0.5039
12-20-37	10	0.5034
2-11-37	10	0.5032
4-8-37	10	0.5054
Average		0.5038

C. Spoon No. 3 used to measure supplement for Pork 39C diet

9-25-37	10	0.5038
11-7-37	10	0.5047
12-16-37	10	0.5042
2-17-37	10	0.5047
4-11-37	10	0.5050
Average		0.5044

D. Spoon No. 4 used to measure supplement for Pork 31 diet

9-23-37	10	0.5033
11-14-37	10	0.5035
12-19-37	10	0.5032
2-16-37	10	0.5024
4-12-37	10	0.5018
Average		0.5028

TABLE 4. PROGRESSION OF GESTATION I IN INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Diet of females	Diet of males	Rat number	Implantation per cent	Placental index	Fertility per cent	Implantation (Appearance of erythrocyte sign)		Length of gestation in days
						Day of occurrence in gestation	Persistence of erythrocytes in days	
Steenbock V.	Steenbock V.	21355	100	100	100	--	--	21
		21357	100	100	100	14	1	22
		21483	100	100	100	--	--	22
		21484	100	100	100	--	--	22
		21504	100	100	100	--	--	23
		21506	100	100	100	--	--	22
		21539	100	100	100	14	1	22
		21558	100	100	100	14	1	22
		21579	100	100	100	16	1	21
		21745	100	100	100	13	3	22
		21749	100	100	100	--	--	22
		21836	100	100	100	14	2	22
		21850	50	100	50	17	1	24
		21852	100	100	100	14	1	23
		22022	100	100	100	15	3	22
Pork 7	Steenbock V.	15306	100	100	100	15	1	22
		15376	100	100	100	--	--	22

TABLE 4. (CONT'D) PROGRESSION OF GESTATION I IN INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork 7 (Cont'd)	Steen-bock V.	15296	100	100	100	15	1	22
		15382	100	100	100	--	--	22
		15390	50	100	50	--	--	22
		15402	100	100	100	--	--	22
		15378	100	100	100	13	2	22
		15537	100	100	100	13	1	22
		15666	50	100	50	--	--	23
		16420	100	100	100	--	--	22
		16650	100	100	100	14	1	22
		15559	50	100	50	--	--	22
		21411	100	100	100	13	1	22
		21508	100	100	100	16	1	21
		21615	100	100	100	--	--	23
		21747	100	100	100	13	1	22
		21879	100	100	100	--	--	24
Pork I	Steen-bock V.	21353	100	100	100	--	--	22
		21458	100	100	100	15	1	22
		21510	50	100	50	--	--	21
		21881	100	100	100	--	--	23
		21977	100	100	100	15	1	22
		21359	100	100	100	14	2	21
		21459	100	100	100	--	--	22
		21512	100	100	100	--	--	23
		21533	50	100	50	13	1	22
		21559	100	100	100	--	--	23
		21611	100	100	100	--	--	22

TABLE 4. (CONT'D) PROGRESSION OF GESTATION I IN INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork I (Cont'd)	Steen-bock V.	21695	100	100	100	15	1	23
		21882	100	100	100	16	1	24
		21978	100	100	100	--	--	22
Pork I	Pork I	21405	100	100	100	13	1	22
		21486	50	100	50	--	--	22
		21581	100	100	100	--	--	23
		22086	100	100	100	12	1	20
		21406	50	100	50	14	2	22
		21487	50	100	50	14	3	22
		21471	100	100	100	--	--	22
		21535	100	100	100	15	1	22
		21561	100	100	100	15	2	22
		21583	100	100	100	--	--	22
		21785	100	100	100	14	2	22
		22088	100	100	100	--	--	23
		22150	100	100	100	14	1	24
Pork 39A	Steen-bock V.	21450	50	100	50	--	--	24
		21528	50	100	50	--	--	22
		21625	100	100	100	15	2	22
		21873	100	100	100	13	3	22
		22003	100	100	100	14	1	22
		21374	100	100	100	14	1	22
		21452	100	100	100	--	--	23
		21529	50	100	50	--	--	22

TABLE 4. (CONT'D) PROGRESSION OF GESTATION I IN INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork 39A (Cont'd)	Steen-bock V.	21548	50	100	50	--	--	22
		21563	100	100	100	16	1	22
		21626	100	100	100	--	--	23
		21756	100	100	100	15	3	22
		21874	50	100	50	15	2	23
		21942	100	100	100	15	2	23
		22004	100	100	100	13	2	22
Pork 39A	Pork 39A	21427	100	100	100	--	--	23
		21476	100	100	100	14	3	22
		21638	100	100	100	15	1	22
		21931	100	100	100	15	2	22
		22104	100	100	100	--	--	22
		21428	100	100	100	--	--	23
		21477	100	100	100	20	1	22
		21550	50	100	50	--	--	21
		21605	50	100	50	14	1	22
		21639	100	100	100	20	2	24
		21840	50	100	50	17	1	22
		21932	100	100	100	14	3	23
		21921	100	100	100	12	3	22
		22108	100	100	100	13	1	22
		22195	100	100	100	13	1	22
Pork 39B	Steen-bock V.	21752	100	100	100	13	4	22
		21779	100	100	100	14	1	22

TABLE 4. (CONT'D) PROGRESSION OF GESTATION I IN INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork 39B (Cont'd)	Steen-bock V.	21865	100	100	100	--	--	21
		21884	100	100	100	15	2	24
		22024	100	100	100	18	1	23
		21754	100	100	100	--	--	22
		21780	100	100	100	15	2	21
		21795	50	100	50	15	1	22
		21803	50	100	50	14	1	22
		21834	100	100	100	13	2	24
		21854	50	100	50	15	1	22
		21860	100	100	100	13	3	22
		21866	100	100	100	--	--	23
		21885	50	100	50	18	2	21
		22026	100	100	100	--	--	23
Pork 39C	Steen-bock V.	21375	100	100	100	15	1	22
		21454	100	100	100	20	1	22
		21525	100	100	100	13	1	22
		22020	100	100	100	11	6	21
		21376	100	100	100	--	--	22
		21456	100	100	100	--	--	22
		21526	100	100	100	13	3	22
		21877	100	100	100	15	1	22
Pork 39C	Pork 39C	21419	100	100	100	15	2	23
		21465	100	100	100	15	4	22
		21936	100	100	100	13	1	22

TABLE 4. (CONT'D) PROGRESSION OF GESTATION I IN INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork 39C (Cont'd)	Pork 39C	22144	100	100	100	--	--	23
		21421	100	100	100	15	2	22
		21466	100	100	100	--	--	23
		21623	100	100	100	--	--	22
		22145	100	100	100	13	1	22
Pork 31	Steen-bock V.	21460	100	100	100	--	--	22
		21585	100	100	100	14	1	22
		21863	100	100	100	12	1	23
		21462	100	100	100	--	--	22
		21546	100	100	100	--	--	24
		21567	100	100	100	14	1	22
		21587	100	100	100	14	1	22
		21692	100	100	100	--	--	22
		21839	100	100	100	13	6	23
		21902	100	100	100	14	1	24
		22194	100	100	100	13	1	22
		22006	100	100	100	14	1	22
		21514	100	100	100	--	--	21
		22008	100	100	100	--	--	22
Pork 31	Pork 31	21408	100	100	100	13	1	22
		21473	100	100	100	14	1	22
		21641	100	100	100	15	1	22
		21409	50	100	50	14	5	22
		21474	100	100	100	14	2	23

TABLE 4. (CONT'D) PROGRESSION OF GESTATION I IN INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork 31 (Cont'd)	Pork 31	21531	33.3	100	33.3	13	3	22
		21547	100	100	100	--	--	22
		21569	100	100	100	13	4	22
		21642	100	100	100	14	1	23
		21694	100	100	100	15	2	24
		22148	100	100	100	--	--	22
		21870	100	100	100	14	1	--

TABLE 5. CONDITION OF LITTER I PRODUCED BY INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Diet of females	Diet of males	Rat number	Per cent of young born dead	Total weight in grams of live young in litter at birth	Number of live young in litter at birth	Total weight in grams of live young in litter 4 days after birth	Number of live young in litter 4 days after birth	Total weight in grams of live young in litter at weaning	Number of live young in litter at weaning
Steen-bock V.	Steen-bock V.	21355	0.0	41	9	43	6	204	4
		21483	0.0	41	8	23	6	235	5
		21504	0.0	25	5	0	0	0	0
		21745	0.0	43	9	38	6	268	6
		21850	0.0	4	1	0	0	0	0
		21357	0.0	40	7	42	5	262	5
		21484	0.0	59	11	38	11	221	5
		21506	0.0	58	12	36	7	275	6
		21539	0.0	43	8	46	7	363	6
		21558	0.0	40	7	0	0	0	0
		21579	0.0	39	8	38	8	240	5
		21749	0.0	40	9	26	4	213	5
		21852	100.0	7	0	0	0	0	0
		21836	0.0	40	9	0	0	0	0
		22022	0.0	53	10	35	9	352	6

TABLE 5. (CONT'D) CONDITION OF LITTER I PRODUCED BY INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork I	Steen-bock V.	21353	0.0	53	10	36	6	226	5
		21458	0.0	47	10	32	7	256	6
		21510	0.0	50	10	31	6	260	0
		21977	0.0	47	9	24	6	106	3
		21359	0.0	46	10	0	0	0	0
		21459	0.0	39	8	35	6	300	6
		21512	100.0	0	0				
		21533	0.0	49	10	43	6	266	6
		21559	100.0	7	1	0	0	0	0
		21611	0.0	31	6	38	6	254	6
		21695	0.0	20	4	9	2	0	0
		21978	0.0	36	8	0	0	0	0
Pork I	Pork I	21405	0.0	34	8	0	0	0	0
		21486	50.0	24	5	0	0	0	0
		21581	0.0	11	2	0	0	0	0
		22086	0.0	22	6	7	2	0	0
		21406	0.0	14	3	0	0	0	0
		21487	0.0	40	8	41	6	267	6
		21471	0.0	50	12	0	0	0	0
		21535	0.0	19	6	21	4	0	0
		21561	0.0	48	10	30	6	278	6
		21583	0.0	31	6	0	0	0	0
		21785	0.0	52	11	18	4	0	0
		22088	0.0	14	3	0	0	0	0
		22150	0.0	27	4	0	0	0	0

TABLE 5. (CONT'D) CONDITION OF LITTER I PRODUCED BY INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork 7	Steen-	15378	0.0	36	6	0	0	0	0
	bock V.	15539	0.0	42	5	18	3	153	3
		15559	0.0	6	11	49	7	281	6
		15666	0.0	44	14	0	0	0	0
		16420	0.0	51	8	0	0	0	0
		16650	0.0	31	6				
		15306	0.0	30	7	46	7	322	6
		15376	16.7	26	8	59	7	318	6
		15296	0.0	49	1	0	0	0	0
		15382	0.0	63	9	0	0	0	0
		15390	0.0	40	10	0	0	0	0
		15402	0.0	62	6	0	0	0	0
		21411	0.0	40	9	34	6	296	6
		21508	0.0	24	5	28	5	266	5
		21615	0.0	40	8	0	0	0	0
		21747	0.0	41	8	39	6	309	6
		21879	0.0	6	1	0	0	0	0
Pork 39 A	Steen-	21450	11.1	39	8	0	0	0	0
	bock V.	21528	0.0	52	10	33	5	166	4
		21625	0.0	36	7	0	0	0	0
		21873	30.0	35	7	0	0	0	0
		22003	0.0	27	5	0	0	0	0
		21374	0.0	35	7	30	6	112	3
		21452	0.0	11	3	0	0	0	0
		21529	0.0	51	10	0	0	0	0

TABLE 5. (CONT'D) CONDITION OF LITTER I PRODUCED BY INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork 39 A (Cont'd)	Steen-bock V.	21548	0.0	51	12	0	0	0	0
		21563	0.0	42	8	39	6	180	5
		21626	0.0	17	3	0	0	0	0
		21756	0.0	16	3	0	0	0	0
		21874	0.0	33	8	0	0	0	0
		21942	0.0	16	3	0	0	0	0
		22004	83.3	4	1	0	0	0	0
Pork 39A	Pork 39A	21427	0.0	26	5	0	0	0	0
		21476	0.0	25	6	7	2	0	0
		21638	0.0	38	8	0	0	0	0
		21931	0.0	45	9	0	0	0	0
		22104	0.0	39	3	0	0	0	0
		21428	0.0	16	10	0	0	0	0
		21477	0.0	45	8	40	6	121	3
		21550	0.0	52	0	0	0	0	0
		21605	11.1	36	7	0	0	0	0
		21639	100.0	0	4				
		21840	0.0	25	6	0	0	0	0
		21932	0.0	20	10	0	0	0	0
		21921	25.0	28	7	0	0	0	0
		22108	0.0	42	9	0	0	0	0
		22195	0.0	23	11	0	0	0	0
Pork 39B	Steen-bock V.	21752	0.0	29	6	0	0	0	0
		21779	0.0	52	10	30	5	193	5

TABLE 5. (CONT'D) CONDITION OF LITTER I PRODUCED BY INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork 39B (Cont'd)	Steen-bock V.	21865	0.0	36	8	25	4	163	4
		21884	75.0	3	1	0	0	0	0
		22024	0.0	7	2	0	0	0	0
		21754	0.0	44	9	47	7	248	6
		21780	0.0	48	10	0	0	0	0
		21795	0.0	45	9	24	6	189	5
		21803	0.0	45	9	22	5	36	1
		21834	50.0	6	1	0	0	0	0
		21854	0.0	35	7	2	1	0	0
		21860	0.0	36	7	26	6	223	6
		21866	0.0	24	5	0	0	0	0
		21885	0.0	37	7	0	0	0	0
		22026	0.0	11	3	0	0	0	0
Pork 39C	Steen-bock V.	21375	25.0	31	6	0	0	0	0
		21454	0.0	36	8	22	6	113	3
		21525	0.0	41	8	38	6	297	6
		22020	0.0	2	1	0	0	0	0
		21376	20.0	36	8	0	0	0	0
		21456	0.0	38	8	0	0	0	0
		21526	0.0	11	11	36	6	277	6
		21877	0.0	30	6	48	6	255	5
Pork 39C	Pork 39C	21419	0.0	27	7	0	0	0	0
		21465	0.0	41	9	17	4	63	2
		21936	0.0	21	5	0	0	0	0
		22144	100.0	0	0	0	0	0	0

TABLE 5. (CONT'D) CONDITION OF LITTER I PRODUCED BY INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork 39C (Cont'd)	Pork 39C	21421	0.0	25	5	23	4	79	2
		21466	100.0	0	0	0	0	0	0
		21623	0.0	35	7	0	0	0	0
		22145	0.0	26	7	0	0	0	0
Pork 31	Steen-bock V.	21460	0.0	42	9	23	6	194	4
		21585	0.0	40	10	0	0	0	0
		21863	16.7	7	2	0	0	0	0
		21462	20.0	25	6	0	0	0	0
		21546	0.0	10	2	0	0	0	0
		21567	0.0	19	5				
		21587	0.0	54	11	0	0	0	0
		21692	0.0	28	5	0	0	0	0
		21839	0.0	26	5	0	0	0	0
		21902	0.0	7	2	0	0	0	0
		22194	0.0	44	8	0	0	0	0
		22006	0.0	48	11	0	0	0	0
		21514	0.0	49	10				
		22008	0.0	27	6	0	0	0	0
Pork 31	Pork 31	21408	0.0	49	10	28	6	80	2
		21473	0.0	17	4	0	0	0	0
		21641	10.0	42	10	0	0	0	0
		21409	0.0	20	5	0	0	0	0
		21474	0.0	34	8	36	6	0	0
		21531	0.0	58	12	41	6	211	6

TABLE 5. (CONT'D) CONDITION OF LITTER I PRODUCED BY INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork 31 (Cont'd)	Pork 31	21547	0.0	26	6	0	0	0	0
		21569	0.0	41	7	34	6	129	5
		21642	0.0	27	16	0	0	0	0
		21694	0.0	15	3	0	0	0	0
		22148	0.0	11	4	0	0	0	0

TABLE 6. VITALITY OF LITTER I PRODUCED BY INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Diet of females	Diet of males	Rat number	Rearing performance in per cent[1]	Per cent of young dying 4 days after birth	Per cent of young dying 4-28 days after birth
Steenbock V.	Steenbock V.	21355	66.7	0.0	33.3
		21483	83.3	25.0	16.7
		21504	0.0	100.0	--
		21745	100.0	33.3	0.0
		21850	0.0	100.0	--
		21357	83.3	28.5	0.0
		21484	83.3	0.0	16.7
		21506	100.0	41.6	0.0
		21539	100.0	12.5	0.0
		21558	0.0	100.0	--
		21579	83.3	0.0	16.7
		21749	66.7	55.6	0.0
		21852	0.0	--	--
		21836	0.0	100.0	--
		22022	100.0	10.0	0.0
Pork I	Steenbock V.	21353	16.7	20.0	16.7
		21458	100.0	30.0	0.0
		21510	100.0	33.3	0.0
		21881	0.0	100.0	--
		21977	50.0	33.3	50.0
		21359	0.0	100.0	--
		21459	100.0	0.0	0.0
		21512	--	--	--
		21533	100.0	30.0	0.0
		21559	0.0	--	--
		21611	100.0	0.0	0.0
		21695	0.0	50.0	100.0
		21882	0.0	--	--
		21978	0.0	100.0	--

[1] $\frac{\text{Number actually reared}}{\text{Number should have been reared}}$

TABLE 6. (CONT'D) VITALITY OF LITTER I PRODUCED BY INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork I	Pork I	21405	0.0	100.0	--
		21486	0.0	100.0	--
		21581	0.0	100.0	--
		22086	0.0	33.3	100.0
		21406	0.0	100.0	--
		21487	100.0	0.0	0.0
		21471	0.0	100.0	--
		21535	0.0	33.3	100.0
		21561	100.0	0.0	0.0
		21583	0.0	100.0	--
		21785	0.0	63.7	100.0
		22088	0.0	100.0	--
		22150	0.0	100.0	--
Pork 7	Steen-	15306	27.3	0.0	100.0
	bock V.	15376	0.0	0.0	100.0
		15296	100.0	--	0.0
		15382	100.0	--	0.0
		15390	100.0	--	0.0
		15402	100.0	--	0.0
		15378	100.0	--	0.0
		15539	40.0	0.0	60.0
		15559	36.4	0.0	100.0
		15666	100.0	--	0.0
		16420	100.0	--	0.0
		16650	69.2	100.0	0.0
		21411	0.0	0.0	100.0
		21508	0.0	0.0	100.0
		21615	100.0	--	0.0
		21747	0.0	0.0	100.0
		21879	100.0	--	0.0
Pork	Steen-	21450	0.0	100.0	--
39A	bock V.	21528	66.7	50.0	20.0
		21625	0.0	100.0	--
		21873	0.0	100.0	--
		22003	0.0	100.0	--
		21374	50.0	14.3	50.0
		21452	0.0	100.0	--
		21529	50.0	30.0	50.0

TABLE 6. (CONT'D) VITALITY OF LITTER I PRODUCED BY INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork 39A (Cont'd	Steenbock V.	21548	0.0	100.0	--
		21563	83.3	0.0	16.7
		21626	0.0	100.0	--
		21756	0.0	100.0	--
		21874	0.0	100.0	--
		21942	0.0	100.0	--
		22004	0.0	100.0	--
Pork 39A	Pork 39A	21427	0.0	100.0	--
		21476	0.0	66.7	100.0
		21638	0.0	100.0	--
		21931	0.0	100.0	--
		22104	0.0	100.0	--
		21428	50.0	100.0	50.0
		21477	0.0	10.0	--
		21550	0.0	100.0	--
		21605	0.0	100.0	--
		21639	0.0	100.0	--
		21840	0.0	100.0	--
		21932	0.0	100.0	--
		21921	0.0	100.0	--
		22108	0.0	100.0	--
		22195	0.0	100.0	--
Pork 39B	Steenbock V.	21752	0.0	100.0	--
		21779	83.3	50.0	0.0
		21865	66.7	50.0	0.0
		21884	0.0	100.0	--
		22024	0.0	100.0	--
		21754	100.0	22.2	0.0
		21780	0.0	100.0	--
		21795	83.3	22.2	16.7
		21803	16.7	44.4	80.0
		21834	0.0	100.0	--
		21854	0.0	85.7	100.0
		21860	100.0	14.3	0.0
		21866	0.0	100.0	--
		21885	0.0	100.0	--
		22026	0.0	100.0	--

TABLE 6. (CONT'D) VITALITY OF LITTER I PRODUCED BY INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork 39C	Steenbook V.	21375	0.0	100.0	--
		21454	50.0	25.0	50.0
		21525	100.0	25.0	0.0
		22020	0.0	100.0	--
		21376	0.0	100.0	--
		21456	0.0	100.0	--
		21526	100.0	9.1	0.0
		21877	83.3	0.0	16.7
Pork 39C	Pork 39C	21419	0.0	100.0	--
		21465	33.3	55.6	50.0
		21936	0.0	100.0	--
		22144	0.0	--	--
		21421	33.3	20.0	50.0
		21466	0.0	--	--
		21623	0.0	100.0	--
		22145	0.0	100.0	--
Pork 31	Steenbook V.	21460	66.7	33.3	33.3
		21585	0.0	100.0	--
		21863	0.0	100.0	--
		21462	0.0	100.0	--
		21546	0.0	100.0	--
		21567	0.0	100.0	--
		21587	0.0	100.0	--
		21692	0.0	100.0	--
		21839	0.0	100.0	--
		21902	0.0	100.0	--
		22194	0.0	100.0	--
		22006	0.0	100.0	--
		21514	100.0	20.0	0.0
		22008	0.0	100.0	--
Pork 31	Pork 31	21408	33.3	10.0	50.0
		21473	0.0	100.0	--
		21641	0.0	100.0	--
		21409	0.0	80.0	100.0
		21474	66.7	12.5	33.3
		21531	100.0	16.7	0.0

TABLE 6. (CONT'D) VITALITY OF LITTER I PRODUCED BY INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork 31 (Cont'd)	Pork 31	21547	0.0	100.0	100.0
		21569	83.3	14.3	16.7
		21642	0.0	100.0	100.0
		21694	0.0	100.0	100.0
		22148	0.0	100.0	---
		21870	0.0	---	---

TABLE 7. PROGRESSION OF GESTATION II IN INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Diet of females	Diet of males	Rat number	Implantation per cent	Implantation (Appearance of erythrocyte sign)	
				Day of occurrence in gestation	Persistence of erythrocytes in days
Steenbock V.	Steenbock V.	21355	100	13	1
		21483	100	--	-
		21504	100	--	-
		21745	100	14	2
		21850	50	17	1
		21357	100	15	1
		21484	100	13	1
		21504	100	15	1
		21539	100	--	-
		21558	100	13	1
		22022	100	13	3
		21579	100	13	2
		21749	100	--	-
		21852	100	14	1
		21836	100	--	-
Pork I	Steenbock V.	21353	100	15	1
		21458	100	--	-
		21510	100	13	5
		21977	100	14	2
		21359	100	15	2
		21459	100	11	1
		21512	100	12	4
		21533	100	16	1
		21559	100	--	-
		21611	100	14	2
		21695	100	--	-
		21882	100	15	2
		21978	100	--	-
Pork I	Pork I	21405	100	--	-
		21486	100	14	2

TABLE 7. (CONT'D) PROGRESSION OF GESTATION II IN INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork I (Cont'd)	Pork I	21581	100	11	2
		22086	100	15	1
		21406	100	16	1
		21487	100	12	2
		21471	100	15	2
		21535	100	16	1
		21561	100	13	5
		21583	100	10	2
		21786	50	13	2
		22088	100	--	-
		22150	100	10	3
Pork 7	Steen-bock V.	21411	100	--	-
		21508	100	13	2
		21615	100	--	-
		21747	100	--	-
		21879	100	15	2
Pork 39A	Steen-bock V.	21450	100	10	1
		21528	100	15	2
		21625	100	13	2
		21873	100	13	1
		22003	50	--	-
		21374	100	13	4
		21462	100	13	1
		21529	100	15	1
		21548	100	15	3
		21563	100	16	4
		21626	100	--	-
		21756	100	14	4
		21874	100	13	3
		21942	100	13	4
		22004	100	15	1
Pork 39A	Pork 39A	21427	100	14	1
		21476	100	17	1
		21638	100	16	1
		21931	100	11	2
		22104	100	15	2

TABLE 7. (CONT'D) PROGRESSION OF GESTATION II IN INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork 39A (Cont'd)	Pork 39A	21428	100	--	-
		21477	100	14	8
		21550	100	13	1
		21606	100	15	1
		21639	100	16	1
		21840	100	14	1
		21932	100	15	3
		21921	100	13	2
		22108	100	17	1
		22195	100	--	-
Pork 39B	Steen-bock V.	21752	100	12	2
		21779	100	15	3
		21865	100	--	-
		21884	100	18	2
		22024	100	14	2
		21754	100	15	2
		21780	100	16	1
		21795	100	18	2
		21803	100	15	1
		21834	100	13	2
		21854	100	--	-
		21860	100	17	2
		21866	100	--	-
		21885	100	11	3
		22026	100	14	1
Pork 39C	Steen-bock V.	21375	100	14	2
		21454	100	14	7
		21525	100	13	2
		22020	100	13	5
		21376	100	14	1
		21456	100	15	1
		21526	100	16	3
		21877	100	13	2
Pork 39C	Pork 39C	21419	100	14	3
		21465	100	13	4
		21936	100	19	1

TABLE 7. (CONT'D) PROGRESSION OF GESTATION II IN INDIVIDUAL RATS MAKING UP THE VARIOUS EXPERIMENTAL GROUPS

Pork 39C (Cont'd)	Pork 39C	22144	100	15	2
		21421	100	13	1
		21466	100	14	3
		21623	100	19	1
		22145	100	--	-
Pork 31	Steen-bock V.	21460	100	13	2
		21585	100	--	-
		21863	100	--	-
		21462	100	13	2
		21546	100	15	1
		21567	100	13	5
		21587	100	16	1
		21692	100	13	2
		21839	100	13	4
		21902	100	13	1
		22194	100	--	-
		22006	100	--	-
		21514	100	15	1
Pork 31	Pork 31	21408	100	12	5
		21423	100	14	1
		21641	100	13	3
		21409	100	11	6
		21474	100	13	3
		21531	100	16	2
		21547	100	--	-
		21469	100	15	4
		21642	100	--	-
		21694	100	15	5
		22148	100	13	2
		21870	100	--	-

TABLE 8. INTRA-UTERINE DEVELOPMENT OF FETI OF FEMALES FED VARIOUS EXPERIMENTAL DIETS (Sick rats omitted)

Diet of females	Diet of males	Rat number	Total no. of feti	Total wt. in gm.	No. normal feti	Per cent of feti resorbed	Total wt. of placentae	Fertilization per cent
Steenbock V.	Steenbock V.	21850	8	26.21	6	25.0	2.25	80.0
		21357	13	58.5	13	0.0	4.96	100.0
		21484	5	2.77	1	80.0	0.90	100.0
		21506	12	---	11	8.3	--	100.0
		21539	13	49.78	12	7.9	5.16	100.0
		21558	13	60.17	12	7.9	5.11	100.0
		21579	13	---	12	7.9	--	99.0
		21606	11	---	9	18.3	--	98.0
		21749	9	47.03	19	0.0	4.11	100.0
		21836	12	56.61	12	0.0	4.73	75.0
		22022	13	18.80	13	0.0	3.09	100.0
Pork I	Steenbock V.	21353	11	---	9	18.2	--	99.8
		21458	10	---	9	10.0	--	100.0
		21510	9	---	7	22.2	--	100.0
		21977	7	---	6	14.3	--	100.0
		21359	12	---	9	25.0	--	100.0
		21459	13	44.14	12	7.9	4.12	100.0
		21512	10	40.14	8	20.0	2.88	92.8
		21695	12	38.43	10	16.7	4.13	84.4
		21559	11	53.81	11	0.0	4.04	100.0
		21533	11	35.61	9	18.2	3.67	99.8
		21611	11	23.93	6	45.5	2.70	100.0
		21882	11	30.59	9	18.2	3.53	78.6
		21978	10	37.57	10	0.0	4.20	99.8

TABLE 8. (CONT'D) INTRA-UTERINE DEVELOPMENT OF FETI OF FEMALES FED VARIOUS EXPERIMENTAL DIETS (Sick rats omitted)

Pork I	Pork I	21405	6	---	6	0.0	--	85.6
		21486	10	---	7	30.0	--	100.0
		22086	7	---	7	0.0	--	100.0
		21406	13	41.53	11	15.4	4.18	100.0
		21487	14	---	3	78.6	--	100.0
		21471	6	---	3	50.0	--	100.0
		21535	8	29.88	7	12.5	3.21	100.0
		21561	13	46.65	13	0.0	5.43	100.0
		21583	12	44.41	12	0.0	4.36	100.0
		21785	13	30.14	7	46.8	3.61	100.0
		22088	10	36.71	9	10.0	4.23	99.6
		22150	4	17.41	4	0.0	2.01	100.0
		22192	9	10.11	3	66.7	1.02	100.0
Pork 39A	Steen-book V.	21450	9	---	6	90.0	--	33.3
		21528	13	---	7	100.0	--	48.3
		21625	10	---	7	100.0	--	30.0
		21873	12	---	7	100.0	--	41.6
		22003	9	17.89	4	90.0	1.51	55.4
		21374	12	33.71	9	92.3	3.50	33.3
		21452	7	18.48	5	100.0	1.99	18.3
		21529	10	11.67	4	99.1	1.69	60.0
		21548	12	27.53	11	100.0	3.70	8.3
		21563	11	11.34	3	100.0	1.31	72.8
		21626	14	---	7	100.0	--	50.0
		21756	13	24.16	5	100.0	2.07	61.6
		21874	13	20.34	7	99.6	2.50	48.3

TABLE 8. (CONT'D) INTRA-UTERINE DEVELOPMENT OF FETI OF FEMALES FED VARIOUS EXPERIMENTAL DIETS (Sick rats omitted)

Pork 39A (Cont'd)	Steen-bock V.	21942	10	---	7	84.5	--	30.0
		22004	11	70.0	4	100.0	0.0	64.3
Pork 39A	Pork 39A	21427	12	---	6	50.0	--	100.0
		21426	11	---	9	18.2	--	100.0
		21638	15	35.03	9	40.0	3.66	100.0
		21931	8	---	7	12.5	--	89.9
		22104	5	---	3	40.0	--	100.0
		21428	9	---	4	55.4	--	100.0
		21429	12	---	11	8.3	--	100.0
		21477	12	23.5	5	58.2	2.10	99.9
		21550	9	34.4	8	11.1	3.10	90.0
		21605	10	20.92	6	40.0	2.62	77.3
		21639	2	---	1	50.0	--	50.0
		21840	12	24.55	7	40.1	2.65	98.9
		21932	11	0.0	0	100.0	0.0	73.3
		21921	9	0.0	0	100.0	0.0	100.0
		22108	10	3.07	1	90.0	0.309	100.0
		22195	14	30.11	5	64.2	3.05	100.0
Pork 39B	Steen-bock V.	21752	6	---	6	0.0	--	100.0
		21779	14	---	8	42.8	--	93.3
		21865	9	---	7	22.2	--	100.0
		21884	6	---	4	33.3	--	85.7
		22024	11	---	0	100.0	--	99.3
		21754	10	25.14	7	30.0	2.22	100.0

TABLE 8. (CONT'D) INTRA-UTERINE DEVELOPMENT OF FETI OF FEMALES FED VARIOUS EXPERIMENTAL DIETS (Sick rats omitted)

Pork 39B (Cont'd)	Steen-bock V.	21780	13	---	9	30.7	--	100.0
		21795	14	33.67	8	42.8	0.39	100.0
		21803	12	12.19	4	66.7	2.14	92.3
		21834	14	30.06	8	42.8	3.08	100.0
		21854	12	37.19	10	16.7	4.67	100.0
		21860	12	3.15	1	98.1	0.40	100.0
		21866	6	26.44	6	0.0	2.35	100.0
		21885	8	12.35	3	62.5	1.14	77.9
		22026	8	28.61	7	12.5	2.97	80.0
Pork 39C	Steen-bock V.	21375	12	20.74	5	58.1	2.51	100.0
		21454	0	0.0	0	100.0	0.0	0.0
		21529	9	---	0	100.0	--	90.0
		22020	10	---	5	50.0	--	100.0
		21376	4	0.0	0	100.0	0.0	66.7
		21456	6	18.61	4	33.3	1.91	100.0
		21526	9	0.0	0	100.0	0.0	100.0
		21877	11	---	9	22.2	--	22.9
Pork 39C	Pork 39C	21419	8	---	4	50.0	--	46.1
		21465	10	---	3	66.7	--	83.3
		21936	12	10.17	3	75.0	1.57	100.0
		22144	11	13.19	4	63.7	2.11	100.0
		21421	7	---	6	14.3	--	70.0
		21466	12	33.44	10	16.7	3.49	100.0
		21623	6	---	0	100.0	0.0	46.1
		22145	6	---	0	100.0	0.0	54.5

TABLE 9. CERTAIN CHARACTERISTICS OF CONDITION OF INDIVIDUAL PREGNANT FEMALES AT END OF EXPERIMENT THAT WERE FED VARIOUS DIETS
(Rats developing pregnancy disorder omitted)

Diet of females	Diet of males	Rat number	Score for general physical appearance	Rectal temperature (deg.F.)	Score for appearance of liver				Number of stomach ulcers
					Yellow	Friable	Mottled	Spongy	
Steen-bock V	Steen-bock V	21355	51	----	1	2	1	0	0
		21357	47	97.1	2	2	1	2	0
		21483	52	96.9	3	3	1	1	0
		21484	38	----	1	1	1	1	0
		21504	52	96.9	2	1	1	1	0
		21506	50	96.9	2	3	1	1	0
		21539	54	96.8	1	2	1	1	0
		21558	48	----	1	3	2	2	0
		21579	52	96.4	2	3	2	1	0
		21745	50	----	*	3	1	2	0
		21749	52	97.4	0	1	1	1	0
		21836	50	96.9	4	3	1	1	0
		21850	54	97.4	1	4	2	3	0
		21852	41	96.9	2	2	1	1	0
		22022	--	----	2	2	1	1	0
Pork I	Steen-bock V	21353	45	97.7	3	2	2	2	0
		21359	54	----	1	2	4	3	0
		21458	47	96.7	2	2	2	2	0
		21459	44	----	4	4	0	1	0
		21510	45	98.1	2	3	1	1	0

*Scored less than 1

TABLE 9. (Cont'd) CERTAIN CHARACTERISTICS OF CONDITION OF INDIVIDUAL PREGNANT FEMALES FED VARIOUS DIETS AT END OF EXPERIMENT

		21512	51	96.7	4	4	2	3	0
		21533	50	97.8	3	3	2	1	0
		21559	51	96.5	3	4	3	3	0
		21611	51	96.9	3	2	3	1	1
		21695	48	96.1	4	4	1	3	1
		21882	32	96.1	3	2	3	1	0
		21977	41	93.4	0	2	1	0	0
		21928	47	96.9	2	3	1	3	0
		21992	54	----	3	3	1	2	0
Pork 39A	Steen-bock V	21374	42	97.1	2	4	2	2	0
		21450	43	96.7	4	3	2	2	0
		21452	48	97.7	1	3	4	2	0
		21528	45	96.9	1	3	3	2	
		21529	45	97.1	3	4	3	1	0
		21548	32	97.4	4	4	1	1	0
		21563	46	97.4	3	4	1	2	0
		21625	45	96.9	2	3	2	2	0
		21626	52	----	3	2	3	3	0
		21756	48	----	2	4	3	2	1
		21873	50	----	4	3	0	1	3
		21874	50	96.9	2	2	1	1	0
		21942	52	96.8	2	3	1	2	0
		22003	47	----	1	4	1	4	0
		22004	47	----	2	3	3	1	0
	Pork 39A	21427	52	96.1	2	3	1	4	0
		21428	54	----	2	1	3	1	0

TABLE 9. (Cont'd) CERTAIN CHARACTERISTICS OF CONDITION OF INDIVIDUAL PREGNANT FEMALES FED VARIOUS DIETS AT END OF EXPERIMENT

		21476	51	----	4	4	2	0	0
		21477	50	96.7	2	3	1	1	0
		21550	46	96.9	4	4	4	3	4
		21605	44	96.9	2	2	0	0	3
		21638	41	----	2	4	1	1	4
		21639	48	97.1	3	2	1	2	0
		21840	42	95.7	3	3	2	1	0
		21921	35	96.9	2	4	2	2	0
		21931	52	97.7	3	2	3	3	0
		21932	28	----	2	3	2	1	0
		22104	56	----	2	4	4	1	0
		22108	41	96.7	3	3	2	2	0
		22195	51	96.4	2	3	1	3	0
Pork 39B	Steen-bock V	21752	43	96.7	1	3	2	1	0
		21754	52	----	3	3	*	1	4
		21779	43	----	2	2	1	2	0
		21780	52	----	4	4	1	2	2
		21795	52	96.1	3	3	3	3	0
		21803	43	97.6	3	3	2	1	0
		21834	48	96.6	3	4	1	2	1
		21854	32	97.9	4	4	*	3	0
		21860	49	97.1	3	4	2	3	0
		21865	49	97.1	2	3	1	3	-
		21866	49	97.4	3	4	2	4	0
		21884	33	97.7	2	3	1	2	0
		21885	43	97.2	0	2	1	0	3

TABLE 9. (Cont'd) CERTAIN CHARACTERISTICS OF CONDITION OF INDIVIDUAL PREGNANT FEMALES FED VARIOUS DIETS AT END OF EXPERIMENT

		22024	47	97.3	3	4	3	2	0
		22026	46	----	4	4	0	2	1
Pork 39C	Steen-bock V	21375	49	----	2	3	1	2	0
		21376	41	----	3	4	2	2	0
		21454	49	96.2	3	4	1	2	0
		21456	48	96.7	2	3	2	2	0
		21525	48	97.8	1	3	2	2	0
		21526	52	97.4	2	1	1	2	0
		21877	53	97.1	2	3	1	2	0
		22020	37	96.1	0	2	1	1	0
Pork 39C	Pork 39C	21419	51	----	4	4	1	1	0
		21421	50	97.4	2	3	2	1	0
		21465	47	96.8	3	3	1	2	0
		21466	50	97.5	4	4	4	4	0
		21623	49	96.9	4	4	1	3	10
		21936	46	97.3	2	3	1	1	1
		22144	48	96.4	2	3	1	4	0
		22145	49	96.7	3	3	1	2	0
Pork 31	Steen-bock V	21460	47	96.3	2	3	4	4	0
		21462	38	96.2	3	4	3	1	0
		21514	32	97.0	3	2	4	2	0
		21546	43	96.9	1	3	3	4	0
		21567	44	97.2	2	1	2	3	2
		21585	48	96.8	4	1	1	1	1

TABLE 9. (Cont'd) CERTAIN CHARACTERISTICS OF CONDITION OF INDIVIDUAL PREGNANT FEMALES FED VARIOUS DIETS AT END OF EXPERIMENT

		21587	52	96.0	1	2	1	4	0
		21619	54	97.1	3	1	3	2	0
		21692	47	97.3	2	3	2	3	0
		21839	46	97.4	3	2	3	2	1
		21863	43	97.1	4	4	2	3	1
		21902	53	----	3	3	3	2	2
		22006	53	96.8	2	2	3	3	0
		22194	49	----	2	3	2	1	0
Pork 7	Steen-bock V	21411	49	97.6	2	2	1	1	0
		21508	48	96.9	4	2	3	4	0
		21615	51	97.3	3	2	4	3	1
		21747	47	96.9	1	1	2	2	2
		21879	49	97.1	1	2	4	5	0
Pork I	Pork I	22150	46	95.6	3	4	1	3	1
		21405	42	----	3	3	4	2	0
		21406	45	----	3	4	1	2	2
		21471	50	----	3	4	2	2	0
		21486	51	97.4	2	4	2	1	0
		21487	47	96.7	3	2	1	1	0
		21535	48	96.7	3	4	2	2	0
		21561	45	97.3	4	4	3	2	-
		21581	46	97.1	0	1	1	2	0
		21583	51	96.7	4	4	4	4	0

TABLE 9. (Cont'd) CERTAIN CHARACTERISTICS OF CONDITION OF INDIVIDUAL PREGNANT FEMALES FED VARIOUS DIETS AT END OF EXPERIMENT

Pork I	Pork I	21785	50	----	3	3	2	1	0
		22086	49	96.9	4	3	2	2	0
		22088	47	92.9	2	3	1	1	0
Pork 31	Pork 31	21408	47	97.4	4	3	4	3	2
		21409	46	----	2	1	3	3	0
		21423	46	93.4	2	3	4	2	0
		21473	51	96.9	2	1	3	1	0
		21474	12	----	2	1	3	3	1
		21531	47	97.3	2	1	4	1	0
		21547	31	96.9	1	1	4	2	0
		21569	43	96.4	3	3	3	3	0
		21641	49	97.8	1	2	2	2	0
		21642	48	97.3	3	2	3	2	1
		21694	43	----	3	3	4	1	0
		21870	41	91.2	1	2	2	2	1
		22148	53	----	3	3	1	2	0

TABLE 10. CERTAIN CHARACTERISTICS OF CONDITION OF INDIVIDUAL VIRGIN FEMALES AT END OF EXPERIMENT THAT WERE FED VARIOUS DIETS

Diet of females	Rat number	Score for general physical appearance	Score for appearance of liver				Number of stomach ulcers
			Yellow	Friable	Mottled	Spongy	
Steen-bock V.	21402	49	1	2	1	1	0
	21403	47	1	1	1	2	0
	21468	42	0	2	0	1	0
	21469	45	2	3	0	1	0
	21479	49	0	1	0	1	0
	21551	42	0	0	0	0	0
	21575	48	0	*	0	0	0
	21629	48	0	2	0	0	0
	21677	40	1	1	1	1	0
	21608	52	2	2	2	2	0
	21609	49	1	3	0	1	0
	21838	38	1	1	2	1	0
	21903	48	1	2	1	0	0
	21905	53	2	3	1	1	0
	22055	50	1	1	1	0	0
Pork I	21433	48	2	2	1	2	0
	21447	42	2	3	2	1	0
	21489	53	2	2	3	2	0
	21490	43	1	2	0	*	0

*Score less than one

TABLE 10. (CONT'D) CERTAIN CHARACTERISTICS OF CONDITION OF INDIVIDUAL VIRGIN FEMALES AT END OF EXPERIMENT THAT WERE FED VARIOUS DIETS

Pork I (Cont'd)	21552	38	1	2	3	0	4
	21573	43	2	3	1	1	0
	21613	50	*	2	1	2	0
	21631	44	1	2	1	2	5
	21633	43	2	1	0	1	1
	21784	34	*	1	*	1	0
	21858	47	2	1	1	1	0
	21859	42	1	2	1	1	0
	21917	43	2	2	1	1	0
	22057	47	2	3	0	3	0
	22059	41	1	4	0	2	0
Pork 7	21441		1	1	2	1	0
	21463	51	1	2	1	1	0
	21617	49	2	3	0	1	0
	21975	48	3	3	0	1	0
Pork 39A	21436	49	2	4	0	1	0
	21495	49	1	3	1	2	0
	21497	47	2	3	1	1	0
	21499	44	0	4	1	3	0
	21554	41	3	4	2	1	0
	21572	46	1	2	1	2	0
	21645	48	1	2	1	1	2
	21647	47	*	2	0	2	0

*Score less than one

TABLE 10. (CONT'D) CERTAIN CHARACTERISTICS OF CONDITION OF INDIVIDUAL VIRGIN FEMALES AT END OF EXPERIMENT THAT WERE FED VARIOUS DIETS

Pork 39A (Cont'd)	21732	36	3	3	2	1	0
	21734	42	2	3	1	2	0
	21806	32	2	3	2	1	0
	21919	39	2	3	2	2	2
	21954	36	2	3	1	3	1
	22064	44	3	3	2	2	0
	22065	40		4	4	1	4
Pork 39B	21781	45	2	3	1	1	0
	21783	46	3	4	1	1	0
	21787	43	2	3	2	1	0
	21797	52	2	4	3	2	0
	21835	38	1	2	0	0	0
	21861	43	2	3	1	0	2
	21862	46	2	3	2	1	0
	21867	44	2	3	2	1	3
	21869	44	3	2	4	4	0
	21894	27	1	1	0	0	5
	21906	42	0	2	1	1	3
	21907	46	1	3	0	0	0
	21923	40	2	2	1	2	0
	22082	46	2	4	1	1	3
	22083	43	3	0	3	3	0

TABLE 10. (CONT'D) CERTAIN CHARACTERISTICS OF CONDITION OF INDIVIDUAL VIRGIN FEMALES AT END OF EXPERIMENT THAT WERE FED VARIOUS DIETS

Pork 39C	21439	52	2	3	2	2	0
	21501	41	2	1	2	1	2
	21502	40	0	1	1	2	0
	21614	54	1	2	2	2	0
	21622	49	2	2	1	1	2
	21649	46	2	3	1	0	3
	21651	49	3	4	2	2	0
	21956	42	2	4	1	1	0
	22062	47	1	4	2	3	0
Pork 31	21429	40	4	1	0	1	2
	21431	46	1	1	4	3	0
	21492	43	2	1	1	2	1
	21493	47	3	4	2	1	0
	21556	44	1	3	0	3	0
	21570	42	1	3	0	1	0
	21627	47	1	3	4	1	2
	21637	47	2	3	1	2	1
	21643	43	3	2	1	3	0
	21791	49	2	4	2	4	0
	21793	46	1	2	1	2	0
	21980	45	0	2	1	0	0

TABLE 11. DAILY WATER CONSUMPTION OF INDIVIDUAL PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS
(Rats developing pregnancy disorder omitted)

Diet of females	Diet of males	Rat number	Consumption of water from 12th to 21st day of gestation period									
			12th	13th	14th	15th	16th	17th	18th	19th	20th	21st
			cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Steen-bock V	Steen-bock V	21355	12	16	15	12	16	16	14	13	18	16
		21357	13	14	14	11	15	*	13	16	16	19
		21483	16	16	17	10	24	17	21	17	14	13
		21484	14	17	*	13	14	14	19	16	17	20
		21504	13	14	14	16	15	17	16	19	17	17
		21506	11	13	12	14	14	18	*	17	26	16
		21539	16	*	11	18	10	17	18	14	19	17
		21558	17	11	16	14	18	20	14	16	17	18
		21579	18	10	17	16	*	16	17	19	16	14
		21745	14	10	18	18	11	18	18	21	19	17
		21749	10	14	14	17	16	17	16	12	17	16
		21836	9	16	16	15	10	4	20	11	*	18
		21850	14	17	17	*	10	17	19	17	15	17
		21852	13	18	14	12	14	19	18	16	13	16
		22022	14	14	16	10	16	20	21	17	17	20

* Water spilled so that accurate measurement was impossible

TABLE 11. (CONT'D) DAILY WATER CONSUMPTION OF INDIVIDUAL PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS

Pork I	Steen-bock V	21353	10	12	*	19	18	21	17	15	18	16
		21359	8	10	19	17	16	13	18	*	14	19
		21458	16	14	17	21	19	23	4	18	16	20
		21459	11	12	20	18	23	16	14	20	23	18
		21510	17	16	21	18	18	*	12	21	16	17
		21512	16	17	*	20	19	17	30	19	19	16
		21533	10	12	16	19	14	*	21	16	20	19
		21559	9	19	10	16	12	19	19	19	21	18
		21611	13	11	16	18	16	23	18	17	24	14
		21695	14	17	19	14	19	13	22	*	16	20
		21882	12	16	17	21	23	19	16	18	14	21
		21977	16	14	15	19	18	9	14	19	19	19
		21978	11	13	7	16	14	17	*	26	18	16
		22192	14	12	18	*	19	16	19	21	10	15
	Pork I	21405	10	14	16	18	18	16	17	21	20	16
		21406	12	17	21	16	16	19	14	16	19	17
		21471	14	18	17	20	14	18	20	20	17	18
		21486	18	19	20	16	12	17	21	20	16	20
		21487	17	14	16	14	21	14	16	14	19	16
		21535	12	19	19	19	19	16	17	19	18	19
		21561	9	23	18	17	23	19	20	23	20	14
		21581	14	18	14	18	18	20	17	19	17	18
		21583	16	16	16	14	16	18	19	18	16	14
		21785	11	17	19	12	19	21	16	17	20	19
		22086	12	19	20	19	20	17	14	16	19	20
		22088	18	17	19	17	21	22	21	12	18	19
		22150	11	18	16	14	17	19	19	20	16	14

TABLE 11.(CONT'D) DAILY WATER CONSUMPTION OF INDIVIDUAL PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS

Pork 7	Steen-bock V	21411	14	18	12	16	14	17	18	20	19	20
		21508	18	14	20	14	18	18	17	21	20	16
		21615	19	21	20	21	16	13	14	16	17	29
		21747	16	16	18	19	20	17	19	18	18	17
		21879	17	19	17	17	21	14	17	18	7	14
Pork 39A	Steen-bock V	21374	16	14	*	19	16	19	17	14	17	20
		21450	19	17	*	17	14	20	19	16	7	21
		21452	18	19	14	16	12	14	18	19	14	18
		21528	14	16	16	18	7	*	18	19	14	17
		21529	7	*	18	11	19	18	16	12	18	16
		21548	*	19	14	12	21	16	*	14	18	16
		21563	16	18	19	18	19	19	20	16	18	19
		21625	12	17	15	13	18	16	16	7	9	20
		21626	10	16	18	18	19	*	17	16	19	14
		21756	11	14	10	12	20	18	16	19	14	16
		21873	14	17	12	19	16	14	10	8	12	19
		21874	16	11	19	17	18	17	17	7	10	12
		21942	17	19	16	16	11	19	20	17	18	14
		22003	10	12	14	19	18	21	7	18	12	18
	Pork 39A	21427	16	14	16	17	14	18	20	18	17	16
		21428	*	8	19	14	16	16	19	19	20	18
		21476	17	17	18	19	9	20	17	16	14	*
		21477	18	9	14	13	10	7	14	15	13	14
		21550	*	14	19	16	11	19	20	18	17	13
		21605	14	16	18	11	16	*	13	12	14	19
		21638	19	12	16	8	19	15	11	*	19	17

TABLE 11. (CONT'D) DAILY WATER CONSUMPTION OF INDIVIDUAL PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS

Pork 39A (Cont'd)	Pork 39A	21639	16	19	14	7	18	17	19	13	11	13
		21840	17	17	19	12	7	14	16	14	16	19
		21921	14	18	18	19	9	19	14	19	19	18
		21931	18	14	16	17	20	20	13	17	18	*
		22104	19	20	14	6	18	10	12	16	14	15
		22108	16	17	20	3	14	14	17	11	12	16
		22195										
Pork 39B	Steen-bock V	21752	16	18	20	18	20	18	20	26	23	21
		21754	18	*	23	19	19	19	24	19	20	18
		21779	10	16	18	17	16	20	16	14	17	19
		21780	12	14	21	20	21	*	29	27	19	22
		21803	19	19	27	21	18	16	17	16	18	19
		21834	6	15	14	17	*	19	14	18	14	16
		21854	14	16	16	18	14	20	16	14	19	19
		21860	17	20	19	14	16	26	18	19	16	17
		21865	20	*	7	19	19	19	19	23	14	13
		21866	16	16	18	17	18	14	24	26	17	7
		21884	14	17	14	16	4	16	19	14	21	20
		21885	12	10	7	19	19	27	18	16	16	19
		22024	12	19	9	18	18	18	22	19	24	17
		22026	14	14	18	19	16	19	21	17	19	20
Pork 39C	Steen-bock V	21375	16	14	7	17	20	18	14	16	16	18
		21376	*	16	23	19	23	19	19	21	19	14
		21454	19	*	14	18	18	17	16	19	10	21
		21456	14	17	19	14	20	16	*	17	21	19
		21525	15	19	21	16	17	18	23	20	18	16
		21526	17	16	18	19	19	15	19	*	17	19
		21877	9	14	16	21	21	19	18	14	19	20

TABLE 11.(CONT'D) DAILY WATER CONSUMPTION OF INDIVIDUAL PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS

Pork 39C (Cont'd)	Pork 39C	21419	18	14	*	20	18	18	14	16	18	20
		21421	*	21	20	18	14	21	19	19	16	17
		21465	14	19	18	14	19	19	*	13	*	18
		21466	16	17	14	16	*	18	23	14	22	19
		21623	19	15	*	23	21	17	19	17	17	*
		21936	18	14	16	13	24	16	20	20	19	17
		22144	17	19	7	19	13	20	16	12	18	18
		22145	18	17	19	17	17	19	*	19	14	19
Pork 31	Steen-bock V	21460	12	12	26	18	18	10	16	29	19	20
		21462	*	16	14	18	17	18	20	12	21	19
		21514	7	7	13	19	8	10	21	9	*	7
		21546	6	14	16	*	19	16	14	10	21	19
		21567	14	17	9	14	27	10	16	14	18	28
		21585	14	18	29	15	18	4	18	19	16	14
		21587	19	17	16	17	11	16	*	19	20	16
		21692	23	19	*	19	9	8	16	16	14	17
		21839	18	16	18	20	17	24	18	4	18	19
		21863	16	12	14	18	6	20	16	14	12	16
		21902	16	14	*	28	19	16	19	19	17	17
		22194	14	19	7	19	7	14	18	16	20	18
		22006	16	18	17	4	19	18	20	16	*	*
	Pork 31	21408	19	18	19	14	19	17	*	20	16	20
		21409	10	12	11	13	18	12	*	17	16	19
		21423	16	14	*	17	19	18	19	18	19	19
		21474	*	20	12	19	*	6	19	16	16	18
		21531	12	11	15	17	6	14	16	14	18	16

TABLE 11. (CONT'D) DAILY WATER CONSUMPTION OF INDIVIDUAL PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS

Pork 31 (Cont'd)	Pork 31	21547	18	16	5	*	17	14	19	9	19	21
		21569	10	16	7	12	18	8	*	20	17	18
		21641	*	12	10	11	16	17	16	*	15	17
		21642	10	16	18	18	15	*	16	14	19	13
		21694	*	19	18	16	*	18	15	18	17	14
		21870	8	*	14	5	16	19	20	13	18	15
		22148	14	16	19	17	17	18	19	16	17	19

TABLE 12. DAILY WATER CONSUMPTION OF INDIVIDUAL PREGNANT FEMALES DEVELOPING SYMPTOMS OF THE PREGNANCY DISORDER

Rat number	Consumption of water from 12th to 21st day of gestation period									
	12th	13th	14th	15th	16th	17th	18th	19th	20th	21st
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
21937	20	19	19	18	20	19	28	30	12	19
21621	14	18	*	16	18	17	17	19	16	16
21876	29	21	19	17	*	20	*	*	*	*
21618	17	19	16	18	17	17	21	27	18	19
21901	18	17	21	14	21	18	19	26	14	17
22008	12	16	18	*	16	22	*	24	17	18
22147	19	21	20	19	20	*	*	*	*	*
21928	27	17	19	20	24	24	27	32	11	14
21929	14	18	17	16	20	21	16	30	16	*
21881	23	14	20	21	14	16	25	27	12	13

* Water spilled so that accurate measurement was impossible

TABLE 13. FAT CONTENT OF LIVERS OF PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS[1] (Sick rats omitted)

Diet of females	Diet of males	Rat number	Fat content of dry liver in per cent
Steenbock V.	Steenbock V.	21357	24.98
		21558	24.00
		21579	20.76
		21539	20.97
		21484	19.20
		21506	21.34
		21836	23.57
		21749	23.24
		21850	---
		22022	20.28
Pork I	Steenbock V.	21512	46.87
		21559	47.51
		21695	48.25
		21359	38.66
		21459	45.34
		21533	38.52
		21611	29.74
		21882	35.74
		21978	42.89
Pork I	Pork I	21583	47.57
		21535	38.32
		21561	46.33
		21487	28.54
		22150	26.44
		21785	40.72
Pork 39A	Steenbock V.	21563	29.26
		21548	49.73
		21452	37.29
		21374	40.02

[1]These data are included through the courtesy of Miss Ethelwyn Wilcox

TABLE 13. (CONT'D) FAT CONTENT OF LIVERS OF PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS (Sick rats omitted)

Pork 39A (Cont'd)	Steenbock V.	21626	45.95
		21529	30.50
		21942	30.94
		21874	40.57
		22003	34.48
Pork 39A	Pork 39A	21428	47.42
		21638	39.24
		21550	47.97
		21427	25.61
		21477	33.21
		21605	56.30
		21921	36.56
		21840	46.00
		21932	26.52
		22108	33.85
Pork 39B	Steenbock V.	21834	46.43
		21754	40.67
		21854	42.76
		21860	31.89
		21885	25.87
		22026	35.67
		21866	46.28
Pork 39C	Steenbock V.	21375	34.76
		21526	28.23
		21877	34.95
Pork 39C	Pork 39C	21419	39.05
		21466	41.88
		21623	26.66
		22145	38.36
Pork 31	Steenbock V.	21514	34.01
		21585	21.43

TABLE 13. (CONT'D) FAT CONTENT OF LIVERS OF PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS (Sick rats omitted)

Pork 31 (Cont'd)	Steenbock V.	21462	31.66
		21546	35.47
		21567	30.48
		21587	26.12
		21839	33.47
		21902	38.42
		21619	24.18
Pork 31	Pork 31	21547	29.51
		21694	38.36
		21474	29.17
		21870	25.79
		21409	26.85
		21641	28.44
		21569	26.82
		21531	24.10

TABLE 14. FAT CONTENT OF LIVERS OF RATS EXHIBITING SYMPTOMS OF THE PREGNANCY DISORDER[1]

Rat number	Fat content of dry liver in per cent
21937	29.18
21621	28.19
21876	26.26
21618	25.30
21901	25.15
22008	28.08
21928	44.34
21708	41.63
21843	25.53
21911	52.35
21706	46.73
21740	38.98
21842	50.56
21940	20.37
21939	26.15

[1]These data are included through the courtesy of Miss Ethelwyn Wilcox

TABLE 15. FAT CONTENT OF LIVERS FROM VIRGIN RATS FED VARIOUS EXPERIMENTAL DIETS[1]

Diet of females	Rat number	Fat content of dry liver in per cent
Steenbock V.	21469	23.27
	21403	20.79
	21479	22.46
	21575	19.69
	21565	20.28
	21609	19.53
	21903	20.31
	21838	18.77
Pork I	21573	24.79
	21433	23.75
	21633	28.79
	21784	26.42
	21490	26.86
	21552	26.60
	22057	26.69
	21917	20.06
	22059	21.14
Pork 39A	21499	25.22
	21436	22.95
	21572	20.89
	21495	25.77
	21647	27.30
	22065	30.58
	21919	21.66
	21732	22.50
	21554	24.17
	22064	24.79
	21954	16.34
Pork 39B	21781	26.22
	21835	24.01
	22083	21.97
	21862	24.42
	21923	21.16

[1]These data are included through the courtesy of Miss Ethelwyn Wilcox

TABLE 15. (CONT'D) FAT CONTENT OF LIVERS FROM VIRGIN RATS FED VARIOUS EXPERIMENTAL DIETS

Pork 39B (Cont'd)	21869	24.07
	21787	23.39
	21797	28.97
	21907	17.37
Pork 39C	21502	23.72
	21614	21.63
	21439	21.69
	21651	22.58
Pork 31	21643	24.68
	21429	24.61
	21637	22.86
	21493	25.67
	21431	23.73
	21793	23.79
	21556	21.31
	21570	23.78
	21980	23.94

TABLE 16. MOISTURE CONTENT OF CERTAIN ORGANS OF PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS (Sick rats omitted)

Diet of females	Diet of males	Rat number	Water content of liver in per cent[1]	Water content of kidney in per cent	Water content of spleen in per cent	Water content of heart in per cent	Water content of mammary gland in per cent
Steenbock V.	Steenbock V.	21749	70.1	77.6	83.4	77.0	70.1
		21850	69.9	76.7	79.6	78.9	77.3
		22022	70.5	77.5	77.3	78.7	66.3
		21357	70.3	--	--	--	--
		21558	71.4	--	--	--	--
		21579	70.4	--	--	--	--
		21539	70.8	--	--	--	--
		21484	70.0	--	--	--	--
		21506	69.9	--	--	--	--
		21836	70.1	--	--	--	--
Pork I	Steenbock V.	21459	64.5	77.6	77.6	76.9	76.4
		21611	68.7	77.7	77.8	71.9	74.1
		21512	61.9	--	--	--	--
		21559	66.0	--	--	--	--
		21695	63.1	77.4	75.2	74.7	75.6
		21359	65.9	--	--	--	--
		21533	65.1	--	--	--	--

[1]These data are included through the courtesy of Miss Ethelwyn Wilcox

TABLE 16. (CONT'D) MOISTURE CONTENT OF CERTAIN ORGANS OF PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS (Sick rats omitted)

Pork I (Cont'd)	Steen-bock V.	21882	67.2	--	--	--	--
		21978	65.1	--	--	--	--
Pork I	Pork I	22150	68.8	89.0	77.5	77.9	23.9
		21583	63.1	78.3	76.4	76.7	37.8
		21561	62.0	76.4	74.1	76.7	32.4
		21487	68.7	--	--	--	--
		21785	64.2	--	--	--	--
Pork 39A	Steen-bock V.	21942	66.8	79.4	83.7	78.1	56.7
		22003	68.3	78.6	77.4	79.2	33.8
		21563	68.2	--	--	--	--
		21548	61.7	--	--	--	--
		21452	65.8	--	--	--	--
		21374	66.0	--	--	--	--
		21626	64.0	--	--	--	--
		21529	69.4	--	--	--	--
		21874	65.4	--	--	--	--
Pork 39A	Pork 39A	21932	69.4	77.6	78.4	76.1	60.5
		21428	65.6	--	--	--	--
		21638	66.8	--	--	--	--
		21550	64.4	--	--	--	--
		21427	69.0	--	--	--	--
		21477	65.5	--	--	--	--
		21605	63.0	--	--	--	--
		21921	66.9	--	--	--	--

TABLE 16. (CONT'D) MOISTURE CONTENT OF CERTAIN ORGANS OF PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS (Sick rats omitted)

Pork 39A (Cont'd)	Pork 39A	21840	63.9	--	--	--	--
		22108	65.7	--	--	--	--
Pork 39B	Steen-bock V.	21854	66.5	77.8	74.3	77.8	64.7
		21866	62.9	77.8	76.8	77.2	76.3
		21834	65.2	--	--	--	--
		21754	65.1	--	--	--	--
		21860	68.2	--	--	--	--
		21885	68.8	--	--	--	--
		22026	66.1	--	--	--	--
Pork 39C	Steen-bock V.	21877	66.8	78.9	81.3	78.7	74.2
		21375	69.1	--	--	--	--
		21526	68.7	--	--	--	--
Pork 39C	Pork 39C	22145	66.2	76.6	80.3	77.1	39.7
		21419	65.5	--	--	--	--
		21466	68.4	--	--	--	--
		21623	69.3	--	--	--	--
Pork 31	Steen-bock V.	21546	68.9	73.6	76.1	77.9	74.3
		21514	69.9	--	--	--	--
		21585	71.1	--	--	--	--
		21462	70.1	--	--	--	--
		21567	70.3	--	--	--	--

TABLE 16. (CONT'D) MOISTURE CONTENT OF CERTAIN ORGANS OF PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS (Sick rats omitted)

Pork 31 (Cont'd)	Steen-bock V.	21587	71.8	--	--	--	--
		21839	70.6	--	--	--	--
		21902	71.4	--	--	--	--
		21619	70.0	--	--	--	--
Pork 31	Pork 31	22148	69.8	75.9	77.6	78.6	76.9
		21547	70.2	--	--	--	--
		21694	70.2	--	--	--	--
		21474	69.2	--	--	--	--
		21870	75.8	--	--	--	--
		21409	71.4	--	--	--	--
		21641	70.8	--	--	--	--
		21569	69.3	--	--	--	--
		21531	69.7	--	--	--	--

TABLE 17. MOISTURE CONTENT OF CERTAIN ORGANS FROM FEMALES EXHIBITING SYMPTOMS OF PREGNANCY DISORDER

Rat number	Water content of liver in per cent[1]	Water content of kidneys in per cent	Water content of spleen in per cent	Water content of heart in per cent	Water content of mammary glands in per cent
21937	72.5	80.4	84.6	78.1	67.4
21621	70.1	82.9	81.7	77.6	79.6
21876	73.8	--	--	--	--
21618	73.3	81.3	83.2	74.3	77.4
21901	71.7	82.4	81.3	77.2	79.6
22008	74.3	--	--	--	--
21928	71.3	83.2	87.2	76.3	76.7
21929	71.1	86.7	79.4	76.8	24.6
21741	71.5	--	--	--	--
21708	69.9	83.4	81.2	77.6	54.7
21843	74.6	--	--	--	--
21911	66.7	--	--	--	--
21965	67.4	--	--	--	--
21841	71.0	81.9	83.7	82.6	79.4
21706	65.3	82.3	82.1	81.7	72.3
21740	69.4	84.3	82.1	77.9	72.8
21842	66.6	--	--	--	--
21940	72.5	79.4	77.4	76.4	27.8
21939	75.1	--	--	--	--

[1]These data are included through the courtesy of Miss Ethelwyn Wilcox

TABLE 18. MOISTURE CONTENT OF ORGANS OF VIRGIN FEMALES FED VARIOUS EXPERIMENTAL DIETS

Diet of females	Rat number	Water content of liver in per cent	Water content of kidney in per cent	Water content of spleen in per cent	Water content of mammary gland in per cent
Steenbock V.	21575	68.7	76.5	73.2	78.6
	21609	69.6	83.0	74.3	77.8
	21677	69.6	72.9	78.4	79.1
	21903	69.1	78.5	79.6	77.0
	21838	68.1	78.7	76.1	77.7
	22055	--	78.7	75.9	77.3
	21469	68.8	--	--	--
	21403	69.0	--	--	--
	21479	68.6	--	--	--
	21555	69.1	--	--	--
Pork I	21917	69.7	79.4	77.2	77.0
	22057	68.3	79.7	81.3	77.4
	22059	69.4	79.1	77.4	77.2
	21573	69.4	--	--	--
	21433	67.3	--	--	--
	21633	68.7	--	--	--
	21784	69.1	--	--	--
	21490	69.5	--	--	--

[1]These data are included through the courtesy of Miss Ethelwyn Wilcox

TABLE 18. (CONT'D) MOISTURE CONTENT OF ORGANS OF VIRGIN FEMALES FED VARIOUS EXPERIMENTAL DIETS

Pork I (Cont'd)	21552	69.7	--	--	--
Pork 39A	21554	59.0	77.9	76.8	77.5
	21732	68.1	77.4	80.3	77.4
	21919	68.3	76.7	81.4	76.6
	21954	70.1	77.4	81.3	78.4
	22064	68.8	78.2	79.1	76.3
	22065	67.0	76.3	81.2	78.9
	21499	68.3	--	--	--
	21436	69.2	--	--	--
	21572	69.3	--	--	--
	21495	68.8	--	--	--
	21647	68.3	--	--	--
Pork 39B	21781	68.6	78.3	76.3	78.9
	21787	69.6	76.9	72.3	76.9
	21862	69.1	77.3	77.2	78.4
	21869	70.1	77.4	76.7	76.9
	21907	69.7	78.1	75.2	77.1
	21923	69.4	77.2	76.1	77.9
	21797	68.9	77.9	74.3	77.8
	22083	68.6	77.9	74.9	79.6
	21835	68.9	--	--	--
	21797	68.9	--	--	--

TABLE 18. (CONT'D) MOISTURE CONTENT OF ORGANS OF VIRGIN FEMALES FED VARIOUS EXPERIMENTAL DIETS

Pork 39C	21502	69.8	78.9	77.3	76.7
	21651	68.6	76.7	80.4	78.2
	21956	--	77.4	81.0	76.4
	21614	69.0	--	--	--
	21439	69.2	--	--	--
Pork 31	21429	69.5	77.9	74.9	76.9
	21431	69.4	78.4	77.8	78.4
	21556	68.9	76.9	76.4	78.5
	21570	69.7	77.8	77.6	78.4
	21637	68.4	79.4	76.9	79.3
	21643	68.2	78.6	76.6	79.8
	21793	69.2	79.4	76.4	79.9
	21980	69.2	77.9	79.4	78.7
	21493	68.9	--	--	--

TABLE 19. WEIGHTS AND ORGAN WEIGHT TO BODY WEIGHT RATIO OF LIVER AND KIDNEY OF PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS (Sick rats omitted)

Diet of females	Diet of males	Rat number	Body weight[1]	Weight of liver[2]	Weight of liver/ body wt.	Weight of kidney	Weight of kidney/ body wt.
			gm.	gm.		gm.	
Steen-bock V.	Steen-bock V.	21357	171	6.0010	0.03509	--	--
		21558	177	6.7854	0.03833	--	--
		21579	174	6.9092	0.03970	--	--
		21539	178	6.7538	0.03894	--	--
		21484	202	6.9673	0.03449	--	--
		21506	180	6.2345	0.03463	--	--
		21836	178	6.8937	0.03873	--	--
		21749	178	7.4713	0.04191	1.4700	0.0082
		21850	206	7.9684	0.03968	1.4992	0.0079
		22022	221	8.1699	0.03698	1.4503	0.0071
Pork I	Steen-bock V.	21512	171	6.6327	0.03878	--	--
		21559	184	7.2059	0.03916	--	--
		21695	174	7.4601	0.04287	1.7146	0.0089
		21359	166	7.3368	0.03817	--	--

[1]Body weight at initiation of second pregnancy
[2]These data are included through the courtesy of Miss Ethelwyn Wilcox

TABLE 19. (CONT'D) WEIGHTS AND ORGAN WEIGHT TO BODY WEIGHT RATIO OF LIVER AND KIDNEY OF PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS (Sick rats omitted)

Pork I (Cont'd)	Steen-bock V.	21459	196	7.2257	0.03686	1.7034	0.0086
		21533	190	7.1430	0.03759	--	--
		21611	181	6.9881	0.05233	1.6471	0.0091
		21882	181	6.6937	0.03698	--	--
		21978	180	6.5103	0.03571	--	--
Pork I	Pork I	21583	159	5.9385	0.03734	1.6961	0.0067
		21535	191	6.6604	0.03587	--	--
		21561	201	7.5362	0.03749	1.7062	0.0085
		21487	209	7.4820	0.03579	--	--
		22150	177	6,4941	0.03668	1.4215	0.0074
		21785	190	7,4517	0.03921	--	--
Pork 39A	Steen-bock V.	21563	184	6.8458	0.03720	--	--
		21548	178	9.4279	0.05396	--	--
		21452	157	6.0642	0.03862	--	--
		21374	169	6.4972	0.03844	--	--
		21626	165	6.4662	0.03919	--	--
		21529	197	7.0213	0.03564	--	--
		21942	164	5.9309	0.03616	1.5671	0.0085
		21874	192	7.7225	0.04022	--	--
		22003	190	7.2451	0.03813	1.4870	0.0078

TABLE 19. (CONT'D) WEIGHTS AND ORGAN WEIGHT TO BODY WEIGHT RATIO OF LIVER AND KIDNEY OF PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS (Sick rats omitted)

Pork 39A	Pork 39A	21428	181	8.5732	0.04736	--	--
		21638	168	6.6013	0.03929	--	--
		21550	187	7.6602	0.04096	--	--
		21427	180	6.1423	0.03412	--	--
		21477	194	7.2065	0.03716	--	--
		21605	186	7.7321	0.04257	--	--
		21921	178	5.9000	0.03315	--	--
		21840	190	6.7694	0.03563	--	--
		21932	188	5.3855	0.02965	1.3718	0.0073
		22108	190	7.4606	0.03927	--	--
Pork 39B	Steen-	21834	164	7.2044	0.03861	--	--
	bock V.	21754	180	6.8125	0.03974	--	--
		21854	173	6.3993	0.03694	1.4819	0.0086
		21860	173	6.4748	0.03761	--	--
		21885	180	6.3790	0.03601	--	--
		22026	186	6.5840	0.03811	--	--
		21866	198	6.3608	0.03611	1.3679	0.0069
Pork 39C	Steen-	21375	146	6.9642	0.04919	--	--
	bock V.	21526	214	7.2438	0.03671	--	--
		21877	187	6.2425	0.0321	1.3587	0.0073

TABLE 19. (CONT'D) WEIGHTS AND ORGAN WEIGHT TO BODY WEIGHT RATIO OF LIVER AND KIDNEY OF PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS (Sick rats omitted)

Pork 39C	Pork 39C	21419	167	6.7310	0.03869	--	--
		21466	161	6.2532	0.03971	--	--
		21623	176	5.7003	0.03611	--	--
		22145	186	7.4775	0.03713	1.5700	0.0084
Pork 31	Steen-bock V.	21514	200	6.9018	0.03451	--	--
		21585	118	3.7352	0.02223	--	--
		21462	160	6.2362	0.03898	--	--
		21546	187	6.5969	0.03528	1.7937	0.0096
		21567	176	7.0175	0.03987	--	--
		21587	178	5.9665	0.03352	--	--
		21839	163	6.6273	0.04066	--	--
		21902	166	7.1455	0.03900	--	--
		21619	182	7.0996	0.03900	--	--
Pork 31	Pork 31	21547	176	7.6060	0.04321	--	--
		21694	161	6.8175	0.04234	--	--
		21474	178	6.7172	0.03773	--	--
		21870	156	6.9997	0.04486	--	--
		21409	180	6.5773	0.03072	--	--
		21641	160	5.4217	0.03388	--	--
		21569	190	6.0633	0.03191	--	--
		21531	217	6.7767	0.03122	--	--
		22148	142	6.2832	0.03217	1.3606	0.0084

TABLE 20. WEIGHTS AND ORGAN WEIGHT TO BODY WEIGHT RATIO OF SPLEEN, HEART AND MAMMARY GLANDS FROM PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS

Diet of females	Rat number	Body weight[1] in grams	Weight of spleen in grams	Weight of spleen/ body weight	Weight of heart in grams	Weight of heart/ body weight	Weight of mammary glands in grams	Weight of mammary glands/ body weight
Steen-bock V.	22022	221	0.5715	0.0025	0.5947	0.0027	5.0112	0.0227
	21749	178	0.3756	0.0021	0.7230	0.0040	4.9371	0.0277
	21850	206	0.4791	0.0023	0.6971	0.0034	5.0304	0.0244
Pork I	22150	177	0.3222	0.0018	0.6791	0.0038	3.7981	0.0214
	21459	196	0.2978	0.0015	0.7432	0.0038	4.6197	0.0213
	21611	181	0.4161	0.0023	0.5142	0.0028	3.1241	0.0173
	21695	174	0.3761	0.0022	0.6147	0.0035	5.6717	0.0326
	21583	159	0.3971	0.0025	0.5791	0.0033	4.6211	0.0291
	21561	201	0.2781	0.0014	0.4961	0.0025	3.9167	0.0195
Pork 39A	21932	188	0.4271	0.0023	0.7143	0.0038	3.0268	0.0161
	22003	190	0.3399	0.0018	0.7651	0.0040	2.5258	0.0133
	21942	164	0.5214	0.0032	0.7621	0.0046	3.6129	0.0220
Pork 39B	21866	198	0.3199	0.0016	0.6738	0.0034	4.1762	0.0211
	21854	174	0.2945	0.0017	0.5851	0.0034	4.0279	0.0231

[1]Body weight at initiation of second pregnancy

TABLE 20. (CONT'D) WEIGHTS AND ORGAN WEIGHT TO BODY WEIGHT RATIO OF SPLEEN, HEART AND MAMMARY GLANDS FROM PREGNANT FEMALES FED VARIOUS EXPERIMENTAL DIETS

Pork 39C	21877	187	0.3792	0.0020	0.7641	0.0041	4.7995	0.0257
	22145	186	0.4203	0.0022	0.8621	0.0046	3.6794	0.0198
Pork 31	22148	142	0.3390	0.0024	0.7224	0.0050	3.2974	0.0232
	21546	187	0.3144	0.0017	0.7408	0.0040	3.7461	0.0200

TABLE 21. WEIGHTS AND ORGAN WEIGHT TO BODY WEIGHT RATIO OF LIVER AND KIDNEYS FROM RATS SUFFERING FROM PREGNANCY DISORDER

Rat number	Body weight in grams	Wt. of liver in grams	Wt. of liver/ body wt.	Wt. of kidneys in grams	Wt. of kidney/ body wt.
21937	135	5.8704	0.04348	1.7649	0.0131
21621	112	4.7628	0.04252	1.8761	0.0167
21876	155	4.7395	0.03057	--	--
21618	166	6.2779	0.03782	--	--
21901	141	6.2698	0.04447	--	--
22008	216	9.2821	0.04397	--	--
21928	141	7.5852	0.05479	--	--
21929	143	--	--	1.8071	0.0127
21940	116	5.6293	0.04852	1.8031	0.0155
21939	136	5.9921	0.04405	1.8341	0.0134
21843	164	8.5506	0.05213	1.7942	0.0194
21911	160	7.2916	0.04556	--	--
21740	175	8.5870	0.04906	1.9473	0.0105
21965	145	8.6563	0.05969	--	--
21881	186	--	--	1.9217	0.0103
21841	193	7.7717	0.04026	1.7432	0.0090
21706	168	8.1575	0.04855	1.7134	0.0101
21708	170	7.4493	0.04323	1.7891	0.0105
21741	171	6.8959	0.06157	--	--
21908	112	--	--	1.6932	0.0151

TABLE 22. WEIGHTS AND ORGAN WEIGHT TO BODY WEIGHT RATIOS OF SPLEEN, HEART AND MAMMARY GLAND FROM RATS SUFFERING FROM PREGNANCY DISORDER

Rat number	Body weight in grams	Wt. of spleen in grams	Wt. of spleen/ body wt.	Wt. of heart in grams	Wt. of heart/ body wt.	Wt. of mammary gland in grams	Wt. of mammary glands/ body wt.
21621	112	0.5201	0.0046	0.7632	0.0068	3.7162	0.0316
21937	135	0.4971	0.0031	0.8712	0.0065	4.9174	0.0219
21929	143	0.5421	0.0036	0.5641	0.0038	4.1762	0.0290
21881	186	0.4749	0.0025	0.7432	0.0047	4.3271	0.0232
21706	168	0.5341	0.0031	0.6741	0.0048	3.7914	0.0226
21740	175	0.6149	0.0035	0.6497	0.0049	3.8975	0.0222
21708	170	0.6211	0.0036	0.7921	0.0042	3.6129	0.0212
21841	193	0.5671	0.0029	0.6421	0.0036	4.7964	0.0248
21908	112	0.5369	0.0038	0.5324	0.0041	4.1012	0.0366
21940	116	0.5341	0.0046	0.7426	0.0045	4.1793	0.0360
21618	166	0.5124	0.0030	0.7431	0.0039	3.9271	0.0236
21901	141	0.5132	0.0032	0.7421	0.0047	4.6721	0.0331

TABLE 23. WEIGHTS AND ORGAN WEIGHT TO BODY WEIGHT RATIOS FOR CERTAIN ORGANS OF VIRGIN FEMALES FED THE VARIOUS EXPERIMENTAL DIETS

Diet of females	Rat number	Body wt. gm.	Wt. of liver[1] gm.	Wt. of liver/ body wt.	Wt. of kid-neys gm.	Wt. of kid-neys/ body wt.	Wt. of spleen gm.	Wt. of spleen/ body wt.	Wt. of heart gm.	Wt. of heart/ body wt.
Steen-bock V.	21479	190	5.7127	0.03006	1.4974	0.0078	0.4001	0.0021	0.6974	0.0038
	21575	170	4.7098	0.02770	1.4361	0.0084	0.3963	0.0023	0.6781	0.0040
	21609	192	5.9061	0.03013	1.4554	0.0075	0.3553	0.0018	0.7019	0.0036
	21677	184	----	-----	1.5013	0.0082	0.3421	0.0018	0.7416	0.0040
	21838	190	5.5811	0.02937	1.4933	0.0078	0.3642	0.0019	0.6794	0.0036
	21903	212	6.1625	0.02906	1.4793	0.0069	0.3941	0.0018	0.7315	0.0034
	22055	193	----	-----	1.4407	0.0074	0.3847	0.0020	0.6976	0.0036
	21469	174	4.8650	0.02795	----	----	----	----	----	----
	21555	176	4.6280	0.02629	----	----	----	----	----	----
Pork I	22059	185	5.1944	0.02808	1.4895	0.0080	0.3300	0.0018	0.6703	0.0036
	22057	205	5.5166	0.02501	1.4069	0.0069	0.3491	0.0017	0.4712	0.0023
	21917	188	4.8135	0.02560	1.5391	0.0082	0.2897	0.0015	0.6331	0.0034
	21573	178	5.6961	0.03200	----	----	----	----	----	----
	21433	181	4.9140	0.02715	----	----	----	----	----	----
	21633	200	6.1940	0.03097	----	----	----	----	----	----
	21784	184	6.3250	0.03438	----	----	----	----	----	----

[1]These data are included through the courtesy of Miss Ethelwyn Wilcox

TABLE 23. (CONT'D) WEIGHTS AND ORGAN WEIGHT TO BODY WEIGHT RATIOS FOR CERTAIN ORGANS OF VIRGIN FEMALES FED THE VARIOUS EXPERIMENTAL DIETS

Pork I (Cont'd)	21490	199	5.6322	0.02830	----	----	----	----	----	----
	21552	169	5.1239	0.03032	----	----	----	----	----	----
Pork 39A	21554	182	4.5494	0.02499	1.4576	0.0080	0.2974	0.0016	0.6892	0.0038
	21732	178	4.7590	0.02673	1.4963	0.0084	0.3312	0.0019	0.6594	0.0037
	21919	192	4.8396	0.02520	1.3423	0.0070	0.3487	0.0018	0.6730	0.0035
	21954	172	4.5672	0.02655	1.5671	0.0091	0.4132	0.0024	0.5794	0.0034
	22064	180	4.8968	0.02720	1.3661	0.0090	0.3132	0.0017	0.6865	0.0034
	22065	178	4.8091	0.02701	1.6142	0.0077	0.3121	0.0018	0.6174	0.0038
	21499	168	4.7233	0.02811	----	----	----	----	----	----
	21436	213	7.3917	0.03740	----	----	----	----	----	----
	21572	182	5.1164	0.02811	----	----	----	----	----	----
	21495	182	5.2748	0.02898	----	----	----	----	----	----
	21647	188	4.1983	0.02233	----	----	----	----	----	----
Pork 39B	21781	188	5.4723	0.02961	1.7983	0.0096	0.4016	0.0021	0.6791	0.0036
	21787	218	5.8526	0.02619	1.6416	0.0075	0.3162	0.0014	0.6431	0.0029
	21862	194	5.1308	0.02864	1.7967	0.0092	0.3981	0.0021	0.7016	0.0036
	21869	220	6.1794	0.02794	1.7819	0.0066	0.1945	0.0009	0.3851	0.0017
	21907	202	5.5337	0.02979	1.7620	0.0087	0.3976	0.0019	0.6872	0.0034
	21923	196	4.8653	0.02769	1.4781	0.0074	0.2961	0.0014	0.6961	0.0031
	21797	222	6.2622	0.02641	1.4442	0.0066	0.2818	0.0013	0.6161	0.0031
	22083	207	5.9638	0.02469	1.8174	0.0087	0.3963	0.0019	0.6673	0.0032
	21835	178	6.4904	0.03179						

TABLE 23. (CONT'D) WEIGHTS AND ORGAN WEIGHT TO BODY WEIGHT RATIOS FOR CERTAIN ORGANS OF VIRGIN FEMALES FED THE VARIOUS EXPERIMENTAL DIETS

Pork 39C	21502	166	----	----	1.5672	0.0094	0.3821	0.0023	0.7841	0.0047
	21651	166	4.5877	0.02811	1.3721	0.0083	0.3741	0.0022	0.7943	0.0048
	21956	178	----	----	1.4761	0.0083	0.3793	0.0021	0.7642	0.0043
Pork 31	21429	201	5.5282	0.02694	1.7062	0.0084	0.3469	0.0017	0.6999	0.0035
	21431	180	5.0586	0.02931	1.5975	0.0088	0.3761	0.0021	0.7321	0.0041
	21556	214	5.8421	0.02729	1.5961	0.0074	0.3161	0.0015	0.6794	0.0032
	21570	198	5.3708	0.02712	1.6245	0.0082	0.2998	0.0015	0.7410	0.0037
	21637	164	4.4945	0.02740	1.7641	0.0087	0.3641	0.0022	0.7461	0.0046
	21643	196	6.0922	0.03102	1.6961	0.0086	0.2991	0.0015	0.7651	0.0039
	21793	194	6.0038	0.03094	1.7063	0.0088	0.3014	0.0016	0.7642	0.0039
	21980	190	5.6328	0.02964	1.6979	0.0089	0.3164	0.0017	0.7421	0.0039
	21493	177	5.0586	0.02857						

1. Section from spleen of normal pregnant control rat (21504) fed Steenbock V diet. X 160.

2. Section from spleen of pregnant rat (21747) fed Pork 7 diet. Three cells show fatty degeneration. X 160.

3. Section from spleen of pregnant rat (21458) fed Pork I diet. X 160.

4. Section from spleen of pregnant rat (21756) fed Pork 39A diet. X 160.

5. Section from spleen of pregnant rat (21795) fed Pork 39B diet. X 160.

6. Section from spleen of pregnant rat (21421) fed Pork 39C diet. X 160.

7. Section of spleen from pregnant rat (21473) fed Pork 31 diet. X 160.

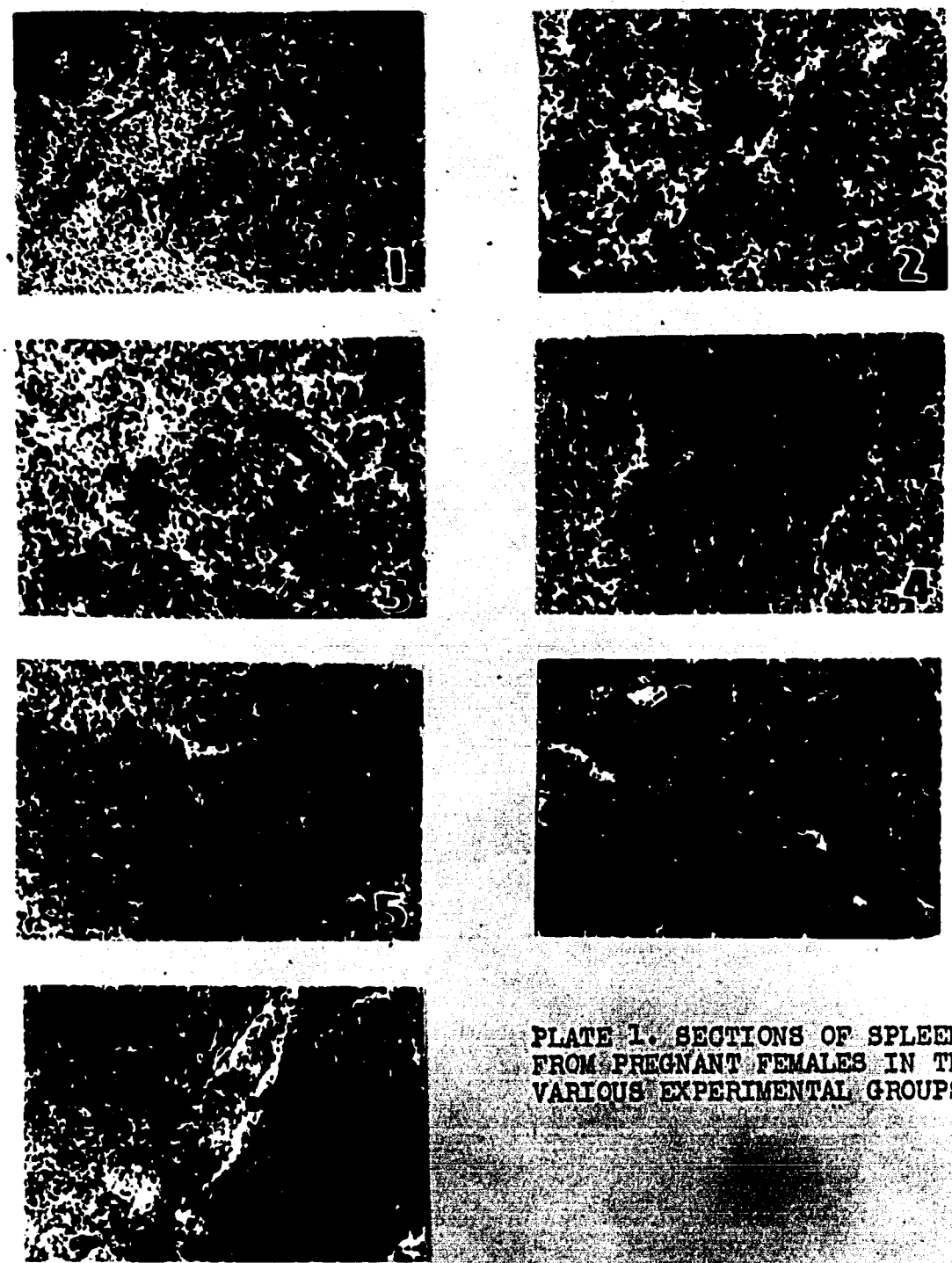

PLATE I. SECTIONS OF SPLEEN FROM PREGNANT FEMALES IN THE VARIOUS EXPERIMENTAL GROUPS

1. Section from spleen of normal control rat (21504) fed Steenbock V diet.

2. Section from spleen of sick rat (21708) fed Pork I diet.

3. Section from spleen of sick rat (21937) fed Pork 39B diet.

4. Section from spleen of sick rat (21618) fed Pork 31 diet.

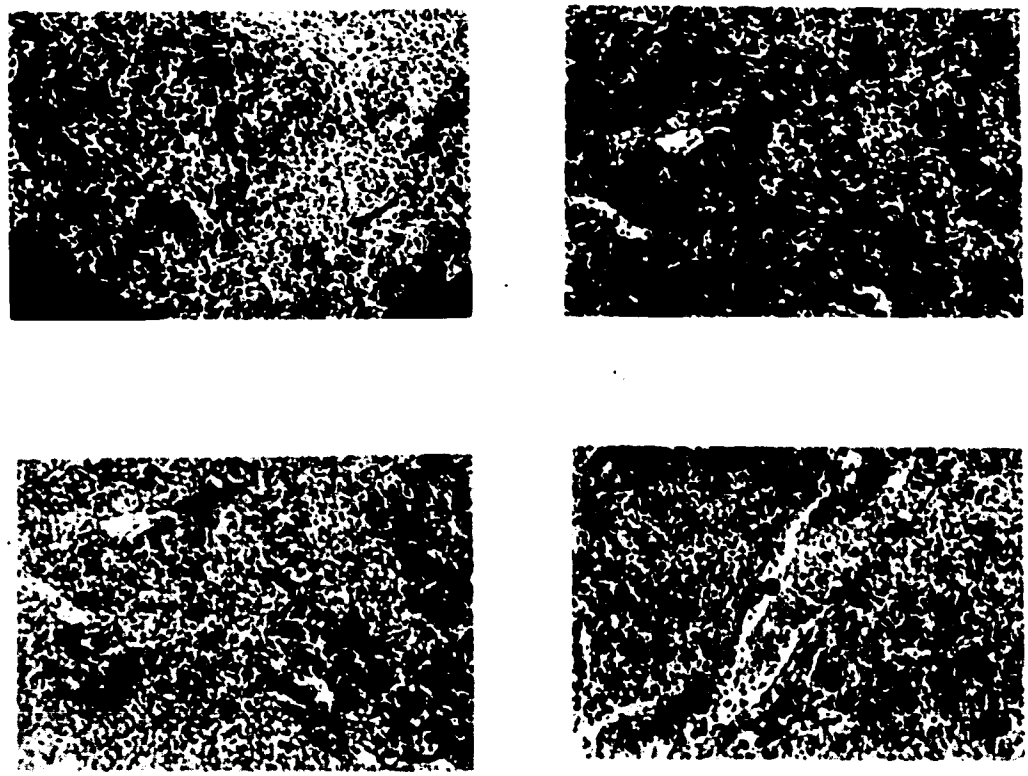

PLATE 2. SECTIONS OF SPLEEN FROM A PREGNANT CONTROL FEMALE AND THREE SICK RATS

1. Section from pancreas of normal pregnant control rat (21504) fed Steenbock V diet. X 160.

2. Section from pancreas of pregnant rat (21747) fed Pork 7 diet. Three cells show fatty degeneration. X 160.

3. Section from pancreas of pregnant rat (21458) fed Pork I diet. X 160.

4. Section from pancreas of pregnant rat (21756) fed Pork 39A diet. X 160.

5. Section from pancreas of pregnant rat (21795) fed Pork 39B diet. X 160.

6. Section from pancreas of pregnant rat (21421) fed Pork 39C diet. X 160.

7. Section of pancreas from pregnant rat (21473) fed Pork 31 diet. X 160.

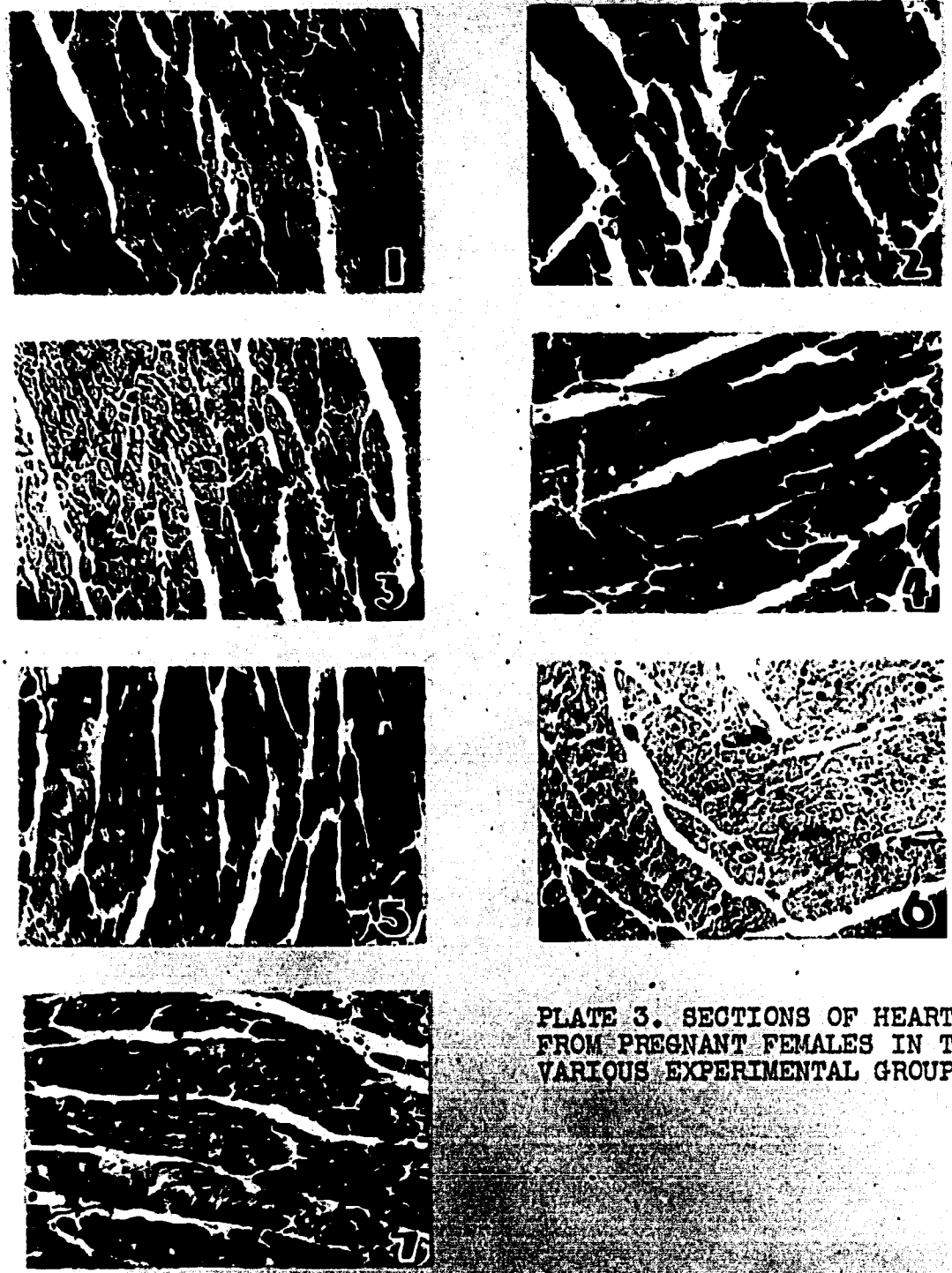

PLATE 3. SECTIONS OF HEART FROM PREGNANT FEMALES IN THE VARIOUS EXPERIMENTAL GROUPS

1. Section from pancreas of normal control rat (21504) fed Steenbock V diet.

2. Section from pancreas of sick rat (21708) fed Pork I diet.

3. Section from pancreas of sick rat (21937) fed Pork 39B diet.

4. Section from pancreas of sick rat (21618) fed Pork 31 diet.

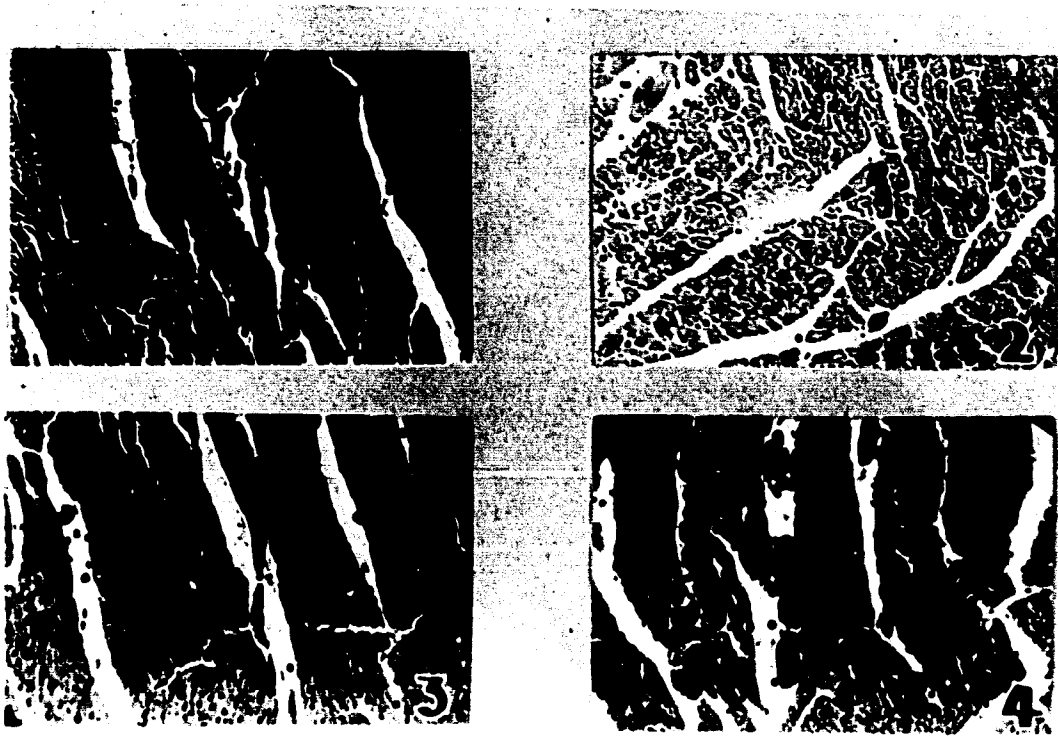

PLATE 4. SECTIONS OF HEART FROM A PREGNANT CONTROL FEMALE AND THREE SICK RATS