NOTE TO USERS

MEAT IN NUTRITION XXI. DISTRIBUTION AND PARTITION OF FATS IN CERTAIN TISSUES OF RATS FED A DIET CONTAINING DRIED AUTOCLAVED PORK MUSCLE

by

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INTRODUCTION AND PURPOSE OF EXPERIMENT

In the last decade biochemists have been increasingly attracted to researches relating to fats, fatty acids, glycolipids, phospholipids, and sterols in order to better understand physiological processes occurring in the body. Although the lipid metabolism of many organs of the body under varying circumstances has been investigated, the study of the changes in the liver and blood has proven most fruitful in yielding information. Many disturbances in the metabolic processes of the body are accompanied by changes in the concentration of the individual lipid constituents of the liver. On the other hand, factors such as the quality of food ingested, toxic drugs, etc., may lead to fat deposition in the liver of such magnitude that the health of the organism is affected. Definite alterations in blood lipids may or may not accompany the changes in the liver.

Workers in the Nutrition Laboratory of the Foods and Nutrition Department of the Iowa State College at present are interested in investigating certain derangements induced in the albino rat by dietary means, that seem to be characterized by a fault in the metabolism of lipids. The abnormalities appeared in the course of an investigation designed to study the rôle of meat in nutrition. A diet containing dried autoclaved pork muscle as the main source of protein

was used in the study. At the time the ration was formulated, it was believed adequate from the nutritional standpoint. A diet to be adequate should provide for the growth and maintenance of the animal, support normal reproduction and lactation, prevent premature aging, allow a normal span of life, and support the species over a successive number of generations. However, instead of maintaining these life functions, the feeding of the pork diet produced a series of nutritional disasters. One of the earlier observations was a failure of the ration to support more than a second generation of animals. All of the latter group were sterile. Dyar (1935) found that growth was retarded and abnormalities occurred in reproductive functions. Difficulties encountered by the rat at parturition were most striking. Other workers in this laboratory have repeatedly confirmed Dyar's findings and have studied certain of the metabolic derangements caused by the pork diet (King, 1936; Wilcox, 1937; Walliker, 1938; Armstrong, 1939; Campbell, 1940; Farrankop, 1941; and Ho, 1941).

Dyar reported that 33 per cent of the rats died during parturition. Similar toxemias of pregnancy have been observed in the sheep and rabbit (Roderick et al., 1932, Greene, 1937). There is, also, a striking resemblance between many of the symptoms characterizing the pregnancy disease in these rats and toxemic pregnancy in human beings.

To all appearances the rats affected were normal until the day of parturition when a most outstanding disturbance was observed. External symptoms noted were: labored breathing, erection of the fur, loss of muscle tone, lethargy, very pale ears, and coldness of the body. Often the animal was unable to support its head which it rested on the food cup. Vaginal hemorrhage was a common occurrence. Hematuria occurred frequently. In some instances, convulsions immediately preceded death; in others, parturition was long and labored. In the latter case the animal invariably died during the birth of the young. Other rats did not show early signs of illness. They were found dead, evidence indicating that they had died in a convulsion.

The necropsy studies made on animals actually ill and sacrificed prior to death were of particular interest. The abdominal cavity contained an abundance of fat. Even though the feti might appear normal and fully developed, they were dead, pale, and cold to the touch. Sometimes the feti had died at different stages of development. Severe intrauterine hemorrhage was often present. Placental connections were not always intact. Hemolysis of fetal blood and thrombi in the umbilical veins were noted by Armstrong (1939). She also observed marked degeneration of the tubular epithelium in the kidney. The blood was pale in color, watery, and very slow to clot.

Abnormalities of the liver were evident. The livers were large, pale, friable, and of a yellowish color indicating a deposition of fat globules in the tissues. Histological studies were made by Armstrong (1939). She found fatty infiltration and fatty degeneration of the hepatic cells in the moribund pork-fed rats. Chemical analysis of the total lipid in the liver showed an abnormally high lipid content in all animals fed the pork diet, whether or not they became sick. The percentage of fat, calculated on the dry basis, in the livers of the control rats was 22 per cent as compared with 40 per cent for the pork-fed animals not getting sick and 47 per cent for the females developing the pregnancy disease.

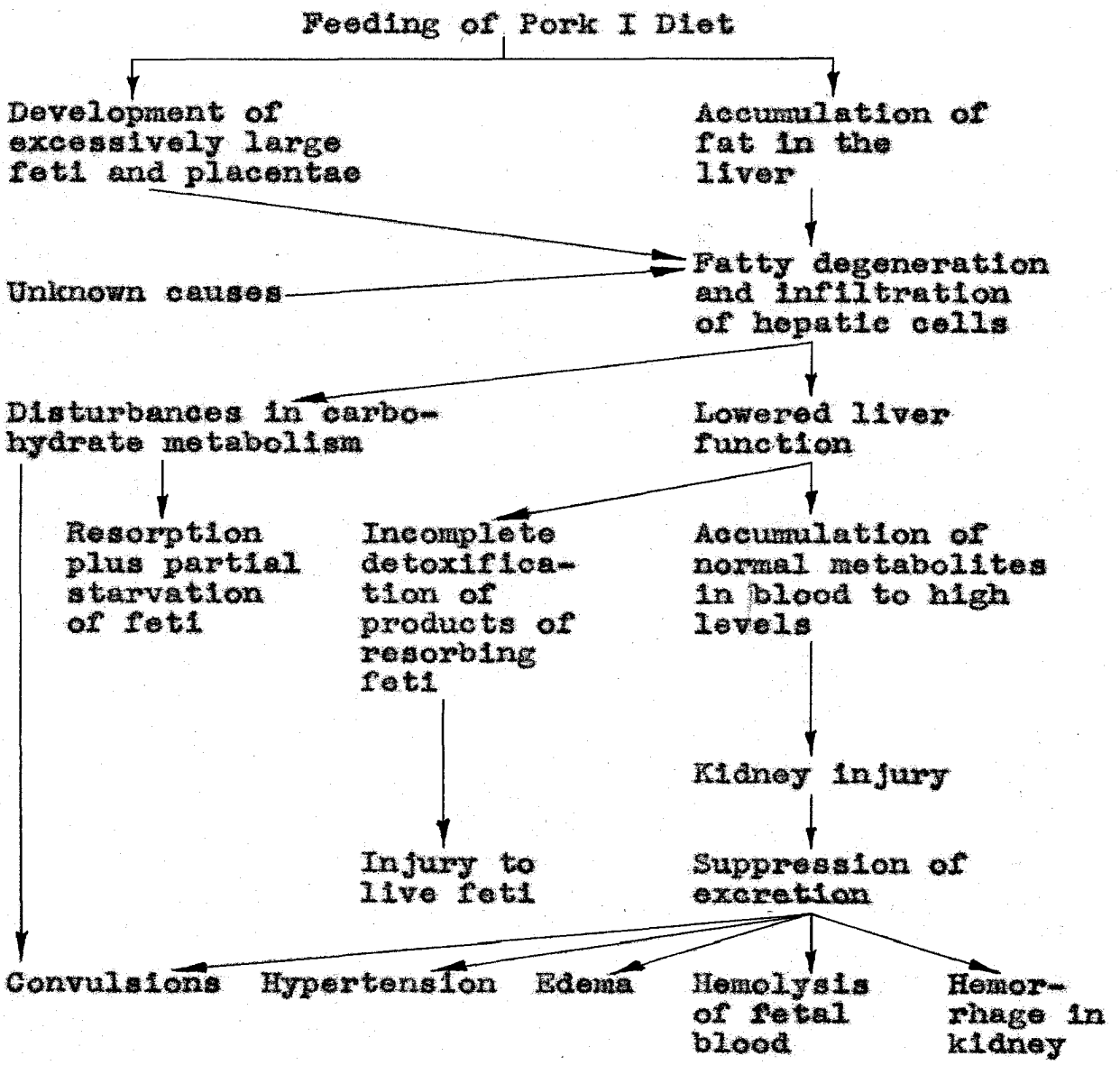
Is there interference with the functioning of the liver when the hepatic cells become loaded with fat? If this does occur, derangements in the functioning of the liver might be associated with the pregnancy disorder. One type of metabolic dysfunction has been described by Farrankop (1941). She found no glycogen in the liver of two toxemic animals and low values in other pork-fed rats that did not develop the disease. Or, under the condition described, may there be a decrease in detoxifying power of the liver with the result that metabolites accumulate in the blood? Edema, hypertension, and convulsions might then follow. Certain of these relationships have been suggested

by Armstrong in a diagrammatic representation (Figure 1) of a theoretical sequence of events that might result in the disease.

The finding that the livers of the pork-fed rats contained twice as much total lipid as did those of the control group provided no evidence of possible changes undergone by the individual components of the lipid. Such changes have physiological significance. Bloor and his collaborators (1930) have demonstrated that the phospholipid content and usually the free cholesterol content of a tissue increases with physiological activity while degeneration and inactivity of the tissue is accompanied by decreasing or low percentages of these lipids and by an increase in cholesterol esters and neutral fat. Later work in Bloor's laboratory, by Boyd (1935b), and by Erickson et al. (1937) has given support to this hypothesis. Thus, variations in the lipid constituents of the liver and blood may be used as an index to measure a disturbance in the metabolism of fat.

Various workers have found that the relation of one lipid fraction to another may depend upon the dietary regime. Fatty livers induced by a high fat, low protein diet (Best, Channon, and Ridout, 1934) are characterized by a large increase in neutral fat while the fatty livers occurring after cholesterol feeding show the greatest percentage increase in cholesterol esters, although a

FIGURE 1. THEORETICAL REPRESENTATION OF SEQUENCE OF EVENTS RESULTING IN ACUTE PREGNANCY DISORDER[a]



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a. Armstrong, W. E.
1939. Meat in nutrition. XV. Certain characteristics of gestational performance in albino rats fed a diet containing dried autoclaved pork muscle. Unpublished Thesis, Ph. D., Library, Iowa State College, Ames, Iowa.

substantial increase in the neutral fat also occurs (Okey and coworkers, 1934).

The fact that Wilcox (1937) prevented the syndrome in the pregnant female by feeding lipocaic, a lipotropic substance extracted from pancreas (Dragstedt et al., 1936) also suggested a positive relationship between fat metabolism and the pregnancy disorder. Therefore the present study of the partition of the lipids in the liver and blood of rats was undertaken to detect, if possible, any derangement of the fat metabolism which might have occurred as a result of feeding the pork-containing diet to pregnant animals.

EXPERIMENTAL PROCEDURE

EXPERIMENTAL GROUPS

Three series of rats have been studied. In Series I, the quantity of total fat in the livers of pregnant females maintained on the pork diet and of pregnant control females reared on the stock ration was determined gravimetrically after alcohol-ether extraction. Virgin control rats matching certain of the animals in each experimental group in regard to age were also studied to determine the effect of pregnancy *per se*. Dyar (1935) found that only 30 to 40 per cent of the pork-fed animals developed the pregnancy disease. Hence, the experimental group fed the ration containing pork divided itself into two sub-groups, *i.e.*, animals that developed a toxic pregnancy and those that did not. A second group of sick females whose basal pork diet had been supplemented with various types of foods was obtained from animals comprising other studies in progress in the laboratory.

A more refined analysis was made of the liver lipids in the group of rats included in Series II. The individual fractions of the liver lipid were determined by the oxidative procedure. As in Series I, data from a pregnant experimental pork-fed group were compared with those obtained from a

pregnant control group. The experimental animals were again divided into two groups, "toxic" and "non-toxic." In addition, a few virgin controls matching specific experimental animals were studied. Also, a small group of toxemic females which had been fed various dietary supplements was available.

The final series, III, was comprised of pregnant animals fed the control stock ration and pregnant rats fed the pork-containing diet, variations in the blood lipid values being compared.

In all series, the control groups received the stock colony ration which will be designated hereafter as Steenbock V. The diet given the experimental groups will be called Pork I.

The plan of the experiment showing the number of animals used and their distribution in the various experimental groups is summarized in table 1. Individual numbers of the rats composing each group are found in tables V and VI in the Appendix.

ANIMALS USED

The animals used in the present investigation were albino rats (*Mus norvegicus albinus*) of Wistar stock, strain A, obtained from the stock colony maintained by the Foods and Nutrition Department at the Iowa State College. These

TABLE 1. SUMMARY OF EXPERIMENTAL PLAN

Series	Name of diet fed	Reproductive status	No. of rats	Lipid fraction analyzed
I	Steenbock V	Pregnant Virgin	9 8	Total lipids
	Pork I	Pregnant Non-toxic Toxic Virgin	 15 8 9	
	Supplemented pork diets	Pregnant Toxic	 21	
II	Steenbock V	Pregnant Virgin	16 3	Total fatty acids, total cholesterol, free cholesterol, phospholipid analyzed. Total lipids, neutral fat, and cholesterol esters calculated from above data
	Pork I	Pregnant Non-toxic Toxic Virgin	 13 13 4	
	Supplemented pork diets	Pregnant Toxic	 4	
III	Steenbock V	Pregnant	8	Total fatty acids, total cholesterol, phospholipid
	Pork I	Pregnant Non-toxic Toxic	 9 1	
Total number of analyses made				141

rats had been inbred by brother matings for about 80 generations. The experimental rats employed during the five years the study was in progress were representatives of animals bred in the interval ranging from the seventy-second to the eighty-first generations.

The ration upon which the stock colony was maintained was a modification of the whole grain diet recommended by Steenbock in 1923. The composition of the modified diet, designated as Steenbock V, has remained the same since 1932 and the quality of the components of the ration has been kept as uniform as possible.

Animals representing the second and third litters of stock colony females were used in the experiment. These were removed from the colony at the time of weaning. Littermate pairs were distributed between the experimental and control groups. Some of the animals developing toxemia on the Pork I ration did not have a littermate control because often they were taken from other groups of rats in the laboratory raised under the same conditions. All of the rats received the stock colony ration until the time of the opening of the vaginal orifice. The diet of the experimental group was then changed to Pork I. The control animals were continued on the Steenbock V ration.

Healthy young littermate males maintained on the stock colony diet were used for breeding these females.

Brother and sister matings were employed in most instances.

The uniformity of the animals used in Series II and III, at the initiation of the experiment, is shown in table 2. Certain data were used as indices for judging uniformity, i.e., average weight of the various groups at weaning, at sexual maturity, and at the time of the initiation of the first pregnancy; and average age at sexual maturity, and at the time of the initiation of the first pregnancy. The data show a surprising uniformity over the four years covered in the analysis. Any differences noted between groups, therefore, probably are not attributable to a lack of homogeneity in the experimental animals used. The values depicting the uniformity of the animals falls within the range noted by Greenwood (1940) in a study of 14 generations of the stock colony. The females in Series I were selected in the same manner that was used in the other series. Therefore, they could be judged just as uniform.

COMPOSITION AND PREPARATION OF DIETS

The Pork I Diet

The diet fed the experimental group had the following composition:

TABLE 2. UNIFORMITY OF ANIMALS USED

Series	Experimental group	No. of animals	Body wt. at weaning	Age at sexual maturity[a]	Body wt. at sexual maturity	Age at initiation of 1st pregnancy	Body wt. at initiation of 1st pregnancy
			gm.	days	gm.	days	gm.
II	Steenbock V						
	Pregnant	16	51.11	41.44	87.56	68.81	140.44
	Virgin	3	52.33	42.33	86.00	-	-
	Pork I						
	Non-toxic	13	53.85	41.92	91.31	68.50	143.08
	Toxic	13	49.54	41.23	88.46	70.23	136.70
	Virgin	4	53.50	43.00	96.25	-	-
III	Steenbock V	8	53.25	41.38	93.25	74.62	150.88
	Pork I	9	52.00	41.78	92.22	75.00	154.57

a. Opening of vaginal orifice.

Canned pork muscle (dried to one-half its original weight)	25 grams
Cornstarch[a]	53 grams
Yeast[b]	5 grams
Agar agar[c]	2 grams
NaCl[d]	1 gram
Salt mixture[e]	4 grams
Butterfat[f]	8 grams
Cod liver oil[g]	2 grams
	100 grams

At the time of its formulation, the diet, largely synthetic except in its source of protein, was believed to meet the requirements of an adequate ration. The experimental evidence in support of the adequacy of the diet has been discussed by Armstrong (1939). However, experience has demonstrated that the diet is deficient in one or more as yet unidentified factors.

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a. Purchased in local market in 140 lb. lots
b. Yeast foam tablet powder purchased from the Northwestern Yeast Co., Chicago, Ill.
c. Bacto-agar purchased from the Difco Laboratories, Inc., Detroit, Mich.
d. Purchased in local market.
e. Osborne, T. B., and Mendel, L. B., J. Biol. Chem., 37, 557-601, 1919.
f. Purchased at Iowa State College Dairy.
g. Refined Norwegian vitamin tested cod liver oil, U.S.P., purchased from the Pearson, Ferguson Co., Kansas City, Mo.

In order to reduce variation in the pork muscle, large quantities (lots ranging from 500 to 750 pounds) were purchased at one time. The green skinned hams were boned, trimmed of all excess fat, and ground once through the medium plate of the meat grinder. One pound of ground meat was weighed and packed into each tin can. After sealing, the cans were processed in the pressure cooker for 65 minutes at 15 pounds pressure. Upon removal from the pressure cooker, the cans were examined for leaks, cooled immediately in cold running water, and stored at room temperature until needed.

When the pork was prepared for use in the diet, all visible fat congealed at the top of the can was removed and the meat placed on a Monel metal tray covered with cheese cloth. On each tray was spread 1000 grams of meat which was then dried to one-half its original weight in a current of air kept below 100 degrees Centigrade. When placed in a warm oven, 65 minutes were required for drying.

Butter was heated for two hours in a double boiler to separate the butter fat. The coagulated protein and salt rose to the surface while the water and other minerals collected at the bottom of the container. The middle layer of pure butter fat was decanted, filtered through a cotton plug in a hot water funnel, cooled, and stored in the refrigerator.

Fresh diet was mixed twice weekly. The necessary butter fat was melted at a low temperature and placed with the other weighed ingredients in a Hobart mixer, and the whole mixed for 25 minutes at first speed and stored in tin cans in the refrigerator.

The Steenbock V Diet

The ration, Steenbock V, regularly fed to the stock colony of the laboratory was given to the control group. The basal portion of the ration consisted largely of casein, a mixture of grains, yeast, and calcium carbonate. The above food was supplemented daily with milk enriched by the addition of trace minerals and cod liver oil. Lettuce and fresh ground beef were offered on alternate days. The exact composition of the diet with its supplements is shown on the next page.

I. Basal diet:

Yellow cornmeal[a]	64.0 grams
Crude casein[b]	5.0 grams
Linseed meal[c]	16.0 grams
Ground alfalfa[d]	2.0 grams
Sodium chloride[e]	0.5 gram
Calcium chloride[f]	0.5 gram
Yeast[g]	1.5 grams
Irradiated yeast[h]	0.5 gram
Wheat germ[i]	10.0 grams
	100.0 grams

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a. Purchased from Grain Storage, Iowa State College.
b. Finely ground, purchased from the Wilkens-Anderson Co., Chicago, Ill.
c. Purchased from Ames Grain and Coal Co.
d. Dehydrated alfalfa leaf meal purchased from Denver Alfalfa Co., Denver, Colo.
e. Purchased from the local market.
f. Purchased from Chemistry Stores, Iowa State College.
g. Yeast foam tablet powder purchased from Northwestern Yeast Co., Chicago, Ill.
h. Irradiated in 200 gm. lots for 11 min. at a distance of 15 in. with a General Electric Uviarc lamp.
i. Type A, purchased from Washburn Crosby Co., Minneapolis, Minn.

II. Supplementary foods

A. Liquified Klim fortified as described below was fed as follows:

1. Male ---------- 12.5 ml. daily
2. Resting female ----- 12.5 ml. daily
3. Pregnant female ---- 25.0 ml. daily
4. Female with litter-- 50.0 ml. daily

B. Lettuce fed three times weekly - 10 grams

C. Raw ground round of beef[a] fed three times weekly on alternate days with lettuce ---------- 5 grams

The liquid Klim was made from dry Klim[b] purchased in sufficient quantities each winter to last throughout the year. Each day's supply was mixed in the proportion of 130 grams of Klim to one quart of distilled water and one teaspoon of cod liver oil[c] in a Hobart mixer at third speed for five minutes. To each quart of milk thus prepared was added 2 ml. of a solution of trace elements. The salt

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a. Purchased in the local market.
b. Powdered whole milk obtained from the Borden Co., New York
c. Refined Norwegian vitamin tested cod liver oil, U.S.P., imported by the Pearson-Ferguson Co., Kansas City, Mo.

solution contained the following trace elements dissolved in 10 ml. of distilled water:

Potassium iodide	0.08 gram
Manganese sulfate	0.316 gram
Potassium aluminum sulfate	0.098 gram
Anhydrous copper sulfate	0.375 gram

CARE OF ANIMALS

Each female rat after reaching sexual maturity was housed separately in a round wire cage with a raised bottom. The cages were set in enameled pans lined with paper towels and placed on a steel shelf in a room maintained at a fairly constant temperature (75 to 80° F.). The paper towels collected the urine and feces, and were changed daily. Food and water were given ad libitum. The food was placed in glass jars wired to the side of the cage with copper wire. Distilled water was provided by the use of glass bubble fountains inserted through openings in the cages. Small porcelain cups which fitted into metal brackets held the milk. Food jars and milk cups were changed three times weekly, washed in strong soap suds, and sterilized in live steam for 20 minutes. The cages, pans, water fountains, and metal holders were washed and sterilized weekly.

The daily routine except for Sunday consisted of changing the paper towel in the pan, observing the general condition of the animal, and providing food and water. A quantity of food slightly in excess of the rat's needs was offered daily with the exception of Saturday when a double portion was given. Each day the uneaten Pork I ration which remained in the cup was discarded to eliminate any possible effects of developing rancidity. The intake of food was recorded every day for the Pork I rats.

Each rat was weighed daily at the time the vaginal smear was observed. The pregnant females were weighed on the twenty-first day of gestation at 10 p.m. and on the twenty-second day at four-hour intervals until parturition occurred. The procedure permitted the detection of early signs of toxicity, and examination and weighing of the litter immediately after birth.

The individual weights of the young and the weight of the female were recorded as soon after birth as possible. Nesting paper was provided for the litter. If any one individual of the litter was dead, its weight was obtained. Then the lungs were removed and placed in water. If the lungs floated, the rat was assumed to have been alive at birth.

On the fourth day, the litters were reduced to six rats. If possible, three males and three females whose

weight most nearly approached the average of each sex in the litter were kept. At various intervals, i.e., when the young were 4, 7, 14, 17, 21, and 28 days old, they were differentiated as to sex and weighed separately. Daily records of the total weight of the litter were kept. The young were weaned at 28 days.

VAGINAL SMEARS

In the rat, the course of the oestrous cycle may be followed by a daily study of the type of cell characterizing the vaginal epithelium (Long and Evans, 1922). The normal female rat will accept mating during the late pro-oestrous and early oestrous stages (stages 1 and 2). In order to know when to mate each rat, cells removed from the vagina were examined daily from the ninth week until the time that the rat was killed.

In taking the smears, the rat was held on her back in the palm of the left hand with the thumb and index finger supporting the head. 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 and examined under the low power of the microscope. Artificial light was used for illumination.

Every effort was made to prevent infection of the

vagina by this manipulation. Immediately after use the rods were put in a strong soap solution. The rods were then washed and placed in test tubes containing a small amount of distilled water. The tubes were sealed with cotton and sterilized at 15 pounds pressure for 15 minutes.

For the determination of the stage of the oestrous cycle the classification of Long and Evans (1922) was used, i.e.,

Stage 1. Epithelial cells only

Stage 2. Epithelial and cornified cells

Stage 3. Many cornified cells

Stage 4. Cornified cells and many leucocytes

Stage 5. Leucocytes, epithelial, and cornified cells

Vaginal smears were studied daily at approximately the same hour and a record kept of the date, time of day, weight of rat, stage of the oestrous cycle, and any changes in the physical condition of the animal. Each female was allowed to pass through one complete cycle before being mated. Then, when stage 1 or 2 was observed, a brother male was placed in the cage with the female. The next day the vaginal smear was examined for sperm cells and the vagina and paper beneath the cage for vaginal plugs. If neither plugs nor sperm were found, the male was not removed until stage 3 was noted.

During gestation the smears were observed from the

twelfth to the sixteenth day for the appearance of red blood cells. Their presence indicated the implantation of the feti in the uterine wall.

Vaginal smears were not observed in any of the virgin rats used.

TREATMENT OF RATS

Before Necropsy

Each pregnant rat was allowed to raise one litter. When oestrus reappeared after the litter had died or was weaned, the rat was mated for a second litter. A vaginal smear was taken at 10 p.m. on the day the male was placed with the female to determine whether mating had occurred. With this procedure we could determine fairly accurately the 21.5 day of pregnancy, the time chosen for killing the rat for study. All pregnant animals, except a few of the sick females, were killed on the 21.5 day $\pm$ 4 hours of their second pregnancy.

The animals were starved to deplete the liver and blood of any fat which might be directly traceable to food fat. Williams *et al.* (1937) starved their rats approximately 15 hours. In the present experiment, it was necessary to consider the effect of starvation on the feti.

A 10-hour starvation period was chosen because it was believed long enough to eliminate the influence of food fat, and short enough not to injure the feti. Also, 10 hours was a time interval that fitted conveniently into the laboratory routine. Hence, the food was removed from the cage at 10 p.m. when the rat was to be killed at 8 a.m. the next day or at 6 a.m. when 4 p.m. was the hour of killing.

The sick rats were not starved inasmuch as many of them showed no signs of illness until shortly before acute symptoms were observed. However, it was believed that the liver fat would be comparable to that from the starved females because the sick animal starved herself. For instance, a group of 10 sick animals ate an average of 2 gm. of food during their last 24 hours of life.

The litter mate virgins were killed when they were approximately the same age as the pregnant females.

All animals were weighed before and after starvation and the weights recorded. A record of the general physical condition of each animal before it was killed was made according to the outline shown in form I in the Appendix.

At Necropsy

Preparation of Liver for Removal

In choosing a method for killing these animals, two

points were given consideration. First, the liver should be free from blood thereby minimizing the possibility of adding the fat of the blood to that in the liver and second, it should be removed as soon as possible after anesthesizing the animal.

In 1934, Enblom working in this laboratory developed a technique of perfusing the liver with a modified Locke's solution. This method was adopted. The preparation of the Locke's solution is given in the Appendix.

The rat was anesthetized by intrapleural injection of a 3 per cent solution of nembutal obtained from the Veterinary Department. The amount injected depended upon the size of the animal. Usually 0.3 to 0.4 ml. was given. When the rat failed to respond to external stimuli, it was laid on its back. An incision was made on the ventral median line extending from the anus to the diaphragm. A transversal cut was made in both sides of the abdominal wall to expose the viscera. Care was taken to avoid cutting the larger blood vessels. The viscera and uterus were pushed to one side and the abdominal aorta exposed. The fascia covering the aorta was carefully removed with a small forceps. The entrance of the portal vein into the liver was then located and a cannula with a threaded tip inserted and tied in place. The cannula had previously been connected by means of gum rubber tubing to an elevated

separatory funnel containing the perfusion solution warmed to body temperature. A hypodermic needle connected to rubber tubing was inserted into the abdominal aorta. The Locke's solution flowed by gravity from the separatory funnel into the portal vein, through the liver and out through the hypodermic needle inserted into the aorta. The blood that was washed from the rat was collected in a beaker containing a small amount of a 3 per cent solution of sodium oxalate. The rate of inflow of the perfusing fluid was adjusted so that it was approximately equal to that of the rate of outflow from the aorta. The perfusion was continued until the liver became very pale and the outflowing fluid colorless.

Because of the weakened condition of many of the sick rats, perfusion of their livers was impossible. In each case, however, the abdominal aorta was cut and free bleeding allowed.

Removal of the Liver

The liver was removed by severing the mesenteric attachments and the blood vessels. The excised liver was freed of adhering fat or connective tissue and blotted free of fluid. A piece of the large lobe, weighing approximately 1 gram, was placed in a weighing bottle, weighed, and then dried to constant weight at 105° C. for

an estimation of moisture content. The remainder of the liver was weighed and put immediately into the freezing unit of an electrical refrigerator. The frozen liver was kept at a temperature of -7° C. until the chemical analysis was made.

Removal of Blood

The rat was anethetized by intrapleural injection of nembutal and the abdominal aorta exposed in the manner described under Preparation of the liver for removal. The blood was drawn into a 10 ml. syringe, transferred to a centrifuge tube, and thoroughly mixed with a small amount of heparin[a] previously placed in the tube. Enough blood was taken from each animal to yield 2 to 3 ml. of plasma. All blood samples were secured within 5 minutes after the animal was anesthetized. Williams et al. (1937) emphasized the necessity of promptness in obtaining blood samples to prevent a rise in blood cholesterol. Heparin was used as the anticoagulant instead of oxalate because a number of investigators have shown that error may be introduced by changes in the lipid content of the plasma brought about by variation in osmotic pressure introduced by the anti-coagulant. Man and Gildea (1932-33) found distinctly lower values for total fatty acids in oxalated plasma than in heparinized plasma and serum. This finding was verified

a. 1 to 50 heparin obtained from Hynson, Westcott and Dunning, Inc., Baltimore, Md.

by the more detailed study of the effect of anticoagulants on blood lipids by Boyd and Murray (1937). Significantly lower values of total and free cholesterol in oxalated plasma were found by Sperry and Schoenheimer (1935) than in either serum or heparinized plasma.

Examination of Other Tissues

The intact uterus was removed from the animal and weighed on a trip balance. It was cut longitudinally and each fetus with its placentae was removed, examined, blotted free from blood and placed in a weighing bottle to be weighed. After the fetus and placentae were weighed together, the placentae was removed and the fetus again weighed. Then the weight of the placentae was obtained by difference. Any resorptions were noted. The stripped uterus was weighed on the trip balance. The number of corpora lutea was recorded.

The relative amounts of fat in the subcutaneous, genital, perirenal, peritoneal, omental, and intermuscular depots were noted. The stomach was opened, washed in running water and examined for ulcers. The middle ear, the base of the tongue and the lungs were inspected for infection. Any abnormal condition was recorded.

CHEMICAL METHODS USED IN LIVER FAT ANALYSIS

Method Chosen

Several methods have been used by different workers to determine the lipid content of biological material. In Series I, the fat in the liver was weighed directly after alcohol-ether extraction. After grinding in sand the livers were first extracted for eight hours in absolute alcohol, then in a 1:1 mixture of alcohol and ether for eight hours, and finally in ether for the same length of time. The extracts were combined and the mixture reduced in volume over a water bath. The last traces of solvent were removed in a vacuum oven and the residue dried to constant weight. The crude fat thus obtained included the fats, sterols, and other alcohol-ether soluble materials in the liver.

Methods for the analysis of the fat fractions in the tissues were needed for Series II. Therefore, methods for the separation of the various lipid fractions were investigated. In 1933 Boyd discussed the advantage of using the same method in the determination of each lipid constituent. Instead of having a summation of errors, that will occur with the use of several different methods, there will be but one group of errors when the procedure is based on the

same principle. This fact became especially important when values for the lipids not estimated directly were calculated from the values of the determined lipids. Therefore, the oxidative procedure was chosen as the method of determining not only the total lipids but its fractions. Total fatty acids, total cholesterol, free cholesterol, phospholipid, iodine number of phospholipid fatty acids and iodine number of the acetone soluble fatty acids were estimated directly according to the method of Boyd (1933) with modifications suggested by Dr. H. H. Williams[a]. The cholesterol ester, neutral fat, and total lipid values were calculated from data obtained in the direct determinations.

A description of methods used in the preparation of the various solutions employed in the following analysis is given in the Appendix.

Extraction of Liver

The liver was prepared for analysis by a modification of the technique of Bloor (1929). The frozen tissue was removed from the weighing bottle and ground in a mortar with one teaspoon of acid-washed sand. The pasty mass was transferred quantitatively to a 200 ml. Erlenmeyer flask. One

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a. The methods for lipid analysis were developed by the author in Dr. Icey Macy Hoobler's Research Laboratory at the Children's Fund of Michigan in Detroit.

ml. of distilled water was added to the mortar and the grinding continued to loosen the adherent material which was then poured into the flask. Two additional ml. portions of water were used to completely clean the mortar. The material in the flask was shaken to break up any large lumps. In some cases the washing of the mortar was done with absolute ethyl alcohol. The weighing bottle was washed with portions of alcohol into the flask. About 85 ml. of a 3:1 mixture of absolute ethyl alcohol and redistilled ethyl ether were added and the mixture heated to boiling. It was then allowed to cool to room temperature and, in most instances, to stand over night.

The alcohol-ether extract was filtered through Whatman's no. 43 fat-free filter paper into a 200 ml. volumetric flask. After rinsing the contents of the filter paper with portions of alcohol-ether, the filter paper and contents were wrapped in two additional filter papers and placed in a Soxhlet condenser for a 5-hour extraction with redistilled anhydrous ethyl ether. The ether extract was then filtered into the above volumetric flask and made up to volume.

Determination of Total Fatty Acids

Total fatty acids, determined by the following procedure, includes total cholesterol. Hence, upon subtracting

the value for total cholesterol the remainder represented the total fatty acid content of the liver.

Duplicate aliquots of the alcohol-ether extract were measured into 125 ml. Erlenmeyer flasks for saponification with 0.1 ml. of saturated sodium hydroxide. Ten ml. of absolute alcohol was added and the whole evaporated on the water bath until no odor of alcohol remained. The last traces of alcohol vapor were swept out by a gentle current of air, leaving a pasty residue. One ml. of (1:3) sulfuric acid and one drop of phenol red were added. The solution was shaken to clump the fatty acids together. The fatty acids were extracted with 5 ml. of petroleum ether which was decanted from the watery residue into a 25 ml. volumetric flask. The extraction was repeated four timeswith about 5 ml. portions of petroleum ether and the mixture heated to boiling each time. The solution was brought to room temperature, filled to the mark and the contents well mixed.

Oxidation

The fatty acids in the sample were oxidized to carbon dioxide and water with dichromate and sulfuric acid, silver serving as the catalyst. The amount of material oxidized is determined by titration of the unused reagent by the iodometric procedure with standard thiosulfate.

Oxidations were conducted in triplicate. Aliquots of the petroleum ether solution which contained from 2 to 4 mg. of lipid were accurately measured into 125 ml. glass stoppered oxidation flasks. The solvent was evaporated on a water bath and the last traces of ether blown out with a gentle stream of air. Five ml. of the sulfuric acid-dichromate reagent was measured into the flask. Next, exactly 3 ml. of 1 normal potassium dichromate was added with rotation in a manner to rinse the oxidizing reagent from the walls of the flask. The flasks were loosely stoppered and placed in a steam heated water bath. After five minutes the stoppers were tightly inserted and the heating continued for 75 minutes. Then the flasks were removed from the water bath, 50 ml. of ice cold distilled water added and the solutions placed in cold water for not less than 20 minutes nor longer than two hours. Bloor in 1928a emphasized the necessity of having the digestion mixture remain definitely brown throughout the heating period to indicate an excess of oxidizing agent. A blank containing all the reagents except the fatty material was prepared and run at the same time under exactly the same conditions as the samples. One blank was run with every three samples.

The solutions were now ready to have the excess dichromate measured by titration. Ten ml. of a 10 per

cent solution of potassium iodide was added to the flask without stirring and a 0.1 normal solution of sodium thiosulfate added from a burette. At first the contents of the flask were gently rotated, later more forceful mixing was used after the iodine content had diminished. When the titration was nearly complete and just before the last tint of yellow disappeared, 2 ml. of starch solution was added. The end point was reached when the dark blue of the starch solution changed to a light blue. The silver caused the formation of a white precipitate which did not interfere with the end point. The blank was titrated in the same way. The difference between the titrations of the blank and the sample represented the amount of 0.1 normal dichromate used by the fatty acids in the sample. The titration difference was multiplied by the correction factor for the thiosulfate since it was not exactly 0.1 normal. Standardization of the thiosulfate is given in the Appendix. The number of ml. of 0.1 normal thiosulfate expressed as ml. of 0.1 normal dichromate used was divided by 3.6 to estimate the milligrams of fatty acid present in the aliquot analyzed. The amount of fatty acid in the liver was calculated. 3.6 ml. of 0.1 normal dichromate is the equivalent of 1 mg. of fatty acids. Bloor (1928a) found this factor, 3.6, very close to the theoretical oxidation values of stearic, palmitic and oleic acids and less than 10 per

cent different from the theoretical value for cholesterol.

The oxidation flasks were cleaned for use by completely filling them with cleaning solution (concentrated sulfuric acid and dichromate) and allowing them to stand over night. After the flasks were rinsed five to seven times in tap water followed by the same number of rinsings in distilled water, they were hung in a wire mesh to dry.

Determination of Phospholipids

Phospholipids were determined on a petroleum ether extract of the alcohol-ether residue obtained after reduction *in vacuo* in an atmosphere of nitrogen according to the method used by Williams *et al.* (1938). Aliquots of 50 or 75 ml. of the alcohol-ether extract were evaporated to dryness under a stream of nitrogen in a vacuum maintained by a Cenco oil pump. All joints in the reduction system were sealed with water. The temperature of the water bath was not allowed to rise above 45° C. The residue was extracted with a 10 ml. portion of petroleum ether which was filtered into a 50 ml. volumetric flask. Additional extractions were made with five portions of petroleum ether, heating the solution to boiling the last three times. The filter paper was washed with five portions of petroleum ether. The solution was cooled to room temperature and made up to volume. Phospholipids, total cholesterol, free

cholesterol, iodine number of phospholipid fatty acids and iodine number of the acetone soluble fatty acids were determined on this extract.

A 15 ml. aliquot of the petroleum ether solution was measured into a 50 ml. centrifuge tube for precipitation of the phospholipids by an excess of acetone which is essentially the method used by Katsura and coworkers (1934). The method of reduction of volume of the solution followed the plan described by Bloor (1929). The volume of the solution in the tube was reduced to 3 ml. by immersion in a beaker containing hot water. A boiling tube, made by fusing about 1/2 in. of melting point tube to the end of a 6 in. length of 2 mm. stirring rod, was placed in the solution to prevent excessive boiling. Next 5 ml. of dry acetone (redistilled from calcium chloride) was added and the volume again reduced to 2½ ml. The phospholipid was precipitated by adding 20 ml. of acetone (redistilled acetone which was kept on calcium chloride) and allowing the solution to stand one-half hour. The boiling tubes were removed carefully and placed in such a manner that no adhering precipitate was lost. The precipitate was separated by centrifuging 7 minutes at 1400 revolutions. If the solution was not clear, the precipitation was repeated with another portion of acetone. This step was necessary in only a few cases. The acetone solution was filtered into a 25 ml. volumetric

flask. The precipitate was washed twice with small portions of acetone followed by centrifugation, dried in a current of nitrogen and dissolved in 10 ml. of warm moist ethyl ether. The boiling tube was placed in the moist ether to dissolve the adhering precipitate. The above filter paper was used for filtering the moist ether extract into a 25 ml. volumetric flask. The tube and later the filter paper were washed with five portions of moist ether. The solutions were made up to volume and aliquots which contained 2-4 mg. of phospholipid were used for oxidation.

The oxidation was carried out as previously described. Three ml. of 0.1 normal dichromate solution was considered equivalent to 1 mg. of phospholipid. This factor was used in the calculation (Bloor, 1929; Boyd, 1931).

Determination of Free Cholesterol

The precipitation of cholesterol by digitonin has been found by many workers to be a precise method for the measurement of cholesterol. By determining the amounts of free and total cholesterol, the value for combined cholesterol can be calculated as the difference between the two values.

A modification of Okey's method described by Turner (1931) was essentially the method used in the determination of the two cholesterol fractions. One extra step was

introduced into the procedure just before the oxidation of the precipitate. Since digitonin has been shown by McEwen[a] as quoted by Kelsey (1939) to precipitate some of the phospholipid, the free cholesterol determinations were made on the acetone fraction.

A 15 ml. aliquot of the acetone solution which contained about 1 mg. of cholesterol was measured into a 15 ml. centrifuge tube and slowly reduced to dryness in a beaker of hot water. Care should be taken to prevent explosive boiling during the evaporation. When the solution just reached dryness, the lipid was dissolved in 4 ml. of absolute ethyl alcohol and 1 ml. of a 1 per cent solution of digitonin in 50 per cent alcohol added. Gentle mixing with a stirring rod aided in precipitation. A blank containing 1 ml. of the digitonin was run at the same time. The ethyl alcohol was evaporated by placing the tube in a beaker of hot water on the water bath. Drying as completely as possible at this point greatly facilitated subsequent washings with ether and water. Three washings with ether to dissolve the lipids remaining after precipitation of the cholesterol were followed by three washings with boiling water to dissolve excess digitonin. Ten ml. of warm

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a. Recently McEwen published his findings. McEwen, H. H., and MacLachlan, P. L., Proc. Soc. Exper. Biol. and Med., 48, 195-197, 1941.

redistilled ethyl ether was added to the dry precipitate without stirring and the tubes were centrifuged 10 minutes at 1600 revolutions. The ether was drawn off through a piece of glass tubing, drawn out to a capillary opening, by suction produced by a water pump. The glass tube was inserted carefully into the center of the centrifuge tube in such a manner that dislocation of any of the precipitate adhering to the sides of the tube was avoided. Two more washings were made with 10 ml. portions of warm ethyl ether which was mixed with the precipitate with a stirring rod and then separated by centrifuging 10 minutes. The last traces of ether were evaporated by allowing the tubes to stand over night at room temperature. The digitonide was washed three times with boiling water. Each time, the digitonide was stirred and centrifuged for 25 minutes at 2400 revolutions. If after the third washing with water, the solution drawn off was foamy showing the presence of digitonin, a fourth washing was necessary. The water was drawn off and the last trace of water removed by evaporation in a steam heated water bath. The dry cholesterol digitonide was dissolved in 5 ml. of boiling absolute methyl alcohol and transferred quantitatively to a glass stoppered oxidation flask. Five 1 ml. portions of boiling methyl alcohol were used to wash out the tubes.

The steps described in the oxidation of total fatty

acids were followed in the oxidation of cholesterol digitonide. The milligrams of cholesterol were calculated with the use of a factor, 10.62 of 0.1 normal dichromate solution being considered the equivalent of 1 mg. of cholesterol digitonide (Okey, 1930).

Determination of Total Cholesterol

The method used in the saponification of the lipid extract for determination of total cholesterol was essentially the same as that described by Kirk et al. (1934). Duplicate aliquots of the petroleum ether extract were measured into saponification tubes which were pyrex test tubes 175 x 22 mm. The solution was reduced to dryness in a beaker of hot water and 2 ml. of saturated alcoholic sodium hydroxide added. Two blanks containing only the alcoholic sodium hydroxide were run simultaneously. The tubes were heated in an electric oven at 85° C. for 2½ hours. After saponification was complete one drop of phenol red was added and approximately 1 normal hydrochloric acid was added drop-wise until the color changed to yellow. The fatty acids were extracted with five 5 ml. portions of petroleum ether, heating to boiling each time. A special suction head was used in transferring the petroleum ether to a special 25 ml. volumetric flask. The solutions were made up to volume. The cholesterol in a 15 ml. aliquot of the petroleum ether solution was

precipitated and oxidized in exactly the same manner that was used in determining the free cholesterol. Care was taken to carefully evaporate the ether solution in the first step.

Determination of Phospholipid Fatty Acid Iodine Number

The method adopted for the determination of iodine numbers was that of Yasuda (1931-32) with some modifications. Iodine numbers obtained on different amounts of oleic acid are found in table VII in the Appendix. The values agreed with those obtained by Yasuda on oleic acid.

Phospholipids were precipitated from the petroleum ether solution, dissolved in moist ethyl ether and transferred directly into a 125 ml. Erlenmeyer flask. Ten ml. of absolute ethyl alcohol, 0.1 ml. of a 0.1 per cent solution of hydroquinone, and two drops of a saturated solution of potassium hydroxide were added and the flask placed on a water bath for saponification. Wilson et al. in 1935 showed that the addition of hydroquinone prevented oxidation of the fatty acids during the saponification and did not itself interfere with the subsequent determinations. Evaporation was allowed to progress until the volume was reduced to about 2 ml. Then nitrogen was admitted and the volume finally reduced under nitrogen to a gummy residue.

Last traces of solvent were swept out with a gentle stream of nitrogen. The residue was neutralized with 1 ml. of (1:3) sulfuric acid and one drop of phenol red added. Extraction with petroleum ether was done as previously described under Total fatty acids. The solvent was filtered into a 25 ml. volumetric flask and made up to volume.

An aliquot of the petroleum ether extract which contained 3 to 4 mg. of fatty acid was used for the analysis of the fatty acid content by the oxidative method and the same sized aliquot was measured into a 50 ml. glass stoppered Erlenmeyer flask to determine the iodine number. After evaporation of the solvent under nitrogen, the fatty acid was dissolved in 2 ml. of anhydrous chloroform. One sample and its blank and a second sample and its blank were prepared. Five ml. of pyridine dibromide solution was added from a microburette to the four flasks in consecutive order. The addition of the reagent to each sample and its blank at the same temperature is important since variations in room temperature easily influence the amount of the reagent in 5 ml.[a] After the first sample had set 15 minutes the four titrations were run immediately. For the titration the stopper was carefully washed off with a

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a. The author wishes to thank Dr. J. A. Schulz for his suggestions and helpful advice.

stream of carbon dioxide-free water and the sides of the flask washed down, 0.5 ml. of a 10 per cent solution of potassium iodide added and 0.02 normal thiosulfate run in drop-wise from a 5 ml. microburette until the iodine color had changed to a straw color. Next, 1 ml. of starch solution was added and the titration continued until the dark blue color changed first to lavender and then to a colorless hue with a sharp end point. The 0.02 normal thiosulfate was standardized against a 0.02 normal iodate solution every day it was used. Typical standardization values are given in the Appendix.

Calculations were made using the formula of Yasuda (1931-32)

$$\frac{(a - b)\, d}{c} \times \frac{1.27}{5} = \text{iodine number}$$

in which a is the titration value for the blank; b the titration value for the sample; c the number of gm. of fatty acids; and d the correction factor for the thiosulfate. The factor 3.6 was used in the calculation of the fatty acid content by the oxidative method.

Determination of the Acetone Soluble Fatty Acid Iodine Number

The iodine number of the fatty acids in the acetone fraction was determined in the acetone mother liquor and washings from the precipitation of the phospholipid by the

method described for the analysis of the phospholipid fatty acids. Aliquots were used which contained 2 to 4 mg. of fatty acid. Since this fraction contained the cholesterol, allowance was made in the calculation by substracting the amount of cholesterol in the aliquot. Yasuda's (1931-32) formula was used for this calculation as follows:

$$\frac{(a - b)\, d - (0.27 \times e)}{c} \times \frac{1.27}{5} = \text{iodine number}$$

in which a is the titration value for the blank; b the titration value for the sample; c the number of gm. of fatty acids; d the correction factor for the thiosulfate; and e the number of mg. of cholesterol in the aliquot.

Calculated Lipids

Values for the other fractions of liver lipids were found by calculation from the results obtained by the above procedures. The amount of combined cholesterol was obtained by subtracting the value for free cholesterol from that for total cholesterol. The cholesterol ester fatty acids were computed as 0.67 times the combined cholesterol (Boyd, 1933). Phospholipid fatty acids were calculated as 0.67 times the phospholipid. The sum of the phospholipid fatty acids plus the cholesterol ester fatty acids was subtracted from the total fatty acids to give the value for the neutral fat fatty acids which make up

95 per cent of the neutral fat. Then the neutral fat was computed. The total lipid was determined as the sum of the phospholipid plus the neutral fat plus the total cholesterol plus the cholesterol ester fatty acids.

CHEMICAL METHODS USED IN BLOOD FAT ANALYSIS

Lipid Fractions Studied

A complete analysis of all the lipid fractions in blood would have been highly desirable. However, the amount of time which could be devoted to this phase of the problem was limited. It was believed that analysis of the total fatty acids, phospholipids, and total cholesterol would indicate changes that were taking place in the blood lipids. At a later date measurement of the free and total cholesterol would be desirable. The relationship between the cholesterol esters and the other fractions could then be studied.

Method Chosen

The oxidative procedure already described was used to determine the amount of total fatty acid and phospholipid in the blood plasma. Total cholesterol was determined by the method described in the booklet, General directions, which accompanied the Klett-Summerson photoelectric

colorimeter. This method is a modification of the method of Bloor, Pelkan, and Allen (1922).

Extraction of Blood

The recommendations for the extraction of blood fat given by Boyd in 1936 were followed. The heparinized blood was centrifuged 40 minutes at a speed of 2400 revolutions per minute. The plasma was slowly added with agitation to 80 ml. of a 3:1 mixture of absolute ethyl alcohol and redistilled anhydrous ethyl ether. A finely divided precipitate was obtained. The extract was heated to boiling with frequent shakings for 5 minutes, allowed to cool to room temperature, filtered and made up to a volume of 100 ml.

Determination of Total Fatty Acids

An aliquot of the alcohol-ether extract of the plasma was used for the determination of total fatty acids which included total cholesterol. The analysis followed the procedure described for the determination of total fatty acids in the liver extract.

Determination of Phospholipids

Phospholipids were precipitated in an excess of acetone and analyzed in the same manner that phospholipids in the liver extract were determined.

Determination of Total Cholesterol

Total cholesterol was analyzed by dissolving the lipid in chloroform, treating the extract with acetic anhydride and concentrated sulfuric acid, and allowing the cholesterol color to develop. The color was read in a Klett-Summerson photoelectric colorimeter using a blue filter. The chloroform, acetic anhydride and sulfuric acid must be of the highest quality.

Triplicate aliquots of the acetone solution were measured into 50 ml. Erlenmeyer flasks and the acetone evaporated on a water bath. A 1 ml. portion of anhydrous chloroform was added to the dry residue, brought momentarily to boiling on the water bath, and then the liquid carefully decanted into a clean dry colorimeter tube. This process was repeated with three more 1 ml. portions of chloroform. The combined extracts were allowed to cool to room temperature before making up to a final volume of 5 ml.

A blank tube containing 5 ml. of anhydrous chloroform was prepared. Exactly 1 ml. of acetic anhydride was added from a burette to the blank and the tubes containing the unknown. The contents of each tube was mixed with a dry glass rod which was removed before placing the tube in the colorimeter. The blank tube was placed in the colorimeter and the instrument adjusted to its zero point. Then the unknown was read in the colorimeter against this zero point.

This reading of the unknown was called the R_1 reading and corrected for any color present in the solution before the cholesterol color was developed by adding the sulfuric acid. The stirring rod was returned to the colorimeter tube and the tube set aside until the standard tube had been prepared.

To prepare the standard tube 5 ml. of a standard solution of cholesterol in chloroform (see Appendix) was placed in a clean dry colorimeter tube and 1 ml. of acetic anhydride added. The contents were mixed with a glass rod.

To both the standard and unknown was added 1 ml. of concentrated sulfuric acid. The contents were well mixed with the glass rods and the solutions placed in a dark place for 15 minutes. At the end of that time the rods were removed and the tubes read in the colorimeter against the blank tube adjusted to the zero point. This reading of the unknown tube is called the R_2 reading.

The cholesterol content of the unknown is obtained from the standard and unknown readings as follows:

$$\frac{\text{mg. cholesterol in 5 ml. standard}}{\text{reading of standard}} \times (R_2 - R_1) = \text{mg. cholesterol in sample}$$

REVIEW OF LITERATURE

FAT METABOLISM IN THE LIVER

The liver has long been known to play an important part in a large number of physiologic activities. The inability of the liver to perform any one of its many functions may be reflected in some derangement of metabolic processes.

The hepatic functions pertinent to the present discussion are concerned with the utilization of food. Mann (1941) lists and discusses these functions as the storage of foodstuffs, the manufacture of food materials, and the regulation of the food supplies to the body. Carbohydrates as glycogen, and fat in several forms can be stored in relatively large amounts in the organ. There is also some evidence that a storage form of protein may be deposited in hepatic tissue.

The importance of the liver in carbohydrate and protein metabolism has been well established for some time. The rôle of this organ in fat metabolism in recent years has assumed major importance as rapidly accumulating data indicate the relationship of hepatic activity to the utilization of fat.

Under normal conditions the amount of fat which is stored in hepatic tissue changes very little. The fat which is brought there is worked over and transported to the tissues. Bloor discussed in 1939 the changes which occur in the fat before it is stored in the liver. Early workers had proposed the hypothesis on purely theoretical grounds that the fat was phosphorylated in the liver. More recent work has substantiated this early assumption. This fact has been studied by many investigators who have used a variety of substances such as the radioactive isotope of phosphorus or certain easily identified fatty acids as tracers. Sinclair (1935a, 1935b) demonstrated by feeding elaidic acid that the phospholipid in the liver is found in two forms, i.e., a structural and a metabolic form. The latter form contained the ordinary food fatty acids and exhibited a rapid turnover. When the dietary supply of unsaturated fat was cut off, the metabolic form of the phospholipid was believed to maintain its high degree of unsaturation by retention of its own fatty acids (Sinclair, 1935b).

The liver, kidney, and intestine have been found by Perlman et al. (1937) to be a group of organs particularly active in phospholipid turnover. Other workers have also noted that the liver ranks high among the organs in the body engaged in phospholipid metabolism. The laying hen has to produce large quantities of phospholipid in the

the rate of phospholipid turnover. It should be pointed out that these workers emphasized the fact that while the phospholipid turnover may be rapid, the phospholipid content of the liver itself is as constant as it is in other tissues.

A recent study on fat metabolism by Barnes et al. (1941) is of interest. They tagged the lipids with fatty acids of strong spectral absorption. Adrenalectomized rats which had been maintained in good physical condition by the administration of salt exhibited an impaired ability to deposit absorbed fat in the neutral fat stores of the liver. No such impairment was noticed in the transport of these acids into the liver phospholipid. Hence, the neutral fat has importance as well as the phospholipid in fat transport.

FATTY LIVERS

Livers which contain an accumulation of fat have been noted by many workers under a great variety of circumstances. The ability of the liver to hold widely varying quantities of fat is one of the most striking characteristics of the organ. However, Mann emphasized in 1941 that the liver is impaired in regard to some of its other functions by any considerable increase in the fat content of the liver, even by an increase produced by physiologic means.

The concentration of total lipid or of any single lipid constituent that is present in the liver at any given time is influenced by many different factors. The species of animal used, type of diet fed including its vitamin content, action of hormones and administration of toxic substances may be mentioned. A detailed outline of the literature in respect to the production, the cure, and the prevention of fatty livers was made by Campbell in 1940. A very brief summary of the most important circumstances under which fatty livers are produced follows:

(1) Fatty livers produced by operative procedure
    (a) Depancreatized dogs maintained with insulin (first reported by Fisher, 1924; Allen et al., 1924; confirmed by many later workers)
    (b) Pancreatic duct-ligated dogs (Ralli et al., 1938; Montgomery et al., 1939)
(2) Fatty livers produced by dietary manipulation
    (a) After feeding a high fat, low protein diet (Best, Channon, and Ridout, 1934)
    (b) After cholesterol feeding (Okey et al., 1934; Best et al., 1934)
    (c) After cystine feeding to rats fed low protein, high fat diets (Tucker and Eckstein, 1937)

(d) After feeding diets which contain or lack certain members of the vitamin B complex (McHenry, 1937; Gavin and McHenry, 1941b)

(3) Fatty livers produced by fasting (Best and Ridout, 1938a)

(4) Fatty livers produced by the administration of poisons (Barrett et al., 1939-40)

(5) Fatty livers produced by administration of hormones, i.e., anterior pituitary extract (Best and Campbell, 1938)

It should also be noted that fatty livers may be associated with certain pathological conditions (Connor, 1938).

The prevention and cure of these various types of fatty livers have been the subject of extensive investigation by many workers. Choline is the substance which has been found to have the most lipotropic activity when used to prevent or cure certain types of fatty livers. It will control the increase in neutral fat found in the fatty livers produced by feeding a high fat, low protein diet or a high cholesterol ration (Best and coworkers, 1934). In the latter type of fatty liver the deposition of cholesterol ester is only partially controlled by choline. It will cause a 60 per cent decrease in cholesterol esters when provided in adequate amounts.

The fatty livers produced by feeding thiamine and

by a pyridoxine deficiency were controlled by choline (Halliday, 1938; Gavin and McHenry, 1940). However, the large accumulation of fat produced in livers by the administration of biotin, another member of the vitamin B complex, was only slightly affected by choline while lipocaic prevented the condition (Gavin and McHenry, 1941b). Extracts of liver, kidney, muscle, wheat germ, yeast, and rice polishings made according to the procedure which had been used for the preparation of lipocaic from pancreas were found to have the same lipotropic effect as lipocaic (Gavin and McHenry, 1941a). Later inositol proved to be as effective as lipocaic in preventing the "biotin" type of fatty liver.

The curative effect of dietary protein in alleviating fatty livers was first observed independently by Best and Huntsman in 1935 and by Channon and Wilkinson in 1935. Later Tucker and Eckstein (1937) and Channon, Manifold, and Platt (1938) proved that methionine was also effective in this respect. The action of methionine was similar to that of choline in decreasing the deposition of neutral fat but differed in that it decreased both the free and ester cholesterol in the "cholesterol" fatty liver (Channon, Manifold, and Platt, 1938; 1940). However, the effect on the neutral fat was only observed when the fat content of the livers of the control animals was high (20 to 25 per

cent) while no effect was noticeable when the amount present did not rise above 15 per cent.

Fatty livers in depancreatized dogs maintained with insulin have been prevented by feeding raw pancreas, choline in adequate amounts, lipocaic (Dragstedt, 1936), an extract of pancreas designated as "Fraction AR" (Entenman and Chaikoff, 1941), and fresh pancreatic juice (Montgomery et al., 1941). At present, the results obtained by the several investigators are at variance as to whether the active principle is an internal or external secretion of the pancreas. It is not within the scope of this discussion to pursue the details of the controversy.

The early experiments in which lipocaic was tested for its effect on fatty livers of rats showed that the substance did not exert any curative action that could not be accounted for by the summation effect of the choline present in the preparation and the dietary protein (Best and Ridout, 1938b; MacKay and Barnes, 1938). After more extensive research, Channon, Loach, and Tristram (1938) concluded that lipocaic did contain a specific lipotropic factor.

SIGNIFICANCE OF CHANGES IN LIPID FRACTIONS

In the Liver

The changes which occur in the liver and in the

various lipid fractions following an accumulation of fat are of interest. A general increase in the size and weight of the liver as it becomes loaded with fat has been noted by many workers. This increase in size is accompanied by a decrease in moisture content. Kaplan and Chaikoff (1936) after making a detailed study of the relation of glycogen, fat, and protein to water storage in the liver, concluded that the decrease in moisture content is due to a dilution of the contents of the liver with fat entering the organ.

Changes in the concentrations of the various lipid fractions of an organ are definitely related to its physiological activity or efficiency. Yet as Bloor et al. stated in 1930 it is not only the concentrations but also the balance between these substances which are of physiological significance. Cholesterol and phospholipid are considered integral and constant components of tissues and are not altered in amount except under extreme changes in the nutritional status of the animal. Neutral fat, on the other hand, has been found to be the most variable lipid component. In 1923b, Bloor studied the lipid content of various beef tissues. He observed that the phospholipid content of a tissue is always characteristic of an organ, not varying in any tissue more than 30 per cent above or below the average value. For example, he found beef liver contained 2.1 to 3.9 gm. of phospholipid per 100 gm. of

tissue. Cholesterol occurs mainly in the free form in normal tissues while cholesterol esters are present in notable amounts only in blood plasma and in degenerating tissues. A study by Bloor et al. (1930) of the variations in the lipid content of the corpus luteum, an organ which passes through periods of activity and regression, demonstrated the fact that the phospholipid content and the free cholesterol to a lesser degree increase during activity of the organ. Associated with retrogression or lowered physiological inactivity are increased concentrations of neutral fat and cholesterol esters. Later, Boyd (1935b) strengthened this hypothesis with results from a study of the lipids in the jelly of Wharton from the umbilical cord, one of the least active of all the body tissues. The inactive tissue contained very little phospholipid and free cholesterol. From the finding that cholesterol esters were absent in the inactive tissue, he concluded that the presence of large amounts of the esters in a tissue indicate a degeneration or retrogression.

The remarkable ability of the liver to maintain a constancy in the absolute amount of phospholipid regardless of the degree of infiltration of fat has been demonstrated. For instance, Channon and his associates (1937) after extensive observations concluded that the total content of liver phospholipid is independent of dietary

choline and the degree of fatty infiltration. Also, the majority of workers agree that after cholesterol feeding no change occurs in total phospholipid (Okey and Yokela, 1936; Loizides, 1938). Another confirmation of the fact that the phospholipids remain remarkably constant in amount despite an enormous increase in total lipid is found in the work of Longenecker and coworkers (1941). They examined fatty livers produced by feeding beef liver extract. Neither does poisoning of the liver by carbon tetrachloride produce much change in the phospholipids (Winters, 1940). The only contradictory evidence found appeared in the work of Beeston and coworkers (1935) who reported an increase in the actual weight of lecithin present in the liver as they increased the percentage of protein in the diet of rats from 5 to 15 per cent.

Variations in the free cholesterol content of the liver under different circumstances follow closely those found in phospholipid values, i.e., few changes are reported in the different types of fatty livers. Free cholesterol apparently is not increased in the "cholesterol" fatty liver (Best et al., 1934; Okey et al., 1934). Finally, in a fatty liver produced by beef liver extract, the percentage of free cholesterol is not appreciably affected (Longenecker and coworkers, 1941).

However, the picture of the cholesterol esters in

varying experimental circumstances does not follow the pattern of phospholipid and free cholesterol. Big increases occur in certain types of fatty livers. The most striking effect is observed in the "cholesterol" fatty liver where the increase in cholesterol esters is greater in degree than that noted in any of the other lipid fractions (Best et al., 1934). These authors also noted a small but definite rise in ester content in the fatty livers produced by high fat feeding. Variations in total cholesterol should follow those found in the ester fraction since no significant changes were found in free cholesterol. Hence, the increase by approximately 5 times in total cholesterol observed by Gavin and McHenry (1941b) in the "biotin" fatty liver may be assumed to be due to cholesterol esters.

In the Blood

The importance of the cholesterol esters in the blood plasma has been discussed by Bloor (1939). Two-thirds or more of the total cholesterol in the plasma is combined with fatty acids as esters. Cholesterol ester is nearly as important as phospholipid in the blood in fatty acid transport. As the cholesterol esters of the blood pass into the tissues they are believed to be deprived of their fatty acids which then are available for use by the cells.

In the experimental animal, certain conditions have produced a reduction in cholesterol esters in the blood. Chaikoff and Kaplan (1934) noted in depancreatized dogs maintained with insulin a storage in cholesterol esters and neutral fat in the liver that was accompanied by a decided decrease of cholesterol esters in the blood. Blood lipid values were reduced to half their normal value. Feeding choline did affect a reduction in liver lipids but did not alter the low blood values. Raw pancreas, lipocaic (Dragstedt et al., 1939), and fresh pancreatic juice (Entenman et al., 1941) have proved effective in maintaining normal concentrations of blood lipids.

Hypolipemia in the blood has also been produced experimentally by injecting an anterior pituitary extract in rabbits (Houchin and Turner, 1939). The decline in blood plasma fat values reached an average of 36 per cent of the initial value within 6 to 8 hours. Fatty livers also resulted after injection of anterior pituitary extract in mice, guinea pigs and to a lesser extent in rats (Best and Campbell, 1938). Hence, again the lowered blood lipid values appear to be associated with fatty livers.

In rats given a diet containing 2 per cent cholesterol, the level of blood cholesterol was elevated to about three times the normal value (Cook and McCullagh, 1939). A fatty liver accompanied this rise in blood fat. This

finding does not agree with that of Chanutin and Ludewig in 1933, who added 2.5 per cent cholesterol to the diet and found blood lipids varied little from those in a normal group.

The lipid values of the blood in a group of rats having fatty livers developed by cystine feeding and in another group of rats receiving methionine were determined by Channon et al. (1940). No differences in values for the two groups of rats were detected. They found no change in the amount of blood fat at any time up to 31 hours after the meal.

CHANGES IN LIVER AND BLOOD LIPIDS IN TOXEMIC PREGNANCY

There is considerable evidence that a derangement in fat metabolism occurs in toxemias of pregnancy. Roderick et al. (1933) reported an enormous elevation in the fat content of livers of pregnant ewes which developed pregnancy disease. They did not find any significant deviations in blood cholesterol, only a slight drop being noted in the sick animals.

The physical state of sheep in flocks where pregnancy disease is prone to develop varies; the ewe may be well fed and underexercised, or it may be undernourished. Its condition seems to bear some relation to the appearance of fatty livers. For example, Roderick et al. (1937) produced fatty

livers in ewes by starvation that corresponded to those found in field cases of pregnancy disease. They concluded that the problem was related to carbohydrate metabolism. As the glycogen was withdrawn from the liver to maintain the blood sugar level, the fat took its place.

Snook (1939-40) also observed fatty infiltration in the livers of ewes developing pregnancy disease. They expressed doubt as to whether the loss of liver function was significant in precipitating the decline of the ketonemic animals because many ewes, which by inference had fatty livers of long standing, went to term successfully. The finding by these workers that livers found in pregnant ewes developing toxemia contained more fat than did the livers of healthy pregnant animals was not confirmed by Dryerre and Robertson (1940-41). They observed no differences.

The toxemia of pregnancy occurring in the rabbit by Greene (1937) is also of interest. The livers of these animals showed widespread fatty infiltration and degeneration. Plasma fat values and whole blood cholesterol values in twelve fatal cases of toxemia were not greatly altered from those obtained in healthy animals. Later Greene (1939) produced a disorder resembling spontaneous toxemia of pregnancy by administration of aqueous anterior pituitary extract. The livers showed fatty degeneration and areas

of necrosis.

Another method of producing toxemia experimentally in the rabbit was employed by Patterson et al. (1938). Rabbits which were completely thyroidectomized, developed convulsions at term and died. A greater lowering of blood cholesterol occurred in these animals than in healthy pregnant rabbits.

Many clinical studies have been made of eclampsia in human beings. However, few detailed reports of laboratory findings are available. Blood lipids in eclamptic patients have been studied in detail by Boyd (1935a). He found the concentration of the lipids varied greatly but there was no significant variation in the value of any single lipid. All values reported for the various fat fractions were within the normal range. However, the phospholipid to cholesterol ratio in the plasma without exception was found to be higher in eclampsia than in normal gestation. Many workers have noted a hypercholesteremia in pregnancy. Slightly higher cholesterol values were noted by Bartholomew and Kracke (1936) and by Patterson et al. (1938). Reduction of the high cholesterol values could be accomplished by giving thyroid extract. Hypothroidism is an important factor in excessive hypercholesteremia. The two latter groups of workers disagree as to details but both support the theory that the hypercholesteremia

is the basis for vascular changes in the placental arteries which result in placental infarction and degeneration. Toxins absorbed from the degenerating placentae might then cause eclampsia.

RESULTS AND DISCUSSION

FAT CONTENT OF THE DIET

In preparing the pork muscle for canning, a considerable portion of the fat present is removed by trimming. By exercising care in this process and later, by removing the layer of congealed fat on the top of the canned meat, the fat content of the Pork I diet may be kept at a minimum. Jones in 1931 found that the diet contained 24 per cent of fat. She weighed the fat directly after alcohol-ether extraction of the entire ration.

In the present study, the total alcohol-ether soluble constituents of only those components of the ration that might contain fat were determined gravimetrically.[a] Total fat, thus defined, made up 13.6 per cent of the Pork I diet. Two contributing factors probably are responsible for the discrepancy between this value and the one reported by Jones. Closer trimming of the fat from the pork muscle and better removal of the congealed fat on the autoclaved meat may account for part of this reduction. Analysis of only the fat-carrying constituents of the diet

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a. The fat content of dried pork, yeast, butter and cod liver oil equivalent to 8 gm. of diet is 1.085 gm.

is also undoubtedly a factor.

It was interesting, also, to secure information regarding the cholesterol content of the diet since the sterol plays a part in the development of fatty livers. Therefore, the quantity of cholesterol was determined. Inasmuch as the percentage of cholesterol in all ingredients except the meat of the diet is negligible, only the dried pork muscle was analyzed.[a] The oxidative procedure was used. The data showed that the free cholesterol content of the diet was 0.031 per cent.

These analyses indicate that the amount of total fat in the diet and of cholesterol in the meat are of a low enough magnitude to eliminate the possibility that dietary lipids alone are responsible for the development of fatty livers.

RELATIVE MOISTURE CONTENT AND SIZE OF LIVERS

Steenbock V Virgins vs. Pork I Virgins

The moist weights of the livers and the relative moisture contents of the organ were studied in the virgin pork-fed rats and the virgin control rats. Animals in Series I and Series II were grouped together for comparison.

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a. 3.727 mg. free cholesterol in 3 gm. dried pork.

Individual weights of the livers from the animals in the two series are given in tables XIV and XV (Appendix). The data are summarized in table 3. Analyses of the significance of the mean differences noted are presented in table 4. The F test of significance was used.[a] (One star indicates that F was significant; two stars, highly significant). In the two groups, the livers did not vary significantly in regard to weight or to moisture content. Thus, in the virgin rat, the effect of feeding the pork diet is not reflected in any change in size of the liver or in its moisture content.

It will be shown later that a deposition of fat, somewhat above normal, occurs in the livers of non-gravid rats fed the pork diet. The finding that liver size is not increased in the Pork I virgin is not in agreement with results reported in the literature regarding the weight of the organ when fatty livers appear. Best and Huntsman (1935) found that the livers may increase to twice their normal size when they become infiltrated with fat.

Virgin vs. Pregnant Rats

A comparison of the weights of the liver of each

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a. Snedecor, G. W.
1938. Statistical Methods, revised ed., Collegiate Press Inc., Ames, Iowa

TABLE 3. AVERAGE MOISTURE CONTENT AND WEIGHT OF LIVERS IN RATS IN SERIES I AND II[a]

Diet	Reproductive status	Number of rats	Weight of liver	Moisture content
			gm.	per cent
Steenbock V	Virgin	11	5.318	68.9
	Pregnant	25	6.900	70.5
Pork I	Virgin	13	5.665	69.7
	Pregnant	28	7.025	65.5

a. The values for Series I and Series II were combined in this table.

TABLE 4. ANALYSIS OF VARIANCE OF MOISTURE CONTENTS, LIPID CONTENTS, AND WEIGHTS OF LIVERS IN VIRGIN RATS

Analyses made	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Weights of livers	Items within the groups	22	10.333	0.470	
	Between group means	1	0.717	0.717	
	Total	23	11.050		1.52
Relative amount of moisture (per cent)	Items within the groups	22	103.41	4.70	
	Between group means	1	4.50	4.50	
	Total	23	107.91		
Relative amount of total lipid (per cent)	Items within the groups	15	82.50	5.50	
	Between group means	1	81.00	81.00	
	Total	16	163.50		14.73**

virgin group in Series I and Series II with similar data characteristic of the corresponding pregnant group is interesting (Table 3). Only the pork-fed animals which did not develop toxemia were included in this analysis. In both the control and experimental groups, the average weights of the livers of the pregnant rats were greater than those of virgins, the variance being highly significant (Table 5).

In pregnancy, the per cent of water in the livers of the control rats did not change from that characteristic of livers in the virgin rat. However, the pregnant females receiving the pork ration had a significantly lower percentage of water in their livers than did the virgin rats fed the same diet (Table 5). For example, the average per cent of moisture in the livers of the virgins was 69.7; in the livers of the gravid females, 65.5.

Thus, the data show that pregnancy is associated with a definite increase in the size of the liver. The phenomenon is apparent in both the experimental and control groups. With the liver enlargement, there is no concomitant change in the moisture content of the organ in the control group. However, the amount of water in the hepatic tissue of the gravid female fed the pork diet is low.

TABLE 5. ANALYSES OF VARIANCE OF LIVER WEIGHTS AND LIVER MOISTURES IN VIRGIN AND PREGNANT RATS

Analyses made	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Weights of liver of Steenbock V rats	Items within the groups	34	19.453	0.572	
	Between group means	1	19.124	19.123	
	Total	35	38.577		33.42**
Weights of livers of Pork I rats	Items within the groups	39	21.514	0.551	
	Between group means	1	16.805	16.805	
	Total	40	38.319		30.47**
Relative amount of moisture - Steenbock V rats	Items within the groups	34	139.35	4.10	
	Between group means	1	21.58	21.58	
	Total	35	160.93		5.26
Relative amount of moisture - Pork I rats	Items within the groups	39	522.24	13.39	
	Between group means	1	160.98	160.98	
	Total	40	683.22		12.02**

Steenbock V Pregnant Rats vs. Pork I Pregnant Rats

The average weights of the livers and the average percentages of moisture present in the organs of the pregnant control and the gravid experimental rats in each series are presented in table 6. The variances in the weights of the livers in different pairs of the groups studied were analyzed. (Tables 7 and 8). For example, the weights of the livers of the toxic and non-toxic rats fed the pork diet were compared; then the liver weights of the control group were compared with those of the pork-fed rats that showed no symptoms of pregnancy disease; and finally, the liver weights of the control group were compared with those of all pork-fed rats, toxic and non-toxic. A similar comparison of differences in percentages of liver moisture, as presented in tables 9 and 10, was made also.

In Series I, the group of toxemic rats receiving the pork diet had heavier livers than did the non-toxic animals. The difference in the average weights of the organ in the two groups is highly significant. However, the data from the larger group of toxic animals in Series II did not confirm this finding.

More variation in liver weight occurred within the groups than between groups when the control animals in either series were compared with the corresponding groups of non-toxic pork-fed rats. Hence, there was no real

TABLE 6. AVERAGE WEIGHT AND MOISTURE CONTENT OF LIVERS OF PREGNANT RATS

Diet	Series	Number of rats	Weight	Moisture content
			mg.	per cent
Steenbock V	I	9	6.909	70.4
	II	16	6.895	70.6
Pork I	I			
Non-toxic		15	6.917	65.3
Toxic		8	8.168	67.7
Non-toxic	II	13	7.149	65.7
Toxic		13	7.726	67.4

TABLE 7. ANALYSIS OF VARIANCE OF MOIST WEIGHT OF LIVERS IN PREGNANT RATS IN SERIES I

Groups compared	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Toxic and non-toxic Pork I rats	Items within the groups	21	7.0551	0.3359	
	Between group means	1	8.1667	8.1667	
	Total	22	15.2218		25.31**
Steenbock V and non-toxic Pork I rats	Items within the groups	22	6.5834	0.2992	
	Between group means	1	0.0004	0.0004	
	Total	23	6.5838		
Steenbock V and all Pork I rats	Items within the groups	30	10.2841	3.4280	
	Between group means	1	9.4361	9.4361	
	Total	31	19.720		2.75

TABLE 8. ANALYSIS OF VARIANCE OF MOIST WEIGHT OF LIVERS IN PREGNANT RATS IN SERIES II

Groups compared	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Toxic and non-toxic Pork I rats	Items within the groups	24	20.746	0.864	
	Between group means	1	2.158	2.158	
	Total	25	22.904		2.50
Steenbock V and non-toxic Pork I rats	Items within the groups	27	24.051	0.891	
	Between group means	1	0.466	0.466	
	Total	28	24.516		
Steenbock V and all Pork I rats	Items within the groups	40	31.160	0.779	
	Between group means	1	5.078	5.078	6.52*
	Total	41	36.238		

TABLE 9. ANALYSIS OF VARIANCE OF RELATIVE MOISTURE CONTENT OF LIVERS IN PREGNANT RATS IN SERIES I

Groups compared	Source of variation	Degrees of freedom	Sum of squares	Mean Square	F
Toxic and non-toxic Pork I rats	Items within the groups	21	135.73	6.46	
	Between group means	1	130.37	30.37	
	Total	22	166.10		4.70
Steenbock V and non-toxic Pork I rats	Items within the groups	22	75.91	3.45	
	Between group means	1	146.95	146.95	
	Total	23	222.86		42.59**
Steenbock V and all Pork I rats	Items within the groups	30	137.74	4.59	
	Between group means	1	148.42	148.42	
	Total	31	286.16		32.34**

TABLE 10. ANALYSIS OF VARIANCE OF RELATIVE MOISTURE CONTENT OF LIVERS IN PREGNANT RATS IN SERIES II

Groups compared	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Toxic and non-toxic Pork I rats	Items within the groups	24	561.64	23.40	
	Between group means	1	17.94	17.94	
	Total	25	579.58		
Steenbock V and non-toxic Pork I rats	Items within the groups	27	582.27	21.56	
	Between group means	1	172.86	172.86	
	Total	28	755.13		8.02**
Steenbock V and all Pork I rats	Items within the groups	40	692.07	17.30	
	Between group means	1	182.68	182.68	
	Total	41	874.75		10.56**

difference in the average weight of the organ in the two groups.

A significant difference in liver weights between the entire group of experimental animals and the control animals was noted in Series II but not in Series I.

Thus, all analyses indicate, on the whole, that feeding the pork diet to pregnant rats does not exert any influence on the size of the liver if pregnancy disease does not develop. If it does appear, liver enlargement may or may not occur. Increase in size may be related to the severity of the symptoms developing in the disease.

The average percentage of moisture in the livers of the experimental rats was significantly lower in both series than it was in livers of either group of the control animals. The average amount of moisture in the organs of the non-toxic pork-fed rats was also lower than it was in the livers of the rats that developed the toxemia. The difference, however, was significant only in Series I.

It may be recalled that dehydration of hepatic tissue marks the change from the virgin to the gravid state in the pork-fed rat. Data in the present section shows that the condition still prevails when "Pork I pregnancy" is compared with normal pregnancy. Apparently then, when the strain of pregnancy is imposed on dietary influence, tissue changes occur.

DETERMINATION OF A PRECISE METHOD FOR THE EXPRESSION OF LIPID VALUES

A survey of the various means used for expressing lipid values obtained in an analysis by different workers in the field show a lack of uniformity. A lipid value may be expressed as the total amount in the liver, as a percentage figure based on either fresh or dry liver weight, or as a percentage figure related to body weight. Beeston et al. (1935) demonstrated, for example, that different values representing the concentration of free cholesterol may be obtained depending upon the base used for comparison.

To determine which of the above procedures represents the most satisfactory method for expressing experimental results, the liver weight, water in the liver, and total lipid in the liver calculated as percentages of body weight were plotted against body weight, the normal pregnant rats composing Series II being used for the study. Scattergrams showing the relationships in the control rats are presented in figure 2. Inspection of these scattergrams shows that relative liver weight and relative water content of liver are positively correlated with body weight. Therefore, the use of fresh liver weight as a base would at once increase variation among individual ratios, perhaps to the point where true differences between groups might not be

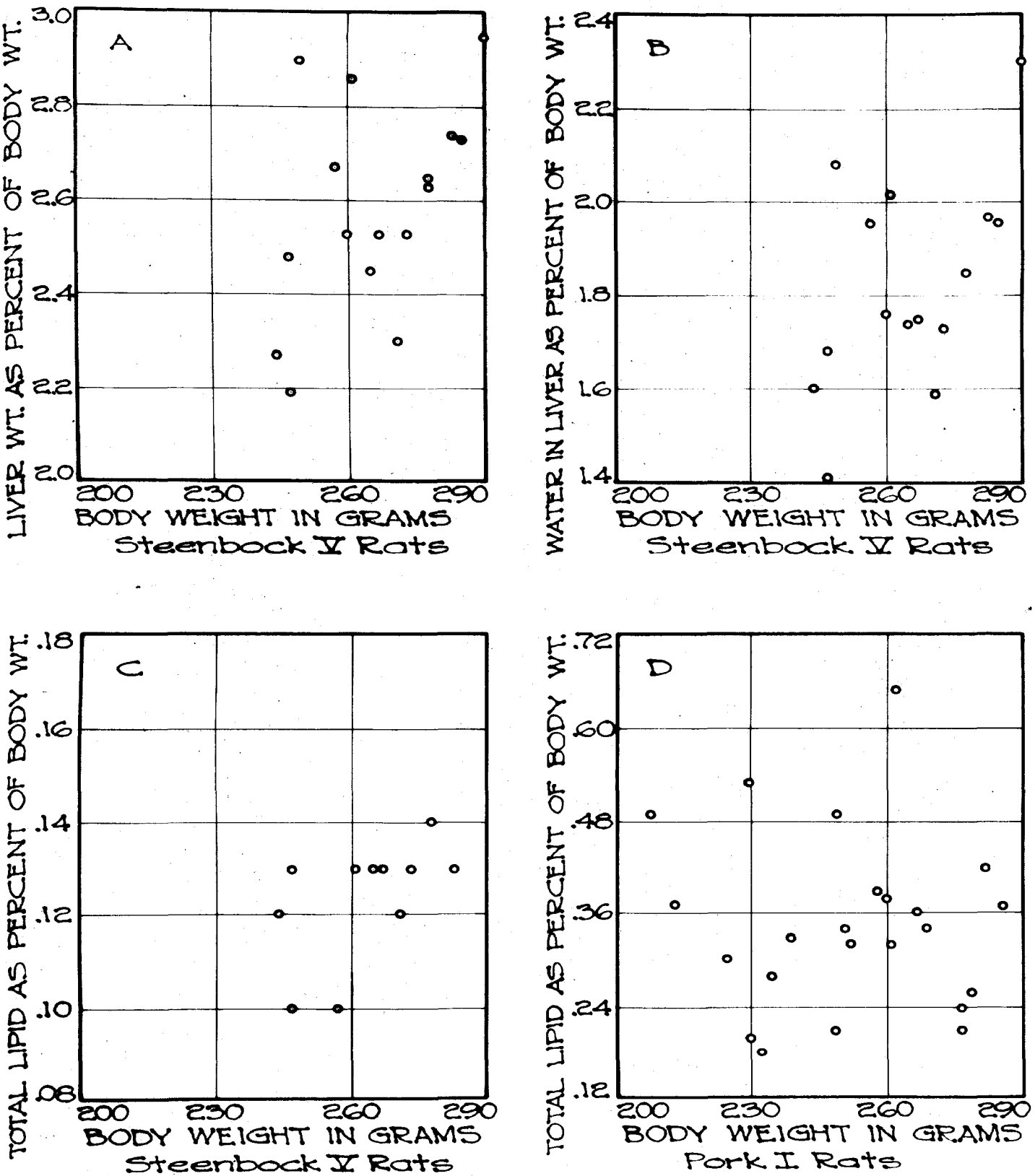

Figure 2. RELATION BETWEEN CERTAIN BODY CONSTANTS IN RATS.

detected. There is no correlation of total lipid with body weight, i.e., the amount of fat in the liver is independent of body weight. As additional evidence, liver lipid values plotted for the group of rats receiving the pork ration also showed no correlation with body weight (Figure 2D). Hence, calculating the lipid content in the liver as a percentage of the 100 gm. rat is a precise method of expression. Channon et al. (1937) believe this base reflects both the percentage increase in fat and the increase in size of the fatty liver.

The amount of active liver tissue of each rat is represented by the weight of the dry-defatted liver. Average values of the dry-defatted liver are summarized in table 11. A statistical analysis of these data, based on

TABLE 11. AVERAGE WEIGHT OF DRY-DEFATTED LIVERS, SERIES II

Diet	No. of rats	Wt. of dry defatted liver
		gms.
Steenbock V	11	1.742
Pork I		
Non-toxic	12	1.607
Toxic	11	1.696

the F test, showed that no significant differences existed

between any two groups studied (Table 12). The uniformity of the values indicates that the lipids can be expressed as a percentage of the dry liver weight. Also, when the values were plotted against each other, the dry-defatted liver weight expressed as a percentage of body weight was found to have no correlation with body weight. Thus, either method, i.e., lipid calculated as a percentage of dry liver weight or calculated in relation to body weight, appears to be equally satisfactory. Therefore, the data reported in this study are presented from both standpoints.

RELATIVE CONCENTRATION OF TOTAL LIPID IN LIVERS

Steenbock V Virgins vs. Pork I Virgins

The average concentration of total lipid in the liver was determined in a group of virgin rats reared on the stock diet and in a group of virgin rats reared on the pork-containing diet. Analytical results are presented in tables XIV and XV (Appendix). The average values for total lipid obtained in the two series of rats (Table 13) are not strictly comparable because the weight of the alcohol-ether soluble substances determined in the livers in Series I includes some substances which are not fat. Hence, this value is slightly higher than the amount of lipid obtained by the oxidative procedure.

TABLE 12. ANALYSIS OF VARIANCE OF DRY-DEFATTED LIVERS IN PREGNANT RATS IN SERIES II

Groups compared	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Toxic and non-toxic Pork I rats	Items within the groups	21	1.236	0.059	
	Between group means	1	0.045	0.045	
	Total	22	1.281		
Steenbock V and non-toxic Pork I rats	Items within the groups	21	0.731	0.035	
	Between group means	1	0.023	0.023	
	Total	22	0.754		
Steenbock V and all Pork I rats	Items within the groups	32	1.475	0.046	
	Between group means	1	0.048	0.048	
	Total	33	1.523		1.05

TABLE 13. AVERAGE CONCENTRATION OF TOTAL LIPID IN LIVER

Series	Diet	Reproductive status	Number of rats	Total lipid in dry liver	Total lipid per 100 gm. gravid rat
				per cent	mg.
I	Steenbock V	Virgin	9	20.6	182.4
		Pregnant	9	22.0	173.7
	Pork I	Virgin	9	25.0	228.3
		Pregnant			
		Non-toxic	15	40.1	390.0
		Toxic	8	46.7	489.2
	Pork I supplemented diet	Pregnant	21	32.5	289.5
II	Steenbock V	Virgin	3	13.6	127.1
		Pregnant	11	15.8	123.3
	Pork I	Virgin	4	21.4	194.7
		Pregnant			
		Non-toxic	12	34.3	340.4
		Toxic	11	33.9	371.2

In Series I, the total lipid concentration was 20.6 per cent for the virgin controls and 25.0 per cent for the experimental virgins, calculated as the per cent of dry liver weight. The variation is highly significant (Table 4). The same difference apparently holds in Series II where a smaller number of animals was studied. Results based on the 100 gm. rat show the same trend. Therefore, the feeding of the Pork I diet to virgin rats is associated with an increase in total lipids.

Virgin Rats vs. Pregnant Rats

The effect of pregnancy *per se* on the total liver lipids was next investigated. Mean values of the concentration of total lipids in livers of virgin and gravid rats fed both diets are presented in table 13. Insignificant differences were noted when the total liver fat in the pregnant and virgin rats of the control groups in Series I were compared (Table 14). A highly significant variance is shown in the liver lipid values in the pregnant and virgin pork-fed rats (40 *vs.* 25 per cent, respectively). Values for the females in Series II showed the same trend but the number of virgins was too small to allow a statistical analysis.

The findings that the virgin rats reared on the pork diet have a significantly higher percentage of total

TABLE 14. ANALYSIS OF VARIANCE OF TOTAL LIPID[a] IN LIVERS OF PREGNANT AND VIRGIN RATS - SERIES I

Groups compared	Source of variation	Degrees of freedom	Sum of square	Mean square	F
Steenbock V rats	Items within the groups	15	47.00	3.13	
	Between group means	1	8.23	8.23	
	Total	16	55.23		2.62
Pork I rats	Items within the groups	22	1022.40	46.47	
	Between group means	1	1276.51	1276.51	
	Total	23	2298.91		27.47**

a. Calculated as a percentage of the dry weight of the liver.

lipid in their livers than do virgins fed the control diet and that the deposition of fat is further increased in the gravid animal, indicate that pregnancy definitely augments disturbances in fat metabolism induced by feeding the Pork I diet.

Steenbock V Pregnant Rats vs. Pork I Pregnant Rats

The average concentration of lipid in the livers of the pregnant control animals was next compared with that in the livers of the pregnant animals reared on the pork-containing diet. The experimental data pertaining to individual rats are reported in tables VIII to XI (Appendix). Mean values for the percentages of total lipid on the basis of dry liver weight and for the amounts in the 100 gm. gravid female are presented in table 13. Both values indicate that in the pork-fed group of Series I, the quantity of liver lipid in the toxemic animals was not significantly different from that in the "healthy" rats (Table 15). In Series II, the average value for the group of toxic rats was higher than that for the non-toxic group when referred to the 100 gm. rat. However, this difference did not prove to be significant. Hence, there is no real difference in the quantities of fat present in the livers of toxic and non-toxic pork-fed rats.

In each series, the non-toxic animals reared on the pork ration had a liver fat content more than double the

TABLE 15. ANALYSIS OF VARIANCE OF TOTAL LIPID[a] IN LIVERS OF PREGNANT RATS IN SERIES I

Groups compared	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Toxic and non-toxic Pork I rats	Items within the groups	21	1275.80	60.75	
	Between group means	1	229.90	229.90	
	Total	22	1495.70		3.78
Steenbock V and non-toxic Pork I rats	Items within the groups	22	786.40	35.74	
	Between group means	1	1831.03	1831.03	
	Total	23	2617.43		51.23**
Steenbock V and all Pork I rats	Items within the groups	30	1296.50	43.21	
	Between group means	1	2909.05	2909.05	
	Total	31	4205.55		67.34**

a. Calculated as the percentage of the dry weight of liver.

amount observed in the control rats, i.e., Series I, 40.1 vs. 22.0 per cent; Series II, 34.3 vs. 15.8 per cent, respectively (See tables 15 and 16 for analysis of variance.). Total lipid related to the 100 gm. gravid female showed the same difference between the control and experimental groups of animals.

Since no significant difference was demonstrated in the lipid contents of the livers of the toxic and non-toxic rats, data derived from all rats that had received the pork diet, were combined, and compared once more with the control group. The differences between the averages for the groups in each series remained highly significant, F in Series I being 67.34; and in Series II, 77.39 (Tables 15 and 16). Thus, the feeding of the Pork I diet causes a marked increase in total lipid in the liver. The development of the toxic state is not associated with any further augmentation of liver fat.

The average number of resorptions observed per female in Series II of the pork-fed group was 1.3; the average number of feti, 9.5; and the average weight of the intact uterus, 51 gm. (Table 17). These data corresponded very well with the normal values. The influence that each variant might have on the amount of total liver lipid was tested by inspection of the individual data. The values showed wide variations with no correlation between the amount of liver fat and the number of live feti, the number

TABLE 16. ANALYSIS OF VARIANCE OF TOTAL LIPID[a] IN LIVERS OF PREGNANT RATS IN SERIES II

Groups compared	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Steenbock V and non-toxic Pork I rats	Items within the groups	21	713.23	33.96	
	Between group means	1	1949.64	1949.64	
	Total	23	2662.87		57.40**
Steenbock V and all Pork I rats	Items within the groups	32	1025.26	32.04	
	Between group means	1	2479.59	2479.59	
	Total	33	3504.85		77.39**

a. Calculated as the percentage of the dry weight of liver.

TABLE 17. AVERAGE WEIGHT OF INTACT UTERUS AND AVERAGE NUMBER OF FETI PER RAT IN SERIES II

Group	Age	Weight	Weight of intact uterus	Number of live feti	Number of dead feti	Number of resorptions
	days	gm.	gm.			
Steenbock V	138	266	57.5	11.1	-	0.03
Pork I						
Non-toxic	127	256	49.4	9.2	-	1.50
Toxic	109	248	53.8	-	9.9	1.10

of resorptions, or the weight of the intact uterus.

It is appropriate at this point to compare the data herein reported with data on typical fatty livers recorded in the literature. The "cholesterol" fatty liver contains approximately 5 times more fat than does the liver of a rat maintained on a mixed grain diet; the fatty liver produced by feeding a high fat diet has 4.7 times more fat than the control liver (Best et al., 1934). The livers produced in the present investigation contained approximately twice the normal content of fat. It appears that the fattiness of the livers produced by feeding the Pork I diet is much less pronounced than that of typical fatty livers described in the literature. However, it must be kept in mind that the "Pork I fatty liver" developed on a diet containing only 13 per cent of fat whereas the diet used by Best contained 40 per cent of fat.

Pregnant Rats Receiving the Supplemented Pork I Diet - Series I

Livers from a group of 21 toxemic females whose basal pork diet had been enriched with various types of food supplements were obtained for analysis. The experimental data including the supplement added to the diet are found in table XVI (Appendix). Armstrong (1939) has described the diets to which lipocaic[a] and the liver extract[b]

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a. Obtained from Eli Lilly Co.,
b. Lilly Liver Extract, no. 343.

were added. The rats receiving the lactoflavin had 50 gamma of Borden's lactoflavin added to the Pork I diet. Raw pork was substituted for the dried autoclaved pork in the case of one rat.

Mean values for the total lipid characterizing the livers of this group are summarized in table 13. It may be noted that the average quantity of liver lipid in the group receiving a supplemented Pork I diet was lower than that in the basal pork-fed groups. The differences as shown by the F test were significant (Table 18). While the supplements added to the Pork I ration seem to have exerted a lipotropic action, liver fat remained at a higher than normal level. The average value for this group of sick animals was 1.5 times higher than that for the control group (32.5 vs. 22.0 per cent, respectively).

The data presented represent the over-all picture. When the quantities pertaining to the effect of individual supplements are examined (Table XVI, Appendix), other interesting points may be noted. Approximately one-half of the rats fed the lipocaic had liver fat values within the normal range but the rest had about the same amount of fat in their livers as had the rats on the basal pork diet. Then, of the six rats fed the Lilly Liver Extract no. 343, all but one showed liver fat values within the normal range. Again, one female in a group of four receiving lactoflavin

TABLE 18. ANALYSIS OF VARIANCE OF TOTAL LIPIDS[a] IN LIVERS OF PREGNANT RATS - SERIES I

Groups compared	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Pork I supplemented diet and toxic Pork I rats	Items within the groups	27	2472.31	91.57	
	Between group means	1	1169.57	1169.64	
	Total	28	3641.88		12.77**
Pork I supplemented diet and non-toxic Pork I rats	Items within the groups	34	2717.91	79.94	
	Between group means	1	501.47	501.47	
	Total	35	3219.38		6.27*

a. Calculated as the percentage of the dry weight of liver.

had a liver normal in respect to fat. Apparently then, the toxic condition can develop without the concomitant development of a fatty liver. In fact, toxic conditions as severe as any noted, appeared in the liver extract-fed group where the fat content of livers with one exception was normal. It can only be concluded that the fatty liver is not the basic cause of the pregnancy disease.

RELATIVE CONCENTRATION OF LIPID FRACTIONS IN LIVERS OF PREGNANT RATS IN SERIES II

Total Fatty Acids and Neutral Fat

The concentrations of the various lipid fractions in the livers of the pregnant experimental and control groups were determined by the oxidative procedure. Mean values for total fatty acids and neutral fat (a calculated value) are given in table 19. Examination of the data shows that 47 per cent of the total fatty acid in the control livers was neutral fat as compared to 88 per cent in the livers of the pork-fed group.

Only data pertaining to the fatty acid fraction were analyzed statistically, because certain facts concerning the data indicate an evaluation of differences in this component will yield not only complete but the most reliable

TABLE 19. AVERAGE CONCENTRATION OF NEUTRAL FAT AND TOTAL FATTY ACIDS IN LIVER

Diet	Neutral fat in dry liver	Neutral fat per 100 gm. gravid rat	Total fatty acid in dry liver	Total fatty acid per 100 gm. gravid rat
	per cent	mg.	per cent	mg.
Steenbock V	7.6	57.0	12.1	121.4
Pork I				
Non-toxic	25.0	251.6	29.2	286.6
Toxic	22.6	273.6	28.3	293.4

information. First, since the neutral fat contributes such a large part to the total fatty acid fraction, values for both constituents will vary in a similar manner; second, the neutral fat figure is a calculated value; and third, data are more complete for total fatty acids than for neutral fat.

The quantities of total fatty acids in the livers of the control and pork-fed groups were first compared (Table 20). The difference, 17.1 per cent, was highly significant. The variance of the relative amounts of total fatty acids present in the livers of the experimental females which developed toxemia and those that were "healthy" was not significant (28.5 vs. 29.2 per cent, respectively). Thus, the data again show that the feeding of the Pork I ration has definitely caused an accumulation of lipids in the liver of the pregnant rat which is not augmented by pregnancy disease. This fat is largely composed of neutral fat.

Some values for the concentration of neutral fat and total fatty acids in the liver of rats as reported by other investigators are compiled in table 21. The normal values, 34 mg. neutral fat and 112 mg. total fatty acid per 100 gm. rat, reported by Loizides (1938) are below the values observed in the control animals of this study, i.e., 57 mg. neutral fat and 112 mg. total fatty acid. Loizides' study,

TABLE 20. ANALYSIS OF VARIANCE OF TOTAL FATTY ACIDS[a] IN LIVERS OF PREGNANT RATS

Groups compared	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Toxic and non-toxic Pork I rats	Items within the groups	24	1020.36	42.51	
	Between group means	1	5.92	5.92	
	Total	25	1025.28		
Steenbock V and non-toxic Pork I rats	Items within the groups	27	658.50	24.39	
	Between group means	1	2117.87	2117.87	
	Total	28	2776.37		86.83**
Steenbock V and all Pork I rats	Items within the groups	40	1045.99	26.15	
	Between group means	1	2770.49	70.49	
	Total	41	3816.48		105.95**

a. Calculated as the percentage of the dry weight of liver.

TABLE 21. VARIATIONS IN CONCENTRATION OF NEUTRAL FAT AND TOTAL FATTY ACIDS IN LIVER AS REPORTED IN THE LITERATURE

Investigator	Date	Diet	Neutral fat		Total fatty acids	
			per 100 gm. rat	per cent[a]	per 100 gm. rat	per cent[a]
Loizides	1938	Normal mixed diet	34 mg.	1.0	112 mg.	-
Best, Channon, Ridout	1934	Grain diet	-	0.40	-	2.76
		High fat diet	-	12.76	-	13.96
		High fat + choline	-	1.09	-	2.98
		2% cholesterol diet	-	9.50	-	12.63
		Cholesterol + choline	-	1.51	-	4.38
Okey et al.[b]	1938	Cholesterol-low diet	-	-	-	4.1
		1% cholesterol diet	-	-	-	10.0
Beeston et al.	1935	Low protein, high fat	-	32.75	-	-
		High protein, high fat	-	4.23	-	-
Channon et al.	1940	Control diet	-	-	1.17 gm.	21.70
		Cystine diet	-	-	1.75 gm.	27.22
		Methionine diet	-	-	0.56 gm.	13.14
Gavin and McHenry	1941b	Control diet	-	-	-	3.6
		Biotin diet	-	-	-	15.6
		Biotin + lipocaic diet	-	-	-	3.7

a. Per cent of moist liver weight.
b. The rats in this study were pregnant.

however, is based on virgin animals.

The only study on pregnant rats included in the table was made by Okey et al. (1938). They found 2.4 times as much total fatty acid in the livers of the rats receiving the cholesterol diet as they did in the control livers. This value corresponds to the 2.4 increase in the liver lipid fraction observed in the pork-fed rats in the present study.

There is a very great increase in total fatty acids in fatty livers produced by other workers in non-pregnant animals, i.e., 4.9 times more than normal total fatty acids for the fatty liver induced by high fat feeding, and 4.5 times more than normal for the "cholesterol" fatty liver (Best et al., 1934), and 4.3 times more for the "biotin" fatty liver (Gavin and McHenry, 1941b).

Phospholipids

The third lipid fraction studied was the phospholipid group. Mean values for the concentration of the fraction in the livers of each test group are given in table 22.

TABLE 22. AVERAGE CONCENTRATION OF PHOSPHOLIPIDS IN LIVER

Group	No. of rats	Phospholipid in dry liver	Phospholipid per 100 gm. gravid rat
		per cent	mg.
Steenbock V	14	7.69	58.9
Pork I			
Non-toxic	13	8.19	76.7
Toxic	13	7.42	74.2

Analysis of variance of the percentages of phospholipid calculated on the basis of dry liver weight showed that, no matter what two groups were compared, the differences within the groups were greater than those between the groups (Table 23). Examination of the individual data shows a wide range of values (Tables XI to XIII, Appendix). Since the livers were stored in the frozen condition, the question arose as to whether variation was due to changes developing during the period of storage. In table XVII (Appendix) the values are grouped according to the length of time that the liver was stored. However, inspection of the data did not show any correlation between the time of storage and the phospholipid value.

It is known that wide variabilities are to be expected in lipid analysis although phospholipid is the most

TABLE 23. ANALYSIS OF VARIANCE OF PHOSPHOLIPIDS[a] IN LIVERS OF PREGNANT RATS

Groups compared	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Toxic and non-toxic Pork I rats	Items within the groups	24	116.35	4.85	
	Between group means	1	3.81	3.81	
	Total	25	120.16		
Steenbock V and non-toxic Pork I rats	Items within the groups	25	89.62	3.58	
	Between group means	1	1.70	1.70	
	Total	26	91.32		
Steenbock V and all Pork I rats	Items within the groups	38	153.68	4.04	
	Between group	1	3.93	3.93	
	Total	39	157.61		

a. Calculated as the percentage of the dry liver weight.

constant of any lipid component of the tissues, not varying more than 30 per cent above or below the average (Bloor, 1928b). In the control group and in the pork-fed animals that were not toxemic, the values fall within a range of 35 per cent above or below the average while the lipid in the livers of the toxemic rats varied over a 60 per cent range. However, if one high figure is omitted, the rest are included in a 45 per cent range. Can the wider range in the phospholipid concentrations noted in the toxic rats be indicative of some disturbance in the metabolism of the substance.

The above analyses were made on the quantities of phospholipids present in the dry liver. Evaluation of the data when the amount of the lipid is expressed in terms of the 100 gm. rat leads to a different interpretation. It was found that the concentration of phospholipid related the 100 gm. rat in the pork-fed groups was significantly higher than that in the control group. Thus, reference to this base would indicate that there is a difference in the quantity of phospholipid in the livers of the two groups. At first, it was thought that this finding might be explained on the basis of the average body weights of the two groups. However, it was found that even though the body weights of the control rats were heavier than those of the experimental animals, (Table 17) the absolute quantity of

phospholipid in the livers of the smaller pork rats was actually greater than in the control animals. The discrepancy cannot be explained.

Results obtained when phospholipid is calculated on dry liver weight are more in line with the data reported by other investigators than they are when calculated on the basis of the 100 gm. rat. As appears to be the case in the present study, no appreciable increase in phospholipid values has been noted by the various workers when the liver becomes fatty (Channon et al., 1937; Okey and Yokela, 1936).

Cholesterol

A complete picture of the cholesterol storage in the liver was obtained by an analysis of the quantity of the free and total cholesterol fractions present and by calculation of the cholesterol ester concentration. The amount of free cholesterol was subtracted from the value for the total fraction to give the combined or ester cholesterol, from which datum, in turn, the cholesterol esters were calculated. Mean values for the cholesterol fractions are given in table 24. In both groups of pork-fed rats, the percentages of the total cholesterol based on dry liver weight were slightly higher than that in the control group. However, the free cholesterol values were low. It is the

TABLE 24. AVERAGE CONCENTRATION OF CHOLESTEROL FRACTIONS IN LIVER

Diet	Total cholesterol in dry liver	Total cholesterol per 100 gm. gravid rat	Free cholesterol in dry liver	Free cholesterol per 100 gm. gravid rat	Ester cholesterol in dry liver	Ester cholesterol per 100 gm. gravid rat
	per cent	mg.	per cent	mg.	per cent	mg.
Steenbock V	0.84	6.35	0.70	5.34	0.13	1.09
Pork I						
Non-toxic	0.97	9.71	0.65	6.06	0.36	3.89
Toxic	0.88	9.21	0.60	6.10	0.30	3.17
Pork I + 1% cholesterol	14.84	-	0.74	-	14.10	-

increase in ester cholesterol that raises the total value.

The significance of the variance of the concentrations of each of the three cholesterol fractions in the control and experimental groups was tested. The difference between the total cholesterol values proved to be insignificant. However, the decrease in the percentage of free cholesterol noted in the liver when the entire pork-fed group was compared with the control animals was significant (Table 25). Accompanying this decrease was a significant increase in ester cholesterol (Table 26). Hence, a disturbance in cholesterol metabolism appears to have occurred.

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, 1928b; Boyd, 1935b). Therefore, the finding that the proportion of free cholesterol in the liver fat of the gravid pork-fed rat is reduced while that of the ester cholesterol is simultaneously increased is indicative of hepatic damage.

No significant differences were noted in the relative concentrations of the three cholesterol fractions in the livers of the toxic and non-toxic groups receiving the pork diet. Increased dysfunction of the liver, therefore, does not accompany pregnancy disease.

TABLE 25. ANALYSIS OF VARIANCE OF QUANTITIES OF FREE CHOLESTEROL[a] IN LIVERS OF PREGNANT RATS

Groups compared	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Toxic and non-toxic Pork I rats	Items within the groups	24	0.49	0.02	
	Between group means	1	0.01	0.01	
	Total	25	0.50		
Steenbock V and non-toxic Pork I rats	Items within the groups	27	0.46	0.01	
	Between group means	1	0.02	0.02	
	Total	28	0.48		2.00
Steenbock V and all Pork I rats	Items within the groups	40	0.57	0.01	
	Between group means	1	0.08	0.08	
	Total	41	0.65		8.00**

a. Calculated as the percentage of the dry liver weight.

TABLE 26. ANALYSIS OF VARIANCE OF QUANTITIES OF ESTER CHOLESTEROL[a] IN LIVERS OF PREGNANT RATS

Groups compared	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Toxic and non-toxic Pork I rats	Items within the groups	21	0.83	0.04	
	Between group means	1	0.03	0.03	
	Total	22	0.86		
Steenbock V and non-toxic Pork I rats	Items within the groups	23	1.11	0.05	
	Between group means	1	0.35	0.35	
	Total	24	1.46		7.00*
Steenbock V and all Pork I rats	Items within the groups	34	1.20	0.04	
	Between group means	1	0.37	0.37	
	Total	35	1.57		9.25**

a. Calculated as the percentage of the dry liver weight.

With one exception, the cholesterol values when calculated on the basis of the quantity of the fraction per 100 gm. gravid rat, indicatedthe trends noted when calculations were made on the basis of dry liver weight. The exception was in the free cholesterol fraction where the data showed an increase. However, the absolute amounts of free cholesterol (see tables XI to XIII in the Appendix) varied in the same way as did the percentages of free cholesterol based on dry liver weight. The control animals were heavier than the pork rats (Table 18) which probably accounts for the lower free cholesterol values referred to the 100 gm. rat in this group.

A comparison of the variation in the cholesterol values reported herein with values taken from the literature should be made. In contrast to the decrease in free cholesterol in the livers of the pork-fed rats, a slight increase in this fraction is reported in the "cholesterol" and "high fat diet" types of fatty livers. Also, the rats reared on the Pork I diet stored 2.8 times as much cholesterol ester and 1.2 times as much total cholesterol as did the control animals. These increases in the fractions correspond fairly closely to the increases in values reported by Best et al. in 1934 (Table 27). In general, the condition described in the present experiment more nearly approaches the "high fat diet" type of fatty liver than any other. In the

TABLE 27. INCREASES IN LIVER CHOLESTEROL REPORTED IN THE LITERATURE

Investigator	Date	Diet	Increase over normal of cholesterol ester	Increase over normal of total cholesterol
Best et al.	1934	High fat	4.4 times	1.4 times
		Cholesterol 2%	120 "	12.5 "
Okey et al.	1938	Cholesterol 1%	23 "	20 "
Gavin and McHenry	1941b	Biotin	-	5 "

"cholesterol" fatty liver, the increase in the quantity of sterol present is of great magnitude. In this study it was found also that the addition of one per cent of cholesterol to the Pork I diet raises the cholesterol esters to a figure 108 times the control value.

Iodine Numbers

The iodine number of the fatty acids in the liver indicates to what extent the fat deposited in the organ is saturated or unsaturated. The phospholipid fatty acid and the acetone soluble fatty acid iodine numbers of the fats characteristic of the livers of the two test groups are summarized in table 28, individual data being shown in tables XVIII and XIX in the Appendix. The phospholipid

TABLE 28. IODINE NUMBERS OF LIVER LIPIDS

Diet	No. of rats	Phospholipid fatty acid I.N.	Acetone in soluble fatty acid I.N.
Steenbock V	10	149	141
Pork I			
Non-toxic	5	153	128
Toxic	7	152	124

fatty acid iodine number relates to the saturation of the phospholipids while the acetone soluble fatty acid iodine number gives an index of the saturation of the neutral fat and the cholesterol esters. The number of determinations is somewhat limited because it was found that the iodine numbers of the acetone soluble fatty acids decreased after the liver was stored six months. Therefore, values obtained from livers that had been stored longer than six months are not reported.

The iodine numbers of the fractions of the liver lipids of the control animals were not significantly different from those of the experimental rats. Thus, the extent to which the fatty acids of the lipids in the liver are normally saturated or unsaturated has not changed following the feeding of the pork diet.

Normal values for non-fatty livers of non-pregnant rats as reported by Blatherwick et al. (1933) are 139 for the phospholipid fatty acids and 128 for the acetone soluble fatty acids; as reported by Williams[a], 158 and 113, respectively. No data on pregnant rats have been found.

RELATIVE CONCENTRATION OF LIPID FRACTIONS IN LIVERS OF VIRGIN RATS - SERIES II

In the discussion just completed that pertained to the concentrations of the various lipid fractions in the livers of pregnant rats, the effect of pregnancy per se was not stressed because the number of analyses of the various components of the liver lipids available for virgin animals was not large enough to justify statistical comparison and because some information in respect to total lipids had already been presented in Series I. However, it may be pertinent at this point to present a compilation of the values for the various lipid fractions in virgin rats, as obtained in this series by the oxidative procedure and to compare them with pregnant values. Mean values are presented in table 29. The total fatty acids, neutral fat, and total and ester cholesterol values were higher in virgin animals receiving the Pork I diet than they were in the

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a. Personal communication from Dr. H. H. Williams.

TABLE 29. AVERAGE CONCENTRATION OF LIPID FRACTIONS IN LIVERS OF VIRGIN AND PREGNANT RATS

Lipid fraction	Virgin				Pregnant	
	Steenbock V		Pork I		Steenbock V	Pork I
	per cent[a]	mg.[b]	per cent[a]	mg.[b]	per cent[a]	per cent[a]
Total fatty acids	9.6	93.0	17.5	161.2	12.1	29.2
Neutral fat	4.7	38.0	14.1	130.4	7.6	25.0
Phospholipids	8.1	76.7	5.9	53.4	7.7	8.1
Cholesterol						
Total	0.71	6.6	0.88	8.10	0.84	0.95
Free	0.70	6.5	0.63	5.75	0.70	0.65
Ester	0.1	0.2	0.25	2.35	0.14	0.28

a. Per cent dry weight of liver.
b. Mg. per 100 gm. rat.

virgin control group while the values for phospholipid and free cholesterol were lower. The importance of the drop in phospholipids is to be questioned because the liver of the pork-fed virgins were analyzed after they had been stored for one year and four months. The values for the different fractions in the control animals compare quite closely with the normal figures of Loizides (1938) which on the basis of the 100 gm. rat are as follows, phospholipid 111 mg., total cholesterol 8-10 mg., ester cholesterol 0.33 mg., neutral fat 34 mg., and total fatty acid 112 mg.

The trends noted regarding the partition of fat in virgin livers were the same as those noted in gravid rats (Table 29). Pregnancy, therefore, induces no marked further change in the components in the lipid in the liver beyond that produced by feeding the pork diet.

RELATIVE CONCENTRATION OF LIPID FRACTIONS IN PREGNANT RATS RECEIVING THE SUPPLEMENTED PORK I DIET - SERIES II

The curative effect of the addition of certain substances to the pork diet was tested in other studies in the laboratory. In these experiments, a few females developed the toxemia in spite of the dietary treatment. The partition of fats in the livers of these rats was studied as well as one rat to whose diet one per cent cholesterol had been added to test the effect of this

substance in precipitating the toxemia. The female used in this analysis had not developed the pregnancy disease. The lipocaic added to the diet of one rat was prepared in this laboratory according to the directions of Dragdstedt. The Desicole supplement represented desicated whole fresh bile and the Cerophyl addition contained the grass juice factor.

The absolute amount of each lipid component is recorded in table XX in the Appendix and the relative concentration of each is given in table 30. The data from the group have not been averaged because the effects of the dietary supplements on the several lipid components varied.

The substitution of raw pork for the dried autoclaved pork or the addition of Desicole to the Pork I ration did not change the picture of the lipid components induced by feeding the basal Pork I diet. Both lipocaic and Cerophyl reduced the ester cholesterol to normal. However, while lipocaic reduced the neutral fat, Cerophyl had no effect. Only lipocaic controlled both the neutral fat and the cholesterol esters and thus, the total lipid in the liver of this animal was normal.

The data concerning the rat with added cholesterol in its diet showed an elevation in all the lipid fractions except phospholipid above those of the animals receiving the pork-containing diet. The rise in cholesterol esters was most marked. These data are in confirmation of the

TABLE 30. RELATIVE CONCENTRATION OF LIPID FRACTIONS[a] IN LIVERS OF PREGNANT RATS RECEIVING THE SUPPLEMENTED PORK I DIET

Supplement	No. of rats	Total lipid	Total fatty acids	Neutral fat	Phospholipid	Cholesterol		
						Total	Free	Ester
		per cent	per cent	per cent	per cent	per cent	per cent	per cent
Lipocaic	1	16.9	13.5	9.3	6.84	0.75	0.70	0.05
Raw pork	1	39.8	35.6	35.8	4.89	0.92	0.51	0.41
Desicole	1	36.6	32.1	29.4	5.75	1.12	0.66	0.46
Cerophyl	1	29.7	25.1	20.2	8.73	0.71	0.62	0.09
Cholesterol	1	60.5	42.9	32.8	4.96	14.84	0.74	14.10

a. Calculated as the percentage of the dry weight of liver.

results noted in preceding sections that the fatty liver produced by feeding the Pork I diet is of a different order than that of the "cholesterol" fatty liver.

RELATIVE CONCENTRATION OF BLOOD PLASMA LIPIDS

The concentrations of total fatty acids, phospholipid, and total cholesterol were determined in blood plasma taken from pregnant animals maintained on the stock diet and from pregnant pork-fed rats. These groups were fasted 10 hours before the blood was drawn on the 21.5 day of pregnancy for analysis. The experimental data are presented in tables XXI and XXII (Appendix), and the average values are summarized in table 31 in the body of the text.

A comparison of the findings (See tables 31 and 32) relating to the concentration of the various lipid fractions in the blood of the control group of gravid animals fed the Steenbock V ration with similar data reported by Boyd (1942) for normal non-pregnant rats shows that pregnancy has caused an approximate doubling of normal values. Lipemia is also characteristic of pregnancy in man (Boyd, 1934; Schwarz et al., 1940) and in guinea pigs (Boyd and Fellows, 1935-36). Hence the high concentration of the various fractions of the blood lipids noted in the control group studied herein are in accord with findings reported for other species during pregnancy.

TABLE 31. AVERAGE CONCENTRATION OF LIPID FRACTIONS IN BLOOD PLASMA OF RATS

Diet	Number of rats	Total fatty acid	Phospho-lipid	Total cholesterol	P/TC
		mg./100 ml.	mg./100 ml.	mg./100 ml.	
Steenbock V	8	448.19	177.83	85.94	2.09
Pork I	9	214.28	87.46	48.67	1.79
Pork I Toxic rat	1	208.51	--	52.49	--

TABLE 32. CONCENTRATION OF LIPIDS IN BLOOD OF NON-PREGNANT ANIMALS AS REPORTED BY VARIOUS INVESTIGATORS

Investigators	Date	Animal	Status of animal	No. of animals	Portion of blood analyzed	Lipid fraction analyzed
						mg./100 ml.
Boyd	1942	Rat	Normal male and female	116	Plasma	152 mg. total fatty acid 82 mg. total cholesterol 83 mg. phospholipid
Cook, McCullagh	1939	Rat	2% cholesterol in diet Normal	3 4	Serum Serum	222 mg. total cholesterol 70 mg. total cholesterol
Chanutin, Ludewig	1933	Rat	2.5% cholesterol in diet Control diet	6 4	Plasma	112 mg. total cholesterol 85 mg. total cholesterol
Chaikoff, Kaplan	1934	Dog	Depancreatized	10	Whole	Total fatty acids, cholesterol and phospholipid decrease 1/2 to 1/3 normal value
Entenman et al.	1939	Dog	Pancreatic duct-ligated	11	Whole	"
Dragstedt, et al.	1939	Dog	Depancreatized	12	Serum	Total lipid 1/2 normal value
Rubin, Ralli	1940	Dog	Depancreatized and pancreatic duct-ligated	4 3	Plasma Plasma	Total fatty acids, total cholesterol and phospholipid values decrease 1/2 to 1/3 normal value

It should be noted that the feeding of the pork ration to pregnant rats causes a profound alteration of the normal picture. In the case of each fraction studied, the average value per 100 ml. of plasma obtained for the control group was twice the corresponding value for the experimental group, i.e., 448 vs. 214 mg. total fatty acids, 178 vs. 87 mg. phospholipid, and 86 vs. 49 mg. total cholesterol in the control and experimental groups, respectively. These differences indicate that feeding the Pork I diet has definitely caused a disturbance in the fat metabolism in pregnant rats.

Boyd (1935a) has written that under normal conditions the ratio of phospholipid to total cholesterol in the blood is fairly constant. In the present experiment, the feeding of the pork diet apparently has not affected this ratio. While the difference between the two P/TC ratios, 2.09 vs. 1.79, might indicate a change in relationship, inspection of the individual data will show that seven of the nine values presented for the experimental group fall within the range of values characterizing the control group.

Only one animal developing pregnancy disease as the result of living on the pork diet was available for study. The various lipid values, in this instance, were very close to the average concentration of each lipid constituent in the blood of the rats that did not become sick upon the dietary regimen. However, additional analyses on toxic

rats are needed to verify or disprove the assumption that the blood lipids are not altered in toxemia.

Some blood lipid values in toxic and non-toxic pregnant animals are compiled from the literature in table 33. In most instances, the blood lipid values remained fairly constant. The drop in values produced by feeding the pork diet, therefore, becomes doubly interesting.

Neither is the change in blood cholesterol in the same direction as that produced by feeding a high cholesterol diet (See table 32 for values reported by Chanutin and Ludewig, 1933; Cook and McCullagh, 1939.). This observation is in agreement with the finding previously mentioned that the changes in the fatty liver produced by feeding the pork diet were not analogus to those in the cholesterol type of fatty liver.

Further survey of the literature shows that the change in blood lipid values produced in this experiment is also exhibited under other experimental conditions. A drop in blood lipids to half their normal values has been observed in depancreatized dogs by several workers (Chaikoff and Kaplan, 1934; Dragstedt et al., 1939; Rubin and Ralli, 1940). A marked fatty infiltration of the liver accompanies the hypolipemia. Entenman et al. (1939) and Rubin and Ralli (1940) note a similar hypolipemia in pancreatic-duct ligated dogs. Dragstedt, however, does

TABLE 33. CONCENTRATION OF LIPIDS IN BLOOD OF PREGNANT ANIMALS AS REPORTED BY VARIOUS INVESTIGATORS

Investigators	Date	Animal	Status of animal	No. of cases	Portion of blood analyzed	Lipid fraction analyzed
						mg./100 ml.
Roderick, Harshfield, Merchant	1933	Sheep	Normal pregnant		Whole	192 mg. total cholesterol
			Sick pregnant		Whole	189 mg. total cholesterol
Greene	1937	Rabbit	Toxemia of pregnancy	12	Whole Whole	Total fat raised slightly Total cholesterol raised slightly
Patterson, Hunt, Nicodemus	1938	Rabbit	Normal pregnant	4	Whole	60 mg. total cholesterol
			Thyroidectomized pregnant (fatal)	3	Whole	53 mg. total cholesterol
		Human	Normal patients	100	Whole	247 mg. total cholesterol
			Eclamptic patients	12	Whole	268 mg. total cholesterol
Bartholomew, Kracke	1936	Human	Normal pregnant	12	Whole	191 mg. total cholesterol
			Eclamptic patients	10	Whole	209 mg. total cholesterol
Boyd	1935a	Human	Normal pregnant	7	Plasma	900 mg. total lipid 205 mg. total cholesterol 248 mg. phospholipid
			Eclamptic patients		Plasma	1018 mg. total lipid 216 mg. total cholesterol 345 mg. phospholipid

not confirm this observation.

Raw pancreas proved effective in remedying both the depressed blood fats and the fatty livers observed by the above investigators. Whether or not the external secretion of the pancreas is also curative is a question that has not yet been settled. Dragstedt believes this is not the case. However, a preparation derived from pancreas tissue, known as lipocaic, is very potent in his hands in raising the lipid values to normal. A sample of lipocaic prepared by Dragstedt was fed by Wilcox in 1937 to a group of females receiving the pork diet. The substance prevented the toxemic syndrome characterized by a fatty liver. The similarity in the blood pictures produced in the pork fed rats studied in the present experiment and in dogs with fatty livers adds validity to the conclusion that a disturbance in fat metabolism occurs in pregnant rats that have been maintained on the Pork I diet since the time of weaning.

This hypothesis, also, has some corroboration in certain statements made by Bloor (1933, p. 152). He has related the concentration of cholesterol ester in the blood with liver damage stating that a drop in ester parallels parenchyma damage. Although only total cholesterol was determined in the present study, examination of the literature indicates that the variations in this constitu-

ent should parallel those of cholesterol ester. For instance, work by Sperry (1936) has demonstrated that the amount of free in total cholesterol in blood serum is very constant under varying circumstances (24.3 to 30.1 per cent). His findings should apply to studies of plasma as well, since Sperry and Schoenheimer (1935) found no significant difference between determinations of total and free cholesterol in serum and heparinized plasma. If this reasoning is true, then it can be concluded that the low lipid values in the blood of pregnant rats fed the pork ration are a direct reflection of the liver injury induced by the diet.

SUMMARY AND CONCLUSIONS

Workers in the Nutrition Laboratory at the Iowa State College have found that the feeding of a supposedly adequate diet containing dried autoclaved pork muscle to rats consistently produces a pregnancy disorder at parturition in approximately one-third of the animals maintained on the ration. A fatty yellow liver is a characteristic finding at necropsy.

The present investigation was undertaken to determine whether any derangement in the metabolism of fat occurs as a sequence of feeding the pork diet to rats. The total quantity of fat present and the relative concentrations of the various lipid fractions in the liver and blood were used as indices of measurement. The relationship of the fatty liver to the pregnancy disease was also studied.

In the analyses relating to the liver, data obtained from pregnant females fed the pork-containing diet were compared with those derived from groups of normal animals maintained on the diet fed to the stock colony of the laboratory. The experimental diet was designated as Pork I; the control as Steenbock V. The group of experimental gravid females, arbitrarily, divided itself into two subgroups, i.e., animals that developed toxic pregnancy and

those that did not. Virgin animals reared on each diet were also studied to determine the effect of pregnancy *per se*.

Each pregnant animal was allowed to rear the first litter. On the 21.5 day of the second pregnancy, after a 10 hour-starvation period, the liver was removed for analysis. The virgins were killed when they were approximately the same age as the gravid rats.

At the time the liver was removed from the animal, a portion of the large lobe was taken for an estimation of the moisture content. The remaining portion of liver was weighed and stored in a frozen condition until the chemical analysis was made. The frozen liver was ground with sand before it was extracted with alcohol and ether.

In one series of experiments, the quantity of total fat in the liver of the test animal was determined gravimetrically from the alcohol-ether extract; in a second series, the oxidative procedure of Bloor was used to estimate the concentrations of total fatty acids, phospholipids, total and free cholesterol in the alcohol-ether extract of the liver. The values for the quantities of total lipid, neutral fat and cholesterol were calculated. The iodine numbers of the phospholipid fatty acids and the acetone soluble fatty acids in the liver lipids were also determined.

The first analysis dealt with the effect of the feeding of the pork diet on the weight of the liver, and on the relative amounts of moisture and fat present. Characteristic differences between the experimental and control group are summarized in table 34. The comparisons clearly demonstrate the following points:

1. Feeding the pork diet to virgin rats produces no change in the weight of the liver or in its moisture content. However, the quantity of total lipid increases.

2. Pregnancy *per se* is associated with a definite increase in liver size in the normal animal. With the liver enlargement, there is no concomitant change in the moisture or lipid content of the organ.

3. In the gravid pork-fed animal the expected enlargement of the liver occurs. However, the diet is responsible for a dehydration of tissue and a deposition of fat greater than that noted in the virgin.

4. The feeding of the Pork I ration to gravid rats causes a dehydration of liver tissue and a deposition of fat in the organ greater than that occurring in normal pregnant animals.

TABLE 34. COMPARISON OF CHANGES IN WEIGHT, MOISTURE CONTENT, AND LIPID CONTENT OF LIVERS IN DIFFERENT GROUPS OF RATS

Analysis	Pork I vs. Steenbock V virgins	Steenbock V gravid vs. Steenbock V virgin	Pork I gravid vs. Pork I virgin	Pork I gravid vs. Steenbock V gravid	Pork I toxic vs. Pork I non-toxic
Liver weight	No change	Greater	Greater	No change	Greater in Series I No change in Series II
Moisture	No change	No change	Lower	Lower	No change
Total lipid	Greater	No change	Greater	Doubled	No change

5. The toxemic condition in the pork-fed rat may or may not be associated with a further increase in liver weight. The condition does not affect either the moisture or the fat content of the organ.

In the second analysis, the fats in the livers of the experimental and control rats were partitioned and the relative concentrations of the various components determined. Changes induced in the various lipid fractions are summarized in table 35. The table shows that the feeding of the Pork I ration to the pregnant rat produces:

1. A definite accumulation of fatty acids in the liver that is largely composed of neutral fat;
2. No change in the proportion of the phospholipids[a];
3. No increase in total cholesterol;
4. A definite reduction in free cholesterol;
5. A definite rise in ester cholesterol;
6. No change in phospholipid fatty acid iodine numbers;
7. No change in acetone soluble fatty acid iodine numbers.

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a. Based on data calculated as the percentage of the dry liver weight.

TABLE 35. CONCENTRATION OF LIPID FRACTIONS IN LIVERS OF PORK-FED RATS COMPARED TO THAT IN THE LIVERS OF CONTROL RATS

Lipid fraction	Concentration on basis of dry liver weight	Significance of differences
Total lipid	2.2 times greater than normal	**
Total fatty acids	2.4 times greater than normal	**
Neutral fat	3.1 times greater than normal	**
Phospholipids	1.1 times greater than normal	-
Total Cholesterol	1.1 times greater than normal	-
Free Cholesterol	1.1 times less than normal	*
Ester Cholesterol	2.5 times greater than normal	**
Phospholipid fatty acid iodine number	the same as the normal value	-
Acetone soluble fatty acid iodine number	1.1 times greater than normal	-

The high concentration of total fatty acids is indicative of a disturbance in fat metabolism. The nature of the disturbance is characterized by an increase in neutral fat and cholesterol esters and a reduction in free cholesterol. Other workers have associated these conditions with a degenerating and inactive tissue. The constancy of the phospholipid fraction demonstrated by other investigators is noted herein. Apparently no marked change in the saturation of the fatty acids occurs. Thus certain aspects of the fat distribution in the liver give evidence of an abnormal metabolism, while others indicate no disturbance of a normal condition.

Pregnancy disease does not change the amounts of the various lipid fractions in the liver from those characteristic of the liver fat in the non-toxic group of animals fed the pork ration.

In the third analysis, the blood lipids in pregnant control animals were compared with those in the blood of gravid rats receiving the Pork I diet. The blood was heparinized and the plasma separated by centrifugation. Total fatty acids and phospholipids were determined in an alcohol-ether extract of the plasma by the oxidative procedure. Total cholesterol was estimated colorimetrically by reading the intensity of the color produced in a Klett-Summerson photelectric colorimeter.

In the case of each fraction studied, the average value per 100 ml. of plasma obtained for the control group was twice the corresponding value for the experimental group. The constancy of the phospholipids and the cholesterol esters, which in the blood are as important as the phospholipids in fat transport, was not maintained. A decrease in the cholesterol ester of the blood reflects hepatic damage.

One pork-fed animal which developed the pregnancy disease had lipid values which were very close to the average concentration of the corresponding lipid constituent in the blood of the non-toxic rat receiving the pork diet.

The fourth analysis of the investigation related to the lipids in the livers of a group of toxemic females which were fed the basal pork diet enriched with various dietary supplements. Approximately one-half of the livers analyzed for total fat in this group of animals were normal with respect to fat. The liver lipids were fractionated in a few instances. Records show that one animal that had received lipocaic in its diet developed the toxemia even though all the lipid components were normal.

Two general conclusions may be drawn from the data reported in the present investigation. They may be stated as follows:

1. Fat metabolism as revealed by analysis of the

lipids in the liver and in the blood is altered in the pregnant rat following the feeding of a diet containing dried autoclaved pork muscle.

2. The development of a fatty liver is not a basic cause of the pregnancy disease as shown by analyses of the lipids in the livers of toxic and non-toxic rats fed the Pork I diet and of toxemic females fed the basal diet enriched with various dietary supplements.

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APPENDIX

PREPARATION AND STANDARDIZATION OF REAGENTS

50 Per Cent Alcohol

50 ml. of 95 per cent ethyl alcohol was diluted to 95 ml. with distilled water.

Pyridine Sulfate-dibromide

8.25 ml. (8 gm.) of purified pyridine and 5.45 ml. (10 gm.) of c.p. concentrated H_2SO_4 were measured into separate beakers containing about 20 ml. of purified glacial acetic acid and cooled. These solutions were combined in a 1 l. volumetric flask. 2.5 ml. (8 gm.) of bromine was dissolved in 20 ml. of glacial acetic acid, added to the flask and the whole volume made up to 1 l. with glacial acetic acid. The solution was made up in sufficient quantities to last a year and stored in a dark bottle. This solution which is about 0.2 N according to the content of H_2SO_4 was diluted to about 0.05 N (in respect to H_2SO_4) with glacial acetic acid before using it. Usually 50 ml. of the 0.2 N solution was measured accurately into a 200 ml. volumetric flask, diluted to the mark and mixed. The dilutions were made at frequent intervals to insure the

stability of the solution.

Standard Cholesterol Solutions

A stock standard was made by dissolving exactly 160 mg. of c.p. cholesterol[a.] in 100 ml. anhydrous chloroform. This solution was kept in the refrigerator.

The working standard was prepared from the stock standard by diluting 1 ml. to 25 ml. with anhydrous chloroform. This standard can be kept several days in the refrigerator. Five ml. of the working standard contained 0.32 mg. of cholesterol.

Several dilutions of the stock standard solution were made and analyzed colorimetrically with the Klett-Summerson photoelectric colorimeter. The readings included the range of readings within which the unknown was expected to fall, table I.

1 N Potassium Dichromate Solution

49 gm. of c.p. $K_2Cr_2O_7$ was dissolved in distilled water and made up to 1 l.

Moist Ethyl Ether

Peroxide-free freshly distilled anhydrous ethyl ether was shaken with a small quantity of distilled water

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a. Cholesterol (Cholesterin) C. P. purchased from A. H. Thomas Co., Philadelphia.

until saturated. Only the fraction containing the first third of the vapor distilled was used. The moist ether was stored in a bottle containing a piece of copper wire to keep it peroxide-free.

Petroleum Ether

A c.p. grade of petroleum ether was allowed to stand over one-tenth its volume of concentrated H_2SO_4 for three days. The solutions were shaken every day. The petroleum ether was distilled and the fraction boiling between 30° and 60° C. was collected.

Approximately 1 N Hydrochloric Acid Solution

88.45 ml. of c.p. concentrated HCl (sp. gr. 1.18) was dissolved in distilled water and made up to 1.

Hydroquinone Solution

A 0.1 per cent solution by weight was made up in 95 per cent ethyl alcohol. This solution was made up fresh each week.

Standard Iodate Solutions

Exactly 3.567 gm. of pure KIO_3 was dissolved in 1 l. of CO_2 free-water. This solution was 0.1 N with respect to I_2 when in contact with an excess of KI and H_2SO_4 and was

used to standardize the 0.1 N $Na_2S_2O_3$.

A KIO_3 solution equivalent to a 0.05 N concentration was made by dilution of the above solution to use in standardizing the 0.05 N $Na_2S_2O_3$. Usually 5 ml. of the original iodate solution was measured accurately into a 25 ml. volumetric flask, diluted to the mark and mixed.

Locke's Solution

The Locke's solution was made according to the directions of Bogniard and Whipple (1932) based on the formula given by Howell (1927).

	gm.
NaCl	9.0
$CaCl_2$	0.25
KCl	0.42
$NaHCO_3$	0.30
Dextrose	50.00

The mixture was dissolved in distilled water and made up to a volume of 1 l.

Phenol Red

A 0.04 per cent aqueous solution by weight was made by dissolving 40 mg. of phenol red in 100 ml. of distilled water. The solution was filtered and stored in the refrigerator to prevent mold growth.

Sulfuric Acid-dichromate Reagent

To 2.5 gm. of $AgNO_3$ dissolved in 12.5 ml. of distilled water in a 50 ml. centrifuge tube was added 2.5 gm. of $K_2Cr_2O_7$ dissolved in about 25 ml. of distilled water. The precipitated silver dichromate was separated by centrifuging and washed twice with water to get rid of nitric acid. The wet precipitate was dissolved in 250 ml. of c.p. concentrated H_2SO_4 with the aid of gentle heating.

Starch Indicator

Two gm. of soluble starch (Merck's Lintner) and 5 mg. of mercuric iodide were triturated with a little water and added slowly to 500 ml. of boiling water. The solution was boiled until clear and after cooling stored in the refrigerator.

Approximately 1 N Sulfuric Acid Solution

28 ml. (98 gm.) of c.p. concentrated H_2SO_4 (sp. gr. 1.84) was dissolved in distilled water and made up to 1 l. The solution was not standardized.

(1:3) Sulfuric Acid Solution

Three parts of distilled water was added to one part of c.p. concentrated H_2SO_4.

Thiosulfate Solutions

CO_2 free-water was prepared in a five gallon bottle by bubbling CO_2 free air through distilled water for three days. Air was drawn through saturated NaOH, a trap to catch any NaOH that might be drawn over, the five gallon bottle of water, and another trap by means of suction produced by a water pump. Approximately 0.1 N thiosulfate was made by adding 1 pound of $Na_2S_2O_3$ to approximately 19 l. of water in the five gallon bottle. The solution was mixed thoroughly and standardized.

Typical standardizations are given in table II. The first standardization was checked against three different solutions of 0.1 N KIO_3. After that the solution was standardized monthly against one iodate solution. The stability of the thiosulfate solution is shown in table III. To 1 gm. of KI dissolved in a little CO_2 free-water in an Erlenmeyer flask was added 25 ml. of 0.1 N KIO_3 and 10 ml. of 1 N H_2SO_4. The sides of the flask were washed down with water and the solution titrated, using 1 ml. of starch solution as the indicator. The number of ml. of 0.1 N iodate was divided by the number of ml. of thiosulfate used to get the factor that was used in the calculation.

The 0.02 N thiosulfate was made by diluting the above solution. Usually 200 ml. of the 0.1 N thiosulfate was

diluted to 1 l. This solution was standardized every day it was used. Typical standardization values are found in table IV. To 3 ml. of 0.02 N iodate in an Erlenmeyer flask was added 1 ml. of a 10 per cent solution of KI, a little water and 2 ml. of 1 N H_2SO_4. Titration was carried out using starch solution for the indicator.

TABLE I. RECOVERY OF CHOLESTEROL IN STANDARD SOLUTIONS

Dilution	Actual quantity of cholesterol present	Cholesterol determined colorimetrically
1	0.128 mg. in 2 ml.	0.131 mg. in 2 ml. 0.121 mg. in 2 ml. 0.130 mg. in 2 ml.
	0.192 mg. in 3 ml.	0.191 mg. in 3 ml. 0.191 mg. in 3 ml. 0.191 mg. in 3 ml.
2	0.256 mg. in 2 ml.	0.258 mg. in 2 ml. 0.258 mg. in 2 ml. 0.258 mg. in 2 ml.
3	0.384 mg. in 3 ml.	0.384 mg. in 3 ml. 0.384 mg. in 3 ml. 0.381 mg. in 3 ml.
	0.576 mg. in 4 ml.	0.564 mg. in 4 ml. 0.570 mg. in 4 ml. 0.570 mg. in 4 ml.
4	0.576 mg. in 4 ml.	0.579 mg. in 4 ml. 0.579 mg. in 4 ml.

TABLE II. STANDARDIZATIONS OF APPROXIMATELY 0.1 N $Na_2S_2O_3$ SOLUTIONS

$Na_2S_2O_3$ solution tested	KIO_3 solution used	Normality of KIO_3	$Na_2S_2O_3$ used	Normality of $Na_2S_2O_3$
			ml.	
1	1	0.1000	25.10 25.10	0.09960
1	2	0.1000	25.10 25.12	0.09956
1	3	0.1000	25.13 25.12	0.09952
2	2	0.1000	24.62 24.63	0.10154
2	4[a.]	0.1000	24.65 24.65	0.10146
2	5[b.]	0.1000	24.63 24.62	0.10154
3	5	0.1000	25.90 25.90	0.09652
3	6[c.]	0.1000	25.92 25.92	0.09645
4	6	0.1000	24.82 24.82	0.10072

a. 3 mo. later than first analysis in group.

b. 8 mo. later than first analysis in group.

c. 4 mo. later than first analysis in group.

TABLE III. CHANGES IN THE CONCENTRATION OF $Na_2S_2O_3$ SOLUTION NO. 1 WITH PROGRESSION OF TIME AS CHECKED AGAINST 0.1 N KIO_3

Time interval	KIO_3 solution used	$Na_2S_2O_3$ used	Normality of $Na_2S_2O_3$
		ml.	
At beginning	2	25.10 25.12	0.09956
After 1 mo.	2	25.13 25.14	0.09944
After 2 mo.	2	25.12 25.10	0.09956
After 3 mo.	2	25.16 25.18	0.09932
After 4 mo.	2	25.16 25.15	0.09936
After 5 mo.	2	25.21 25.22	0.09913
After 6 mo.	2	25.22 25.23	0.09913
After 7 mo.	2	25.22 25.22	0.09913

TABLE IV. STANDARDIZATIONS OF APPROXIMATELY 0.02 N $Na_2S_2O_3$ SOLUTIONS

$Na_2S_2O_3$ solution tested	Time interval	Normality of KIO_3	$Na_2S_2O_3$ used	Normality of $Na_2S_2O_3$
			ml.	
1	0	0.02	2.922 2.925	0.02052
1	16 da.	0.02	2.962 2.961	0.02026
1	26 da.	0.02	2.962 2.962	0.02026
1	8 da.	0.02	2.969 2.968	0.02022
2	0	0.02	3.104 3.104	0.01933
2	21 da.	0.02	3.106 3.105	0.01932
3	0	0.02	2.755 2.755	0.02162
3	12 da.	0.02	2.750 2.750	0.02182

TABLE V. INDIVIDUAL NUMBERS OF RATS IN SERIES I

Steenbock V		Pork I			Supplemented Pork diets
Pregnant	Virgin	Pregnant		Virgin	Pregnant
		Non-toxic	Toxic		
21357	21403	21359	21599	21552	21712
21484	21469	21459	21706	21573	21708
21506	21479	21487	21740	21433	21841
21539	21551	21512	21842	21490	21843
21558	21575	21533	21928	21633	21911
21579	21609	21535	21929	21784	21965
21749	21838	21559	25808	21917	21621
21836	21903	21561	26165	22057	21876
22022		21583		22059	21937
		21611			21618
		21695			21870
		21785			21901
		21882			21939
		21978			21940
		22150			22008
					22302
					22322
					22325
					22476
					25908
					26053
Total					
9	8	15	8	9	21

TABLE VI. INDIVIDUAL NUMBERS OF RATS IN SERIES II AND SERIES III

Series II						Series III	
Steenbock V		Pork I			Supplemented pork diet	Steenbock V	Pork I
Pregnant	Virgin	Pregnant		Virgin	Pregnant	Pregnant	Pregnant
		Non-toxic	toxic				
23782	25841	23779	23811	25840	24439	30369	30494
23887	25886	23885	24229	25885	25935	30495	30786
23947	25981	23889	24296	26101	25962	30599	30840
23950		23946	24418	26133	27995	30693	30877
24103		23949	27687			30787	30901
24106		24102	27930			30902	30937
24160		24158	28251			31025	30954
24164		24162	28255			31051	31136
24230		25883	28256				31162
24291		28337	28435				
25839		28864	28842				
25884		30622	28862				
25979		30711	28894				
27683							
27692							
27693							
Total 16	3	13	13	4	4	8	9

TABLE VII. IODINE NUMBERS OF OLEIC ACID AS DETERMINED WITH DIFFERENT QUANTITIES OF THE FATTY ACID

Oleic acid	Iodine number[a]
mg.	
9.04	89
	89
6.26	88
	89
4.52	89
	90
3.13	90
	88
2.46	89
	89

a. Theoretical value for oleic acid is 90 (Yasuda, 1931-32).

TABLE VIII. CONCENTRATION OF TOTAL LIPID IN LIVERS OF PREGNANT CONTROL RATS OF SERIES I

Rat number	Age	Weight	Moist wt. of liver	Dry wt. of liver	Total lipid
	days	gm.	gm.	gm.	mg.
21357	152	243	6.001	1.782	445.3
21558	132	269	6.785	1.939	465.5
21579	154	257	6.909	2.046	424.9
21539	158	261	6.754	1.971	413.3
21484	162	224	6.967	2.096	402.7
21506	170	256	6.234	1.878	400.9
21836	121	287	6.894	2.061	486.0
21749	149	247	7.471	2.234	519.2
22022	170	297	8.170	2.458	498.4
Av.	152	260	6.909	2.052	450.8

TABLE IX. CONCENTRATION OF TOTAL LIPID IN LIVERS OF PREGNANT NON-TOXIC PORK-FED RATS OF SERIES I

Rat number	Age	Weight	Moist wt. of liver	Dry wt. of liver	Total lipid
	days	gm.	gm.	gm.	mg.
21512	121	248	6.633	2.526	1183.9
21559	117	265	7.206	2.446	1162.3
21695	128	258	7.460	2.754	1328.6
21359	121	234	6.337	2.166	837.7
21459	152	266	7.226	2.561	1161.2
21533	170	258	7.143	2.492	960.0
21611	175	249	6.988	2.187	650.6
21785	139	243	7.452	2.670	1087.2
21882	131	248	6.694	2.197	785.2
21978	128	257	6.510	2.228	955.7
21583	117	228	5.938	2.190	1041.6
21535	138	243	6.660	2.362	905.1
21561	154	275	7.536	2.863	1362.5
21487	172	244	7.482	2.343	725.7
22150	158	230	6.494	2.031	537.1
Av.	141	250	6.917	2.401	979.0

TABLE X. CONCENTRATION OF TOTAL LIPID IN LIVERS OF PREGNANT TOXIC PORK-FED RATS OF SERIES I

Rat number	Age	Weight	Moist wt. of liver	Dry wt. of liver	Total lipid
	days	gm.	gm.	gm.	mg.
21928	94	227	7.585	2.179	966.3
21929	92	259	6.871	1.988	670.6
21599	95	271	8.278	3.103	1424.6
21706	113	263	8.158	2.832	1319.1
21740	124	273	8.587	2.626	1023.6
21842	99	271	7.866	2.629	1329.4
25808	104	284	8.924	2.918	1503.6
26165	85	222	9.078	2.882	1790.2
Av.	101	259	8.168	2.644	1253.4

TABLE XI. CONCENTRATION OF LIPID FRACTIONS IN LIVERS OF PREGNANT CONTROL RATS OF SERIES II

Rat no.	Weight	Moist wt. of liver	Dry wt. of liver	Total lipid	Total fatty acids	Neutral fat	Phospholipid	Cholesterol		
								Total	Free	Ester
	gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
23887	247	6.121	1.981	313.2	233.1	102.9	175.6	16.16	15.30	0.86
23782	278	7.321	2.161	-	228.4	-	155.0	-	14.13	-
23947	260	6.577	1.996	-	198.1	-	164.9	-	16.44	-
23950	247	5.404	1.918	256.2	186.3	101.4	122.4	24.37	12.38	11.99
24230	278	7.355	2.217	387.9	283.8	128.1	242.0	17.80	17.80	0.00
24291	257	6.866	1.816	261.7	206.0	141.2	104.6	14.08	11.47	2.61
24103	271	6.219	1.903	312.7	250.6	182.8	111.9	16.11	13.25	2.84
24160	285	7.770	2.172	-	266.0	-	152.3	-	14.14	-
24164	290	8.542	1.851	-	231.4	-	-	14.35	12.98	1.37
24106	249	7.230	2.029	-	214.7	-	-	16.56	11.06	5.50
25884	261	7.470	2.184	348.9	267.7	164.8	162.4	19.42	15.99	3.43
25839	244	5.544	1.632	284.7	215.4	118.7	152.5	12.97	12.25	0.72
25979	267	6.758	2.080	349.1	265.1	152.1	178.5	17.64	16.02	1.62
27693	283	7.739	2.163	375.7	277.9	132.3	227.2	-	16.25	-
27683	273	6.907	2.180	345.1	280.6	215.0	114.0	15.82	16.16	0.00
27692	265	6.492	1.878	341.0	275.1	198.1	127.6	13.96	11.82	2.14
Av.	266	6.895	2.010	325.1	323.3	148.9	156.5	16.60	19.04	2.76

TABLE XII. CONCENTRATION OF LIPID FRACTIONS IN LIVERS OF PREGNANT NON-TOXIC PORK-FED RATS OF SERIES II

Rat no.	Weight	Moist wt. of liver	Dry wt. of liver	Total lipid	Total fatty acids	Neutral fat	Phospholipid	Cholesterol		
								Total	Free	Ester
	gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
23779	257	6.682	1.964	-	542.1	-	205.1	-	22.85	-
23889	258	7.423	2.656	1017.1	892.4	807.7	176.2	26.20	15.74	10.46
23885	230	7.166	2.284	505.9	411.0	328.0	130.1	35.61	17.34	18.27
23946	233	6.137	1.859	413.7	320.4	192.3	202.1	16.94	13.44	3.50
24102	269	7.058	2.278	910.3	788.5	678.9	208.6	18.99	13.27	5.72
23949	249	6.384	1.980	531.8	443.5	352.6	156.7	18.90	13.79	5.11
24162	261	5.535	2.302	841.9	746.2	693.6	121.3	21.03	12.18	8.85
24158	277	5.792	2.296	674.9	583.8	499.0	161.7	12.74	10.60	2.14
25883	262	8.451	3.856	1698.9	1488.5	1355.6	262.3	56.03	18.84	37.19
28337	249	7.783	2.911	1221.5	1086.1	1011.6	175.5	26.93	15.72	11.21
28864	235	7.319	2.302	665.5	550.3	420.6	218.5	22.03	15.52	6.51
30622	260	9.279	2.457	992.4	845.9	691.4	277.3	20.50	15.68	4.82
30711	286	7.934	2.639	1061.5	917.2	779.5	259.3	19.76	15.31	4.45
Av.	256	7.149	2.445	877.9	739.7	650.9	196.5	24.64	15.41	9.85

TABLE XIII. CONCENTRATION OF LIPID FRACTIONS IN LIVERS OF PREGNANT TOXIC PORK-FED RATS OF SERIES II

Rat no.	Weight	Moist wt. of liver	Dry wt. of liver	Total lipid	Total fatty acids	Neutral fat	Phospholipid	Cholesterol		
								Total	Free	Ester
	gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
23811	239	8.429	2.500	-	640.5	-	176.8	-	16.94	-
24296	260	6.857	1.956	-	421.4	-	246.2	-	14.47	-
24229	277	8.387	2.238	593.5	479.2	347.8	212.6	26.75	17.36	9.39
24418	225	6.393	2.071	667.5	586.8	537.5	108.9	17.77	12.91	4.86
27687	282	8.432	2.933	1195.2	1050.9	951.3	207.8	28.16	16.36	11.80
27930	208	7.843	2.698	1017.3	894.4	805.2	186.8	20.94	14.67	6.27
28251	251	6.443	2.200	841.1	738.5	656.5	167.8	14.43	10.89	3.54
28435	230	7.798	2.744	1219.8	1086.2	1018.8	164.7	28.31	16.30	12.01
28894	239	7.734	2.512	800.3	678.7	552.9	223.4	20.18	14.62	5.56
28862	279	7.406	2.294	737.6	619.9	497.0	214.3	22.22	16.22	6.00
28842	267	7.620	3.310	956.2	727.9	666.3	254.4	27.66	16.00	11.66
28256	213	8.656	2.538	782.9	693.3	651.8	101.7	23.46	14.63	8.83
28255	252	8.436	2.723	795.0	697.4	631.7	133.9	20.25	14.00	6.25
Av.	248	7.726	2.517	873.3	916.5	647.0	184.9	22.74	15.03	7.83

TABLE XIV. CONCENTRATION OF TOTAL LIPID IN LIVERS OF VIRGIN RATS OF SERIES I

Diet	Rat number	Age	Weight	Moist wt. of liver	Dry wt. of liver	Total lipid
		days	gm.	gm.	gm.	mg.
Steenbock V	21469	174	174	4.865	1.517	353.2
	21403	195	194	5.146	1.596	351.9
	21479	188	190	5.713	1.795	403.3
	21575	203	170	4.710	1.473	290.1
	21551	205	176	4.628	1.428	289.7
	21609	220	196	5.906	1.798	351.4
	21903	209	212	6.162	1.905	387.0
	21838	218	190	5.581	1.781	334.3
	Av.	202	188	5.339	1.662	342.6
Pork I	21573	140	178	5.696	1.741	431.8
	21433	158	181	4.914	1.605	381.3
	21633	160	200	6.194	1.938	558.1
	21784	152	184	6.325	1.955	516.7
	21490	184	199	5.632	1.719	461.8
	21552	198	169	5.124	1.554	413.5
	22057	178	205	5.517	1.750	467.2
	21917	193	188	4.814	1.457	292.2
	22059	176	185	5.194	1.592	336.6
	Av.	171	188	5.490	1.702	428.8

TABLE XV. CONCENTRATION OF LIPID FRACTIONS IN LIVERS OF VIRGIN RATS OF SERIES II

Diet and rat no.	Weight	Moist wt. of liver	Dry wt. of liver	Total lipid	Total fatty acids	Neutral fat	Phospholipid	Cholesterol		
								Total	Free	Ester
	gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Steenbock V										
25886	182	6.714	2.050	292.7	214.2	98.6	179.3	14.37	13.90	0.47
25841	171	4.905	1.473	198.1	135.0	52.4	155.4	10.15	10.10	0.05
25981	171	4.166	1.378	178.9	140.7	98.9	69.4	10.37	10.00	0.37
Av.	175	5.262	1.634	223.2	163.3	76.6	134.7	11.63	11.33	0.30
Pork I										
25885	193	5.765	1.987	513.3	434.0	369.9	111.7	23.98	12.51	11.47
25840	184	5.252	1.643	279.7	224.0	179.9	78.5	11.71	11.00	0.71
26101	188	6.494	1.602	350.9	296.8	248.8	86.8	12.92	9.55	3.37
26133	187	6.720	1.629	325.5	262.4	186.4	124.9	12.63	10.15	2.48
Av.	188	6.058	1.715	367.4	304.3	246.2	100.5	15.31	10.80	4.51

TABLE XVI. CONCENTRATION OF TOTAL LIPID IN LIVERS OF PREGNANT RATS RECEIVING THE SUPPLEMENTED PORK I DIET - SERIES I

Rat number	Supplement	Age	Weight	Moist wt. of liver	Dry wt. of liver	Total lipid
		days	gm.	gm.	gm.	mg.
21712	Lipocaic	100	175	5.224	1.445	420.0
21708	"	148	240	7.449	2.244	934.1
21843	"	100	218	8.551	2.167	553.2
21911	"	99	250	7.292	2.430	1272.4
21965	"	130	244	8.656	2.818	1333.1
21841	"	136	294	7.772	2.257	851.0
21937	"	93	218	5.870	1.615	471.4
21621	"	85	181	4.763	1.426	402.0
21876	"	98	220	4.740	1.242	326.1
21618	Liver extract	109	250	6.578	1.758	444.7
21901	" "	101	233	6.270	1.772	445.8
21870	" "	91	242	7.000	1.696	437.5
21939	" "	88	214	5.629	1.403	286.0
21940	" "	90	192	5.992	1.636	427.8
22008	" "	147	290	9.282	2.389	671.1
22302	Lacto-flavin	88	228	6.912	2.282	286.8
22325	"	88	222	6.338	1.868	786.5
22322	"	94	271	9.054	2.982	1315.6
22476	"	91	240	6.118	1.947	766.0
25908	Milk	227	290	10.067	2.968	1064.1
26053	Raw pork	124	159	6.561	1.950	795.8
Av.		111	232	6.958	2.014	680.5

TABLE XVII. RELATIVE CONCENTRATION OF PHOSPHOLIPID AND LENGTH OF TIME OF STORAGE OF LIVER

Length of time of storage of liver	Pork I rats		Steenbock V rats
	Non-toxic	Toxic	
	Per cent	Per cent	Per cent
0 days to 1 week	9.49 8.73 11.29 9.82	9.34 8.90	7.44 9.99
2 weeks	6.03	7.08 7.68	-
3 weeks	10.44	7.07 6.93	-
1 month	-	7.62 6.00	5.23 6.79
2 months	6.63 6.80	- -	8.87 9.34
5 months	-	5.10	7.17 8.58
6 months	5.70 10.87	4.01	8.26
7 months	9.16 7.92 5.27 7.04	-	-
8 months	-	12.59	-
9 months	-	9.50	-
10 months	-	-	5.76 10.92
11 months	-	-	5.83 7.01

TABLE XVIII. IODINE NUMBERS OF LIVER LIPIDS IN PREGNANT CONTROL RATS IN SERIES II

Rat Number	Phospholipid fatty acid I.N.	Acetone soluble fatty acid I.N.
23837	149	-
23782	153	134
23947	150	132
23950	140	162
25834	162	148
25839	137	149
25979	-	126
27693	151	141
27683	152	140
27692	147	141
Av.	149	141

TABLE XIX. IODINE NUMBERS OF LIVER LIPIDS IN PREGNANT PORK-FED RATS IN SERIES II

Group	Rat number	Phospholipid fatty acid I.N.	Acetone soluble fatty acid I.N.
Non-toxic	23889	149	-
	28337	154	128
	28864	150	118
	30622	159	134
	30711	153	133
	Av.	153	128
Toxic	27687	159	121
	27930	144	119
	28251	147	125
	28435	154	121
	28894	150	123
	28862	152	129
	28842	155	128
	Av.	152	124

TABLE XX. CONCENTRATION OF LIPID FRACTIONS IN LIVERS OF PREGNANT RATS RECEIVING THE SUPPLEMENTED PORK I DIET - SERIES II

Supplement and rat no.	Weight	Moist wt. of liver	Dry wt. of liver	Total lipid	Total fatty acids	Neutral fat	Phospholipid	Cholesterol		
								Total	Free	Ester
	gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Lipocaic 24439	235	7.272	2.078	351.9	279.8	193.5	142.1	15.56	14.47	1.09
Raw pork 25935	199	6.511	2.352	937.1	837.8	798.8	115.0	21.75	11.91	9.84
Desicole 25962	286	8.268	2.536	927.2	813.2	744.7	145.9	28.53	16.62	11.91
Cerophyl 27995	300	8.052	2.644	784.5	662.8	533.2	230.8	18.92	16.51	2.41
Cholesterol 28572	250	9.088	4.366	2639.6	1872.0	1434.2	145.1	647.80	32.33	615.27

TABLE XXI. CONCENTRATION OF LIPID FRACTIONS IN BLOOD PLASMA IN CONTROL RATS

Number of rat	Age	Weight	Total fatty acid	Phospho-lipid	Total cholesterol	P/TC
	days	gm.	mg./100 ml.	mg./100 ml.	mg./100 ml.	
30599	118	265	602.94	204.24	83.96	2.43
30495	139	250	758.09	--	85.71	--
30369	168	260	390.13	240.28	81.39	2.95
30693	115	265	262.77	159.88	74.35	2.15
30902	138	253	444.41	182.38	88.77	2.05
30787	155	298	99.83	132.49	75.54	1.75
31025	161	274	773.58	194.50	114.76	1.69
31051	160	268	253.80	131.03	83.05	1.58
Av.	144	267	448.19	177.83	85.94	2.09

TABLE XXII. CONCENTRATION OF LIPID FRACTIONS IN BLOOD PLASMA IN PORK-FED RATS

Number of rat	Age	Weight	Total fatty acid	Phospho-lipid	Total cholesterol	P/TC
	days	gm.	mg./100 ml.	mg./100 ml.	mg./100 ml.	
30494	152	288	235.48	133.76	51.00	2.62
30840	121	255	431.07	122.70	60.76	2.02
30901	143	243	143.59	86.31	39.00	2.21
31136	118	257	249.58	63.05	48.26	1.31
31162	114	236	92.82	45.89	40.94	1.12
30877	152	251	234.43	57.77	33.09	1.74
30937	153	280	94.72	78.59	45.51	1.73
30786	174	285	156.12	89.41	57.97	1.54
30954	174	302	290.72	109.63	61.46	1.78
Av.	144	289	214.28	87.46	48.67	1.79
Sick rat 30806	187	255	208.51	--	52.49	--

FORM 1. CONDITION OF FEMALE RATS AT NECROPSY (EXTERNAL CONDITION)

Rat no._____ Diet no._____ Description of diet_____
Age rat (in days) _____ Necropsy date _____ Post starvation period[a].
Pregnancy no. _____ Hour _____ Food_____
Day of gestation period____ Period of starvation Quantity_____
Wt. before starving_____ Hour initiated_____ Length of period_____
Wt. after starving_____ Hour terminated_____

Physical condition[b].
General_____Alert_____Paws pinkish_____ Eyelids
Fat_____Gaunt_____Eyes pink_____ Inflamed_____ Infected_____

Muscle tone
General_____Abdominal_____ Respiration
Sniffy_____ Wheezy_____
Palpitations_____

Gait
Dragging_____Elevated_____ Exudates[c].
Sprawling_____Awkward_____ Nasal_____ Anal_____
Oral_____ Vaginal_____

Hair
Clean_____Smooth_____ Hematuria_____
Creamy_____Thick_____
Fine_____ Remarks:_____

Tail
Clean_____Smooth_____
Discolored_____Sores_____

a. Not checked in this study.
b. In recording the degree to which any condition is present use a scale ranging from minus (-) to four plusses (++++).
c. Indicate character of exudate.

FORM 2. CONDITION OF FEMALE RATS AT NECROPSY (INTERNAL CONDITION)

Rat no._____ Diet no._____ Description of diet_____

Fat Depots[a.]
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:_____

Corpora lutea
No. in left ovary_____ right_____
Color_____

Fetal sites, no. of_____

Live feti, no. of_____

Resorptions, 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_____
_____
_____

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