1946

Meat in nutrition: XXVII. Concentration of urea nitrogen, calcium and plasma protein in the blood of pregnant rats fed a diet containing partially dried, autoclaved pork muscle

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NOTE TO USERS

MEAT IN NUTRITION. XXVII. CONCENTRATION OF UREA NITROGEN, CALCIUM, AND PLASMA PROTEIN IN THE BLOOD OF PREGNANT RATS FED A DIET CONTAINING PARTIALLY DRIED, AUTOCLAVED PORK MUSCLE

by

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A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Nutrition

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Iowa State College
1946

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INTRODUCTION AND REVIEW OF LITERATURE

BACKGROUND OF PROBLEM

Meat occupies an important place in the dietaries of the people of our nation. Per capita consumption, as quoted in Agricultural Statistics 1943, was 133.6 lb. in 1939; 142.0 lb. in 1940. Records show that meat is widely used in Iowa. Following a survey of the food consumption habits of 145 farm families, Nelson, Hoyt, McLaughlin, and Morgan in 1935 reported that the average consumption of meat per adult male unit per year was 173 lb., certain families consuming as much as 500 lb. per man per year. Because meat represented such an important commodity in the state, nutritionists in the Foods and Nutrition Section of the Iowa Agricultural Experiment Station undertook a study in 1928 of the nutritional effectiveness of meat diets in a controlled experimental situation. Very little was known then of the effects on the animal organism of continued maintenance on a diet in which meat furnished the main source of protein. The preliminary experiments reported by Nelson and her co-workers (1930) demonstrated the inadequacy of such diets when fed to the albino rat. They showed that the needs of the animal organism for the

maintenance of a good nutritional state were very complex indeed, and that much still remained to be learned regarding a diet that would support normal reproduction and lactation. In view of these considerations, workers in the laboratory decided to repeat and enlarge the preliminary experiment. The efficacy of two kinds of meat, i.e., pork and beef, as the main source of protein in the ration was tested. Each kind, autoclaved and dried to one-half its original weight, was incorporated into an otherwise synthetic ration, yeast furnishing the vitamins of the B-complex. The experiment was set up to study the nutritional history of rats maintained on the various diets. Plans were made to observe six successive generations of animals throughout their entire life cycles.

At the time the rations were formulated, they were believed to be adequate from the nutritional standpoint. An adequate diet must provide for the growth and maintenance of body tissue, support normal reproduction and lactation, protect the animal from infection, prevent premature aging, allow for a normal span of life, and permit the propagation of the species from generation to generation. Judged by these criteria, neither the beef nor the pork diets when formulated to contain 15 per cent protein were adequate, as complete reproductive failure resulted.

When the same diets were modified so as to contain 30 per cent of protein, the ration containing beef supported the experimental animals through six generations with a relatively small number of reproductive failures, but the pork ration did not. Each original lot of test animals was composed of 15 rats. The 15 animals in the pork series produced 528 progeny, those in the beef-fed group, 3,652. These results suggested that pork might be deficient in a factor (or factors) essential for normal reproduction and lactation in the rat and that beef contained this factor (or factors) in a limited amount (Swanson, Armstrong, and Nelson, 1943).

EFFECTS OF FEEDING THE PORK-CONTAINING DIET

The general character of the response of the experimental animal to the test diet was so interesting that a series of more detailed experiments were initiated. Many of the studies were carried out by graduate students in the section, the present investigation being the most recent in the sequence. As a result of this continuous effort a surprisingly large amount of interesting data have been accumulated. To give background to the study herein reported, a brief resumé of the findings to date seems important.

In one of the earliest of these studies, the effect of feeding to the albino rat the diet containing pork at a level equivalent to 15 per cent of dietary protein, was studied carefully throughout the three generations that the species survived. There was an accelerating effect on sexual development, indicated by the early age at which the vaginal orifice opened and at which the first oestrus cycle was established. The oestrus cycles were of normal length during the early months of life, but later became abnormally long. A more or less permanent cornification of the epithelial cells of the vaginal mucosa not traceable to a deficiency of vitamin A, was characteristic. Cessation of sexual activity occurred early in life. Fewer and smaller litters were produced by rats fed the pork diet than by animals in the control group, with 55 per cent of the young born alive dying within one week after birth. Besides being smaller in size, the young rats appeared very red in color and the skin was often rough and scaly. Dark spots, resembling bruises, occurred quite frequently on the head and limbs. Investigations showed that the spots were clots of blood, indicating subcutaneous hemorrhage (Pressley, 1941). Those young that survived the first week of life were usually reared, but their weight records indicated that they were decidely

undernourished during the lactation period. Of great interest was the fact that one-third of the maternal animals fed the pork ration died at parturition of a disease that had many of the characteristics of toxemia of pregnancy and eclampsia in the human being (Dyar, 1935).

Gross observations at necropsy, histological studies, and biochemical analyses of certain tissues of the pork-fed animals on the day of parturition proved most interesting especially when compared with the picture characterizing control animals. For instance, the livers of the animals given the pork diet were pale, friable, and of a yellowish color indicating a deposition of fat globules in the tissue. King (1936) verified the presence of a large proportion of fat by staining the tissue. Chemical analyses of the liver showed a considerable increase in the quantity of total liver lipids present in the organ of the virgin pork-fed rat over that in the organ of a similar control animal (Wilcox, 1942). The increase in fatty constituents was due largely to an increase in the neutral fat fraction. Pregnancy <u>per se</u> had little effect on the fat content of the liver of the control animal, but it definitely augmented the disturbances in fat metabolism brought about by the ingestion of the pork-containing diet. As fat was deposited in the liver, there was a significant

loss in its moisture content (Wilcox, 1942; Weedman, 1942). Hepatic damage was indicated by the finding that with a reduction in the proportion of free cholesterol in the liver fat of the gravid pork-fed rat, a simultaneous increase in cholesterol ester occurred (Wilcox, 1942).

Histological studies by Armstrong (1939) showed a cloudy swelling of the hepatic cells in both virgin and pregnant pork-fed rats, and although pregnancy *per se* produced moderate swelling of the cells the abnormality was more severe in the gravid pork-fed rat than in the normal pregnant female. Livers extirpated from virgins fed the pork-containing diet contained a significantly higher percentage of glycogen than those from control animals, but interestingly, gravid pork-fed animals showed considerably lower than normal liver glycogen values (Farrankop, 1941).

The kidney was larger than normal in rats maintained on the pork-containing diet (Armstrong and Swanson, 1943). In a series of histological studies, Armstrong (1939) showed that the renal epithelium was mildly damaged in all pork-fed animals. There was stratification of the cytoplasm with condensation at the periphery of the cells and hyaline degeneration.

Feeding the pork-containing diet to pregnant rats

did not seem to affect the concentration of sugar in the blood (Ho, 1941). Later work by Anderson (1943) and Caminong (1945) indicated, however, that the glucose tolerance curve of the pregnant rat fed the diet containing pork was diabetic in character.

Total fatty acids, phospholipids, and total cholesterol in the blood were reduced to one-half the normal concentration in a study reported by Wilcox (1942). She seems to believe that the low lipid values of the blood are related to the deposition of abnormally high concentrations of fat in the liver.

An elevation in non-protein nitrogen content of the blood, above that expected in normal pregnancy was also noted (Barnhart, 1942).

TOXEMIC PREGNANCY IN THE RAT

Perhaps one of the most interesting manifestations of feeding the pork-containing diet was the unusual syndrome that developed in some of the animals in either the first or second gestation on the day of parturition. Many of the symptoms observed were similar to those of "toxemia of pregnancy" in other animals, i.e., guinea pigs, rabbits, sheep, and human beings.

The disease was first described by Dyar in 1935 and

later, in detail, by Armstrong and Swanson (1943). To all appearances the animals developing the disorder were normal until the twenty-first or twenty-second day of gestation. Then a sudden and striking disturbance appeared, which invariably led to the death of the animal. Often when the animals were observed they appeared perfectly normal, but were found dead one-half hour later. One of the first symptoms observed as the disease developed was a loss in muscle tone, which made the animal feel soft to the touch. The feet and ears became pale and cold, and the hair stood erect. Hematuria and vaginal hemorrhage occurred frequently. Dyspnea was evident and the skin and mucous membranes became increasingly cyanotic as the disorder became more acute. Sometimes a frothy discharge came from both the mouth and nose. Shivering, gnashing of the teeth, and convulsions often preceded death. In some instances, the abnormality took another form. If this occurred, labor was long and protracted with the animal dying during the birth of the young. Usually, however, death occurred before any young were born, but when birth did occur the young were born dead.

A careful study of the gains in weight of the animals during gestation revealed an abnormally sharp increase in their average weight near the eighteenth day of pregnancy.

Water consumption, prior to this time, had been greater than normal reaching its peak also at the eighteenth or nineteenth day. Water intake fell abruptly during the last two days of gestation. Measurement of urinary output (Lefebvre, 1941) revealed that excretion of urine was definitely diminished in the toxic condition.

Necropsy studies of the toxic animals sacrificed just prior to death showed abnormalities of the liver. They were swollen, friable, and extremely yellow in color. The kidneys were enlarged and congested with blood. Often the pleural and abdominal cavities contained considerable fluid. The blood seemed to be concentrated in the visceral vessels, and congestion of blood in the uterine vessels was especially characteristic. Often blood was found free in the uterus, the hemorrhage apparently originating at the base of the placentae. Frequently the placenta seemed very loosely attached to the uterine wall. Sometimes the feti died at different stages of development; in other animals, the feti were fully developed but were dead and very anemic in appearance. The blood itself was very pale in color, watery, and slow to clot. The prothrombin time in a series of seven sick animals ranged from 25.6 to 300+ seconds. Only one animal of the group showed the normal prothrombin time of 25-26 seconds; prothrombin times of

all of the others were considerably longer, the blood from one animal failing to clot even after five minutes (Pressley, 1941).

Histological studies of hepatic tissue from the toxic animals showed a marked fatty degeneration accompanied by varying degrees of fatty infiltration (Armstrong, 1939). The regions of focal necrosis were so prevalent that the characteristic hepatic structure nearly disappeared. The kidneys of the sick animals showed extensive hemorrhage. The glomeruli were normal but the tubular epithelium was almost completely destroyed. No signs of infection were observed. More extensive renal damage was evident in those animals which had been sick several hours before death than in those in which death had occurred very suddenly. Pancreatic tissue was often edematous, in which case the acini were contracted and deeply stained. The connective tissue supporting the acini was destroyed. Blood in the placenta, the umbilical vein, the liver, and the heart of the fetus showed varying degrees of hemolysis, and the umbilical veins contained thrombi so large that they nearly blocked the vessels.

The adrenal glands were hemorrhagic, congested, and necrotic with occasional evidence of fat disturbance, autolysis and hyalinization (Molsberry, 1943). The amount

of damage to the tissues seemed to depend upon the duration and severity of the toxemia.

Biochemical studies of the tissues of these animals revealed additional evidence concerning the abnormalities present in the toxic condition. Analyses of the maternal livers for glycogen (Farrankop, 1941) showed that these organs were completely depleted of this constituent, and that fetal livers contained very low amounts. While the glucose tolerance curves of both toxic and non-toxic animals were diabetic in character, utilization of intraperitoneally injected glucose was considerably slower in the sick rats than in the pork-fed animals not developing the symptoms (Anderson, 1943; Caminong, 1945). The concentration of non-protein nitrogen in the blood of the toxic rats was considerably greater than that found in the non-toxic group (Barnhart, 1942). The faulty fat metabolism, however, as indicated by the analyses of the lipids in the liver and in the blood, was not further deranged in the toxic condition. As might be expected from the gross observations, increased water consumption and diminished urinary output, analyses showed a definite hydration of certain body tissues (Weedman, 1942).

TOXEMIC PREGNANCY IN OTHER ANIMALS

The analogue of the syndrome observed in rats fed a diet containing pork as the main source of protein has been observed in other animals.

The Guinea Pig

Smith in 1913 briefly described a syndrome occurring in female guinea pigs which led to death just before the birth of the young or shortly after parturition. Frequently the difficulty was associated with pneumonia. The animals became suddenly quiet, the hair was ruffled and death occurred within one or two days. The condition was rarely observed in early pregnancies. Post mortem examinations revealed extreme fatty degeneration of the liver. This organ was exceedingly friable, a uniform grayish yellow color, and in some instances almost white. Microscopic examination of the tissue revealed a mass of fat globules. Lung tissue also showed extreme fat infiltration of the alveolar cells. The other constant finding was an empty stomach. Smith believed that this disease was a "cold weather phenomenon" as it occurred most frequently in the winter months.

Later, Foley (1942) gave a complete account of a similar syndrome in the pregnant guinea pig. In contrast to

Smith's finding, the disease appeared in the summer months. Symptoms usually appeared two or three days before death occurred. In some cases, however, the onset was sudden and the animals died within 24 hours after the first appearance of signs of the condition. A ruffled coat and loss of appetite were the first symptoms observed. The animals became increasingly lethargic, finally prostrate, and dyspnea was evident. The eyes were dull and a yellowish crusty mass formed along the eyelids. None of the animals showing the typical symptoms recovered. There was a higher incidence of cases among dams pregnant for the first or second time than in those which had passed through these pregnancies successfully.

At necropsy, rich accumulations of fat were observed in all the depots of the body, particularly in the abdominal cavity. The liver was yellow in color, somewhat swollen, friable, and soapy to the touch. In nearly all cases, the contents of the gall bladder were granular and bright yellow. It was interesting that the right auricle of the heart was often filled with a milky fluid, which, microscopic examination showed, contained many fat globules. The stomach was usually empty. Feti <u>in utero</u> in dams that died before parturition appeared to have been dead before the death of the dam occurred and congestion in the walls

of the uteri was evident.

The general finding in histological examinations of liver tissue was widespread fatty infiltration and degeneration, but occasionally areas of necrosis and/or hemorrhage were observed. Although changes in kidney tissue were not common, marked fatty degeneration of the tubular structures and cortical necrosis were observed in some instances.

The Rabbit

Other investigators have observed a similar syndrome in rabbits. As reported by Greene (1937), the disease occurred not only in pregnancy but also post partum and in pseudopregnant females. The symptoms and clinical course of the disorder in the rabbit were variable, but usually one of three general conditions prevailed: 1) a typical acute toxic condition, which usually terminated in death within a few hours, 2) a less acute disturbance, which existed for a week or more and was followed by recovery, and 3) an asymptomatic disorder, which in a few instances led to sudden death.

Typical acute symptoms in some instances were preceded by signs of malaise for three or four hours. Predominating signs of the attack were dyspnea, and the odor

of acetone in the breath. In some animals extreme thirst was noted, in others, however, water intake was diminished. In all cases, complete anuria was the rule. The rabbits suffered a loss of normal vigor and activity, and sat hunched in a corner of the cage with roughened coats and dull lusterless eyes. The ears were cold and flow of blood in the marginal veins could be stopped with slight pressure. In some cases convulsions occurred. Other animals remained lethargic and in a comatose stage with relaxed sphincters, dilated pupils and widespread muscular asthenia. Terminal manifestations often were cyanosis and marked respiratory distress with the appearance of a discharge from the nostrils. The period during which obvious toxic symptoms were observed was generally only a few hours in duration. Often the condition of the female changed from apparent excellent health to complete prostration in one-half hour. Scarcely ever were the symptoms prolonged for more than one day. Necropsy findings, however, indicated that the disease was well advanced before clinical signs were apparent.

In non-fatal cases, the syndrome was mild and not usually specific. The illness persisted for a week or more with loss of appetite and some loss of weight due to muscular wasting, diminished fluid intake and decrease in

urinary output. Occasionally, rapid respiration, dilated pupils, or a slight cyanotic tinge about the lips and nares were observed. Recovery was gradual and left no external signs of disability.

The first indication of the fatal asymptomatic disorder was death of the animal, while in the midst of nest building or other physiological activity. There were no outward signs of the condition of the animal.

Necropsy studies of all animals that died of the disorder showed increases in fat in the normal depots. Even in those animals in which loss of appetite had been a pronounced symptom, there was no appreciable diminution in the fat stores.

The liver was uniformly yellow in color or yellow with irregular red blotches. All lobular differentiation was lost and surface markings were absent. The cut surface of the liver was greasy to the touch and the tissue had a pulpy consistency. Occasionally, the walls of the gall bladder were thickened and its contents granular.

In many cases abnormalities in the kidneys were not observed, in others they were very striking. The most common alterations were depressed scarred areas, increase in size and paleness in appearance. The capsule was stretched and easily stripped showing a bulging cortex of

a yellow color with pink blotches. The urinary bladder was almost always empty.

Pleural and pericardial cavities contained an abnormal amount of fluid, which in many instances was blood stained and gelatinous in consistency. Congestion and edema were commonly found in the lungs.

The adrenals were small, pale yellow in appearance, and extremely soft in consistency.

The hypophysis was always greatly enlarged, and in several instances the posterior half of the gland was replaced by a large cyst containing a colorless fluid. Outside of being wet and edematous, the brain showed no abnormality.

A white milky fluid containing a large amount of fat filled the right auricle and the vena cavae. The heart muscle was pale and flabby.

The feti were usually dead and frequently showed liver changes comparable to those found in the mother. The ovaries of all rabbits dying with toxic symptoms contained large corpora lutea.

In some instances there was extensive necrosis of the pancreas and neighboring adipose tissue. Occasionally, petechial hemorrhages were found on the surface of the large intestine.

Microscopic examination revealed that in the majority of cases liver alteration consisted of widespread fatty infiltration and degeneration. In those animals in which fatty changes were not marked, focal areas of necrosis represented the predominating lesion. Kidney abnormalities were generally degenerative in character and varied from fatty tubular changes to complete necrosis.

The adrenals showed very wide fascicular zones, which often occupied the entire width of the cortex. Considerable fatty degeneration with necrosis appeared at the inner boundary of the cortex.

In contrast to the degenerative character of the lesions in other organs the hypophysis showed the presence of "definite productive changes".

On the basis of chemical analysis of the blood, rabbits displaying the acute toxic form of the disease could be divided into three groups: 1) those animals showing a marked hypoglycemia, 2) those showing a hyperglycemia, and 3) those having approximately normal values. The concentration of non-protein nitrogen, urea nitrogen, and creatinine was greatest in the hyperglycemic group, although these values were markedly elevated in all groups.

The quantity of calcium in the serum was below normal, that of inorganic phosphate high. In general, the

amounts of fat and of cholesterol in the blood were greater than normal. The concentration of serum protein varied; in several instances it was somewhat high, in others low.

Studies of apparently normal animals which later died with toxic symptoms, brought out the interesting fact that a disordered metabolism was reflected in the chemical blood picture only for a short period before an individual exhibited any signs of the condition, also that the degree of variation from normal gave no positive indication of the severity of the developing disorder or of the proximity of death.

Later (1938), Greene reported that although the disease might occur in resting animals or in those that had given birth to young, the greatest percentage of the cases developed during the last week of gestation. Incidence of the disease was greatest during the late winter and early spring. Greene also brought out that hereditary factors related to race and certain constitutional variations might be associated with increased susceptibility to the disorder.

The Sheep

The ewe is another animal affected with pregnancy disease. It occurs largely in the late winter or early

spring shortly before time of lambing. Many investigators have published reports on the nature of the disorder. Although the condition has been given many different names, "pregnancy disease" seems to be the most favored.

A striking similarity may be noted in the symptoms described by various observers (M'Fadyean, 1924; Dimock, Healy, and Bullard, 1928; Dill, 1932; Roderick and Harshfield, 1932; Dimock, Healy, and Hull, 1934; Elder and Uren, 1935; Bergman, 1935). Multiple pregnancies appeared to be a predisposing factor (M'Faydean, 1924; Dill, 1932; Mills, 1932; Roderick and Harshfield, 1932; Elder and Uren, 1935; Bergman, 1935). Bergman (1935) reported that in 53 cases of pregnancy disease, 43 ewes carried twin lambs, five had triplets, and only five had single lambs. When the condition occurred in animals carrying single lambs, the young were quite large in size and well-developed (Mills, 1932; Elder and Uren, 1935). If ewes aborted during the early stages of the disease or before the ewe became too weakened, rapid recovery resulted (Dimock, Healy, and Bullard, 1928; Mills, 1932; Dill, 1932; Elder and Uren, 1935; Roderick, Harshfield, and Hawn, 1937). Pregnancy disease did not occur after parturition (Elder and Uren, 1935).

In the field, the first noticeable evidence of the disorder was the observation that the ewe lagged behind

the rest of the flock. She was unable to climb out of a ditch or walk up a steep hill. A stiffness in the hind quarters and an unsteady gait were apparent. If enclosed, the ewe stood apart and did not eat. Although the animal finally suffered a complete loss of appetite, she was apparently very thirsty, drinking water frequently even after she was unable to rise (Dimock, Healy, and Bullard, 1928; Roderick and Harshfield, 1932; Elder and Uren, 1935).

As the disease progressed there was evidence of nervousness, irritability, and grinding of the teeth. The ewe showed a tendency to walk in circles or to stand with the head pressed against some object in the pen. An affected animal was dull, paid little attention to people, and took little voluntary exercise. The eyesight was affected, the ewe eventually becoming blind (M'Fadyean, 1924; Dimock, Healy, and Bullard, 1928; Dill, 1932; Mills, 1932; Dimock, Healy, and Hull, 1934; Elder and Uren, 1935; Bergman, 1935).

After the ewe went down, she could not rise without assistance. The head was often turned around to the side of the body. As the disorder progressed, the animal became prostrate and the head was protruded and drawn back. Frothy discharge from both the mouth and nares was noted. Upon close examination, acetone breath was evident. The

respiratory movements were accelerated, but later, as Dimock, Healy, and Hull (1934) observed, breathing became shallow.

Still later the animal passed into almost complete coma and had convulsions or spasms at irregular intervals, especially when disturbed. Finally death occurred. The course of the disease was from one to several days (Dimock, Healy, and Hull, 1934; Elder and Uren, 1935; Bergman, 1935; Clark and Groenewald, 1941).

Post mortem examination of the liver, showed that it was uniformly enlarged, soft, friable, and mottled, with yellowish or grayish irregular-shaped areas. In general, the liver had a pale bloodless color and a cooked appearance (Dimock, Healy, and Bullard, 1928; Roderick and Harshfield, 1932; Dill, 1932; Dimock, Healy, and Hull, 1934; Elder and Uren, 1935). When studied microscopically, varying degrees of degeneration with marked fatty infiltration were noted (Roderick and Harshfield, 1932; Bergman, 1935; Elder and Uren, 1935; Clark and Groenewald, 1941). M'Fadyean (1924) and Elder and Uren (1935) observed that some fatty infiltration occurred in the livers of normal pregnant ewes, but that pregnancy disease aggravated the condition.

Dimock and associates (1928, 1934) found the kidneys

were "enlarged", "swollen", soft, dark in appearance and gorged with blood. Edema was noted in the kidney by Bergman (1935) and when the capsule was cut the renal tissue protruded (Elder and Uren, 1935). Abnormal changes in the glomeruli occurred only occasionally, but complete disintegration of the cytoplasm of the cells lining the tubules was found in practically every case (Dimock, Healy, and Hull, 1934).

In most cases the digestive tract was empty or nearly so (Roderick and Harshfield, 1932; Elder and Uren, 1935). All other organs appeared normal (M'Fadyean, 1924; Dill, 1932; Roderick and Harshfield, 1932; Mills, 1932; Elder and Uren, 1935).

If sick ewes were killed in advanced stages of the disease and necropsied, the feti usually were found alive (M'Fadyean, 1924; Dimock, Healy, and Bullard, 1928; Roderick and Harshfield, 1932; Elder and Uren, 1935). Embryonic development seemed normal. Analyses of maternal hepatic tissue showed an excessive deposition of fat and a very low concentration of glycogen. Study of the fetal liver revealed that the glycogen reserves of the fetus were just as depleted as those of the maternal animal, but that fatty infiltration had not taken place (Roderick, Harshfield, and Hawn, 1937; Underwood, Robinson, and

Conochie, 1943).

Studies of the blood of ewes with pregnancy disease proved that acidosis was present (Dimock, Healy, and Bullard, 1928). Hypoglycemia was generally characteristic (Roderick and Harshfield, 1932; Roderick, Harshfield, and Hawn, 1937; Clark and Groenewald, 1941). In the main, the concentration of phosphorus in the blood was above normal. Blood calcium however, was often low, although some maximal and normal values were also noted (Dimock, Healy, and Hull, 1934). Later Underwood, Robinson, and Conochie (1943) reported that no significant disturbance of calcium metabolism occurred in pregnancy toxemia, which supported the findings of Roderick and Harshfield (1932) and other investigators. Non-protein nitrogen values gave very little evidence of retention of nitrogenous metabolites (Roderick and Harshfield, 1932).

The Human Being

When related to the human being the term, toxemia of pregnancy, has been applied to a group of disorders, which have been observed in pregnant women. Classification of the various disorders has been exceedingly difficult for totally different pathological conditions may be accompanied by identical clinical signs. Various attempts have

been made to differentiate the conditions observed. The classification accepted by the American Committee on Maternal Health is as follows: 1) hypertensive disease, 2) renal disease, 3) preeclampsia, mild and severe, 4) eclampsia, 5) vomiting of pregnancy, and 6) unclassified (Williams, 1941). Although many of the diseases in this list are often recognized as separate entities, a reading of the literature indicates that some investigators are inclined to believe that many of the disorders are really forerunners of the severe condition, eclampsia.

Usually the changes characteristic of toxemia develop during the last trimester of pregnancy and rapidly disappear after delivery. In some instances, however, they appear soon after delivery (Dexter and Weiss, 1941). The incidence of toxemia seems to have a relationship to geographical area (McIlroy, 1936). Reports show that eclampsia occurs most often, in the United States, in those areas where nutritional deficiency diseases are common. Seasonal variation in frequency of the disorder may be due to nutritional influences as prices of fresh foods are highest during the winter months and it is at that time that malnutrition and incidence of eclampsia are said to increase (Ross, Perlzweig, Taylor, McBryde, Yates, and Kondritzer, 1938; Siddall and Oberlin, 1940).

Many different conditions have been reported to be predisposing factors of toxemia. Ross, et al. (1938) state, "We have been struck with the number of patients with eclampsia who have been in a very poor state of nutrition." It is most interesting that the animal protein in the diet of the patients studied by these investigators consisted chiefly of pork. The importance of a good nutritive state as a preventive measure is emphasized by McIlroy (1936) and by Kumer and Nath (1940). Reports in the literature seem to agree that toxemia occurs much more frequently in primigravida than in multigravida, and that multiparity is an important predisposing factor. Colvin and Bartholomew (1939), however, believe that more emphasis should be placed upon youth rather than multiparity as a predisposing factor in toxemia of pregnancy. Prepregnant hypertension and generalized edema, which precedes or is concomitant with the onset of toxemia may also be important conditions contributing to toxemia (Dexter and Weiss, 1941).

The present author believes that the experimental condition, as it is produced in the white rat, is analogous to the syndrome that may appear in gravid women. For this reason, the general symptoms and abnormal changes which occur just preceding and during human eclampsia, as reported by different investigators, have been summarized for purposes of comparison and study in table 1.

Table 1. Typical clinical symptoms of eclampsia in human beings

Physiological changes	Characteristic changes observed	References*
General		
Body weight	rapid increase, greater than normal	1,8,9,16,17,28,30
Edema	ankles and legs affected, later eyelids, trunk, and then lungs and brain	1,2,3,4,6,8,9,10,11,13,15, 16,19,20,26,27,28,29,30
Arterial pressure	considerably elevated	1,2,3,4,5,6,8,9,10,15,17, 19,20,26,27,28,29,30,31
Basal metabolic rate	high	1,3,25,29
Symptoms referable to nervous system	nausea and vomiting	1,3,6,15,19,20,27
	headache	1,2,3,4,8,15,17,19,20,27, 28,29
	visual disturbances	1,2,4,8,15,17,19,27,28,29
	dizziness	1,4,8,15,17,19,27,28
	epigastric pain	1,17,27,28
	lassitude	1,2,15,17,19,27
	coma	1,2,19,20,27,28
	convulsions	1,2,4,9,10,11,15,19,20,27, 28
Urinary constituents		
Quantity of urine	volume decreased	1,2,3,15,20,29,30
Specific gravity	normal	19
	high	1,2,28
Red blood cells and bacteria	absent in preconvulsive stage	1,28
Albumin and casts	present	1,2,3,4,8,9,10,11,17,19,20, 27,28,29,31
Hemoglobinuria	present	1,2
Ammonia	concentration lower than normal	2,27
Chlorides	" " " "	2,20,27,30
Urea nitrogen	" " " "	2,3,19,27
Thiamin	" " " "	18

* Numbers below refer to references that appear at the end of the table.

Table 1. (cont'd) Typical clinical symptoms of eclampsia in human beings

Concentration of blood constituents		
Uric acid	elevated	1,2,11,12,14,19,27
Calcium	low to normal	1,2,12,19
Blood sugar	normal or decreased	12
	generally increased	2,6,14,19
Cholesterol	increased	11,12,19,21
Non-protein nitrogen	high	1,2,19,21,26,27,28
Urea nitrogen	high	2,11,14,19,21,26,27
Carbon dioxide	combining power less than normal	1,2,11,21
Chlorides	high	2,27,30
Sodium	high	29,30
Lactic acid	increased	2
Creatine and creatinine	high	19,27
	normal	2
Phosphorus	increased	2,11,19
Acidosis	present	2,3,11
Plasma protein	usually low	1,8,13,16,17,19,29
Globulin, euglobulin, fibrinogen	increased at the expense of albumin	2
Magnesium	low	2
Organs		
Placenta		
Thiamin content	lower than normal	18
Lesions	premature syncytial degeneration	1,22,23,24,25
Liver		
Fatty infiltration	present	20,33
Lesions	necrosis and hemorrhage	1,2,3,7,29
Kidney		
Fatty degeneration	diffuse and regular	32
Lesions	degeneration of glomeruli and/or changes in the convoluted tubules	1,2,3,7,29,32

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ANALYSIS OF REPRODUCTIVE FAILURE IN RATS

It has been stated by McIlroy (1936) that reproduction is the most complicated and most important function in the physiology of every species. Due to its very complexity, however, much still remains to be learned of the physiological changes which take place in normal pregnancy, and of the influences which may exaggerate these normal changes and turn a condition of well-being into one that is pathological. The term "toxemia" has been used loosely to indicate the condition in pregnancy in which alterations of normal changes occur. Actually, the term itself is a misnomer when used in connection with pregnancy, for no toxins or specific poisons have been isolated. A definite toxic condition, however, may result as a reflection of the primary injury. The literature indicates that there is much disagreement as to just what the predisposing factors or causes of toxemia of pregnancy may be; some obstetricians even call eclampsia the "Disease of Theories".

Although there is great similarity in the symptoms and pathological changes which occur in animals and man affected by toxemia of pregnancy, gynecologists seem to believe that the toxemia described in herbiverous animals does not correspond to the disease recognized in man and

that the syndrome in man has not been reproduced in animals (Dexter and Weiss, 1941). Now, however, a syndrome has been produced experimentally by dietary means in the rat, an omniverous animal, which in many respects is surprisingly similar to toxemia of pregnancy in the human being. It is very important that the identity of the syndrome as it occurs in the rat be established in relation to the human disorder. If it is its analogue, the production of the disease experimentally offers an unparalled opportunity for such study of the disorder that may result eventually in a solution of the "riddle of eclampsia" and toxic pregnancy. The importance of the experimental animal in the study of disease cannot be too highly emphasized. For example, many of the facts that we know today concerning diabetes came about through experimental production of the disorder in the dog.

Since the syndrome is produced experimentally in the rat by dietary manipulation, some factor or factors either present or lacking in the diet probably is/are responsible for pathological changes that terminate in symptoms resembling those of toxemia. Because certain abnormal changes occurred in the animals fed the pork-containing diet which did not develop the typical toxic symptoms, it seems reasonable to believe that a dietary deficiency rather than

the presence of some toxic substance is the causative agent. This hypothesis is further supported by the findings that certain substances, when added to the diet, completely prevented or reduced the number of animals succumbing to toxemia. Small doses of fresh liver or lipocaic (obtained from Dragstedt's laboratory) when added to the diet completely prevented the occurrence of toxic pregnancy. Beef muscle substituted for pork at a level equivalent to 30 per cent dietary protein seemed to exert a cumulative effect as no animals showed toxic symptoms after the first generation in a study covering six generations of rats.

It is known that dietary deficiencies of recognized food nutrients produce pathological signs through metabolic derangements that represent the primary lesion and it would appear that this is also the case in toxemia of pregnancy in the rat. Some obstetricians also believe that certain of the symptoms associated with the disease in the human being are the result of metabolic aberrations and not the cause of toxemia as others suggest. That the complications of pregnancy are due to some deficiency in nutrition, as early (1936) emphasized by McIlroy, have had interesting confirmation in the work of Ebbs, Tisdall, and Scott (1942), Interim Report of the People's League of Health (1942),

and Burke, Beal, Kirkwood, and Stuart (1943).

The processes involved in metabolism are so complex and interrelated that it is difficult to determine just where the derangement originates and which of the abnormal changes observed are the result of that primary derangement. It is evident, however, in the pregnant rat that the metabolism of protein, of fat, of carbohydrate, and of water are all affected by the deficiency.

The liver has been called the "watchdog of metabolism". It functions in neutralizing and detoxifying poisonous agents, preparing nutrients absorbed from the intestine for utilization, transforming end products of metabolism, and acting as a storehouse for various essential substances. When adverse conditions are imposed, it is not surprising that this organ seems to show the first evidence of serious damage.

A considerable portion of absorbed fat appears promptly in the liver, and normally this organ takes an important part in the metabolism of the lipids. The change of fat to phospholipid in the liver appears to be a necessary step for its later use. Lipids in excess of the body needs are stored in the fat depots. If poisons enter the body, if starvation occurs, if a hyperactivity of the pituitary occurs, or in the case of diabetes stored fat is

returned to the liver, often faster than it can be worked up. Definite fatty infiltration was found in the liver after feeding of the pork-containing diet, in both the non-toxic and toxic animals. Total lipids in the livers of the toxic animals was only slightly greater than the concentration found in non-toxic rats. Analysis of fat in the hepatic tissue revealed that there is a definite increase in neutral fat and cholesterol esters and a reduction in free cholesterol. This finding is indicative of hepatic damage for increased quantities of cholesterol ester in a tissue are associated with inactivity or degeneration while free cholesterol is considered an integral component of normal tissue (Bloor, 1928; Boyd, 1935). The iodine number of fatty acids in the livers of pork-fed animals was definitely lower than in the control animals, indicating an undue accumulation of saturated fat. Analysis of the blood showed a marked decrease in lipid values and Wilcox (1942) seems to believe that this finding is a direct reflection of the liver injury induced by the diet. Supplementing the pork diet with raw pork in place of the autoclaved pork muscle, liver extract, lacto-flavin, milk, or lipocaic (obtained from Eli Lilly Co.) definitely reduced, though in varying degree, the accumulation of fat in the liver, but did not prevent the occurrence of the

toxic syndrome. The average liver fat value of the group of rats fed these various supplements, however, was 1.5 times higher than that characteristic of the control group (Wilcox, 1942).

With the increase in fat content in the liver there is a definite decrease in the amount of glycogen present. Although the difference in fat content in the livers of toxic and non-toxic rats was very small, the difference in amount of glycogen in the livers of the two groups was considerable. Rats exhibiting typical toxic symptoms showed no liver glycogen (Farrankop, 1941). Glucose tolerance curves were diabetic in character for both non-toxic and toxic rats, but animals showing toxic symptoms exhibited a considerably slower utilization of the injected carbohydrate. The increased deposition of fat in the liver may have lowered the ability of this organ to store glycogen. The fact that there is definite destruction of hepatic tissue as well as fatty infiltration in the toxic condition might also explain why no glycogen was detected in the livers of animals exhibiting toxic symptoms. Secretions of the pituitary, adrenal glands, and the pancreas also influence storage of glycogen in the liver. The action of these different secretions, however, are so interrelated and the secretions, themselves, so complex

that it is difficult to determine the effect of any one gland.

Evans, Meyer, Simpson, and Reichert (1932) discovered that diabetes can be produced in normal intact animals by the injection of anterior pituitary extracts, and Richardson and Young (1938) have shown that specific injury to the Langerhans tissue is produced in this way. The acinar cells, however, are well preserved (Campbell and Best, 1938). That the functional activity of the hypophysis is influenced by the composition of the diet is shown by the fact that in hypophysectomized rabbits, fed a high carbohydrate diet, the injection of Young's glycotropic factor of the anterior pituitary results in impairment of sugar tolerance and insulin sensitivity similar to that observed when normal animals are given a low carbohydrate diet (Himsworth and Scott, 1938).

Long and White (1938) from studies employing animals in a semi-diabetic state believe that adrenalectomy improves diabetes in the same way as does hypophysectomy. They have shown that hypophyseal participation in the carbohydrate mechanism demands the presence of functional adrenal cortical tissue, and that the pituitary gland stimulates the production of cortin. Atrophy of the adrenal cortex follows the removal of the anterior pituitary

(Fisher, Ingram, and Ranson, 1938). In the normal animal an excess of the hormone from the adrenal cortex leads to an accumulation of stored carbohydrate by preventing the breakdown of any glycogen originally present (Long, 1942). Although the adrenal glands in the pregnant pork-fed rat do not show degenerative changes until toxemia of pregnancy is acute (Molsberry, 1943), it is possible that the resulting adrenal insufficiency may be a contributing factor to the depletion of glycogen in the liver of these animals.

In as much as the addition of a pancreatic extract (lipocaic) to the basal pork ration had prevented the pregnancy disorder in the rat (Wilcox, 1937), changes in the pancreas might be responsible for the condition. Histological sections of the gland, however, appeared normal except in a few rats in which the pancreas was enclosed in a jelly-like substance. The acini of such glands were contracted and stained very deeply and the connective tissue supporting the lobules was broken. The islets, however, appeared normal and fat necrosis was not present (Armstrong, 1939). Channon, Loach, and Tristam (1938) found that extracts of pancreas prevent fat deposition in the liver to a greater extent than can be attributed to their choline or protein content so it would appear that

some other substance in the pancreas is involved in the prevention of fat deposition in the liver. The work of Ralli, Rubin, and Present (1938) on dogs suggests that the substance present in the pancreas, which is effective in reducing fatty infiltration in the liver, is an external secretion rather than a hormone. If this is the case, the histological picture of contracted acini and broken connective tissue in the pancreas would indicate degeneration and decreased secretion of the substance active in inhibiting fatty infiltration in the liver.

An edematous condition is quite characteristic of toxemia and gross observation at necropsy in the rat reveal marked edema of the pancreas in some cases. The abdominal cavity was filled with fluid and often the pleural cavity was filled with a considerable amount of a serous fluid. A water discharge from the mouth and nares was also noted. Moisture determinations of tissues from a toxic pork-fed rat showed a decided increase in the moisture content of brain, kidney, liver, and muscle (Weedman, 1942). Work by Fiessinger, Gajdos, and Panayotopoulos (1938) with rabbits indicates that there may be a definite tissue factor in liver which regulates the transference of ingested water. Injury to the liver seems to interfere with this mechanism.

The pituitary and adrenal glands are also known to

influence water metabolism. Liu and Noble (1938) have shown that renal damage and functional derangement may readily be induced in rabbits by the injection into the renal artery of relatively small doses of the pressor principle of the posterior pituitary gland. The posterior lobe also exerts an antidiuretic effect, for removal of this lobe in the rat is followed by prolonged polyuria even in the absence of the anterior lobe. An antidiuretic hormone is also produced in the anterior pituitary (Chen and Geiling, 1943; White, Heinbecker, and Rolf, 1942). Depressed urinary excretion in toxemia, therefore, might be attributed to a hyperactivity of either or both the anterior and posterior pituitary. The antidiuretic substances appear in the urine of normal persons, but are found in larger amounts in the urine from patients with toxemia of pregnancy. The placentas of these patients also contained larger quantities of these substances than placentas from normal women (Ham and Landis, 1942).

Adrenal insufficiency also results in decreased urinary excretion and in increased capillary permeability. The experimental animal becomes lethargic and dyspneic, the body temperature falls and muscular weakness develops. Histological sections of adrenal glands from toxic rats show hemorrhage, congestion and necrosis of the cortex

with occasional evidences of fat disturbance, autolysis, and hyalinization. Congestion of blood in the medulla is an almost constant finding in these animals (Molsberry, 1943). It has been suggested by Silvette and Britton (1938) that the adrenal cortal hormone acts as a physiological antagonist to the antidiuretic principle of the posterior pituitary in its influence upon the excretion of water and sodium chloride. A disturbance in water balance always follows adrenalectomy.

A definite decreased kidney function has been demonstrated in eclampsia (Kariher and George, 1943). In the toxic rat much renal tissue is destroyed; the extent of damage depending on the length of time the animal was sick. Histological examination of the kidneys of these animals by Armstrong (1939) showed extensive hemorrhage within the tubules and nearly a complete destruction of renal epithelium. Large deeply-stained nuclei were evident, but the cytoplasm was so fragmented that the cell outlines were completely lost. Congestion was prominent in the renal blood vessels. With the exception of the glomeruli the hemorrhage apparently occurred throughout the organ, but no signs of infection could be seen. Except for marked hyperemia, the glomeruli appeared normal. Even those pork-fed rats which did not develop toxemia showed

definite renal injury. Measurement of the volume of urine from toxic rats showed that the quantity excreted was decreased in this condition. The urine contained considerable albumin (Lefebvre, 1941).

Whether renal injury is the cause or the result of toxemia of pregnancy is still a matter of discussion. From recent reports in the literature it seems probable, however, that the breakdown in renal function is not the primary lesion in the condition, but may be the cause of the accumulation of metabolic end products in the blood. Determinations of non-protein nitrogen intthe blood by Barnhart (1942) showed a decided increase in this constituent in toxemia of pregnancy. Pork-fed rats that did not develop toxemia also showed an elevation in non-protein nitrogen in the blood above the level normally expected in pregnancy.

The placenta is another organ which undergoes degenerative changes in toxemia. The characteristic lesion in the human being consists primarily of a premature aging of the placenta. In the final stage there is a disappearance of all nuclei from the syncytial layer, leaving the villus surrounded by a thin layer of hyaline material. There is also a marked congestion of the villus blood vessels (Tenney and Parker, 1940). Placentae from toxic rats were

much less vascular than normal and many of the sinuses were devoid of blood (Armstrong, 1939). The cause of these degenerative changes is still unknown, but there is no doubt that the placenta acts as an additional endocrine organ and is closely associated with the pituitary and the ovary (McIlroy, 1936).

Hypertension is characteristic in toxemia of pregnancy in women. Teel and Reid (1937) report that a study of the vascular pathology in fatal cases of eclampsia, as seen for example in the liver, kidney, and adrenals, gives a clear indication of the marked narrowing of the arterioles, leading to ischemia, hemorrhage, and not infrequently thrombosis. Prolonged ischemia as a result of arteriolar spasm might conceivably lead to permanent damage of the functional unit supplied. Eastman (1937) also seems to believe that eclampsia is an affectation of all the small terminal arterioles. Westphal and Sievert (1938) view essential hypertension as due to a derangement of the pituitary and adrenal glands and claim that a pressor substance is found in the blood of such patients. The presence of a high concentration of a pressor hormone in the circulation with hyperactivity of the posterior pituitary in the toxic condition has been postulated by Mukherjee (1941). Swingle and his collaborators (1938)

have shown that adequate doses of adrenal cortical hormone prevent the fall in blood pressure and circulatory collapse in animals given massive doses of adrenalin or other shock inducing procedures.

To date blood pressure determinations have not been made on rats developing toxemia of pregnancy. In view of the interesting implication of the above studies concerning hypertension, it seems important that this aspect of the problem be investigated, especially since pathological changes in the adrenal cortex have been observed in toxic rats.

Blood from rats exhibiting toxic symptoms was pale and watery in appearance. Prothrombin times of this blood were considerably longer than normal (Pressley, 1941). This finding is further evidence of hepatic damage in toxemia for the liver is known to be the site of the formation of prothrombin.

From this discussion, it is evident that the pituitary gland plays an important part in physiology and metabolism, and that abnormal activity by this gland would have far reaching effects. It is significant that in rabbits affected with toxemia of pregnancy the pituitary was the only affected organ which did not show degenerative changes, but instead showed changes indicative of

increased function (Greene, 1937). Subcutaneous and intraperitoneal injections of pituitary preparations in rabbits produced a disorder which resembled the spontaneous disease, toxemia of pregnancy, in many particulars (Greene, 1939).

The fact that the functional activity of the pituitary is influenced by the composition of the diet might explain why fat accumulates in the liver after the feeding of the pork-containing diet. The other abnormal degenerative changes in toxemia could be attributed to depressed liver function as postulated by Armstrong (1939). However the action of pituitary extracts on other organs and systems besides the liver cannot be ignored.

No studies of the pituitary gland in toxic rats have as yet been made nor any attempts to produce toxic symptoms by injection of pituitary extracts in rats. In view of the wide influence of the pituitary in body mechanisms it seems worthy of consideration for future work in relation to toxemia of pregnancy in rats.

PURPOSE OF EXPERIMENT

The syndrome of toxic pregnancy in the albino rat produced experimentally in the Nutrition laboratory of the Iowa State College by the feeding of a ration containing partially dehydrated pork muscle as the chief source of protein has been described in the previous section. An attempt has been made in earlier investigations to define the symptoms not only in terms of gross abnormalities, but also on the basis of biochemical and histological findings. To date, abnormalities in nitrogenous constituents of the blood and in water balance have been indicated but not completely studied. No work has been done on the concentration of inorganic constituents in the blood stream in the toxic animal. In the present investigation an attempt has been made to amplify information along these lines.

As previously cited, Barnhart (1942) found that feeding of the pork-containing diet effected an elevation of the concentration of non-protein nitrogen constituents in the blood of pregnant rats and that their concentration was abnormally high in the blood of animals that developed the pregnancy disease. In as much as urea nitrogen normally constitutes approximately 50 per cent of the total non-

protein nitrogen in the blood, the extent to which this value is elevated may be regarded as an index of the degree of functional impairment in the kidney. The concentration of urea nitrogen in the blood of healthy and toxic rats, therefore, was determined to ascertain whether or not the increase in non-protein nitrogen, noted by Barnhart, was associated with a change in urea nitrogen content.

It was indicated in previous sections that edema is a characteristic finding in toxemia of pregnancy in both the rat and the human being. A low concentration of plasma protein has been cited in the literature as one of the causes of edema. Plasma proteins are formed in the liver. Armstrong (1939) has shown that while the cells of the livers of the non-toxic animals are intact those of the livers of the sick animals are severely damaged. A break in liver function is also indicated in the studies relating to carbohydrate metabolism. The defect is apparent in pork-fed animals exhibiting no toxic symptoms, as well as in those in which acute symptoms develop. It seemed worth while therefore, to obtain information on plasma protein concentration in healthy and sick rats.

Hoffman (1941) has stated that in conditions of low serum protein concentration the quantity of calcium present in serum is also low. Findings presented in table 1 show that in human eclampsia the concentration of serum

calcium is generally normal with only a few cases of slightly subnormal values. Some investigators, however, who have studied pregnancy disease in sheep believe that hypocalcemia is definitely associated with toxemia. It seemed important, therefore, to determine the concentration of serum calcium in the toxic rat. If correlated with a low concentration of plasma protein, some light might be shed on the nature of the metabolic disturbance occurring in toxic pregnancy.

During the years that the project relating to experimental toxemia in rats has been in progress, many facts and observations have been recorded by the various investigators. It seemed important at this time to assemble the facts, and to relate them to each other and to observations regarding the human syndrome as reported in the literature. This the present investigator has attempted to do.

EXPERIMENTAL PROCEDURE

EXPERIMENTAL PLAN

The rats studied were divided into two groups, i.e., the control and experimental. The animals comprising the control group were maintained on the stock colony ration, which will be designated as Steenbock V, and animals making up the experimental group received the diet containing pork, which will be called Pork I. Each of these groups was made up of two sub-groups, one containing pregnant animals, the other virgin rats. In many cases, littermates were represented in each of the four sub-groups. The experimental plan thus provided an opportunity to evaluate the effect of dietary manipulation in terms of physiological changes normally associated with pregnancy itself.

The quantity of urea nitrogen, plasma protein, and serum calcium was determined in the blood of these animals. The pregnant animals were sacrificed when 21.5 days pregnant, the virgin animals when they were of approximately the same age as the gravid rats. In the early part of the experiment the blood was examined for urea nitrogen only, laterone sample of blood was analyzed for the three constituents. In cases where insufficient blood was obtained

from the animals to carry out all three determinations, the amount of blood obtained determined which constituent was estimated.

A summary of the experimental plan showing the number of animals used in each analysis and their distribution in the control and experimental groups is presented in table 2. Tables showing the individual laboratory numbers of the rats composing each group are found in the Appendix.

ANIMALS USED

The animals employed in the present investigation were albino rats of Wistar stock, Strain A, obtained from the stock colony maintained by the Nutrition Laboratory at the Iowa State College. These rats had been inbred by brother and sister mating for about 90 generations, and only the young of second and third litters of the stock colony females were used in the experiment. Greenwood (1940) showed that more young as well as larger animals were reared from these litters than from any other litter. For example, the mean weaning weight of the young in litter 2 was 49.77; in litter 3, 49.83; and in litter 1, 46.95.

The stock colony animals were maintained on a ration called Steenbock V. The composition of the diet has

Table 2. Summary of experimental plan

Group	Name of diet fed	Reproductive status	No. of rats used in analyses		
			Urea nitrogen	Plasma protein	Serum calcium
Control	Steenbock V	Pregnant	36	9	7
		Virgin	29	13	8
Experimental	Pork I	Pregnant			
		Non-toxic	31	11	8
		Toxic	4	2	4
		Virgin	30	13	8
Total number of analyses made, 213					

remained nearly the same since 1932, and the quality of the components of the ration has been kept as uniform as possible.

The young rats used in the experiment were removed from the colony at the time of weaning, and littermates were distributed between the control and experimental groups. Some of the animals developing toxemia did not always have a littermate control, but they originated in other groups of rats raised under similar conditions. Until the time of the opening of the vaginal orifice, all the rats ate the stock colony diet. From then on, the experimental group received the Pork I ration, and the control animals, the Steenbock V diet.

The uniformity of the animals used in the study was examined, data being presented in table 3. Indices used for judging uniformity were: average weight at weaning, and average ages and weights at sexual maturity and at the time of the initiation of the first pregnancy. Data describing the animals used in successive years are shown in the Appendix. The rats were remarkably uniform during the four years that the investigation was in progress. It is worthy of note that the values of the indices fall within the range reported by Greenwood (1940) in a study of 14 generations of normal rats in the stock colony. Any

Table 3. Uniformity of animals

Group	Name of diet fed	Reproductive status	No. of animals	Av. body wt. at weaning	Av. age at sexual maturity*	Av. body wt. at sexual maturity	Av. age at initiation of 1st pregnancy	Av. body wt. at initiation of 1st pregnancy
				gm.	days	gm.	days	gm.
Control	Steenbock V	Pregnant	36	52	43.1	93	68.1	138
		Virgin	33	50	42.1	89	----	---
Experimental	Pork I	Pregnant						
		Non-toxic	33	52	42.6	92	67.0	136
		Toxic	3	54	39.0	86	68.7	149
		Virgin	34	52	41.2	90	----	---

* Opening of vaginal orifice

differences, therefore, noted in groups of experimental data probably are not due to a lack of homogeneity in the animals.

COMPOSITION AND PREPARATION OF DIETS

The Steenbock V Diet

Steenbock V, the regular stock colony ration, was given to the control group. This ration is a modification of a formula recommended by Steenbock in 1923. The basal portion of the diet was supplemented daily with milk to which trace elements and fish liver oil had been added. Due to war time restrictions, fresh vegetables, lettuce, cabbage, or carrots, were offered the first three days of the week and raw ground round of beef the next three days. Previous to 1942, meat and vegetables had been fed on alternate days. The composition of the diet with its supplements is shown on the next page.

I. Basal diet

Yellow cornmeal[1]	64.0 grams
Crude casein[2]	5.0 grams
Linseed meal[3]	16.0 grams
Ground alfalfa[4]	2.0 grams
Sodium chloride[5]	0.5 grams
Calcium chloride[6]	0.5 grams
Yeast[7]	1.5 grams
Irradiated yeast[8]	0.5 grams
Wheat germ[9]	10.0 grams
	100.0 grams

In the summer of 1944 some rats maintained on the Pork I diet developed symptoms characteristic of polyneuritis. Upon investigation it was discovered that the last shipment of yeast from the Northwestern Yeast Co. had been

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[1]Purchased from Grain Storage, Iowa State College
[2]Finely ground, B3F, purchased from the Casein Co. of America, Div. of Borden Co., New York City
[3]Purchased from Ames Grain and Coal Co.
[4]Dehydrated alfalfa leaf meal purchased from Denver Alfalfa Milling and Products Co., Lamar, Colorado
[5]Purchased from the local market
[6]Purchased from Chemistry Stores, Iowa State College
[7]Yeast foam tablet powder purchased from Northwestern Yeast Co., Chicago, Ill., and from Pabst Sales Co., Chicago, Ill.
[8]Irradiated in 200 gm. lots for 11 min. at a distance of 15 in. with a General Electric Uviarc lamp
[9]Type A, purchased from Washburn Crosby Co., Minneapolis, Minn.

sent in cardboard containers instead of metal, which was the usual custom. The yeast powder had hardened and had to be ground before it could be incorporated into the diets. Analysis of this yeast revealed that it contained 6.4 gamma per gram, approximately one-half as much thiamin as previous shipments. The next lot of yeast was obtained from Pabst Sales Co. The thiamin content of this yeast was 186.6 gamma per gram.

II. Supplementary foods

A. Five grams of raw ground round of beef[1] fed three times weekly

B. Ten grams of fresh vegetables, raw carrots, cabbage, or lettuce fed three times weekly

C. Liquified Klim fed as follows:

1. Each male and resting female, 12.5 ml. daily

2. Each pregnant female, 25.0 ml. daily

3. Each female with a litter, 50.0 ml. daily

Each day's supply of Klim was mixed in the proportion of 130 grams of dry Klim[2] to one quart of distilled water

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[1]Purchased in the local market
[2]Powdered whole milk (Klim) obtained from the Borden Co., New York

and one teaspoon of fish liver oil[3] in a Hobart mixer at third speed for five minutes. Two ml. of a solution of salts of certain trace elements were added to each quart of milk. The salt solution contained the following chemicals dissolved in distilled water and made up to a volume of 1000 ml.:

KI	0.80	grams
$MnSO_4$	3.16	grams
$K_2Al_2(SO_4)_4$	0.98	grams
$CuSO_4 \cdot 5\ H_2O$	4.075	grams

The Pork I Diet

The composition of the diet fed the experimental group is shown on the next page.

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[3]Feeding oil containing 1000 U.S.P. units of vitamin A, 400 A.O.C. units of vitamin D, obtained from the Pearson-Ferguson Chemical Co., Kansas City, Missouri

Canned pork muscle (dried to one-half its original weight)	25 grams
Cornstarch[1]	53 grams
Yeast[2]	5 grams
Agar agar[3]	2 grams
Sodium chloride[4]	1 gram
Salt mixture[5]	4 grams
Butter fat[6]	8 grams
Fish liver oil[7]	2 grams
	100.0 grams

In 1945 due to war time restrictions, agar agar could not be obtained and an equivalent amount of Ruffex[8] was substituted.

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[1]Purchased in wholesale lots
[2]Yeast foam tablet powder purchased from Northwestern Yeast Co., Chicago, Ill., and from Pabst Sales Co., Chicago, Ill.
[3]Bacto-agar purchased from the Difco Laboratories, Inc., Detroit, Michigan
[4]Purchased in the local market
[5]Osborne, T. B. and Mendel, L. B., J. Biol. Chem., 37, 557, 1919
[6]Purchased at Iowa State College Dairy
[7]Feeding oil containing 1000 U.S.P. units of vitamin A and 400 A.O.C. units of vitamin D, purchased from the Pearson-Ferguson Chemical Co., Kansas City, Missouri
[8]Purchased from Fisher Scientific Co., Chicago, Ill.

The green skinned hams were purchased as pairs in large numbers to reduce variation in the pork material. As much as 1000 lbs. of meat often was purchased at one time. The hams were boned, trimmed of all excess fat, and ground once through the medium plate of the meat grinder. The ground meat was then packed in tin cans and sealed. Each can contained approximately 500 grams. The meat was processed in pressure cookers for 65 minutes at 15 pounds pressure. Upon removal from the pressure cookers, the cans were examined for leaks, cooled immediately in cold water, and stored at room temperature until needed.

Butter fat was prepared by heating butter for two hours in a double boiler, skimming the coagulated protein and salt off the top, decanting the middle layer of pure butter fat, and filtering it through several layers of cheese cloth in a hot water funnel.

In preparation of the diet, the outside layer of visible congealed fat in the can of pork was removed. The meat was thoroughly mixed with the liquor surrounding it, and approximately 1000 grams placed on a Monel tray covered with cheese cloth. After being dried to one-half its original weight in a current of air kept below 100 degrees Centigrade for approximately 65 minutes, the pork was added to the dry ingredients, butter fat, and fish liver oil.

All the constituents were mixed in a Hobart mixer for 25 minutes at first speed and stored in the refrigerator. Fresh diet was mixed twice weekly.

CARE OF ANIMALS

Upon reaching sexual maturity, each female rat was housed separately in a round wire-mesh cage with a raised bottom. The cages placed on steel shelves stood in enameled pans lined with paper towels. The paper towels collected the urine and feces. Food and distilled water were given ad libitum in glass containers. Small porcelain cups held the milk. The food jars and milk cups were changed three times weekly, and cages, pans, water fountains, and metal holders weekly. All equipment was washed in strong soap suds and all except the glass fountains were sterilized in live steam for 15 minutes.

Each day, except Sunday, the paper towels in the pans were changed, the general condition of the animal examined, and food and distilled water provided. The quantity of food given daily was slightly in excess of the animal's needs. On Saturday a double portion was fed. Each day, uneaten Pork I diet which remained in the food jar was discarded to eliminate possible effects of developing rancidity. Food intake for rats fed the Pork I diet was recorded

daily.

Vaginal smears were examined daily and the weights of the rats were recorded at that time. On the twenty-first day of gestation, each female was weighed at 10 P.M. and at four-hour intervals on the following day. This procedure facilitated the early detection of signs of toxemia, and examination and weighing of the young soon after birth.

As soon after birth as possible, the individual weights of the young and the weight of the female were obtained. If any of the litter were dead, individual weights were recorded and a portion of the lung removed and placed in water. If the tissue floated, the young rat was assumed to have been alive at birth (Webster, 1930). In this test a fairly large portion of the lung has to be used in order to overcome the effect of surface tension.

The weight of the total litter was recorded daily and four days after birth the number of rats in the litter was reduced to six. In deciding which young to retain, whenever possible, three males and three females whose weights approached the average of each sex in the litter were kept. When the young were 4, 7, 14, 17, 21, and 28 days old, they were differentiated as to sex and weighed separately. The young were weaned when they were 28 days old.

VAGINAL SMEARS

The normal female rat will accept mating during either the late proestrus or the early oestrus stage of the oestrus cycle. In order to know when to mate each rat, the course of the oestrus cycle was followed by daily examination of cells removed from the vagina. Inspection of the smears was begun four weeks after the date of weaning and continued daily until the time that the rat was killed.

Small glass rods, 2 mm. in diameter, with fire-polished ends were used in removing the sample of cells from the vaginal wall. The cells adhering to the tip of the rod were placed in a drop of distilled water on a clean slide. They were then examined under the low power objective of a microscope, with artificial light as the source of illumination.

Immediately after use the rods were placed in a strong soap solution. Later, the rods were washed and placed in test tubes containing a small amount of distilled water. After the tubes were sealed with cotton, they were sterilized at 15 pounds pressure for 15 minutes. This routine prevented the possibility of infection of the vagina.

The classification of Long and Evans (1922) was used to determine the stages of the oestrus cycle, which they

divide as follows:

Stage 1. Epithelial cells only,

Stage 2. Epithelial and cornified cells,

Stage 3. Many cornified cells,

Stage 4. Cornified cells and leucocytes, and

Stage 5. Leucocytes, epithelial, and cornified cells.

Stage 1 represents proestrus in the cycle and stage 2, oestrus.

Vaginal smears were examined at approximately the same hour each day and a record was kept of the date, time of day, weight of the rat, kind of cells present in the smear, stage of the oestrus cycle, and any changes in the physical condition of the animal. When inspection of vaginal smears was initiated, each female was allowed to pass through one complete oestrus cycle before being mated. When stage 1 or 2 appeared for the second time, a brother male was placed in the cage with the female. On the following day, the vaginal smear was examined for sperm cells and the vagina and the paper beneath the cage for vaginal plugs. If neither sperm nor plugs were found, the male was not removed until stage 4 or 5 was noted in case the previous vaginal smear had been misinterpreted.

From the twelfth to the sixteenth day of gestation

the vagina was examined for the presence of blood and the smear for the appearance of red blood cells. The presence of either one or both indicated the implantation of the feti in the uterine wall.

PREPARATION OF RATS FOR ANALYSIS

Before Necropsy

The pregnant rats were killed for study 21.5 days after the initiation of the second pregnancy. Each rat after raising one litter or after the litter had died was mated when the next oestrus appeared. In order to determine fairly accurately when the second pregnancy had progressed for 21.5 days, a vaginal smear was taken at 10 P.M. on the day the male was placed with the female to find out whether mating had occurred on that day. All pregnant animals, except some of the sick females, were killed when 21.5 days ± four hours pregnant.

The animals were starved for 10 hours prior to killing. This period was chosen because it was believed long enough to eliminate the influence of the ingestion of food and short enough not to injure the feti. Also, 10 hours was a time interval that fitted conveniently into the laboratory routine. The ration, therefore, was removed from the

cage at 10 P.M. when the rat was to be killed at 8 A.M. on the next day, or at 7 A.M. when 5 P.M. was the hour of killing.

Sick animals were not starved as many of them showed no signs of illness until shortly before acute symptoms were observed. However, examination of the food consumption records showed that the sick animal starved herself as most of the animals consumed only 1 or 2 grams of food during their last 24 hours of life.

Littermate virgins were killed when they were approximately the same age as the pregnant females.

Weights of all animals before and after starvation were recorded, and a record of the general physical condition before killing was made of each animal according to form 1 shown in the Appendix.

At Necropsy

Each rat was anesthetized by an intrapleural injection of a 3 per cent solution of sodium pentobarbital. The amount injected was 0.1 ml. per 100 gm. of body weight. As soon as the rat failed to respond to external stimuli, it was laid on its back, and an incision was made on the ventral median line from the pelvic girdle to the diaphragm. Transverse cuts were made on both sides of the abdominal

wall, care being taken to avoid cutting the larger blood vessels.

The abdominal aorta was exposed postrenally by pushing the viscera and uterus aside. Fat and fascia covering the artery were removed carefully with a small forceps. A 20 gauge B-D Yale needle attached to a 5 ml. syringe of the same make was inserted into the vessel, and 5 to 6 ml. of blood were withdrawn. Two ml. of the blood were quickly delivered into an oxalated centrifuge tube, agitated gently, and used for urea nitrogen and total plasma protein analyses. The remaining blood was delivered into an unoxalated tube and used for serum calcium analysis. Preparation of the oxalated tubes for receiving the blood is given in the Appendix.

Observations of the internal condition of the animal were made according to form 2 in the Appendix. The weights of the intact uterus and of the uterus minus the feti were also recorded.

METHODS OF ANALYSES

Urea Nitrogen

The first chemical method used for the determination of the concentration of urea nitrogen in the blood was a

modification of the method described by Karr in 1924. The procedure as it was finally developed employed 1) the use of the Haden modification (1923) of the Folin Wu precipitation procedure for the preparation of a protein-free blood filtrate; 2) the use of a commercial preparation of urease instead of the Jackbean preparation described by Karr, and 3) direct nesslerization of the digest and determination of the intensity of the resulting color in a Klett-Summerson photoelectric colorimeter.

Because a commercial preparation of urease, in tablet form, was to be used in place of the Jackbean preparation suggested by Karr, it was necessary to determine the amount of urease that could be used without producing a turbidity and yet completely convert urea to ammonia. The enzyme solution was prepared by dissolving one-fourth of a Squibb 0.1 gm. urease tablet in 1 ml. or organic-free distilled water. The necessity of adding a solution of gum ghatti was also tested. Data are presented in the following table.

Table 4

Determination of appropriate amount of commercial urease to be used in the Karr analysis of urea nitrogen

Solution	Amt. of urease soln. used	Calculated amt. of urea nitrogen present	Urea nitrogen as determ'd by analysis	Recovery of urea nitrogen
	drops	mg./100 ml.	mg/100 ml.	per cent
5 ml. urea standard plus gum ghatti	2	0.9000	0.9317	103.52
5 ml. urea standard	2	0.9000	0.8816	97.96
5 ml. urea standard plus gum ghatti	1	0.9000	0.8736	97.07
5 ml. urea standard	1	0.9000	0.8971	99.68

Since the addition of gum ghatti did not seem to show any beneficial effect, it was not used in this procedure. In view of the above data 1 drop of the urease solution was considered an adequate amount of enzyme for the Karr procedure.

If sufficient blood was available, 3 ml. of oxalated blood, measured with a pipette (U.S.B.S.), were transferred to a 50 ml. beaker. Then 8 volumes of 1/12 N sulfuric acid were added to the blood. Laking and darkening occurred rapidly. With stirring, 1 volume of 10 per cent sodium tungstate was added making a final dilution of the blood of 1:10. After thorough mixing, the solution was filtered

through a C. Schleicher and Schull No. 590 filter paper to remove the precipitated protein.

Five ml. of the 1:10 protein-free blood filtrate, 1 drop of buffer solution, and 1 drop of urease solution were placed in a 25 ml. volumetric flask. The solution was mixed gently and placed in a water-bath at 50 degrees Centigrade for 15 minutes. At the end of that time, the solution was diluted to volume with organic-free distilled water and thoroughly mixed. Ten ml. of the diluted solution were transferred to a colorimeter tube and nesslerized with 1 ml. of Nessler's solution prepared according to the directions of Koch and McMeekin (1924). The intensity of the color was determined within the next two minutes in the Klett-Summerson photoelectric colorimeter. If the nesslerized solution is held longer than this, it becomes cloudy making it impossible to secure a reading. Determinations were made to 0.5 of a scale division.

Blank and standard determinations were made in the same way with 5 ml. of organic-free distilled water substituted for the blood filtrate in the blank, and 5 ml. of a standard urea solution, containing the equivalent of 45 mg. per cent of urea nitrogen instead of the blood filtrate for the standard.

All colorimetric readings were made in matched

colorimeter tubes with the colorimeter set at 0 with distilled water. Calculations were made according to the following equation: $\frac{45}{\text{(reading of standard - reading of blank)}} \times$ (reading of unknown - reading of blank) = mg. per cent urea nitrogen

Preparation of solutions used in this procedure are presented in the Appendix.

Although this procedure gave a relatively good recovery of nitrogen from a standard urea solution, occasionally some of the samples and even the blanks became cloudy before the intensity of the color could be determined. The only reagent which seemed capable of causing this irregularity was the enzyme solution. A study of the literature indicated that other workers had experienced this difficulty (Hoffman, 1941). To eliminate the trouble, Hoffman suggested that the enzyme be added to whole blood before deproteinization. His procedure was tested.

Five-tenths ml. of blood was accurately measured and transferred to a test tube (15 by 150 mm.). The same amount of urease solution was added to the blood and the tube was rotated horizontally to assure thorough mixing. After stoppering with a clean rubber stopper, the tube was placed in a water-bath at 45 degrees Centigrade for 15 minutes. Following the incubation period, 7 ml. of organic-free distilled

water were added to the blood and the solution well mixed. When laking was complete, 1 ml. of 7.5 per cent zinc sulfate solution was added with stirring, and then 1 ml. of 0.374 N sodium hydroxide. The mixture was shaken vigorously and placed in a beaker containing just enough boiling water to reach the level of the contents of the tube. The upper part of the tube acted as a condenser, therefore, the volume of liquid was not reduced appreciably. After two minutes in the boiling water, the tube was removed and cooled to room temperature. The contents of the tube were then filtered through ash-free filter paper (Whatman No. 40).

Exactly 2 ml. of the filtrate were transferred to a 25 ml. test tube and 16 ml. of organic-free distilled water and 2 ml. of Nessler's solution added. After mixing by inversion, the intensity of the color developed was determined in the Klett-Summerson photoelectric colorimeter, as described in the previous procedure.

The blank in this instance was prepared by treating a sample of blood first with sulfate and sodium hydroxide, and then with urease. The contents of the tube were heated immediately in the boiling water-bath without previous incubation.

Calculation of the quantity of urea nitrogen present in the aliquot was made from a regression of milligrams of

urea nitrogen on photoelectric colorimeter readings. The regression curve is given in figure 1, and the original data on which the curve is based appear in the Appendix.

In the formulation of the curve, four different standard solutions of urea containing approximately 80 mg. of urea nitrogen per 100 ml. were used. Each solution was diluted so as to contain 8, 16, 32, and 64 mg. of urea nitrogen per 100 ml. The quantity of urea nitrogen in duplicate samples of the 16 solutions was determined. Check analyses were run in several instances making the total number of determinations, 12. The regression was calculated. The regression coefficient obtained, i.e., 0.9939 indicated the linear relation between the scale readings and the concentration of urea nitrogen.

In order to determine the accuracy of the method, a series of dilutions from a standard solution of urea was set up and analyzed. Also, various amounts of a urea standard were added to blood and the resulting mixtures analyzed. As a check on technique, the determination involving blood and urea mixtures was carried out at three different times during the study with the use of different samples of blood. The data obtained are presented in table 5. The average recovery of urea nitrogen from biological material was 98.20 per cent.

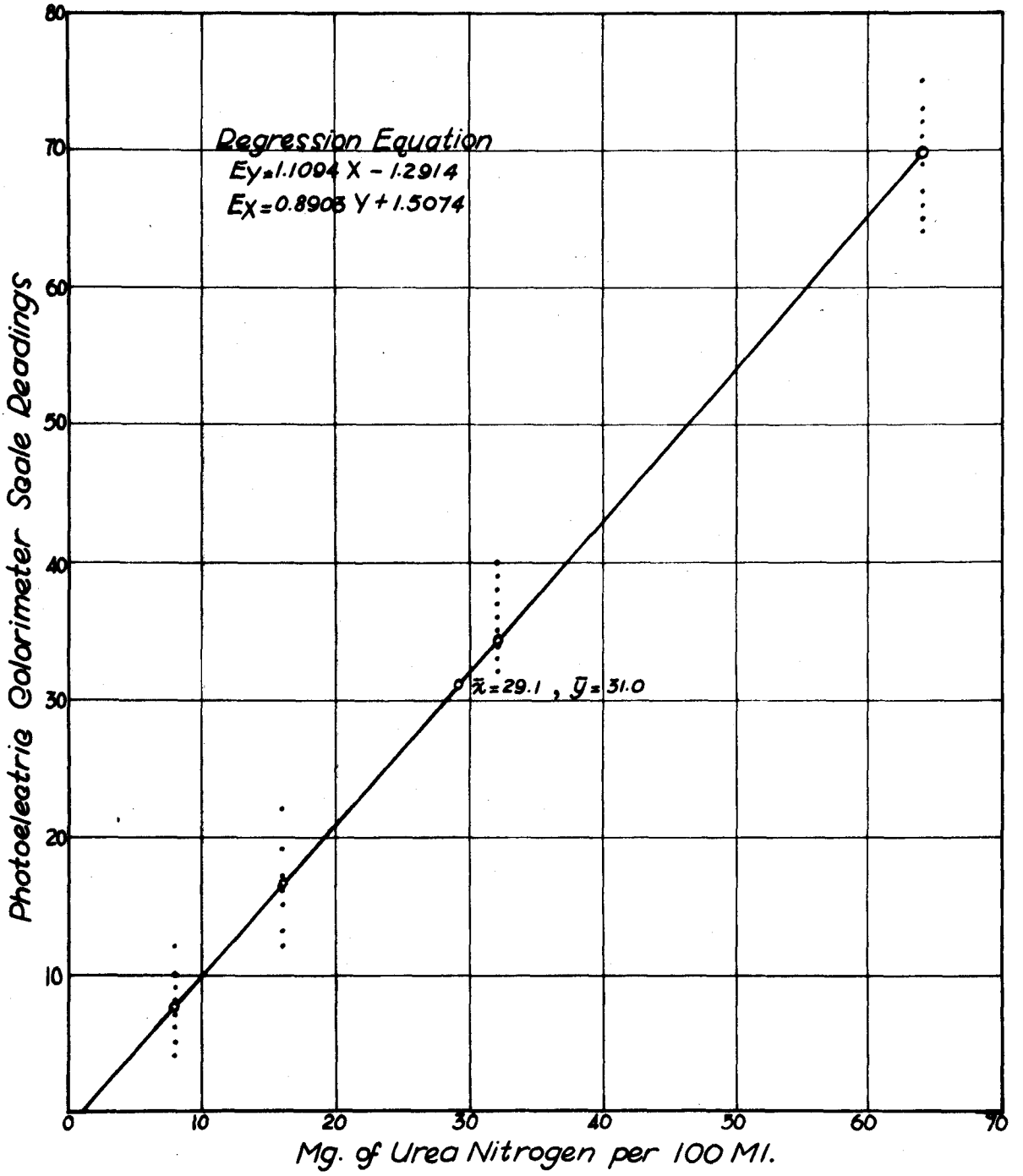

FIG. 1. REGRESSION OF MG. OF UREA NITROGEN PER 100 ML. OF SOLUTION ON SCALE READINGS OF PHOTOELECTRIC COLORIMETER

Table 5. Recovery of urea nitrogen from standard solutions of urea and from solutions representing mixtures of blood and standard urea solutions analyzed according to the Hoffman method

Date	Solution	Conc. of urea N in vol. of standard solution used	Calculated amt. of urea nitrogen	Urea nitrogen as determined by analysis	Recovery of urea nitrogen	Average recovery in each series
		mg.	mg./100 ml.	mg./100 ml.	per cent	per cent
May 1944	0.5 ml. urea standard	0.4000	8.0000	7.7395	96.74	
	0.5 ml. urea standard	0.8000	16.0000	15.7522	98.45	
	0.5 ml. urea standard	1.6000	32.0000	31.7776	99.31	
	0.5 ml. urea standard	3.2000	64.0000	62.9381	98.34	98.21
Oct. 1944	0.5 ml. of blood A			9.5201		
	0.4 ml. of blood plus 0.1 ml. standard soln.	0.0800	23.6161	24.6552	104.96	
	0.3 ml. of blood plus 0.2 ml. standard soln.	0.1600	37.7121	34.5115	91.51	
	0.25 ml. of blood plus 0.25 ml. standard soln.	0.2000	44.7601	44.2418	98.84	
	0.2 ml. of blood plus 0.3 ml. standard soln.	0.2400	51.8080	49.5836	95.71	
	0.1 ml. of blood plus 0.4 ml. standard soln.	0.3200	65.9040	62.9381	95.50	97.30

Cont'd on next page

Table 5. (cont'd) Recovery of urea nitrogen from standard solutions of urea and from solutions representing mixtures of blood and standard urea solutions analyzed according to the Hoffman method

Date	Solution	Conc. of urea N in vol. of standard solution used	Calculated amt. of urea nitrogen	Urea nitrogen as determined by analysis	Recovery of urea nitrogen	Average recovery in each series
		mg.	mg./100 ml.	mg./100 ml.	per cent	per cent
Feb. 1945	0.5 ml. of blood B*			27.7713		
	0.4 ml. of blood plus 0.1 ml. standard soln.	0.800	38.2170	35.4018	92.63	
	0.3 ml. of blood plus 0.2 ml. standard soln.	0.1600	48.6628	49.5836	101.89	
	0.25 ml. of blood plus 0.25 ml. standard soln.	0.2000	53.8857	51.3642	95.32	
	0.2 ml. of blood plus 0.3 ml. standard soln.	0.2400	59.1085	62.0478	104.97	
	0.1 ml. of blood plus 0.4 ml. standard soln.	0.3200	69.5543	66.4993	95.61	98.08
March 1945	0.5 ml. of blood C			13.0813		
	0.4 ml. of blood plus 0.1 ml. standard soln.	0.0800	26.4650	26.4358	99.89	
	0.3 ml. of blood plus 0.2 ml. standard soln.	0.1600	39.8488	38.9000	97.62	
	0.2 ml. of blood plus 0.3 ml. standard soln.	0.2400	53.2325	52.2545	98.16	
	0.1 ml. of blood plus 0.4 ml. standard soln.	0.3200	66.6163	67.3896	101.16	99.21

* Animal not subjected to 10-hour starvation period before bleeding.

Protein Content of Plasma

Both chemical and physical methods for determining the quantity of protein in plasma have been reported in the literature. The latter are based on certain physical characteristics of blood. It was found that specific gravity of the plasma paralleled the protein content very closely. The determination of specific gravity, therefore, became the basis for one method of analysis for the concentration of plasma proteins. Barbour and Hamilton (1926) developed a technique for the estimation of specific gravity, the principle of which involved timing the fall of a drop of body fluid of known size, through a definite distance in a mixture non-miscible with the fluid. Sensitivity of the falling drop method as determined by Barbour and Hamilton (1926) is 1×10^{-4}.

The relationship between specific gravity and protein content of plasma may be expressed by the equation of a straight line

$$P = 343(G - 1.0070)$$

in which P represents the grams of total protein per 100 ml. of plasma and G its specific gravity, calculated as specific gravity 20°C./20°C. (Moore and Van Slyke, 1930). Investigations by Nugent and Towle (1934) have shown that variations in the albumin and globulin ratio do not affect

the relationship to a measurable extent.

Protein content of plasma was determined in the present study by means of a La Motte falling drop densiometer (figure 2), and the procedure followed was essentially that of Barbour and Hamilton (1927). In their procedure heparin was used as the anticoagulant. In this study, it seemed desirable to use potassium oxalate in order to reduce the number of manipulations involved in the collection of blood. Its use meant that the sample for the determinations of urea nitrogen and protein content could be collected in the same tube. The use of oxalate also aided in conserving the amount of blood available for analysis. Before the use of oxalate was adopted, it seemed well to determine whether the specific gravity of plasma from oxalated blood was different from the specific gravity of plasma from heparinized blood. In this experiment, a known amount of oxalate was added to one-half of a sample of blood and heparin to the other half. After centrifugation the specific gravity of each sample of plasma was determined by the falling drop method. The specific gravities of both samples of plasma were identical, i.e., 1.0266.

In all of the determinations, a precise method was followed in oxalating the tubes used for collecting the blood. The solution of potassium oxalate containing 8 mg.

of potassium oxalate per ml. was prepared by accurately weighing 0.8 gm. of the oxalate and dissolving in organic-free distilled water. The resulting solution was diluted to a volume of 100 ml. with the solvent. One-half ml. of this solution was accurately measured and transferred to a 15 ml. centrifuge tube. The tube was rotated in such a way that a thin film of oxalate was spread on the walls of the tube in the region which the volume of blood (2 ml.) would occupy. The solution was then evaporated. Each tube was prepared just a short time before the blood was drawn for analysis.

After 1.5 ml. of the oxalated blood had been removed for the determination of the quantity of urea nitrogen, the remaining 0.5 ml. of blood was centrifuged for 15 minutes. The resulting plasma was drawn into an especially designed, calibrated dropping pipette. Care was exercised to prevent sucking in of air with the plasma. After the outer surface of the pipette had been wiped clean, the level of the plasma was adjusted to the upper mark. Again, no plasma was left adhering to the outer surface of the pipette. Then the pipette was placed in the special holder with the tip of the pipette below the surface of the non-miscible liquid. The plasma was forced down to the lower mark on the pipette by means of the drop compressor and the falling

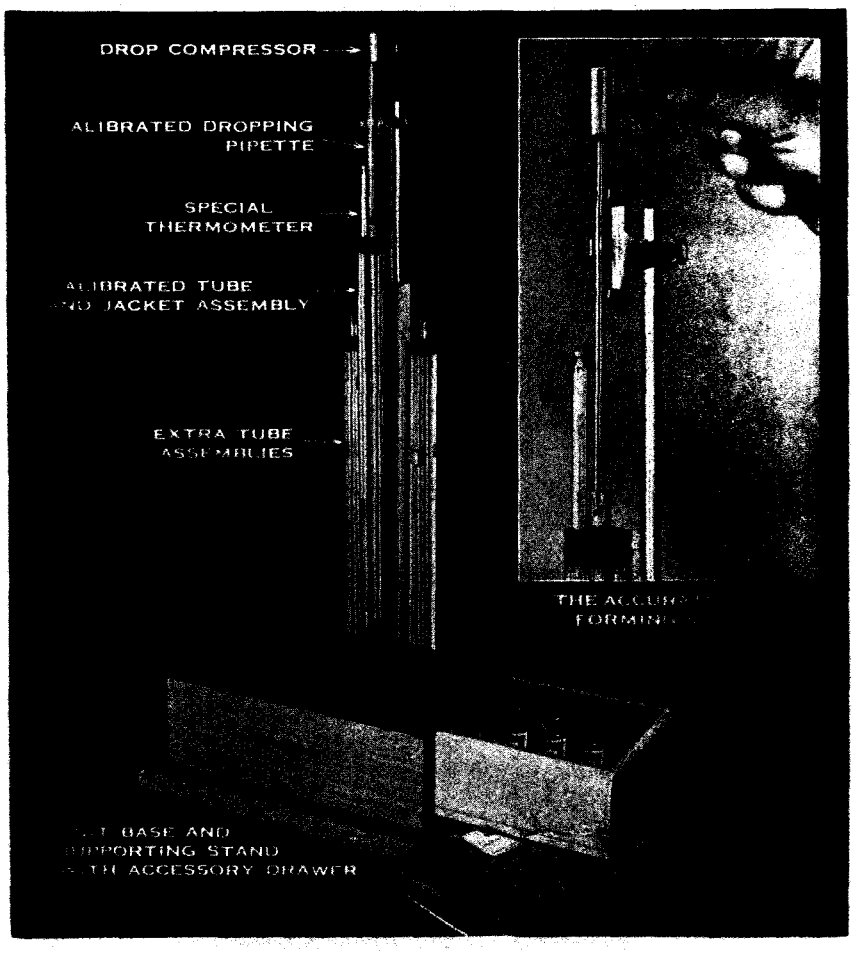

FIG. 2. LA MOTTE FALLING DROP DENSIOMETER

drop tube assembly lowered to release the drop of plasma thus formed. Time required for the drop to fall between the two graduations on the tube containing the non-miscible liquid was accurately determined with a precision timer and the temperature of the water-bath surrounding the non-miscible liquid was noted. This procedure was repeated at least three times for each sample of plasma. The falling time of a drop, similarly measured, of standard potassium sulfate solution having a specific gravity of 1.0150 at 20 degrees Centigrade was immediately determined in the same way.

The apparent density difference between the standard and non-miscible fluid, and between the plasma and non-miscible fluid were determined from an alignment chart (figure 3) using the falling drop times and temperatures of the samples. True density difference between the standard and plasma was obtained by algebraically subtracting the apparent density difference of the standard from the apparent density difference of the plasma. By adding the known specific gravity of the standard to the true density difference between the standard and plasma the density of the plasma was obtained. Protein content per 100 ml. of plasma was then calculated according to the formula:

$$P = 343\ (G - 1.0070)$$

Serum Calcium

A micro-method of analysis was chosen for the estimation of the quantity of calcium in serum in order to permit the determination of urea nitrogen, plasma proteins, and calcium in each sample of blood. In some cases, however, the volume of serum available was not sufficient to analyze duplicates. The procedure used was essentially that described by Holt and Callow in 1943.

Each sample of serum was filtered through a fritted-glass filter-stick (medium porosity) into a micro-beaker*. A measured volume of each filtrate (approximately 1 ml.) was transferred to a micro-beaker and 0.18 ml. of 0.5 per cent ammonium oxalate was added. The beaker was rotated to effect thorough mixing of the solutions. After 3 to 4 hours the liquid was filtered through a filter-stick and the precipitate in the beaker washed with 0.9 ml. of a 2 per cent ammonia solution, 0.18 ml. being added at a time and the beaker sucked dry after each addition. The precipitate was then dissolved in 0.4 ml. of 5N sulfuric acid and the resulting solution transferred through the filter-

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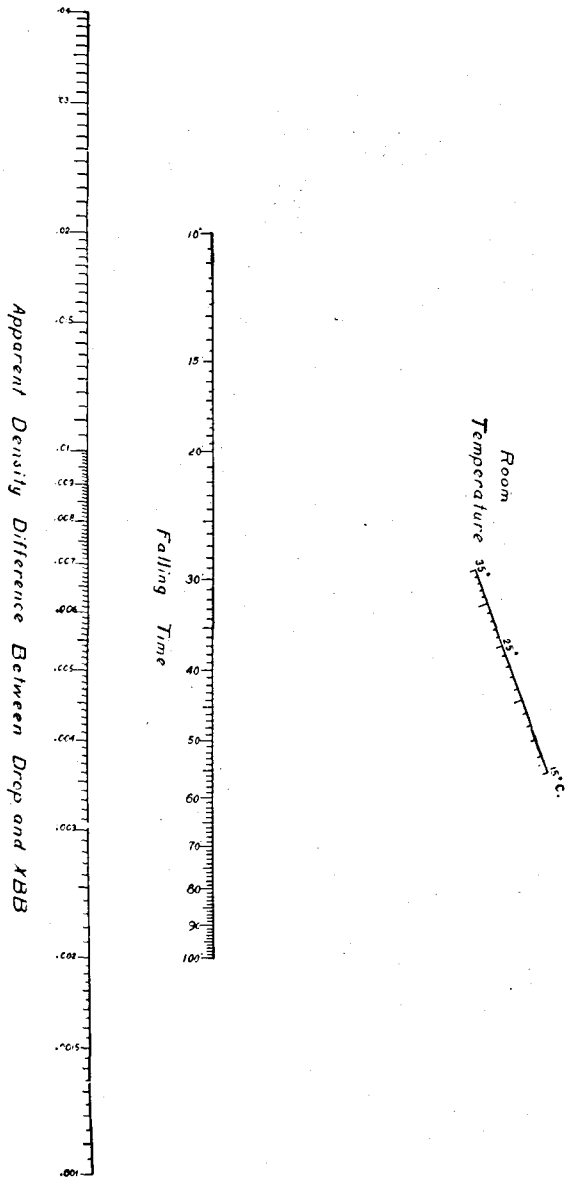
* Emich filtration apparatus, Johns, I. B., 1941, Laboratory Manual of Microchemistry, p. 21. Burgess Publishing Co., Minneapolis.

Alignment Chart for LaMotte Falling Drop Densiometer

After H. G. BARBOUR & W. F. HAMILTON
(*J. Biol. Chem. 1926-69-625*)



This chart is used for calculating specific gravity. A straight line (preferably a taut thread such as is supplied with this unit) connecting the observed falling time and the room temperature* reading intersects the density scale at the proper point.

* *This refers to water jacket temperature.*

Fig. 3. ALIGNMENT CHART

stick to a second micro-beaker. After heating the solution in boiling water for 3 minutes, it was titrated with approximately 0.01N potassium permanganate, prepared according to the method described by Halverson and Bergeim (1918). The permanganate solution was restandardized each time a set of serum samples was analyzed. Since the volume titrated is small a micro-burette with a very fine tip is essential for delivering the 0.01N permanganate solution.

A blank prepared by substituting 1 ml. of organic-free distilled water in place of serum filtrate was treated in the same way as the serum filtrate.

In cases where 0.6 ml. of serum or less was available for analysis, it was necessary to adjust the volume of the sample to 1 ml. with organic-free distilled water. Without this adjustment in volume, abnormally high values were obtained.

A series of dilutions of a standard solution of calcium acetate and two mixtures of serum and standard solution were analyzed and the quantity of calcium recovered determined. The data obtained are presented in table 6.

Table 6. Recovery of calcium in various dilutions of a standard solution and two mixtures of serum and standard calcium solution (containing 30 mg. per cent of calcium)

Solution	Actual quantity of calcium present	Calcium determined
1	0.060 mg. in 1 ml.	0.058 mg. in 1 ml. 0.061 mg. in 1 ml.
2	0.090 mg. in 1 ml.	0.089 mg. in 1 ml. 0.085 mg. in 1 ml. 0.088 mg. in 1 ml.
3	0.120 mg. in 1 ml.	0.121 mg. in 1 ml. 0.120 mg. in 1 ml.
4	0.150 mg. in 1 ml.	0.148 mg. in 1 ml.
1 ml. of serum A		0.136 mg. in 1 ml.
1 ml. of serum A plus 0.5 ml. of standard soln.	0.286 mg. in 1.5 ml.	0.273 mg. in 1.5 ml.
0.2 ml. of serum A		0.063 mg. in 0.2 ml.
0.2 ml. of serum A plus 1 ml. of standard soln.	0.363 mg. in 1.2 ml.	0.359 mg. in 1.2 ml.

RESULTS AND DISCUSSION

Many indices have been used by workers in the Nutrition laboratory at the Iowa State College to measure the effect of feeding a diet containing partially dried autoclaved pork muscle to pregnant rats. In the present study, the concentration of urea nitrogen, of plasma protein, and of serum calcium in the blood was studied. In evaluating the significance of the concentration of urea nitrogen, plasma protein, and serum calcium in the blood of the experimental animals, it was necessary to establish the effect of pregnancy on the three constituents under normal and experimental circumstances. This end was accomplished by determining the quantity of each of the constituents in the blood of pregnant and virgin animals in the control and pork-fed groups. The effect of food intake had been eliminated by a starvation period of approximately 10 hours prior to the time of analysis.

UREA NITROGEN

Effect of Pregnancy on Concentration of Urea Nitrogen in Blood

The quantity of urea nitrogen in the blood of a small group of rats was determined by a modification of the pro-

cedure described by Karr (1924). This procedure was then replaced by a method reported by Hoffman (1941), and the Hoffman method was used throughout the rest of the study. In order to determine whether the values obtained by the two methods were comparable, an analysis of variance was made of the quantities of urea nitrogen present in the blood of pregnant and virgin rats in the control and experimental groups, as determined by both methods (table 7). The differences were not significant; therefore, the values obtained by both methods were grouped together for final comparisons.

The average concentration of urea nitrogen in the blood of control gravid rats was 14.0 mg. per cent; that in the blood of the control virgin rats, 16.0 mg. per cent (table 8). Analysis of variance (table 9) shows that the difference is highly significant. Pregnancy *per se*, therefore, causes a reduction in the concentration of urea nitrogen in the blood.

The depressing effect of pregnancy on the urea nitrogen values in the blood of rats is in line with findings described in comparable studies with human beings. In normal pregnancy, the urea nitrogen value in blood is lower than is usual for the non-pregnant individual (Cantarow and Trumper, 1939; Williams and Wills, 1929). This reduc-

Table 7. Analysis of variance of concentrations of urea nitrogen in the blood of pregnant and virgin rats of the control and experimental groups as determined by two different methods

No. of rats		Groups compared	Source of variation	Degrees of freedom	Mean square	F
Karr	Hoffman					
10	26	Pregnant control rats, Karr method (mean, 13.78) and pregnant control rats, Hoffman method (mean, 14.07)	Items within the group	34	5.23	
			Between group means	1	0.65	
			Total	35		8.05
9	21	Pregnant pork-fed rats, Karr method (mean, 14.36) and pregnant pork-fed rats, Hoffman method (mean, 16.26)	Items within the group	28	13.19	
			Between group means	1	22.67	
			Total	29		1.72
6	23	Virgin control rats, Karr method (mean, 13.79) and virgin control rats, Hoffman method (mean, 16.62)	Items within the group	27	46.99	
			Between group means	1	38.28	
			Total	28		1.23
4	25	Virgin pork-fed rats, Karr method (mean, 11.27) and virgin pork-fed rats, Hoffman method (mean, 15.00)	Items within the group	27	12.07	
			Between group means	1	47.60	
			Total	28		3.94

Table 8. Average concentration of urea nitrogen in the blood of virgin and pregnant rats

Group	Diet	Reproductive status	Number of rats	Urea nitrogen*
				mg./100 ml.
Control	Steenbock V	Pregnant	36	14.0 ± 2.3**
		Virgin	29	16.0 ± 2.4
Experimental	Pork I	Pregnant		
		Non-toxic	30	15.7 ± 3.4
		Toxic	4	39.6 ± 5.5
		Virgin	29	14.5 ± 3.7

* The data on which the averages are based appear in table 6 in the appendix.

** Standard deviation

Table 9. Analysis of variance of concentrations of urea nitrogen in the blood of pregnant and virgin rats of the control and experimental groups

Groups compared	Source of variation	Degrees of freedom	Mean square	F
Pregnant control rats and virgin control rats	Items within the groups	63	5.45	
	Between group means	1	67.11	
	Total	64		12.31**
Pregnant non-toxic pork-fed rats and virgin pork-fed rats	Items within the groups	57	13.43	
	Between group means	1	21.84	
	Total	58		1.63

** Highly significant

tion in the concentration of urea nitrogen has been ascribed to both fetal and maternal demands (Harding, 1925). During pregnancy the gravid animal undergoes a general gain in tissue reserve, which is new protein laid down to meet the requirements of the growing fetus and the needs of future lactation. These new protein reserves are manufactured from amino acids which in the non-gravid individual are ordinarily deaminized.

Cantarow and Trumper (1939) suggest that the reduction in the concentration of urea nitrogen in the blood might also be attributed to altered kidney function in pregnancy; however, studies of renal function during normal pregnancy have yielded discordant results. It is also possible that the enormously increased vascularity of the pelvic organs and increased size of the uterus may result in significant changes in the rate and volume of blood flow through the kidneys, thereby affecting the elimination of urea in the urine.

In view of these findings, the pregnant control rat seems to be responding to the emergencies of pregnancy much as the human being does.

In the pregnant rats fed the diet containing pork, the picture is quite different from that found in normal pregnancy. The average concentration of urea nitrogen in the blood of the experimental, non-toxic, gravid rats was 15.7 mg. per cent; that in the blood of experimental virgin rats, 14.5 mg. per cent (table 8). It is interesting that the

physiological variation between individual rats, as measured by the size of the standard deviation, is of the same magnitude in the virgin and pregnant animals of the control group (table 8). The variation is definitely greater in all groups representing the pork-fed animals, which suggests that the feeding of the Pork I diet might be associated with a tendency toward a break in normal metabolic processes.

Analysis of variance (table 9) showed that the difference in the concentrations of urea nitrogen in the blood of the virgin and pregnant animals was not significant. However, the individual data (table 6, Appendix) show that in almost every case the concentration of urea nitrogen in the blood of the gravid animals was slightly greater than in the blood of the virgin rats. The average concentrations of urea nitrogen in the experimental groups indicate that pregnant rats maintained on the Pork I diet do not show the characteristic depression in the concentration of urea nitrogen in the blood that pregnancy normally produces.

Appraisal of the data obtained from pregnant control and pregnant, non-toxic, experimental groups (table 8) indicates that the difference between the average urea nitrogen values, 14.0 mg. per cent for the pregnant group fed the control diet and 15.7 mg. per cent for the pregnant

pork-fed rats, is significantly higher than normal (table 10). The difference, however, between the average urea nitrogen values in the blood of the virgin rats fed the two diets was not significant (table 10). Evidently, the feeding of the Pork I diet effects an elevation of urea nitrogen only in the blood of the pregnant animal.

Barnhart in 1942 determined the total non-protein nitrogen concentration in the blood of groups of rats similar to those studied in the present investigation. Her data, together with those obtained in the present study, are recorded in table 11. It may be noted that in the pork-fed, non-toxic, pregnant rats the average concentration of non-protein nitrogen in the blood was only slightly lower than the average concentration of this constituent in the blood of the virgin animals, indicating that an abnormally high concentration of non-protein nitrogen in the blood is associated with pregnancy in the pork-fed rats. This same phenomenon was noted in comparing the concentrations of urea nitrogen in the blood of pregnant and virgin pork-fed animals.

Peters and Van Slyke (1931) report that normally in human beings urea nitrogen represents approximately 37 per cent of the total non-protein nitrogen of whole blood. Whenever abnormally high concentrations of non-protein

Table 10. Analysis of variance of concentrations of urea nitrogen values in the blood of pregnant and virgin rats fed different diets

Groups compared	Source of variation	Degrees of freedom	Mean square	F
Pregnant control rats and pregnant non-toxic, pork-fed rats	Items within the group	64	8.91	
	Between group means	1	47.28	
	Total	65		5.31*
Virgin control rats and virgin pork-fed rats	Items within the group	56	9.62	
	Between group means	1	35.34	
	Total	57		3.67

* Significant

Table 11. Average concentrations of non-protein nitrogen and urea nitrogen in the blood of virgin and pregnant rats

Group	Diet	Reproductive status	Non-protein nitrogen		Urea nitrogen		Ratio of urea nitrogen to non-protein nitrogen
			No. of rats	Concentration	No. of rats	Concentration	
				mg./100 ml.		mg./100 ml.	
Control	Steenbock V	Pregnant	10	36.8	36	14.0	0.38
		Virgin	5	42.3	29	16.0	0.38
Experimental	Pork I	Pregnant (non-toxic)	10	35.5	30	15.7	0.44
		Virgin	9	37.5	29	14.5	0.38

nitrogen occur, urea nitrogen constitutes by far the greater proportion of the elevated value (Cantarow and Trumper, 1939). The same relationship seems to exist in rats, as shown by the data presented in table 11. In normal virgin and pregnant rats, the proportion of urea nitrogen to total non-protein nitrogen is 38 and 38 per cent respectively. In the virgin pork-fed rat, the ratio also is normal (38 per cent). When pregnancy occurs, however, the concentration of urea nitrogen in relation to non-protein nitrogen increases to 44 per cent in the non-toxic animal. Thus, the relative concentrations of total non-protein nitrogen and urea nitrogen and the relative proportions of each of these constituents in virgin and pregnant pork-fed rats suggest an abnormal protein metabolism in the experimental group or the presence of definite renal impairment.

It is interesting to speculate why the rats fed the Pork I diet do not show the drop in concentration of urea nitrogen in the blood, characteristic of pregnancy. Apparently some mechanism has been disturbed that is concerned with the amount of urea nitrogen appearing in the blood. Several different hypotheses might explain the phenomenon.

It is possible that there is some disturbance in nitrogen metabolism which brings about increased catabolism

instead of the increased anabolism characteristic of normal pregnancy. This disturbance would not allow the normal building up of protein reserves necessary for future lactation and fetal requirements and the concentration of urea nitrogen in the blood would be increased. Data reported by other investigators in the laboratory support this hypothesis. For example, the young of maternal rats fed the Pork I diet are smaller than those belonging to the control rats, and pork-fed females are not consistently able to raise their young. In those instances where lactation was established, the mother sacrificed her own tissues during the interval (Gray, 1936). In contrast to the loss in body weight of approximately 8 gm. from the fourth to the seventeenth day of lactation in the pork-fed females, the Steenbock V rats gain 9 gm. in the same period. Campbell in 1940 reported that the average weight of young rats, alive at birth, in the Pork I group was 4.8 gm., of the rats in the control group 5.1 gm. Also, only one-half as many rats were born alive in the pork-fed group as in the control. In the present study, when the females in the experimental and control groups were matched according to the number of live feti present, the blood urea nitrogen values for the pork-fed rats were higher than those determined for the control animals (See table 12). The concen-

Table 12. Average concentration of urea nitrogen in blood of control and experimental rats arranged according to the number of live feti found in the uteri at necropsy

No. of rats		No. of feti	Av. concentration of urea N	
Steenbook V	Pork I		Steenbook V	Pork I
			mg./100 ml.	mg./100 ml.
2	4	7	15.6	16.4
2	2	8	15.9	15.6
3	3	9	13.8	20.1
12	5	10	13.3	16.2
9	3	11	14.8	16.5
5	3	12	12.4	15.3

tration of urea nitrogen in the blood of the pork-fed rats showed no relationship to the number of live feti present; however, there was a progressive decrease in urea nitrogen values characteristic of the control rats as the number of feti increased. These findings would seem to indicate that in the pork-fed pregnant animal anabolism of protein is not increased as it is in the control animal. Here again, is a suggestion of a fundamental abnormality, basic perhaps to the appearance of toxic pregnancy.

Renal efficiency must also be considered, for when the capacity of the kidney to excrete urea nitrogen is reduced an elevation of this constituent occurs in the blood (Best and Taylor, 1939). In the present study, only one gross sign of renal damage was found in the non-toxic pregnant test rats at necropsy. Swelling of the kidney, as judged by a lack of coincidence in the halves of the kidney after the organ was bisected, was observed in approximately 60 per cent of the pregnant experimental animals against 40 per cent of the pregnant control rats. This phenomenon was observed in approximately 30 per cent of the virgin rats in both groups. In pregnancy the stress on the kidney is increased, but the kidneys of the control rats are still able to excrete urea nitrogen normally; the kidneys of the pork-fed rats, however, may not be excreting urea nitrogen

normally, for the concentration of this constituent in the blood is higher than normal. Determination of the concentration of urea nitrogen in urine excreted by these animals might shed some light on the factor responsible for the abnormally high concentration of urea nitrogen in the blood. Low values for urinary urea nitrogen would indicate that impairment of renal function was the causative factor. High values for urinary urea nitrogen, however, associated with the high concentration of urea nitrogen in the blood would indicate that increased protein catabolism was taking place. The determination of urea nitrogen in the urine excreted by the pork-fed animals seems worthy of consideration for future study.

Another explanation for the absence of the expected reduction of urea nitrogen in the blood of pregnant pork-fed rats is brought out by work by Lefebvre (1941). She reported that non-toxic, pregnant rats fed the pork-containing diet show no renal dysfunction as judged by the ability of the kidney to concentrate urine and withhold albumin, but there was a significant reduction in urine volume. This reduction in urine volume may be responsible for the elevation of urea nitrogen in the blood, because when urine volume is reduced below the augmentation limit the rate of urea excretion varies directly as the

square root of the urine volume when the blood urea remains constant (Best and Taylor, 1939).

Urea Nitrogen Concentration in Blood in Toxemic Pregnancy

Of the pregnant rats fed the pork-containing diet, six developed the typical symptoms of pregnancy toxemia, and necropsy findings were similar to those described in the section in the introduction on toxemic pregnancy in the rat. Urea nitrogen values in the blood were determined in four of these animals. In every case, the concentration of urea nitrogen was abnormally high, the average value 39.6 mg. per cent being 2.6 times as high as the average concentration determined for non-toxic pork-fed rats. Urea nitrogen values for the individual rats were 34.5, 37.1, 46.0, and 40.7 mg. per cent. The pregnancy disease, therefore, is accompanied by an increase in the concentration of urea nitrogen in the blood. These findings are in agreement with the high concentration of non-protein nitrogen in the blood of toxic rats as reported by Barnhart (1942). She reports an average concentration of 76.5 mg. per cent. Urea nitrogen to non-protein nitrogen ratios calculated from data presented by Barnhart and data determined in the present study, suggested a definite abnormality in nitrogen

metabolism even in those rats that did not exhibit toxic symptoms. The urea nitrogen/total non-protein nitrogen ratio in the blood was 6 per cent above normal, i.e., 38 vs. 44 per cent. The increase in the ratio of the concentration of these constituents in the blood of the toxic rat, 52 per cent, is, therefore, not unexpected. In the blood of the normal pregnant rat, the percentage of unidentified non-protein nitrogen constituents is 62 per cent, in the toxic rat, 48 per cent. This observation indicates that the increase in the concentration of non-protein nitrogen in the blood of the toxic rat is due to the increase in the concentration of urea nitrogen and not due to an increase in uric acid, creatinine, or undetermined nitrogen fractions. As cited in the previous section Cantarow and Trumper report that, in human beings, elevations of total non-protein nitrogen in the blood are generally due to an increase in the concentration of urea nitrogen. The relation of urea nitrogen to non-protein nitrogen is often used as an indication of the degree of toxemia in human beings (McIlroy, 1936).

The liver undoubtedly plays the predominant role in the production of urea as shown by studies with hepatectomized dogs and in cases of extensive hepatic and renal damage in man. Severe hepatic damage has been shown to

occur in toxic rats by Armstrong (1939); however, decreased hepatic function should result in low concentration of urea nitrogen in the blood and this is not the case in toxic rats, as shown by the above average value. It does not seem possible; therefore, that hepatic dysfunction is associated with the high concentration of urea nitrogen in the blood of the sick rats.

It is interesting that in human cases of toxemia, examination of the blood for urea nitrogen content is used in differentiating hepatic and renal toxemias. In liver dysfunction the concentration of urea nitrogen is low, but in renal impairment the concentration of this constituent is high (McIlroy, 1936).

Renal insufficiency has been postulated in the previous section as the possible cause of the lack of depression in urea nitrogen values in the blood of pregnant, non-toxic, pork-fed rats, and it might also explain the abnormally high urea nitrogen values in the blood of the toxic rats. The only abnormality noted in gross examinations of the kidneys of the non-toxic pregnant animals receiving the pork-containing diet was some swelling of the organ. In the toxic rat, however, the kidneys were so hyperemic that little or no differentiation between the anatomical structures was discernible. Histological studies by Armstrong (1939) con-

firm the findings noted in gross observations of the two groups. Renal lesions in the non-toxic animals were mild and never approached the severity of those occurring in the toxic animals. It seems reasonable to believe, then, that with the increased damage that occurs in the kidney in the toxic rats, renal efficiency is further decreased and as a result abnormally high concentrations of urea nitrogen appear in the blood. However, Armstrong showed that the damage in the kidney of the toxic rat involved only the tubular structures as the glomeruli appeared normal. With tubular damage one would expect the concentrating power of the kidney to be reduced and as a result, a low concentration of urea nitrogen in the blood. However, as shown in the present study, the concentration of urea nitrogen is not decreased but is increased in the blood of the toxic rat. This suggests that some other factor may be responsible for the rise in the concentration of urea nitrogen in the blood in the toxic animal. It is worthy of note, that two pork-fed gravid rats not exhibiting external symptoms of toxemia, but upon necropsy examination showing evidence of very yellow, friable livers and swelling and congestion in the kidneys, did not have abnormally high concentrations of urea nitrogen in the blood.

As previously cited, Lefebvre suggested that water

retention is characteristic of the pregnant pork-fed rat. This water retention has been considered a possible factor responsible for the absence of a fall in the concentration of urea nitrogen in the blood of pregnant pork-fed rats. Water retention may also be a predisposing cause for the high urea nitrogen values observed in the toxic rats, for the abnormally large gain in weight made by the sick rats, the appreciably greater water consumption during gestation by these animals, and the presence of large quantities of fluid in the abdominal and pleural cavities as reported by Armstrong and Swanson (1943) point to considerable water retention in these animals.

PROTEIN CONTENT OF PLASMA

Effect of Pregnancy on Concentration of Protein in Plasma

The average concentration of total protein in the plasma of control gravid rats was 5.93 gm. per 100 ml.; that in the plasma of the control virgin rats, 7.72 gm. per 100 ml. (table 13). Analysis of variance (table 14) shows that the difference is highly significant. Pregnancy *per se*, therefore, seems to cause a reduction in the total protein content of the plasma.

The decrease in the concentration of plasma protein in normal pregnancy has also been found to occur in human beings (Best and Taylor, 1939; Strauss, 1935). This

Table 13. Average concentration of protein in plasma of virgin and pregnant rats

Group	Diet	Reproductive status	Number of rats	Concentration of protein in plasma*
				gm./100 ml.
Control	Steen-bock V	Pregnant	9	5.93 ± 0.81**
		Virgin	13	7.72 ± 1.01
Experi-mental	Pork I	Pregnant		
		Non-toxic	11	6.53 ± 1.21
		Toxic	2	4.98
		Virgin	12	8.23 ± 1.02

* The data on which the averages are based appear in table 7 in the appendix.

** Standard deviation

Table 14. Analysis of variance of concentrations of protein in plasma of pregnant and virgin rats of the control and experimental groups

Groups compared	Source of variation	Degrees of freedom	Mean square	F
Pregnant control rats and virgin control rats	Items within the groups	20	0.85	
	Between group means	1	17.10	
	Total	21		20.12**
Pregnant non-toxic pork-fed rats and virgin pork-fed rats	Items within the groups	21	1.24	
	Between group means	1	16.47	
	Total	22		13.28**

** Highly significant

decrease has been attributed, in part at least, to the appreciably increased plasma hydration that normally occurs during pregnancy. It has been shown, however, that not only the concentration but also the total amount of circulating plasma protein is diminished. Melnick and Cowgill (1937) believe that synthesis of body proteins in the fetus constitutes a drain upon the maternal organism which is of primary importance in causing the lowered plasma protein concentration characteristic of pregnancy.

Studies made by Weedman (1942) with rats, raised under conditions similar to those employed in the present study, showed that the average percentage of water in the blood of the pregnant control rats was significantly higher than that in the blood of non-gravid rats given the same diet. This finding suggests that the decrease in concentration of plasma protein in the pregnant rat as determined in the present study might be due only to the hydration of the plasma, which normally occurs in pregnancy. The amount of hydration of the blood in pregnancy (2.9 per cent in Steenbock V rats, 2.8 per cent in Pork I rats) was calculated from the average figures reported by Weedman. The value calculated for the concentration of plasma protein as influenced by hydration was 7.5 gm. per cent in the Steenbock V rats, 8.0 gm. per cent in the Pork I rats. Comparison of these

values with values obtained for the protein content (5.9 gm. per cent for the Steenbock V rats, 6.5 gm. per cent for the Pork I rats) in the present study indicate that the diluting effect of hydration in pregnancy is not great enough to account for all of the reduction observed in the concentration of plasma protein in pregnant rats.

In view of these findings, the pregnant control rat again appears to respond to the demands of pregnancy much as does the human being.

In the experimental group, the concentration of protein in plasma was similar to that determined in the control group. The average concentration of protein in the plasma of pork-fed, non-toxic, gravid rats was 6.53 gm. per 100 ml.; that in the plasma of pork-fed virgin animals, 8.23 (table 13). Analysis of variance (table 14) shows that the difference is highly significant. Pregnancy *per se*, therefore effects a reduction in protein content of plasma in the pork-fed rats similar to that observed in the control animals.

Analysis of the data obtained from the rats fed the control and pork-containing diets indicates that the difference between the average plasma protein values, 5.93 gm. per 100 ml. for the pregnant control group and 6.53 gm. per 100 ml. for the non-toxic, pregnant pork-fed group,

is not significant (table 15). The difference between the average plasma protein values of the virgin rats fed the two diets also is not significant. Apparently, the feeding of the pork-containing diet has no effect on the concentration of protein in the plasma, and changes in this constituent do not represent a primary causal symptom of the disorder.

Protein Concentration in Plasma in Toxemic Pregnancy

The concentration of protein in plasma was determined in two of the rats that developed toxemia. The values obtained were very similar, i.e., 4.87 and 5.08 gm. per 100 ml. of plasma. Both rats exhibited the signs of severe toxemia and necropsy findings revealed internal conditions similar to those described in the section on toxemic pregnancy in the rat. The concentration of plasma protein as determined in the toxic rats indicates that there is a decrease in the protein content of plasma in toxemic pregnancy. This finding is in line with studies of eclampsia in human beings (table 1).

In the two rats, described in a previous section (Urea Nitrogen Concentration in Blood in Toxemic Pregnancy), as exhibiting no external signs of toxemic pregnancy, but whose livers and kidneys showed gross abnormalities.

Table 15. Analysis of variance of concentrations of protein in plasma of pregnant and virgin rats fed different diets

Groups compared	Source of variation	Degrees of freedom	Mean square	F
Pregnant control rats and pregnant non-toxic pork-fed rats	Items within the groups	18	1.10	
	Between group means	1	1.83	
	Total	19		1.66
Virgin control rats and virgin pork-fed rats	Items within the groups	23	1.04	
	Between group means	1	1.62	
	Total	24		1.56

suggestive of the toxic condition, the concentration of total plasma protein was within the normal range, 6 to 8 gm. per 100 ml. of plasma. This finding seems to indicate that the reduction in the concentration of plasma protein in the toxic rat is a fairly rapid process.

The origin of plasma proteins seems to be in the liver, as indicated by the occurrence of hyproteinemia in liver damage produced by poisoning with carbon tetrachloride or by hepatectomy (Berryman and Bollman, 1943). Histological studies by Armstrong (1939) show that the cells of the livers from non-toxic pork-fed animals remain intact though they are interspersed with fat, while liver cells in the toxic rats show an actual cell degeneration. The fact that the concentration of plasma protein in the non-toxic rat was not significantly different from that observed in the control gravid animal seems to indicate that production of plasma protein by the liver is not seriously impaired until actual cell degeneration in the liver takes place.

Edema is a very prominent clinical feature of reduced plasma protein concentration. The hydration of tissue characteristic of toxemic pregnancy in the rat is indicated by the increased water content of the kidney, muscle and liver in the gravid animals exhibiting toxic symptoms (Weedman, 1942). In these animals a considerable amount

of fluid is also found in the peritoneal cavity (Armstrong and Swanson, 1943). Although the degree of hypoproteinemia in toxic rats as indicated in the present study may not in itself be sufficient to produce edema to the extent that it seems to exist in the toxic syndrome, it probably favors the development of water retention by the tissues in the presence of some other contributing factor such as increased capillary permeability or increased capillary pressure or a combination of the two. This phenomenon has been reported by Strauss (1935, 1939) as occurring in toxemia of pregnancy in human beings.

SERUM CALCIUM

Effect of Pregnancy on Concentration of Calcium in Serum

In both the control and experimental rats the effect of pregnancy per se, on the concentration of calcium in serum was the same. The average concentration of calcium in the serum of control gravid rats was 8.45 mg. per cent; that in the serum of the virgin control rats 10.16 mg. per cent (table 16). Analysis of variance (table 17) shows that the difference is highly significant. Pregnancy per se, therefore, causes a reduction in the concentration of serum calcium. This phenomenon also occurs in human beings (Cantarow, 1933; Coons and Blunt, 1930; Peters and Van Slyke, 1931). Reports in the literature show that during

Table 16. Average concentration of calcium in serum of virgin and pregnant rats

Group	Diet	Reproductive status	Number of rats	Calcium in serum*
				mg./100 ml.
Control	Steenbock V	Pregnant	7	8.45 ± 1.17**
		Virgin	11	10.16 ± 0.72
Experimental	Pork I	Pregnant		
		Non-toxic	9	9.26 ± 1.17
		Toxic	4	9.77 ----
		Virgin	11	10.80 ± 1.18

* The data on which the averages are based appear in table 8 in the appendix.

** Standard deviation.

Table 17. Analysis of variance of concentrations of calcium in serum of pregnant and virgin rats of the control and experimental groups

Groups compared	Source of variation	Degrees of freedom	Mean square	F
Pregnant control rats and virgin control rats	Items within the groups	16	0.83	
	Between group means	1	12.43	
	Total	17		14.98**
Pregnant non-toxic pork-fed rats and virgin pork-fed rats	Items within the groups	18	1.17	
	Between group means	1	11.74	
	Total	19		10.03**

** Highly significant

pregnancy, calcium is retained by the maternal organism. The amount of calcium retained is more than can be accounted for by fetal utilization and may represent the establishment of a reserve supply (Coons and Blunt, 1930). This storage of calcium is associated with a tendency toward a diminution in the concentration of serum calcium. The mechanism underlying these changes is not clearly understood (Cantarow and Trumper, 1939).

A similar reduction in the concentration of serum calcium was observed in the experimental pregnant rats. The difference between the average concentrations of serum calcium in the non-toxic pregnant and virgin animals in this group (table 16) was highly significant (table 17). Appraisal of the data given in table 16 indicates that the difference between the average concentrations of serum calcium, 8.45 mg. per cent for the pregnant group fed the control diet and 9.26 mg. per cent for the non-toxic pregnant pork-fed rats, is not significant (table 18). When virgin animals fed the two diets are compared in the same manner as were the pregnant rats, analysis of variance shows that the data representing the concentrations of serum calcium in the two groups, i.e., 10.16 and 10.80 mg. per cent, are not significantly different (table 18). Apparently, feeding of the pork-containing diet does not affect the concentration

Table 18. Analysis of variance of concentrations of calcium in serum of pregnant and virgin rats fed different diets

Groups compared	Source of variation	Degrees of freedom	Mean square	F
Pregnant control rats and pregnant non-toxic pork-fed rats	Items within the groups	14	1.09	
	Between group means	1	2.53	
	Total	15		2.32
Virgin control rats and virgin pork-fed rats	Items within the groups	20	0.96	
	Between group means	1	2.23	
	Total	21		2.32

of calcium in the serum of either the pregnant or virgin animals.

Calcium Concentration in Serum in Toxemic Pregnancy

The concentrations of calcium in the serum of four rats that developed typical symptoms of severe toxemia were determined in the present study. Sufficient blood was obtained from two of these animals so that the concentrations of urea nitrogen and plasma protein also could be determined.

The values obtained for serum calcium were within the normal range, 8 to 12 mg. per cent in every case, the average value being 9.77 mg. per cent. These findings are in agreement with many reports in the literature on eclampsia in the human being (table 1). Some investigators, however, have reported subnormal values for serum calcium in eclampsia and in pregnancy toxemia in sheep. Others have reported increased serum calcium values in this condition. The majority of the papers examined by the present investigator reported normal calcium levels in eclampsia. Peters and Van Slyke (1932) also state that in pregnancy toxemias serum calcium appears to be unaltered.

Because of the fact that about one-half of the total serum calcium is bound in some way to the plasma protein,

alterations in the plasma protein concentration may be expected to result in a similar change in the concentration of serum calcium. This has been found to be the case both clinically and experimentally (Hoffman, 1941; Cantarow and Trumper, 1939). From the data given for control and non-toxic experimental pregnant and virgin rats, it can be seen that concentrations of both plasma proteins and serum calcium are lowered in the normal pregnant state. In the toxic animals, however, the picture is different. The concentration of plasma protein is reduced below the value occurring normally in pregnancy and the concentration remains at non-pregnant levels.

Reports in the literature (Best and Taylor, 1939; Cantarow and Trumper, 1939) indicate that the albumin fraction of plasma protein is of much greater importance than globulin or fibrinogen in the calcium-binding action. The fact that the concentration of serum calcium in the toxic rats was not reduced in the presence of reduced plasma protein values in the same animals might indicate that the amount of albumin fraction was normal and that the reduction in total protein was due to a decrease in globulin and fibrinogen fractions.

In this connection, too, it should be pointed out that the albumin fraction, due to the small size of its molecule,

exerts a greater osmotic pressure than either globulin or fibrinogen. A decrease in this fraction, therefore, is considered an important factor in water retention in the tissues. As cited previously, the studies by Weedman (1942) indicate that water retention in the tissues occurs in toxic rats, which might lead to the conclusion that the concentration of albumin in the plasma is seriously reduced. The calcium values determined in toxic rats do not seem to support this statement. Some other factor such as increased capillary permeability might be responsible for the accumulation of fluid in the tissues. Greater capillary permeability might also account for the seeping of fluid into the abdominal and pleural cavities and thereby for the lowered concentration of plasma protein. Cantarow (1938) observed a rapid diminution of the protein of the blood of dogs after removal of ascitic fluid, which contained large amounts of protein.

In relation to this problem, the influence of the parathyroid gland cannot be ignored, although the mechanism of the action of the hormone of this gland is not clearly defined. The primary fundamental effect of this hormone appears to be to increase the diffusible fraction of serum calcium (Cantarow, 1938). If this is the case, the concentration of serum calcium could be within the

normal range even though the protein-bound calcium was decreased. There is evidence in the literature that diminution of the blood calcium concentration directly stimulates the secretory activity of the parathyroid glands (Patt, Wallerstein, and Luckhardt, 1942; Patt and Luckhardt, 1942). It has also been suggested that the pituitary secretions may influence parathyroid activity (Smith, 1927; Collip, 1940). Studies with injections of parathyroid hormone show that repeated administration of large doses of this hormone result in the development of a functional insufficiency of the renal tissue resulting in a slight increase in serum calcium, an increase in serum phosphate, and an increase in the concentration of the non-protein nitrogen constituents in the blood (Cantarow, 1933; Schmidt and Greenberg, 1935). Might hyperparathyroidism be an important factor in the toxic syndrome? If so, it seems that the pituitary might again be implicated, particularly if it exerts some hormonal control on the activity of the parathyroid gland. Because of the far-reaching effects of the hormones produced by these glands, the effect of their administration might be worthy of future study in connection with toxemia of pregnancy in rats.

SUMMARY AND CONCLUSIONS

A disorder of pregnancy resembling the so-called toxemia of pregnancy in human beings has been noted among rats by workers in the animal research laboratory of the Nutrition Section of the Iowa Agricultural Experiment Station. This disorder did not occur among gravid rats fed the usual stock ration but made its appearance among pregnant females fed a pork-containing diet, designated as Pork I. An attempt has been made, herein, to assemble the characteristic symptoms and pathological findings of the gestational disturbance in rats, as reported by other investigators in the laboratory, and to relate these observations to each other and to appropriate experimental conditions reported in the literature.

An absence of a depression in total non-protein nitrogen constituents in the blood, normally found in pregnancy, is characteristic of pregnant non-toxic pork-fed rats. Abnormally high concentrations of this fraction characterize the blood of rats fed the Pork I diet when pregnancy disease develops. Another typical finding in toxemic pregnancy is increased hydration of tissues, with abdominal and pleural cavities also containing considerable amounts of free fluid.

The present investigation was undertaken to determine whether an increase in urea nitrogen was the fraction responsible for the increase in total non-protein nitrogen in the blood, and whether a lowered concentration of plasma proteins might be responsible for the increase in moisture content of the tissues. Prior to the present investigation, no work had been done on the concentration of inorganic constituents in the blood of the toxic animals. In the present study, the effect of feeding the pork-containing diet on the concentration of serum calcium and the concentration of this constituent in the serum of those animals developing toxic pregnancy was determined.

When young albino rats, Wistar strain A, had reached sexual maturity, each rat was assigned to one of four experimental groups and fed the specific diet chosen for that group. The groups were classified as follows:

1. Virgin rats fed the stock colony ration, Steenbock V, which served as the control diet,
2. Virgin rats given the experimental Pork I diet,
3. Pregnant females fed the Steenbock V ration,
4. Pregnant rats fed the Pork I diet.

Blood was drawn from the abdominal aorta at the beginning of the twenty-second day of the second pregnancy in the case of gravid rats and from virgins when they were

of approximately the same age as the pregnant animals. A portion of the blood was oxalated and used for the determination of the respective concentrations of urea nitrogen and plasma protein. The rest of the blood was allowed to clot and the concentration of calcium in the serum determined. At necropsy, the physical condition of the rats was noted and the organs examined in a systematic fashion.

Group comparisons were made of average values for the concentrations of urea nitrogen, plasma protein, and serum calcium. These comparisons showed two things, i.e., the effect of pregnancy per se and the influence of the feeding of the pork diet.

Results may be summarized as follows:

A. Effects of pregnancy, per se;
   1. A highly significant decrease in the concentration of urea nitrogen in the blood,
   2. A highly significant decrease in the concentration of protein in the plasma,
   3. A highly significant decrease in the concentration of serum calcium.

B. Effect of feeding the pork diet;
   1. A significant increase in the concentration of urea nitrogen in the blood of the pregnant animal over that characteristic of the control

pregnant animal,

2. No significant change in the concentration of urea nitrogen in the blood of the virgin animal,
3. No significant change in the concentration of protein in plasma in either virgin or pregnant animals,
4. No significant change in the concentration of calcium in serum in either virgin or pregnant animals.

The only significant change of the constituents studied in the non-toxic rats, which could be associated with the feeding of the Pork I diet was a lack of the depression of the concentration of urea nitrogen in the blood of the pregnant rats.

In pregnant pork-fed rats that developed the toxic syndrome, however, the concentration of urea nitrogen in the blood was abnormally high, the concentration of plasma protein was lower than that determined for the gravid control rats, and the concentration of serum calcium was approximately the same as that determined for the non-gravid control rats.

Thus it can be concluded that the feeding of the diet containing pork does not cause abnormal changes in the concentration of plasma protein or serum calcium in the blood

of albino rats, either in the virgin state or during uneventful pregnancy. Following the feeding of this diet, however, the characteristic depression expected in pregnancy of the urea nitrogen in the blood does not occur. There is clear evidence of a break in normal metabolic processes related to the utilization of nitrogen following the feeding of the pork diet. That this disturbance is a fundamental contributing factor to the appearance of the toxic syndrome seems assured because it appears in the nontoxic as well as the toxic animal.

An attempt has been made to correlate the findings in the present study with those of other investigators in the laboratory who have studied pregnancy disease as it is produced by dietary manipulation in the albino rat.

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ACKNOWLEDGEMENTS

The author wishes to express her appreciation to Dr. Pearl P. Swanson for her inspiration during the years spent in investigating the problem and for her guidance in the preparation of this manuscript.

Grateful acknowledgement is also made to Dr. Ethelwyn Wilcox and Mary Barnhart Kooi for their assistance in the initiation of the research and to the other workers in the laboratory for their generous assistance.

A P P E N D I X

TABLE OF CONTENTS OF APPENDIX

PREPARATION OF REAGENTS

Organic-free Water

Ordinary distilled water to which an alkaline permanganate solution had been added to a deep rose color was re-distilled. A Kjeldal connecting bulb was inserted into the distillation apparatus to prevent the spraying of the alkaline permanganate solution into the distillate. The first portion (one-fourth) of the distillate was discarded. About two-thirds of the remainder was collected and stored in a tightly stoppered flask.

Alkaline Permanganate Solution

The alkaline permanganate solution (Mason, 1917) was prepared by adding 200 gm. of KOH and 8 gm. of pure $KMnO_4$ crystals to 1250 ml. of distilled water. The mixture was boiled to a final volume of 1000 ml., cooled, and stored in a dark glass bottle tightly stoppered.

Sodium Hydroxide, 0.1 N

A saturated solution of NaOH was prepared by shaking 110 gm. of best quality NaOH with 100 ml. of distilled water in a tightly stoppered Erlenmeyer flask. The mixture was then allowed to stand until the Na_2CO_3 settled,

leaving a clear solution of NaOH. This solution contains about 75 gm. NaOH per 100 ml. (Hawk and Bergeim, 1937). A 0.1 N solution was prepared by dilution of the saturated solution and titrating against $KHC_8H_4O_4$ as a primary standard with a 1 per cent alcoholic solution of phenolphthalein as the indicator. The exact normality was determined.

Hydrochloric Acid, 0.1 N

Nine ml. of concentrated HCl, approximately 12 N, was diluted to 1000 ml. with organic-free water. The exact normality was determined by titrating against standard NaOH with phenolphthalein as the indicator.

Sodium Hydroxide, 10 per cent

133.33 ml. of a saturated solution of NaOH were diluted with organic-free water to 1 l. The percentage concentration was checked by titrating against standard HCl with phenolphthalein as the indicator. The exact percentage was calculated and the solution rediluted to make it 10 per cent. The percentage concentration of the NaOH was then redetermined.

Nessler's Solution

Nessler's solution was prepared according to the directions given by Koch and McMeekin (1924). 22.5 gm. of

iodine were dissolved in 20 ml. of organic-free water containing 30 gm. of potassium iodide. After the solution was complete, 30 gm. of pure metallic mercury were added and the mixture was shaken. To keep the mixture from getting hot the flask was held in running tap water during the shaking process. This was continued until the supernatant liquid had lost all of the yellow color due to the iodine. The supernatant aqueous solution was decanted and a few drops were tested by adding to 1 ml. of starch indicator. Unless the blue color of the starch-iodine complex is obtained, the solution may contain mercurous compounds. If the starch test was negative a few drops of an iodine solution of the same concentration as the one first used were added until a faint excess of free iodine could be detected with the starch test. Then the solution was diluted to 200 ml. with organic-free water, and mixed well. The entire solution of HgI_2 was added to 975 ml. of an accurately prepared 10 per cent NaOH solution and thoroughly mixed. The solution cleared upon standing.

Starch Indicator

One gm. of soluble starch (Merck's Lintner) and 5 mg. of HgI_2 were added to 1 l. of organic-free water. The solution was boiled until clear, cooled, and stored in a glass-stoppered bottle in the refrigerator.

Sulfuric Acid, 1/12 N

2.5 ml. of concentrated sulfuric acid, C. P., (arsenic acid and nitrogen-free) were diluted to 1 l. with organic-free water and mixed thoroughly. The normality of the solution was tested against standard NaOH and the exact normality calculated. The solution was rediluted to 1/12 N and restandardized.

Sodium Tungstate, Approximately 10 per cent

Ten gm. of sodium tungstate (Merck's according to Dr. Folin) were dissolved in slightly warm water. After cooling to room temperature, the solution was diluted to 100 ml.

Oxalated Centrifuge Tubes

Potassium oxalate in a concentration of 0.2 per cent is effective in preventing the coagulation of blood (Peters and Van Slyke, 1932). Two solutions of potassium oxalate were prepared so that 0.5 milliliter was equivalent to 4.00 mg. or 6.66 mg. of oxalate when dried in a 15 ml. centrifuge tube. These quantities were adequate to prevent the clotting of 2 or 3 ml. samples of blood, respectively. After 0.5 ml. of the proper oxalate solution had been transferred to the centrifuge tube, the tube was rotated in such a way as to spread a thin film of oxalate

solution on the walls of the tube in the region which the volume of blood would occupy. After drying, a thin layer of oxalate lined the tube.

Buffer Solution

The buffer solution was prepared according to the directions given by Karr (1924). 14 gm. of sodium pyrophosphate were dissolved in a small quantity of 0.5 N H_3PO_4 and then diluted to 100 ml. with the solvent.

Phosphoric Acid, 0.5 N

Twenty ml. of 85 per cent ortho phosphoric acid were diluted to 1 l. The normality of the solution was checked by titrating against standard NaOH with phenolphthalein as the indicator, to the first faint pink color. The exact normality of the solution was calculated, and the acid was diluted to a substantially correct 0.5 N solution. The acid solution was then restandardized.

Urease Solutions

For Karr Method of Analysis

One-fourth of a 0.1 gm. tablet of Squibb urease was ground in a mortar and dispersed in 1 ml. of organic-free water. A fresh solution of urease was prepared for each sample of protein-free filtrate.

For Hoffman Method of Analysis

A 0.1 gm. tablet of Squibb urease was ground in a mortar with 10 ml. of approximately 0.05 M Na_2HPO_4. A fresh urease solution was prepared for each sample of blood and was used before 2 hours had elapsed after preparation.

The urease solution was not allowed to come in contact with mercury salts as these inactivate the enzyme. The test tubes used for incubation, therefore, were not of the same type as those used for the nesslerization.

Sodium Acid Phosphate, Approximately 0.05 M

8.9 gm. of $Na_2HPO_4 \cdot 2H_2O$ were dissolved in organic-free water and diluted to a volume of 1 l.

Standard Solutions of Urea

For Karr Method of Analysis

The stock standard solution of urea was prepared by dissolving 0.643 gm. of pure urea in organic-free water and diluting to a volume of 1 l. One ml. of chloroform was added as a preservative. This solution contained 0.3 mg. of urea nitrogen per ml.

The working standard solution of urea was prepared by diluting 15 ml. of the stock standard solution to 100 ml. with organic-free water and mixing thoroughly. Five ml.

of this solution corresponds to a 1:10 blood filtrate containing 45 mg. per cent urea nitrogen.

For Hoffman Method of Analysis

The stock solution of urea was prepared by dissolving 1.7144 gm. of pure urea in organic-free water and diluting to a volume of 1 l. Two drops of toluene were added as a preservative. This solution contained 80 mg. urea nitrogen per 100 ml.

Zinc Sulfate, Approximately 7.5 per cent

75 ml. of a stock 10 per cent solution of zinc sulfate were diluted to 100 ml. with organic-free water.

Sodium Hydroxide, 0.5 N

26.66 ml. of a saturated solution of sodium hydroxide were diluted to 1 l. with organic-free water. The normality of the solution was determined by titrating against standard HCl with phenophthalein as the indicator. The exact normality was calculated.

Sodium Hydroxide, 0.374 N

75 ml. of the 0.5 N sodium hydroxide solution were diluted to 100 ml. The normality of the solution was determined by titrating against standard HCl with phenol-

phthalein as the indicator. The exact normality was calculated.

Potassium Permanganate, Approximately 0.01 N

The 0.01 N potassium permanganate solution was prepared according to directions given by Halverson and Bergeim (1918):

1. 0.4 gm. of pure $KMnO_4$ crystals were dissolved in 1 l. of organic-free water in a Florence flask which had been rinsed with the same water.
2. The solution was digested at or near the boiling point for 36 hours using a reflux condenser.
3. The solution was then cooled and allowed to stand overnight.
4. After rinsing a 3 inch Büchner funnel lined with ignited asbestos and a filter flask with organic-free water, the permanganate solution was filtered with gentle suction.
5. The filtered solution was stored in a glass-stoppered brown bottle free from organic matter.
6. After standing from 2 to 3 days, the permanganate solution was standardized against standard $Na_2C_2O_4$ solutions.

Sulfuric Acid, Approximately 5 N

13.88 ml. of 36 N H_2SO_4, C. P., (arsenic acid and

nitrogen-free) were diluted with organic-free water to 100 ml. and mixed thoroughly.

Ammonia, Approximately 2 per cent

3.41 ml. of concentrated ammonium hydroxide, C. P., (58.6 per cent NH_3 by weight) were diluted to 100 ml. with organic-free water.

Zinc Sulfate, 10 per cent

17.375 gm. of $ZnSO_4 \cdot 7\ H_2O$ were dissolved in organic-free water and diluted to 100 ml. A trace of precipitate may form on long standing, but it settles to the bottom and may be ignored.

Form 1. Condition of female rats at necropsy (external condition)

Rat no._____ Diet no._____ Description of diet_____
Age of rat (in days)_____ Necropsy date_____ _____
Pregnancy no._____ Hour_____
Day of gestation period_____ Period of starvation
Wt. before starving_____ Hour initiated_____
Wt. after starving_____ Hour terminated_____

Physical condition*
General_____ Alert_____ Paws pinkish_____ Eyelids
Fat_____ Gaunt_____ Eyes pink_____ Inflamed_____ Infected_____

Muscle tone
General_____ Abdominal_____ Respiration
Sniffy_____ Wheezy_____
Palpitations_____

Gait
Dragging_____ Elevated_____ Exudates**
Sprawling_____ Awkward_____ Nasal_____ Anal_____
Oral_____ Vaginal_____

Hair
Clean_____ Smooth_____ Hematuria_____
Creamy_____ Thick_____
Fine_____ Remarks:_____
_____
_____

Tail
Clean_____ Smooth_____
Discolored_____ Sores_____

* In recording the degree to which any condition is present use a scale ranging from minus (-) to four plusses (++++).

** Indicate character of exudate.

Form 2. Condition of female rats at necropsy (internal condition)

Rat No._____ Diet No._____ Description of diet_____

Fat Depots*
Subcutaneous_____ Perirenal_____
Peritoneal_____ Genital_____
Omental_____ Intermuscular_____

Liver
Yellow_____ Mottled_____
Friable_____ Spongy_____

Kidneys
Cortex, color_____ friable_____
Medulla, color_____ friable_____
Pelvis, color_____ friable_____

Pancreas, any gross abnormalities:_____

Corpus Lutea
No. in left ovary_____ right_____
Color_____

Fetal Sites, No. of_____

Live Feti, No. of_____

Resorption, No. of_____

Stomach Ulcers
Number_____ Severity_____
Condition of the lungs:

	Infection	Atelectosis	Emphysema
Lobe 1.			
2.			
3.			
4.			
5.			

Pus pockets:
Ovary_____
Placental Sites_____
Ear_____
Base of the Tongue_____

Teeth
Straight_____ Orange_____

Remarks_____

*Use a scale ranging from minus (-) to four plusses (++++) in so far as possible in recording the degree to which any condition is present.

Table 1. Uniformity of animals over the four-year period of investigation

Year	Experimental group	No. of animals	Av. body wt. at weaning	Av. age at sexual maturity*	Av. body wt. at sexual maturity	Av. age at initiation of 1st pregnancy	Av. body wt. at initiation of 1st pregnancy
			gms.	days	gms.	days	gms.
1941-42	Steenbock V						
	Pregnant	10	52	45.1	97	67.3	133
	Virgin	6	54	39.7	89	----	---
	Pork I						
	Pregnant	9	52	45.7	99	67.6	135
	Virgin	5	54	39.2	87	----	---
1942-44	Steenbock V						
	Pregnant	17	52	41.9	93	67.9	144
	Virgin	14	50	42.2	88	----	---
	Pork I						
	Pregnant						
	Non-toxic	13	52	40.9	91	64.7	137
	Toxic	2	54	39.5	86	69.5	156
	Virgin	16	52	40.9	91	----	---
1944-45	Steenbock V						
	Pregnant	9	51	43.0	90	69.1	134
	Virgin	13	50	43.2	91	----	---
	Pork I						
	Pregnant						
	Non-toxic	11	52	42.2	88	69.2	135
	Toxic	1	52	38.0	86	67.0	135
	Virgin	13	51	42.2	90	----	---

* Opening of vaginal orifice

Table 2. Individual laboratory numbers of rats used in analysis of urea nitrogen in blood

Steenbock V		Pork I		
Pregnant	Virgin	Pregnant		Virgin
		Non-toxic	Toxic	
31055	33008	31054	36547	33009
31075	33037	31074	37070	33046
31133	33045	31148	38218	33087
31174	33086	31173	20*	33102
31224	33101	31253		33209
31234	33299	31259		33221
31260	33340	31267		33341
31268	33457	31299		33408
31300	33580	33105		33581
33120	33827	33339		33722
33301	37018	33361		37016
33338	37073	33460		37071
33409	37106	33521		37104
33459	37135	33689		37111
33472	37198	33725		37133
33520	37215	37103		37196
33724	37766	37110		37213
37017	37788	37132		37765
37072	37832	37212		37787
37105	37848	37763		37831
37134	38309	37829		37847
37197	38424	38310		38311
37214	38445	38389		38391
37764	38464	38425		38426
37786	38470	38447		38446
37830	38516	38465		38466
37846	38684	38471		38472
38308	38772	38517		38518
38390		38682		38683
38423		38773		38774
38444				
38463				
38469				
38515				
38681				
38771				

* Sprague Dawley Stock

Table 3. Individual laboratory numbers of rats used in determining protein content of plasma

Steenbook V		Pork I		
Pregnant	Virgin	Pregnant		Virgin
		Non-toxic	Toxic	
38308	38309	38310	38218	38311
38390	38392	38389	20*	38391
38423	38424	38425		38426
38444	38445	38447		38446
38463	38464	38465		38466
38469	38470	38471		38472
38515	38516	38517		38518
38681	38684	38682		38683
38771	38772	38773		38774
	39042	39155		39043
	39156	39160		39157
	39161			39162
	39283			39284

* Sprague Dawley Stock

Table 4. Individual laboratory numbers of rats used in analysis of serum calcium

Steenbook V		Pork I		
Pregnant	Virgin	Pregnant		Virgin
		Non-toxic	Toxic	
38423	38424	38389	38218	38391
38444	38445	38425	38836	38426
38463	38464	38447	39192	38446
38469	38470	38465	20*	38466
38515	38516	38471		38518
38681	38684	38682		38472
38771	38772	38773		38683
				38774

* Sprague Dawley Stock

Table 5. Concentration of urea nitrogen in various dilutions of standard solutions of urea and scale readings on the photoelectric colorimeter

Solution number	Sample number	Concentration of urea nitrogen in sample	Number of test	Scale reading on photoelectric colorimeter
		mg./100 ml.		
I	1	8	1	9
			2	7
	2	16	1	17
			2	15
	3	32	1	34
			2	32
	4	64	1	66
			2	64
	5	8	1	10
			2	9
	6	16	1	15
			2	15
	7	32	1	37
			2	38
	8	64	1	71
			2	75
	9	8	1	5
			2	7
	10	16	1	12
			2	15
	11	32	1	33
			2	32
	12	64	1	69
			2	69
	13	8	1	9
			2	12
	15	16	1	22
			2	19
	16	32	1	37
			2	39
	17	64	1	73
			2	72
	18	8	1	4
			2	7
	19	16	1	15
			2	15
	20	32	1	33
			2	32

Table 5 (Cont'd). Concentration of urea nitrogen in various dilutions of standard solutions of urea and scale readings on the photoelectric colorimeter

Solution number	Sample number	Concentration of urea nitrogen in sample	Number of test	Scale reading on photoelectric colorimeter
		mg./100 ml.		
	21	64	1	69
			2	75
	22	8	1	10
	23	16	1	16
	24	32	1	33
	25	64	1	65
II	1	8	1	7
			2	6
	2	16	1	16
			2	16
	3	32	1	36
			2	32
	4	64	1	71
			2	64
III	1	8	1	8
			2	8
	2	16	1	16
			2	16
	3	32	1	33
			2	33
	4	64	1	66
			2	66
IV	1	8	1	8
			2	8
	2	16	1	16
			2	16
	3	32	1	34
			2	32
	4	64	1	69
			2	69
	5	16	1	16
			2	16
			3	16
			4	16
			5	17

Table 5 (Cont'd). Concentration of urea nitrogen in various dilutions of standard solutions of urea and scale readings on the photoelectric colorimeter

Solution number	Sample number	Concentration of urea nitrogen in sample	Number of test	Scale reading on photoelectric colorimeter
		mg./100 ml.		
	6	32	1	33
			2	33
			3	40
			4	36
			5	34
			6	37
			7	36
			8	35
			9	32

Ey= 1.1094x - 1.2914
Ex= 0.8903y + 1.5074
(y= scale reading; x= mg. urea N.)

Table 6. Concentration of urea nitrogen in the blood of control and experimental rats

Steenbock V				Pork I			
Pregnant		Virgin		Pregnant		Virgin	
Rat No.	Urea N	Rat No.	Urea N	Rat No.	Urea N	Rat No.	Urea N
	mg./100 ml.		mg./100 ml.		mg./100 ml.		mg./100 ml.
31055	17.82	33008	16.34	31054	13.79	33046	9.19
31075	15.15	33037	11.45	31074	13.93	33087	14.66
31133	13.52	33045	12.86	31132	15.49	33102	11.55
31174	11.20	33086	11.83	31148	10.22	33221	9.68
31224	15.18	33101	14.68	31173	15.99	33209	13.08
31234	15.54	33827	15.55	31233	13.82	33341	13.08
31260	11.89	33299	14.86	31259	16.56	33408	16.64
31268	10.30	33340	14.86	31267	15.00	33581	10.41
31300	13.62	33457	16.64	31299	14.46	33722	17.53
33120	13.53	33580	12.64	33105	9.97	37016	15.75
33301	14.86	37018	18.42	33339	17.53	37071	13.97
33338	13.97	37073	18.42	33361	14.86	37104	12.19
33409	13.08	37106	15.17	33460	26.44	37111	16.64
33459	12.19	37198	21.98	33689	14.86	37133	19.31
33472	15.75	37215	16.64	33725	17.53	37196	22.87
33520	14.86	37766	20.20	*36547	34.45	37213	8.63
33724	14.86	37788	16.64	*37070	37.12	37765	14.86
37017	20.20	37832	15.75	37103	11.30	37787	16.64
37072	16.64	37848	15.75	37132	17.53	37831	16.20
37105	14.86	38309	16.64	37212	18.42	37847	14.86
37134	13.08	38392	15.75	37763	13.08	38311	12.19
37197	17.53	38424	20.20	37829	19.31	38391	12.19
37214	13.08	38445	15.75	38310	16.64	38426	13.97
37764	13.08	38464	16.64	*38218	46.02	38446	13.97
37786	13.08	38470	13.08	38389	16.64	38466	11.30
37830	10.41	38516	14.86	38425	16.64	38472	13.97
37846	9.52	38684	18.42	38447	7.29	38518	24.66

Table 6 (Cont'd). Concentration of urea nitrogen in the blood of control and experimental rats

Steenbock V				Pork I			
Pregnant		Virgin		Pregnant		Virgin	
Rat No.	Urea N	Rat.No.	Urea N	Rat No.	Urea N	Rat No.	Urea N
	mg./100 ml.		mg./100 ml.		mg./100 ml.		mg./100 ml.
38308	13.97	38772	15.75	38465	16.64	38683	13.08
38390	13.08			38471	14.86	38774	16.64
38423	16.64			38517	14.86		
38444	15.75			38682	16.64		
38463	14.86			38773	16.64		
38469	12.19			*S.D. 20	40.68		
38515	10.41						
38681	13.97						
38771	13.97						
Average	13.99		16.03		15.18		14.47

* Developed toxic symptoms -- concentrations of urea nitrogen in the blood of these rats not included in average value

Table 7. Concentration of protein in plasma of control and experimental rats

Steenbock V				Pork I			
Pregnant		Virgin		Pregnant		Virgin	
Rat No.	Plasma protein	Rat No.	Plasma protein	Rat No.	Plasma protein	Rat No.	Plasma protein
	gm/100 ml.		gm./100 ml.		gm./100 ml.		gm./100 ml.
38308	6.83	38309	8.68	*38218	4.87	38311	7.72
38390	6.69	38392	7.72	38310	5.69	38391	7.24
38423	5.76	38424	6.55	38389	5.87	38426	9.12
38444	6.72	38445	7.65	38425	6.48	38446	6.31
38465	6.28	38464	7.03	38447	6.41	38466	7.24
38469	6.21	38470	5.87	38465	5.18	38518	8.64
38515	5.21	38516	7.85	38471	8.40	38683	9.26
38681	5.01	38684	6.65	38517	6.52	38774	7.34
38771	4.63	38772	7.75	38682	6.17	39043	8.85
		39042	8.33	38773	4.90	39157	8.61
		39156	9.36	39155	7.82	39162	9.64
		39161	7.82	39160	8.44	39284	8.78
		39283	9.09	*S.D. 20	5.08		
Average	5.93		7.72		6.53		8.23

* Developed toxic symptoms

Table 8. Concentration of calcium in serum of control and experimental rats

Steenbock V				Pork I			
Pregnant		Virgin		Pregnant		Virgin	
Rat No.	Serum calcium	Rat No.	Serum calcium	Rat No.	Serum calcium	Rat No.	Serum calcium
	mg./100 ml.		mg./100 ml.		mg./100 ml.		mg./100 ml.
38423	8.44	38424	9.26	*38218	10.40	38391	12.77
38444	10.54	38445	11.14	38389	9.47	38426	11.35
38463	6.62	38464	9.27	38425	8.71	38446	9.77
38469	8.75	38470	10.42	38447	8.93	38466	9.41
38515	8.31	38516	9.34	38465	8.58	38472	11.44
38681	8.64	38684	10.73	38471	8.21	38518	11.87
38771	7.88	38772	9.74	38682	11.28	38683	12.15
		39042	11.33	*38836	10.40	38774	10.77
		39156	10.01	39155	9.98	39043	9.53
		39161	10.23	39160	8.61	39157	9.73
		39283	10.28	*39192	8.26	39162	9.96
				*S.D. 20	10.90		
Average	8.45		10.16		9.26		10.80

* Developed toxic symptoms

Table 9. Concentration of urea nitrogen in blood of control and experimental rats according to number of live feti present in the uteri at necropsy

Diet	Rat No.	No. of feti	Concentration of urea nitrogen	Av. concentration of urea nitrogen
			mg./100 ml.	mg./100 ml.
Steenbock V	31234	7	15.54	
	38444	7	15.75	15.6
	31224	8	15.18	
	37072	8	16.64	15.9
	31300	9	13.62	
	33301	9	14.86	
	37764	9	13.08	13.8
	31174	10	11.20	
	31260	10	11.89	
	33409	10	13.08	
	33472	10	15.75	
	33520	10	14.86	
	33724	10	14.86	
	37197	10	17.53	
	37830	10	10.41	
	37846	10	9.52	
	38308	10	13.97	
	38469	10	12.19	
	38515	10	10.41	
	38681	10	13.97	13.3
	31055	11	17.82	
	31075	11	15.15	
	33120	11	13.53	
	33338	11	13.97	
	37105	11	14.86	
	37214	11	13.08	
	37786	11	13.08	
	38423	11	16.64	
	38463	11	14.86	14.8
	31133	12	13.52	
	31268	12	10.30	
	33459	12	12.19	
	37134	12	13.08	
	38390	12	13.08	12.5
	38771	13	13.97	14.0
Pork I	37110	0	17.53	
	37132	0	23.76	20.7
	37103	2	11.30	11.3

Table 9 (Cont'd). Concentration of urea nitrogen in blood of control and experimental rats according to number of live feti present in the uteri at necropsy

Diet	Rat No.	No. of feti	Concentration of urea nitrogen	Av. concentration of urea nitrogen
			mg./100 ml.	mg./100 ml.
	38447	4	7.29	7.3
	31148	5	10.22	
	31233	5	13.82	12.0
	37763	6	13.08	
	38517	6	14.86	14.0
	33689	7	14.86	
	33725	7	17.53	
	37212	7	18.42	
	38471	7	14.86	16.4
	31299	8	14.46	
	38682	8	16.64	15.6
	33339	9	17.53	
	33460	9	26.44	
	38310	9	16.64	20.2
	31054	10	13.79	
	31259	10	16.56	
	38389	10	16.64	
	38465	10	16.64	
	38773	10	16.64	16.1
	31074	11	13.93	
	37829	11	19.31	
	38425	11	16.64	16.6
	31132	12	15.49	
	31173	12	15.99	
	31267	12	15.00	15.5