1941

Meat in nutrition. XVII, Concentration of sugar in blood of pregnant rats fed a diet containing dried autoclaved pork muscle

Florence Pen Ho
Iowa State College

NOTE TO USERS

This reproduction is the best copy available.

UMI®

MEAT IN NUTRITION. XVII. CONCENTRATION OF SUGAR IN BLOOD

OF PREGNANT RATS FED A DIET CONTAINING DRIED AUTOCLAVED

PORK MUSCLE

by

Florence Pen Ho

A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject Nutrition

Approved:

In charge of Major work

Head of Major Department

Dean of Graduate College

Iowa State College
1941

TABLE OF CONTENTS

TABLES

INTRODUCTION

Blood is one of the circulating fluids of the body. It has many functions. In connection with the investigation herein reported, two are of especial importance. For example, blood serves as the main vehicle for transporting nutrient substances from the alimentary canal to the tissues. Also, it removes the waste products of metabolism from the tissues to the organs of excretion. Therefore, the blood picture may reflect the metabolism of the individual if certain physiological conditions are controlled.

Workers in the Nutrition Laboratory of the Foods and Nutrition Department at the Iowa State College have developed a semi-synthetic diet known as Pork I which contains dried autoclaved pork muscle as its main source of protein. They have fed this diet to albino rats in an attempt to elucidate the role of meat in nutrition. Many pregnant animals fed this diet appear to be in perfect health until the twenty-first or twenty-second day of gestation. Then the rats become suddenly ill, dying before they can give birth to their litters. The symptoms exhibited by these sick animals coincide with those of human eclampsia. About 33 per cent of the rats fed the ration containing pork die in this manner. The onset of the disease is rapid. Rats apparently normal at 10 p.m. were found dead in the morning, and those in good condition at 8 a.m. were sometimes dead at noon.

It may be interesting to describe in detail the syndrome

which occurred when the pregnant females failed to give normal birth

to their litters. The animals showed a rapid loss of muscle tone,

and became very weak and listless. They flattened out on the floor

of the cage, and had no strength to support their heads. The body

temperature fell. The animals were cold to touch, and their fur

bristled. Their ears, eyes, and paws were pale. Vaginal hemorrhage

and hematuria were observed in varying degrees of severity. Many of

the animals died during a convulsion. Occasionally, one or more

young were born dead, but more often, no young at all were born. If

the sick rats were killed just before death seemed imminent, and

examined, evidences of hemorrhage were found in the alimentary tract,

kidney, and uterus. In most instances the feti were large and well

developed. They were invariably dead. In less acute cases of

pregnancy disease, incompletely developed feti were sometimes found.

Resorptions were found occasionally.

Among the internal disturbances observed, a yellow and friable

liver was the most prominent. This phenomenon occurred in all the

animals receiving the basic pork diet, whether they became sick or

not. Histological analyses of the livers of the sick animals

(Armstrong, 1959) revealed a marked constant fatty degeneration and

fatty infiltration of the hepatic cells. The pathological changes

were more grave in the animals developing the acute pregnancy disorder.

Chemical analysis, also, indicated that the livers of the pork-fed

rats had much higher fat content than those of the normal control ani-

mals, i.e., 39.7 and 22.0 per cent, respectively. Similar patho-
logical changes have been observed in the livers of other species,
especially in eclampsia of human beings.

From all the data presented, it may be concluded that the
ingestion of the basal pork-containing diet results in an injury to
the liver. The accumulation of fat in the hepatic cells may inter-
fere with the ability of the liver to synthesize or store glycogen.

Armstrong (1939) notes that on the basis of the relation that
exists between the level of blood sugar, convulsions, and coma,
research dealing with the carbohydrate metabolism of the animals fed
the basic pork-containing ration might yield important data. These
considerations led to the initiation of a study of carbohydrate
metabolism. The glycogen phases were undertaken by Farrankop (1941),
and the investigation herein reported deals with the concentration
of glucose in the blood of pregnant rats fed a diet containing dried
autoclaved pork muscle. Perhaps the results obtained in studying
this problem can be applied to the situation in China where inade-
quate diets, some containing large proportions of pork, are consumed,
and where many women die from toxic pregnancies.

GENERAL PLAN OF THE EXPERIMENT

Do the pregnant rats fed Pork I diet utilize carbohydrate as efficiently as the rats receiving Steenbock V diet? Armstrong (1939) and other investigators have shown that fatty degeneration and infiltration were marked in the livers of rats fed the Pork I diet, and these changes were accentuated by pregnancy. It is conceivable that a fatty liver may interfere with either glycogenesis or glycogenolysis and thus influence the concentration of sugar in the blood.

The experiment described herein was, therefore, initiated in an effort to determine the sugar content of the blood in pregnant rats fed a supposedly adequate diet containing dried autoclaved pork muscle as the main source of the protein. This diet was called "Pork I". In order to achieve this purpose, the blood sugar concentration in rats receiving the Pork I ration was compared with that in rats fed "Steenbock V", the ration routinely fed the stock colony. Two series of the experiments were planned for the present investigation.

The object in setting up the first experimental series was to determine whether or not the concentration of sugar in the blood at the termination of pregnancy differed in experimental rats fed Pork I diet from that in control rats fed Steenbock V diet. However, since pregnancy itself affects the blood sugar levels, it was necessary to obtain comparable data on pregnant and virgin animals

receiving the same rations. Therefore, in the first series of
the experiment, both virgin and gravid females fed the two experi-
mental diets were studied.

The two pregnant groups consisted of 11 pairs of sister mate
animals. One of each pair of sister mates was fed the control
stock ration, Steenbock V, and the other was given, instead of the
Steenbock V ration, the Pork I experimental diet. The rats were
placed in these diet groups when sexual maturity was attained.
Both the control and experimental animals were mated with the
brother mates fed the stock ration.

In the selection of the specific life period for the study of
the concentration of sugar in blood, many factors were considered.
Previous investigations in the nutrition laboratory at Iowa State
College showed that the length of the gestation period of the rats
fed the Pork I ration ranged from 22 to 24 days (King, 1936;
Wilcox, 1937). In order to obtain data concerning the condition of
the animals just prior to the birth of the litter, the pregnant
females were killed on the 21.5 day of the second gestation. It
was believed that the effect of the diet upon the physiological
functions might be more pronounced at this time than at the end of
first pregnancy. The plan also gave an opportunity to observe the
progress of the first pregnancy and the condition of the young at
birth, etc.

In order to minimize the various influences which might affect
the sugar level of the blood all the pregnant animals were observed

under standard conditions. The rats were starved for 13 hours so as to deplete the concentration of the sugar in blood to a constant low level. Then, 4 gm. of their regular diet was fed at the end of the fasting period. The rats were stunned four hours after the food was offered by a blow on the head. The blood was drawn immediately for analysis. This autopsy was done as close to the 21.5 day of pregnancy as possible.

In the virgin group of the first series of the experiment, 13 females received the stock ration and served as the control and the 13 of their sister mates fed the Pork I ration served as the experimental animals. The virgins were killed when their age averaged that of the pregnant rats at the time of analysis. Experimental conditions for the virgins were standardized in the same way as was the case in the pregnant animals.

It seemed that the different kinds of diet fed in the post starvation period might introduce variation in results. The digestibility and absorbability of the two diets might well be unlike, because the composition of the two diets was so different. Therefore, a second series of experiments was planned in which carbohydrate utilization was followed after the feeding of a pure sugar. This plan eliminated many variables.

The object of the study in the second experimental series was to determine whether absorption and utilization of sugar was normal after feeding glucose to rats reared on the Pork I diet following a starvation period.

In order to determine the basal levels of the blood sugar
when the rats were 21.5 days pregnant in the second gestation,
eight animals fed the Pork I experimental diet were starved for
13 hours, and killed at the end of this time. The blood and in-
testinal tract were analyzed for sugar and reducing substances,
respectively. Nine pregnant rats receiving the Steenbock V ration,
served as the control group. Analytical procedures for these rats
were standardized in the same manner as for the experimental animals.

Two groups were fed glucose. The experimental group fed the
Pork I diet contained 10 rats; the other group reared on the
Steenbock V diet and serving as the controls contained 10 rats also.
All the animals were standardized by fasting for 13 hours, then
2.5 cc. of a 50 per cent glucose solution were fed at the end of
starvation by means of a stomach tube. The rat was killed four
hours after the forced feeding and the blood and intestinal tract
were then analyzed for glucose and reducing substances, respec-
tively.

The difference between the amount of glucose fed and the
amount recovered from the intestinal tract represents the amount of
substance absorbed. These analyses permitted evaluation of the
concentration of sugar in the blood of rats in terms of the actual
quantity of sugar entering the blood stream.

The general plan of the experiment is summarized in table 1.

TABLE 1.   DISTRIBUTION OF ANIMALS IN VARIOUS EXPERIMENTAL GROUPS

Series in the experiment	Reproductive status	Diet of animals	Post starvation period			Number of animals
			Food	Quantity	Length of period	
I	Pregnant	Steenbock V (control)	Steenbock V	4 gm.	4 hours	11
		Pork I (experimental)	Pork I	4 gm.	4 hours	11
	Virgin	Steenbock V (control)	Steenbock V	4 gm.	4 hours	13
		Pork I (experimental)	Pork I	4 gm.	4 hours	13
II	Pregnant	Steenbock V (control)	---	---	---	9
	Pregnant	Pork I (experimental)	---	---	---	8
	Pregnant	Steenbock V (control)	50% glucose solution	2.5 cc.	4 hours	10
	Pregnant	Pork I (experimental)	50% glucose solution	2.5 cc.	4 hours	10

EXPERIMENTAL PROCEDURE

ANIMALS USED

General

The animals used in the present investigation were albino rats (Mus norvegicus albinus) of Wistar stock, strain A, inbred by brother and sister matings for 78 generations. The last 28 generations were reared in the Laboratory of the Nutrition Department of Iowa State College. The stock colony received a modification of Steenbock's mixed grain diet (Steenbock, 1923). The ration, permanently formulated in 1932, is known as "Steenbock V ration." The constituents of the diet were kept as constant as possible from season to season and from year to year (Swanson, Stevenson, and Nelson, 1938). Timson (1932) showed that the increments in weight at successive age intervals in different generations of the stock animals were nearly identical. In regard to the growth and maturity of the stock colony, Greenwood (1940) showed that the first oestrus occurred at the expected range of ages, indicating that at the beginning of the experiment, the groups of rats were as uniform as could be expected.

The young animals from each litter produced by the females of the stock colony were reduced in number to eight on the fourth day after birth, and were weaned when 28 days old. At the time of

weaning two young female animals taken from the second litter were housed together in a round wire mesh cage with a raised bottom. These young animals were fed the stock ration until the time of rupture of the vaginal membrane. When this occurred, each rat was placed in an individual cage and fed the specific experimental diet of the group to which it had been assigned.

Uniformity of Animals Used in Experiment

Graham, in 1939, showed that the hemoglobin content of the blood of the young representing the second litter is superior to that of individuals making up later litters. Therefore, rats from the second litters of the stock colony females were used as experimental animals for the most part.

In order to compare results obtained from animals composing the experimental groups and the control groups, the animals must be as uniform as possible at the beginning of the experiment. Certain characteristics were used as indices for judging the uniformity of the animals. These were body weight at birth, body weight at weaning, age at sexual maturity, body weight at sexual maturity, body weight when study of vaginal smears was initiated, age at the first positive mating, and body weight at the first positive mating. The groups used in the study are described in terms of these characteristics in table 2. All groups were very uniform in regard to age and body weight at the beginning of the experiment. The data coincide with other data previously obtained

TABLE 2. AVERAGE UNIFORMITY OF ANIMALS USED IN THE EXPERIMENT

	Experimental groups		No. of animals	Body wt. at birth	Body wt. at weaning	Age at sexual maturity	Body wt. at sexual maturity	Body wt. when study of vaginal smears was initiated	Age at the first positive mating	Body wt. at the first positive mating
				gm.	gm.	days	gm.	gm.	days	gm.
Series I	Pregnant groups	Steenbock V (control)	11	4.9	49.5	40.6	86.4	120.6	75.0	146.4
		Pork I (experimental)	11	4.9	47.4	43.3	87.9	117.7	75.7	150.0
	Virgin groups	Steenbock V (control)	13	5.1	52.9	36.1	86.2	---	---	---
		Pork I (experimental)	13	5.1	53.7	37.8	87.6	---	---	---
Series II	Starved groups	Steenbock V (control)	9	5.0	50.7	40.3	81.4	117.8	70.1	141.1
		Pork I (experimental)	8	5.0	51.3	41.8	82.6	116.8	65.8	133.1
	Glucose fed groups	Steenbock V (control)	10	5.1	51.5	40.8	85.8	123.3	71.1	147.1
		Pork I (experimental)	10	5.0	50.7	40.7	84.7	115.0	71.9	136.1

in the laboratory (Dyer, 1935; King, 1936; Wilcox, 1937; Walliker, 1938; Armstrong, 1939; and Campbell, 1940).

The virgin female rats used as the controls of the pregnant animals in the study were not litter mates, because of the insufficiency of the animals available for distribution to the experiment. However, in comparing the data presented in table 2, no appreciable difference can be detected in these rats except in regard to the age at sexual maturity. They matured three or four days earlier than did the animals that were used in the pregnancy studies.

Young, healthy brother males from the same litter were used in mating in order to avoid variation that might result from confused use of mates at different stages of sexual development.

COMPOSITION AND PREPARATION OF DIETS

Two diets were used in this investigation. They were designated as Steenbock V and Pork I. The composition, preparation, and character of each diet are described below.

Steenbock V Ration

Steenbock V is the name of the ration fed to the rats of the stock colony from which the experimental animals were obtained. Also, the rats belonging to the positive control groups were fed this ration throughout the entire experimental period. The diet

is a modification of a mixed whole grain diet originally described by Steenbock in 1923.

Steenbock V ration consisted of two parts; a basal portion and a supplementary portion. The composition of the basal portion is shown below:

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

100.0 gm.

The supplementary portion consisted of milk fortified with trace elements and cod liver oil, ground lean beef, and fresh lettuce. The preparation of each supplement may be described as follows:

1. The milk supplement was made from a dried whole milk powder, known as Klim. It was purchased from the Borden Company, New York, in sufficient quantities to last one year. Each lot purchased came from one day's run at the factory. The Klim was liquefied daily by mixing 130 gm. of the dried powder with one quart of distilled water in a Hobart mixer for five minutes at

third speed. The milk was then fortified with one teaspoon of cod-liver oil* and 2 cc. of a mixed salt solution of trace elements. The salts were mixed in the following proportions and dissolved in 100 cc. of distilled water:

Potassium iodide................0.080 gm.

Manganese sulfate..............0.316 "

Potassium aluminum sulphate...0.098 "

Anhydrous copper sulphate.....0.875 "

The liquefied and fortified Klim was fed daily in the following manner:

a. Each female with a litter received 50 cc. daily;

b. Each pregnant female received 25 cc. daily;

c. Each male and each resting female received 12.5 cc. daily.

2. The meat supplement was raw beef round which had been freshly ground and purchased from a local market. The meat was measured with a calibrated teaspoon containing five grams. Three portions were given to each rat per week.

3. The lettuce was obtained chiefly from the Memorial Union Cafeteria and the Institutional Tea Room. It consisted mainly of the outer green leaves of head lettuce. After it had been thoroughly washed with running cold tap water, 10 gm. of the fresh crisp leaves were fed to each rat three times a week, alternating with the meat supplement.

---

*Refined Norwegian vitamin tested cod-liver oil, U.S.P., imported by the Pearson-Ferguson Company, Kansas City, Missouri.

Pork I Ration

The Pork I diet had the following composition:

Canned pork muscle (dried to one-half its
original weight)...................... 25 gm.

Cornstarch[1]................................ 53 "

Yeast[2]..................................... 5 "

Agar agar[3]................................. 2 "

Sodium chloride[4]........................... 1 "

Salt mixture[5].............................. 4 "

Butter fat .... 8 "

Cod-liver oil[6]............................. 2 "

<div align="right">100 gm.</div>

The diet was semi-synthetic on account of the pork muscle and
yeast used. In order to maintain the quality of the protein as
uniform as possible, large quantities of fresh pork hams were
purchased, i.e., 300-1000 pounds at one time. The hams were boned
and trimmed of all excess fat before grinding through a medium
plate. One pound of the ground meat was weighed and packed into a

---

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

No. 2 tin can. After carefully sealing, the cans were processed in the pressure cooker for 65 minutes at 15 pounds pressure. The cans were then cooled immediately in cold running water and examined carefully for leaks. They were stored at room temperature until needed.

When the cans were opened for use, all the fat on the top of the meat was removed and discarded. Then 1000 gm. of meat were spread on an aluminum tray covered with cheese-cloth, and dried to one-half its original weight in a warm current of air in an oven kept at a temperature of 85 to 95 degrees Centigrade. Approximately one to two hours were required for drying.

The butter fat used in this basal ration was prepared from butter purchased at the Iowa State College Dairy. Four pounds of butter were melted in a double boiler for two hours without stirring. At intervals, the scum of coagulated protein and salt was removed from the surface. The water and other minerals collected at the bottom of the container. The middle layer of the pure butter fat was decanted and filtered through a cotton plug in a hot-water jacketed funnel. The butter fat was then cooled and stored in the refrigerator.

The basal ration was mixed fresh twice each week in a Hobart mixer for 25 minutes at first speed, and then packed in tall tin cans and stored in the refrigerator.

The adequacy of this basal ration was discussed in detail by Dyer in 1935. Armstrong, in 1939, believed that theoretically, it

was adequate in all known dietary essentials.

CARE OF ANIMALS

Each female rat was housed in an individual round wire mesh cage upon attainment of sexual maturity. The cages were set in shallow enameled pans which were lined with paper towels. In order to prevent the animals from eating the excreta, cages with raised wire mesh floors were used so as to allow a dropping of the feces and urine to the paper towels which were changed daily.

The food was given _ad libitum_ in a one-half pint glass jar which was wired to the side of the cage. Distilled water was offered from a fountain attached to the outside of the cage. The food dishes were changed three times each week; the water fountains, twice; and the cages and enameled pans, once. All the equipment was carefully washed and sterilized before being used again.

The quantity of Pork I ration offered daily was 2 to 5 gm. more than the rat was expected to eat. A double portion of food was given to the rats on Saturday in order to reduce the work on Sunday. The amount of food consumed was recorded daily and the uneaten food was discarded each day. By this regulation, the development of rancidity and excessive waste were prevented. No food records were kept for the animals fed the Steenbock V ration.

There were three sets of records for each rat; one was the daily food consumption and weight record. In keeping the weight

record, the pregnant rat was weighed daily from the twelfth day of gestation until one week after the birth of the litter. In the second record, day by day weights were kept in connection with the micro-scopic examination of the vaginal smears. In the third record, the weights of the rats were recorded from the twenty-first day of gesta-tion to parturition at four-hour intervals. Also, after the litter arrived, the individual weights of all the young were recorded on the same record sheet in order to be able to study the fluctuations of the maternal body weight in relation to the body weight of foeti. In any case if some young appeared to be dead, the weights were recorded and then the lungs were removed and tested. If the lungs floated when placed in water, it was believed that the fetus had been born alive and had drawn air into its lungs. If the lungs sank to the bottom of the water, the fetus was believed to have been born dead.

In order to standardize the nursing records of the lactating females, the number of the young in each litter was reduced to six on the fourth day after birth. If possible, three females and three males whose weight most nearly approached the average weight of the litter were retained. If the number of young born was less than six, the female rat was given the chance to rear the entire litter. All the young were weighed daily as a group and also weighed separately as females and males on the fourth, seventh, fourteenth, seventeenth, nineteenth, twenty-first, and twenty-eighth days.

The laboratory was maintained at a fairly uniform temperature during the regular academic year when the experiment was conducted, ranging from 76 to 86 degrees Fahrenheit. The room was well ventilated by two big electric fans. The fresh air was pulled from the hall, filtered through a double cheese-cloth which was tacked on the outside of the ventilator in the door opening into the laboratory. No direct draft blew on the animals. The floor was mopped with soapy water and swept with a compound of paraffin oil and sawdust on alternate days. These precautions reduced the incidence of the lung infections and mortality of the young.

VAGINAL SMEAR TECHNIQUE

The female reproductive system of certain species has a well marked functional rhythm called the oestrous cycle. It is characterized by regular, periodic, and coordinated histological changes in every portion of the reproductive tract. These changes reveal themselves especially in the growth, degeneration, and regeneration of the epithelium of the uterus and vagina, and in the growth and rupture of the ovarian follicles with consequent formation of corpora lutea of ovulation. Therefore, Long and Evans (1922) recommended that the rhythmical changes occurring in the oestrous cycle of the female rat be followed by the microscopic examination of the vaginal contents. Five different histological pictures depicted by examination of the vaginal smear were used as criteria

for judging each stage in the cycle. The histological characterization of each stage is given below:

Stage 1. Epithelial cells, the pro-oestrous period.

Stage 2. Epithelial and cornified cells, the oestrous period.

Stage 3. Many cornified cells.

Stage 4. Cornified cells and many leucocytes, the met-oestrous period.

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

When the vaginal smear indicates either stage 1 or 2, the female rat is in heat and will accept mating. In order to determine the time at which the female should be mated, vaginal smears were examined daily at approximately the same hour. The date, time of the day, weight of rat, the stage of the oestrous cycle, and pertinent remarks were recorded.

In taking the smear, the back of the rat was rested on the palm of the left hand of the technician and the head was held between the thumb and index finger. A sterile, fire-polished glass rod (about 6 inches in length and 1/16 to 1/8 inch in diameter), held between the thumb and index finger of the right hand was gently inserted into the vagina after curling the tail of the rat around the fifth finger of the right hand. The cells removed from the vagina by the tip of the rod were placed on a glass slide, which contained a drop of distilled water. The freshly-made smear was then examined under the low power objective of a microscope.

A substage electric light equipped with a blue ground glass filter was put under the condenser for illumination.

A separate glass rod was used for each animal. All the glass rods were soaked in a strong soap solution after using, then washed with a brush and rinsed with distilled water before placing in a pyrex test tube, which contained a small amount of distilled water.

In order to prevent a possible spread of vaginal infection, the test tubes were then plugged with cotton and sterilized at 15 pounds pressure for 20 minutes before they were used again.

Vaginal smear studies were initiated when the females reached the age of 56 days. All the animals were allowed to pass through one complete oestrous cycle before they were mated. Brother males fed the stock ration were used for mating. The presence of sperm in the smear or of a plug in the vagina or on the paper towel under the cage were taken as signs of the positive mating. If the mating failed, the male was left in the cage until stage 5 appeared; then he was removed. After the onset of pregnancy, the smears were checked carefully for the presence of red blood cells from twelve to fourteen days. Their occurrence indicates implantation of the embryo. Sometimes this phenomena was observed with the naked eye, free blood appearing in the vaginal opening or on the rod. No vaginal smears were studied in the virgin females.

CALCULATION OF TIME FOR AUTOPSY

The female animals of the present study were observed from the beginning of sexual maturity until the 21.5 day of the second pregnancy. At this time the rat was killed by a blow on the head of the animal and autopsy was carried out immediately.

In order to check the time for killing the animal so as to make it come as near the 21.5 day as possible, certain estimations were made. For instance, if a male was put into the female's cage on the first day at 8 a.m. and sperm and the vaginal plug were found the next morning at the same time, the animal had mated within the 24 hour interval. But records show that most of the rats mate in the late afternoon or in the evening. For the convenience of calculation, we therefore assumed that rats mated on the average at 11 p.m. From this estimation, 11 a.m. on the twenty-first day of gestation of the pregnancy would be the right time for execution.

The animals were standardized under controlled conditions, by starving for 13 hours. A definite amount of food was fed at the end of this time and the rat was killed four hours after. For example, food was removed from the cage at 6 p.m. on the twentieth day of gestation, and the specified amount of food fed the next morning at 7 a.m. The animal was ready to be killed by 11 a.m. on the same day. By this well-defined arrangement, the chemical analyses for the concentration of glucose in the blood and in the intestinal tract may be completed in the course of a day's work, and thus any changes that might occur in the samples is avoided.

All the virgin females were killed when they were the same age as the pregnant animals fed the same ration.

AUTOPSY TECHNIQUE

Drawing of Blood

Factors Affecting the Concentration of Sugar in Blood

It is generally recognized that the mechanism by which the concentration of sugar in the blood is controlled is very sensitive and very effective. For this reason, Stinchfield (1937) claims that blood sugar determinations are valueless unless the blood is drawn and examined with special precaution. When the rats were killed in the present investigation, several precautions were taken to prevent fluctuations in blood sugar levels. The following factors, if uncontrolled, may cause changes in the concentration of sugar in the blood.

Environmental temperature

Peters and Van Slyke (1931) have stated that the earlier observers all agree that the blood sugar of warm-blooded animals may be increased by exposure to cold, because severe chilling produces a rapid glycogenolysis. De Langen and Schut (1918) have made an interesting study of the white inhabitants of Java. They found high fasting blood sugar values in the residents near the sea shore. When the same individuals moved inland to a mountainous and cooler region, the hyperglycemia disappeared, only to reappear again when these people returned to the tropical coast.

Emotional and nervous disturbances

It is known that the emotional state, such as pain, fear, or anger, of an individual, may so affect the blood sugar that hyperglycemia may develop. Peters and Van Slyke (1931) have suggested that the emotional response apparently involves two reactions: (1) destruction of muscle glycogen, perhaps to meet the demands of muscular activity evoked by the emotional disturbance; (2) mobilization of glycogen from the liver. The latter was demonstrated by Harris and Ingle (1937). Normal rats, for example, showed an increase in the level of blood sugar in response to emotional excitation, whereas rats whose adrenal medulla had been destroyed by either the enucleation or transplant method showed a decrease in blood sugar under identical experimental conditions. Animals whose adrenal medulla had been destroyed showed a marked increase in the level of blood sugar when epinephrine was injected.

The effect of general anesthesia

Epstein and his associates (1916) reported that nitrous oxide and ether anesthesia used in surgical procedures produced a well-marked hyperglycemia in almost every case. Ross (1918) found that hyperglycemia may be produced in dogs by either ether or morphine anesthesia. Atkinson and Ets (1922) also demonstrated that the sugar content in the blood of dogs increased during ether anesthesia. Stander and Radelet (1926) showed that the use of ether, chloroform, nitrous oxide, or ethylene in general anesthesia produced a marked

hyperglycemia. The hyperglycemia was also induced in rabbits (Underhill and Sprunt, 1927) by doses of amytal anesthesia whether given by mouth, subcutaneously, or intraperitoneally. Within a few minutes following administration of an anesthetic (Roudin, Vars, Goldsmidt, and Klengensmith, 1938), a hyperglycemia occurs in dogs as well as in rats, which nearly always increases during the period of anesthesia. Anesthesia produces asphyxia and delays the oxidation of the glucose in the blood, which, therefore, accumulates and results in hyperglycemia.*

The effect of hemorrhage

Severe hemorrhage also results in hyperglycemia. It is quite incomprehensible why this should be so. Epstein and Baehr (1914) have suggested that we may assume that it is the result of a physiological effort on the part of the body to maintain the rate of sugar supply to the tissues at an approximately constant level. In this connection, Tatum (1920) has stated that the effective cause of sugar change in the blood appears to be a disturbance in the acid-base balance in tissue cells, which is fairly well reflected in corresponding changes in the general circulation. The chief seat of action is probably the liver, for this is the location of glycogen storage most readily affected. It seems that hemorrhage produces hepatic asphyxia in such a way that acids

---

*Personal interview with Dr. E. A. Hewitt, Associate professor of Veterinary Physiology, Division of Veterinary Medicine.

accumulate in liver cells and there promote glycogenolysis result-

ing in an increase in the concentration of sugar in the blood.

Method Used

To minimize the influence of the factors presented above,

conditions for killing the rats were standardized in so far as

was possible. The room temperature ranged from 76 to 86 degrees

Fahrenheit. The animals were used to handling by the technicians.

No fear, anger, or other nervous disturbances were observed even

when the rat was grasped for killing. As previously described, the

rat was killed by a sharp blow on the back of the head. The rat was

reduced to a state of unconsciousness and no anesthetics were

needed for the operation. Occasionally, if the blow fell anterior

to the occipital region, nasal hemorrhage occurred. This probably

not only gave rise to a higher blood sugar level, but it was also

impossible to draw a sufficiently large amount of blood for analysis.

Immediately after stunning, one person held the front legs and

lightly lay the rat down on the operating table with the ventral

side up. No pressure was exerted on the throat or chest thereby

preventing an anoxemia that might disturb the blood sugar level.

Then, a second person seized the hind legs and the tail of the rat

and stretched them down firmly. The third operator quickly made

an incision along the ventral median line beginning from a point

about one inch above the vaginal orifice to another just below the

diaphragm. The abdominal cavity was enlarged by cuts made

transversely from the edges of the incision. The visible blood
vessels were avoided so as to reduce the loss of blood. The in-
ternal organs were then pushed aside and the fat split by pressing
with the fingers. This procedure exposed the aorta. The fascia of
the aorta was removed with a fine forceps. The vessel was then
ready for the drawing of the blood. A No. 18 hypodermic needle,
sharpened to a fine edge with a short bevel (about 45 degrees) and
attached to a mineral oil coated 5 cc. syringe, was used for draw-
ing the blood. The operator placed the fingers of the left hand
on both sides of the aorta in order to hold the rat firmly. The
needle was then rapidly inserted horizontally into the aorta. If
the needle was properly placed, the force of the blood pressure was
sufficient to fill the syringe without suction. Occasionally the
vessel contracted so violently after it had been entered that the
blood flow was entirely shut off. After a moment, however, the
vessel again dilated permitting a free flow of blood. The volume of
blood obtained from a pregnant rat was usually around 3 cc.

Prevention of Coagulation of Blood

When the needle was withdrawn, the whole blood was introduced
into an oxalated test tube and was gently shaken. The tube was
then stoppered with a cork and placed into a beaker containing
cold water and kept cool in the refrigerator. In order to reduce
serious alterations in the constitution of the blood and to prevent
hemolysis, only enough salt was used to insure prevention of

coagulation. A modified technique described by Peters and Van Slyke (1931) was used in this investigation. The authors state that 3 mg. of potassium oxalate will prevent the coagulation of 1 cc. of blood. Therefore, 3 cc. of blood will need 9 mg. of oxalate. A 4.5 per cent solution of refined, powdered potassium oxalate was made, and 0.2 cc. of this solution was measured into a 12.5 mm. by 1.5 mm. test tube. By rotating the tube, the oxalate was spread in a film on its walls. The tube was then dried in a stream of air. This procedure insured uniform rapid mixing of anticoagulant and blood, prevented the formation of small clots, and reduced the danger of hemolysis which may occur when blood stands in contact with slowly dissolving larger crystals of oxalate.

Removal of Intestinal Tract

Reasons for Removing Tract

The fate of food in the animal body may be divided into four steps: first, digestion and absorption from the intestinal tract; second, transport to the tissues via the blood; third, assimilation and deposition in the tissues; and fourth, elimination in the urine and feces. The first and second steps are comparatively easy to follow quantitatively and form the content of this investigation. In one series, absorption was determined by feeding a known amount of glucose to the rats by stomach tube. After a given

time, the rat was killed, the blood was drawn and the amount of

glucose remaining in the intestine was determined quantitatively.

The difference between the amount fed and the amount recovered from

the intestinal tract is then the amount of glucose absorbed.

Method Used

A modified Cori method (1925) for the removal of the intestinal

tract was used that may be described as follows:

The rat was weighed before starvation was started. Solid food

was then taken out of the cage but the water was allowed as usual.

After 13 hours, the rat was again weighed and the glucose solution

was fed by stomach tube. A No. 8 rubber urethral catheter was satis-

factory for this purpose. The catheter was plunged into boiling

water for a minute. This procedure not only made it more flexible

but also less permeable to liquids. One end of the catheter was

tightly fitted over a hypodermic needle which was attached to a

syringe containing glucose. The syringe had a metal plunger and

metal fitting. Generally, 2.5 cc. of 50 per cent glucose solution,

warmed to 40 degrees C., were given.

At the time of feeding, one person held the rat with her left

hand. A small wooden mouthpiece with a hole in the middle was in-

serted into the mouth between the tongue and upper mandible in order

to prevent the incisors of the rat from occluding and puncturing the

catheter. The tube then was wet and passed down the rat's oesophagus

with a to and fro rotary movement by a second person. Still another

assistant held the hind legs firmly to prevent kicking. A mark was

made at the four inch point on the catheter to indicate the depth to

which the catheter had to be introduced in order to reach the stomach.

The tube slipped more easily if the rat was kept on its back. The

more relaxed the rat, the easier was the procedure. The tube seldom

entered the trachea, but if such an accident occurred it was im-

possible to insert the catheter to the marked place on the tube.

Under such circumstances as suggested by Reineoke and his associates

(1939), the tube should be gently withdrawn without delay. In success-

ful feedings, none of the rats showed signs of oesophageal irrita-

tion.

As soon as the glucose solution was delivered, the catheter was

carefully withdrawn from the oesophagus and the distal portion of

the catheter was immediately placed in a United States Bureau of

Standards' one-liter volumetric flask. The other end of the tube

was detached from the hypodermic needle, was washed and rinsed with

a stream of distilled water, and then the washings were diluted to

the mark and the whole mixed well.

Cori believes it is not necessary to give a constant amount of

sugar per unit of body weight. In order to give each rat an amount

of glucose according to its weight, different amounts of glucose

have to be introduced into each rat. This involves a rather high

error in the amounts of fluid measured. It is also difficult to set

the plunger with the desired degree of accuracy at a certain gradua-

tion mark of the syringe. On the other hand, if each rat is fed the

same amount of glucose, the method is simplified to a great extent,
and the glucose can be fed with a high degree of accuracy. The amount
of glucose thus introduced at each feeding was determined by deliver-
ing into a liter volumetric flask the same amount of glucose solution
as was given to the rat. The solution was then diluted to the mark,
mixed well, and analyzed.

Duke (1937) stated that a function of the large intestine of
the carnivores is mainly to return water to the blood that has been
poured out by the digestive glands and to act as a reservoir for
the waste materials that constitute the feces. Best and Taylor
(1937) also indicate that the absorption of food materials such as
fat, protein and carbohydrate is practically confined to the small
intestine; that the caecum and colon absorb water and salts. There-
fore, the caecum and colon portion or the whole large intestine was
left out. As soon as the rat was killed and the blood was drawn,
ligatures were placed around the oesophagus and the ileo-caecal
region just above the caecum. The stomach and the whole small in-
testine were carefully detached from the mesentary and placed in a
beaker. The whole gastro-intestinal tract was held with a forceps,
cut and slit open with a pair of small scissors. The instruments
were then carefully rinsed off and the intestine washed with succes-
sive portions of hot, distilled water with the help of a policeman.
Generally, 200-225 cc. of water were used and the washings decanted
into a 250 cc. volumetric flask were cooled, made up to the mark,
and well mixed.

General Observations at Autopsy

Before the animal was executed, the individual physical condition, muscle tone, manner of walking and respiration, appearance of hair, tail, and eyelids, and the character of exudates were recorded according to the scheme shown in form 1 in the appendix. The weight and age of the rat, the time of starvation, the amount of food fed after starvation, and the time for autopsy were all described on the same sheet. After the blood had been drawn and the intestinal tract had been removed, the other internal organs were examined, and the data recorded as shown in the outline of form 2 in the appendix.

The intact uterus of a pregnant rat was cut at the oviducts and the cervix, and the whole organ was put in a watch glass and weighed immediately. Then the uterus was split open, and the feti and placentae removed. The uterus was weighed again. The difference between the weight of the intact uterus and the weight of the uterus is the weight of the feti and placentae. The number of the live feti and the number of the corpora lutea in each ovary were also recorded. The development of the feti was described clearly.

The distribution of the amount of fat was indicated by plus and minus signs (- to ++++) in the different regions, i.e., subcutaneous, peritoneal, omental, perirenal, genital, and intramuscular.

A section of the liver was next examined. The color, the consistency, and the appearance of the organ were recorded.

The kidneys were then removed and trimmed from all adhering fat, and split longitudinally. The cut surface was blotted; the color and

consistency of the cortex, medulla, and pelvis were described. The pancreas was examined for any abnormalities.

The stomach was next removed and cut open and freed from its contents by washing with cold water. The number and the severity of any ulcers present were noted.

The condition of the lungs was examined for evidences of infection, atelectasis, and emphysema in the different lobes. The ovary, placental sites, ear, and base of the tongue were also observed for symptoms of infection. The appearance of teeth was recorded also.

The same general procedure was followed in so far as possible at the autopsy of the virgin animals.

CHEMICAL ANALYSIS

Choice of Method

In 1775, Dobson recognized that blood may contain a sugar-like substance (Myers, 1924). However, its presence in normal blood was not discovered until seventy years later by the noted French physiologist, Claude Bernard, who has made many of the classic observations on carbohydrate metabolism. From this time, very few studies appeared until 1915. Since, an enormous amount of work on methods used in the study of blood sugar has been done through the cooperation of the chemists and clinicians.

Many of the procedures thus far proposed have taken advantage of the activity of glucose in reducing salts of certain of the heavy metals in hot alkaline solution. This property is not specific for glucose. It is shared by many other substances that are found in appreciable concentration in normal blood (Peters and Van Slyke, 1932), for example, creatinine, uric acid, glutathione, and ergothionine. Somogyi (1927) believes that glutathione and ergothionine represent most of the non-glucose reducing material in the blood. In general, it appears that the method which gives a high degree of accuracy and indicates most nearly the true glucose content of blood is the one by which glucose can be recovered quantitatively not only in pure solution, but also when it is added to blood and which, at the same time, will give the lowest value when applied to blood.

In order to study a phase of carbohydrate metabolism in the present experiment as indicated by the concentration of sugar in the blood, the simple, rapid, and inexpensive procedure, devised by Somogyi (1930), was chosen. In this operation, the non-sugar reducing substances in the blood are first precipitated, so that the protein-free filtrates give true sugar values with alkaline copper solution. Letonoff (1934) confirmed Somogyi's belief that his procedure yields true values for reducing sugar and prevents glycolysis.

The alkalinity of the iodometric copper reagents influences the oxidation of sugar (Shaffer, and Somogyi, 1933, and Somogyi, 1937), i.e., high alkalinity leads to diminished reduction equivalents, and

inversely, the lower the alkalinity, the slower the sugar oxidation, although the final amount of cooper reduced is higher. Therefore, Somogyi's low alkalinity reagent (1937) for the determination of sugar was selected. With it, very small quantities of sugar, even as little as 0.01 mg. of glucose, can be determined accurately by the copper-iodometric technique. With it, a linear proportionality between the amounts of sugar and the copper reduced can be established.

Reagents and Their Preparation

The solutions described below have been used for analyzing the pure glucose solutions and the glucose present in the blood and intestinal tract.

Copper Reagent

The composition of the low alkalinity copper reagent modified by Somogyi in 1937 may be described as follows:

Sodium carbonate (anhydrous).................... 25.0 gm.

Rochelle salt .... 25.0 gm.

Cupric sulphate (crystal) .... 4.0 gm.

Sodium bicarbonate .... 20.0 gm.

Sodium sulphate (anhydrous, analytical grade)200.0 gm.

Potassium iodide .... 1.5 gm.

Potassium iodate (normal solution) .... 6.0 cc.

The carbonate and Rochelle salt are dissolved in about 800 cc. of distilled water in a 2000 cc. beaker; then 40 cc. of a 10 per cent cupric sulphate solution are introduced by pipette through a long stem funnel extending well below the surface of the liquid, the

solution being stirred. This is followed by the addition of the bicarbonate, sulfate, and iodide and all are dissolved by stirring. The solution is heated to boiling, kept boiling for exactly 30 seconds, cooled, and transferred quantitatively to a one-liter volumetric flask. After the addition of normal potassium iodate, the solution is diluted to one liter and mixed well. The solution is allowed to settle one to two days. The impurities are completely removed by filtering through a dry filter paper into the five-liter brown stock bottle, mixed, and stoppered. If the solution is protected from strong light, it will remain unchanged for a year or two.

Sodium Thiosulphate

An approximately 0.1N thiosulphate stock solution was made and standardized by titration of the standard KIO_3 solution. To prepare 0.005 N thiosulphate, the amount required to give 500 cc. of exactly 0.005N solution, is calculated from the normality factor of the 0.1N solution. Then 10 cc. of the 0.01 KIO_3 solution should titrate exactly 20 cc. of the 0.005N thiosulphate; 5 cc. of the copper reagent should titrate the same as the volume of the normal iodate added per liter. The details of preparation, standardization and the explanation of reaction of thiosulphate, potassium iodate and all the other reagents are described in the appendix.

Procedure for Determining Glucose

The ratio of copper reduced to glucose oxidized depends not only upon the composition of the reagent but upon all conditions maintained during the heating period. It is necessary to standardize these conditions and to standardize the reagent with known amounts of the glucose under the exact conditions used in analysis. If changes are introduced (Shaffer and Somogyi, 1933), the reagents must be re-standardized under the new conditions.

Since the investigation has been in progress for three years, the reagents were made twice. The standardization of each set was performed with a series of chemically pure glucose solutions (standard dextrose samples obtained from the Bureau of Standards), freshly made and containing a known amount of glucose. Each series of glucose solutions was diluted proportionally to different con-centrations for determinations. The procedure used in the investigation is the following:

Five cc. of the glucose solution was measured accurately into a pyrex test-tube (25 x 200 mm.) by means of U.S.B.S. pipette. The pipette was allowed to drain for 30 seconds and the last drop of the solution was removed by touching the tip of the pipette to the side of the flask. Then 5 cc. of the copper reagent was added accurately in a manner that rinsed the glucose solution from the walls of the test-tube. Triplicate or duplicate determinations of each dilution were made; in addition, two blanks using distilled water instead of glucose solution, were always treated in the same way as the sample. The solutions were mixed by gentle shaking, and were covered with

glass bulbs in order to prevent convection currents with resultant oxidation by air during heating and cooling. The tubes were placed in a metal basket and held in position by crossed wires to reduce the agitation during boiling. The basket was then put in a vigorously boiling water bath and boiled for exactly 20 minutes, as certified by an interval timer.

At the end of 20 minutes, the flame was turned off immediately, and the basket and the tubes were removed to a pan of cold water and cooled to 30° C. A thermometer was used to check the temperature and then the wire basket was removed from the water. The tubes were then ready for acidifying with 5 cc. of N H_2SO_4. Each tube was gently shaken, in order to dissolve any precipitate. The bulb and walls of the test-tube were rinsed with a stream of distilled water before starting each titration. During the titration, a small flat foot-like glass rod was used for the purpose of stirring. Near the end of the titration, when the solution appeared straw colored, 1 cc. of starch solution (See appendix.) was added as the indicator, and the titration continued until the end point was reached. A burette calibrated by the U. S. Bureau of Standards was used in the titration.

Calculation and the Establishment of Glucose-Thiosulphate Linear Regression

The titration value was subtracted from the heated blank titration and the titration difference corrected by a factor if the thiosulphate was not exactly 0.005N. For example, since solution I_1 made from solution I (Table 3) contained 100 mg. of glucose in

TABLE 3. VOLUME OF 0.005N THIOSULPHATE EQUIVALENT TO
DIFFERENT QUANTITIES OF GLUCOSE AS DETERMINED IN 1939

Series of the solution	Solution number	Mg. of glucose in 5 cc. of solution	Glucose equivalent in cc. of 0.005n $Na_2S_2O_3$	Average glucose equivalent in cc. of 0.005n $Na_2S_2O_3$
I	I_1	0.5000	4.168 4.148 4.188	4.161
	I_2	0.5000	4.207 4.128 4.128	
	IA	0.2500	2.679 2.119 2.159	
	I_2A	0.2500	2.178 2.188 2.178	2.190
	VA*	0.2500	1.970 2.180 2.070	
	IB	0.1000	0.810 0.860 0.870	
	I_1B	0.1000	1.010 1.030 0.980	0.923
	I_2B	0.1000	0.905 0.935 0.905	

TABLE 3 (cont.) VOLUME OF 0.005N THIOSULPHATE EQUIVALENT TO DIFFERENT QUANTITIES OF GLUCOSE AS DETERMINED IN 1939

Group		Glucose	Determinations	Mean
I (cont.)	I_C	0.0500	0.450, 0.440, 0.440	
	$I_1{}^C$	0.0500	0.280, 0.540, 0.550	0.461
	$I_2{}^C$	0.0500	0.448, 0.468, 0.477	
	I_D	0.0250	0.300, 0.300, 0.340	
	$I_2{}^D$		0.358, 0.348, 0.378	0.337
	I_E	0.0100	0.160, 0.200, 0.180	
	$I_3{}^E$	0.0100	0.219, 0.229, 0.199	0.198
II	II A	0.3125	2.582, 2.672, 2.448	2.565
	II B	0.1250	1.011, 1.021, 1.001	1.011
	II C	0.0625	0.470, 0.480, 0.440	0.464
	II D	0.0312	0.310, 0.320, 0.290	0.310

TABLE 3 (cont.) VOLUME OF 0.005N THIOSULPHATE EQUIVALENT TO
DIFFERENT QUANTITIES OF GLUCOSE AS DETERMINED IN 1939

II (cont.)	II E	0.0125	0.140 0.160 0.130	0.143
III	III A	0.2825	2.462 2.362 2.272	2.366
	III B	0.1130	0.941 1.091 0.951	0.994
	III C	0.0565	0.530 0.550 0.480	0.520
	III D	0.0282	0.310 0.330 0.300	0.314
	III E	0.0113	0.130 0.170 0.190	0.164
IV	IV	0.4500	3.499 3.299 3.099	3.511
	IV$_1$	0.4500	3.599 3.589 3.469	
	IV$_2$	0.4500	3.670 3.531 3.521	
	V B*	0.4500	3.590 3.620 3.650	

TABLE 3 (cont.) VOLUME OF 0.005N THIOSULPHATE EQUIVALENT TO
DIFFERENT QUANTITIES OF GLUCOSE AS DETERMINED IN 1939

IV (cont.)	IV_1B	0.4000	3.098 3.128 3.228	3.266
	IV_2B	0.4000	3.392 3.380 3.372	
	V C*	0.3000	2.410 2.380 2.500	2.430
	IV D	0.2250	1.859 1.819 1.799	1.826
	IV E	0.0900	0.760 0.760 0.700	0.740
	IV F	0.0450	0.380 0.390 0.400	0.390
	IV G	0.0225	0.230 0.250 0.280	0.253
	IV H	0.0090	0.160 0.150 0.200	0.170

* The fifth solution employed is not listed separately but with other
  solutions of exactly the same concentration. The data are so
  arranged because means were calculated from solutions of the
  same concentration.

1000 cc. of solution, 5 cc. of this solution should contain 0.5 mg. of glucose. The titration value of 1.66 cc. of thiosulphate was subtracted from the blank titration of 5.83 cc. of thiosulphate. The titration difference of 4.17 cc. of 0.004998N thiosulphate was then corrected to a normality of exactly 0.005N. Thus 4.168 cc. of 0.005N thiosulphate was found equivalent to 0.5 mg. of glucose in the analysis of the first aliquot.

Five different standard glucose solutions were made in 1939, and called solutions I, II, III, IV, and V. They contained 100, 125, 113, 90, and 150 mg. of glucose per 1000 cc. of solution respectively. Each standard glucose solution was diluted to 1/2, 1/3, 1/5, 1/10, 1/20 or 1/50 of its original concentration. Three lots of each dilution were then measured for the standardization. The averages of the corrected titration values of 0.005N thiosulphate obtained with different amounts of glucose are listed in Table 3. These values were used as the bases for the computation of the regression. The short-cut method of computation in regression was used in the calculation (Snedecor, 1940). The calculation showed that the estimated value (symbol E) of 0.005N thiosulphate may be expressed by the following regression equation:

$$E = 7.958X + 0.077$$

where X is equivalent to the mg. of glucose present, or

$$X = \frac{E - 0.077}{7.958}$$

Titration values corresponding to the amounts of glucose were calculated from the equation presented above and a regression line established as shown in Figure I.
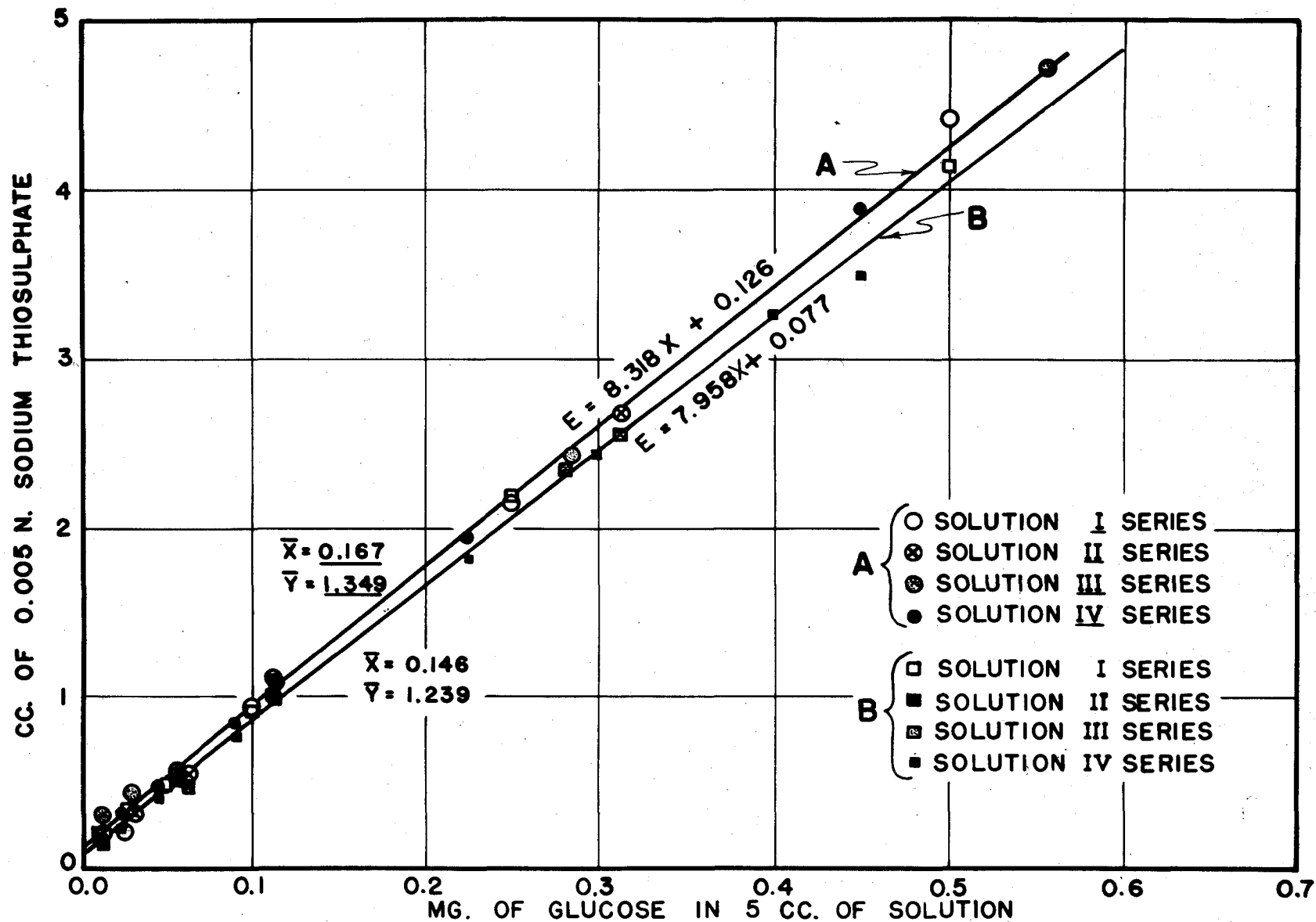
FIGURE 1. REGRESSION OF VOLUME OF 0.005 N SODIUM THIOSULPHATE SOLUTION ON QUANTITY OF GLUCOSE

It has been noted previously that the useful range of the reagent extended down to 0.01 mg. of glucose contained in 5 cc. of solution and reached 0.50 mg. as the upper limit. The present investigation confirmed these essential facts as laid down by Somogyi (1937).

The reagents made in the fall of 1940, were also standardized with approximately the same dilutions of the standard glucose solutions. They are indicated as I, II, III, and IV. The average of the corrected titration values of 0.005N thiosulphate and the corresponding amounts of glucose are shown in Table 4. The regression equation calculated from the data is shown below:

$$E = 8.318 \ x + 0.126, \text{ or}$$

$$X = \frac{E - 0.126}{8.318}$$

From the regression equations presented, it appears that the reduction equivalent of reagents made in 1940 is generally higher than that of reagents made in 1939. The change may be due to a change in the alkalinity, or perhaps, the technique of the investigator improved. However, the two regression lines are parallel and the standard deviations of the estimated values as calculated from the formula, $Se = Vy \cdot x \ (\frac{1}{n} + \frac{x^2}{Sx^2})$, are very small and fall in with each other. The regression line representing the correlation of the glucose and the titration value is shown in Figure 1.

Both regression equations were used as standards for the calculation of the glucose present in the blood, the reducing substances present in the intestines, and the amount of glucose fed to each animal determined in vitro and in vivo.

TABLE 4. VOLUME OF 0.005N THIOSULPHATE EQUIVALENT TO
DIFFERENT QUANTITIES OF GLUCOSE AS DETERMINED IN 1940

Series of the solution	Solution number	Mg. of glucose in 5 cc. of solution	Glucose equivalent in cc. of 0.005n $Na_2S_2O_3$	Average glucose equivalent in cc. of 0.005n $Na_2S_2O_3$
I	I	0.5000	4.419 4.429 4.429	4.426
	I A	0.2500	2.154 2.144 2.184	2.160
	I B	0.1000	0.930 0.930 0.950	0.937
	I C	0.0500	0.566 0.516 0.627	0.570
	I D	0.0250	0.314 0.314 0.314	0.314
	I E	0.0100	0.172 0.222 0.243	0.212
II	II A	0.3125	2.750 2.579 2.750	2.693
	II B	0.1250	1.052 1.072 1.224	1.116
	II C	0.0625	0.637 0.637 0.627	0.634

TABLE 4 (cont.)   VOLUME OF 0.005N THIOSULPHATE EQUIVALENT
TO DIFFERENT QUANTITIES OF GLUCOSE AS DETERMINED IN 1940

II (cont.)	II D	0.0312	0.394 0.374 0.384	0.384
	II E	0.0125	0.212 0.212 0.212	0.212
III	III	0.5650	4.773 4.773 4.753	4.766
	III A	0.2825	2.408 2.489 2.448	2.448
	III B	0.1130	1.173 1.031 1.031	1.078
	III C	0.565	0.639 0.647 0.629	0.638
	III D	0.0282	0.427 0.435 0.427	0.430
	III E	0.0113	0.324 0.283 0.303	0.303
IV	IV	0.4500	3.994 3.893 3.812	3.900
	IV A	0.2250	1.830 2.002 2.033	1.955
	IV B	0.0900	0.880 0.880 0.839	0.866

TABLE 4 (cont.)   VOLUME OF 0.005N THIOSULPHATE EQUIVALENT
TO DIFFERENT QUANTITIES OF GLUCOSE AS DETERMINED IN 1940

IV (cont.)	IV C	0.0450	0.475 0.496 0.496	0.489
	IV D	0.0225	0.314 0.324 0.324	0.320
	IV E	0.0090	0.152 0.182 0.172	0.168

Deproteinization

Preparation of Reagents

Solution I. 12.5 gm. of $ZnSO_4 \cdot 7H_2O$ were dissolved in water, 125 cc. of 0.25N H_2SO_4 were added, and the mixture diluted with water to 1 liter.

Solution II. 0.75N NaOH.

The two solutions were so related that when 50 cc. of solution I were titrated with solution II, 6.70 to 6.80 cc. were required to produce a permanent pink color with phenolphthalein. The titration was carried out by slow addition of the sodium hydroxide with continuous shaking.

Procedure

A modified Somogyi's zinc procedure (1930) for the preparation of 1:20 blood filtrate was used. One volume of blood was laked in 10 volumes of water. Eight volumes of solution I were then added, with gentle shaking. The reagent was sucked in and blown out several times through the pipette in order to rinse off the blood adhering on the walls. The solution was well mixed in a 50 cc. volumetric flask. The pipette was soaked and washed with dilute ammonium hydroxide solution. Then one volume of solution II was added to the 50 cc. flask. The solution was shaken vigorously, and filtered through dry filter paper after standing a few minutes.

Somogyi's other two procedures (1930) for making blood filtrates (1:20 and 1:40 dilutions) were also tried, but the

procedure just described showed certain advantages over the others such as: simplicity of the measurements, more rapid filtration, and the dilution yielded a filtrate of a concentration that made the reduction equivalents fall in the most accurate region of the linear regression line.

In order to compare the relationship of the glucose value of the blood and the amount of glucose absorbed, the intestinal content of the animals must be treated in the same manner as the blood. Therefore, in this analysis, 50 cc. of the well mixed, cooled intestinal contents (total 250 cc.) were measured into a 100 cc. volumetric flask to which 8 cc. of the solution I had been added. It was mixed well. Then 1 cc. of the solution II was added, the solution was diluted to mark, the flask shaken thoroughly and the solution filtered through dry filter paper after a few minutes.

The Accuracy of the Methods

Glucose Recovery Test

As previously described, the method which indicates most nearly the true glucose content of the blood is that one which will permit the recovery of glucose quantitatively not only in pure solution but when it is added to blood (Peters and Van Slyke, 1932). At the same time, it should give the lowest values when applied to blood alone. To ascertain whether analyses of the blood filtrate prepared by the modified method described by Somogyi (1930 and 1937) represents the true sugar, the reduction values of blood filtrate

with and without the addition of known amounts of glucose were
studied. The recovery data are presented in Table 5.

TABLE 5. RESULTS OF THE GLUCOSE-RECOVERY TEST

Trials	Co. of blood fil-trate	Co. of dis-tilled water	Co. of glucose solu-tion	Average glucose equiva-lent in cc. of $0.005N$ $Na_2S_2O_3$	Mg. of glucose present in the 5 cc. aliquot	Mg. of glucose in blood-glucose solution on basis of separate analysis	Theoretical amount of glucose present
1	2.00 ——— 2.00	3.00 2.00 ———	——— 3.00 3.00	0.70 3.72 4.40	0.078 0.456 0.543	0.078 0.456 0.534	——— 0.450 0.528
2	3.00 ——— 3.00	2.00 3.00 ———	——— 2.00 2.00	1.22 2.43 3.67	0.144 0.296 0.451	0.144 0.296 0.440	——— 0.300 0.444

Somogyi (1927) has allowed a maximum deviation of 4 mg. per
cent in terms of glucose. In the present experiment, the average
error was 0.12 mg. per cent, calculated on the basis of separate
analyses and 0.3 mg. per cent when calculated on the blood-glucose
solution. Therefore, the glucose recovery test has proved that
the modified Somogyi's technique is satisfactory for the glucose
analyses in the present investigation.

Checking the Accuracy of the Reagents and Filter Paper

In the early summer of 1940, the glucose determinations of the
blood of some rats were running unusually high. However, the blank
titrations were the same as usual. This indicated that the copper
solution and the distilled water were not the factors causing the
elevation of the reduction value. Certain factors entering into

the processes of anti-coagulation, precipitation, or filtration must have been involved. Therefore, an aliquot of each reagent used was made up to volume and tested for sugar. The solutions were filtered through samples of old and new filter paper. The results are presented in Table 6. It is evident that the filter paper was the factor causing the unusually high reduction value. Therefore, all data obtained from the blood filtered through the old paper in that summer were discarded, in order to prevent the fluctuation. The results apparently were not at fault. We suspected the NaOH solution because it contained some precipitated Na_2CO_3. However, it is interesting to note that when 1 cc. of 0.75N NaOH diluted in 20 cc. of solution, the 5 cc. of the solution gave the same titration value as the unfiltered blank (6.00 cc.).

TABLE 6. TEST OF REAGENTS AND FILTER PAPER USED IN ANALYSIS

Testing conditions	Cc. of 0.005N sodium thiosulphate used in titration of 5 cc. of the solution					
	1 cc. of potassium oxalate in 20 cc. of dilution	8 cc. of $ZnSO_4$ solution in 20 cc. of solution	1 cc. of 0.75N NaOH in 20 cc. of dilution	$ZnSO_4$ + NaOH in 20 cc. of dilution	$K_2C_2O_4$+ $ZnSO_4$+ NaOH in 20 cc. of dilution	Blank dis-tilled water 5 cc.
1939 reagents						
Not filtered	5.88	5.75	6.00	----	----	6.00
Filtered through old filter paper	----	----	----	4.40	4.00	4.90
Filtered through new filter paper	----	----	----	5.80	----	----
1940 reagents						
Filtered through old filter paper	----	----	----	4.50	----	----
Filtered through new filter paper	----	----	----	5.78		5.70

RESULTS AND DISCUSSION

CONCENTRATION OF SUGAR IN BLOOD OF

RATS IN EXPERIMENTAL SERIES I

Pork I Virgin vs. Steenbock V Virgin Rats

In accord with the usual custom, all the blood sugar values were calculated from the regression equation and expressed in terms of mg. of glucose per 100 cc. blood. The results of the individual determinations of the sugar in the blood of the two groups of rats are tabulated in tables I and II in the appendix. Table 7 shows the average concentration of glucose in the blood of virgin rats fed the control Steenbock V ration and virgin animals receiving the Pork I experimental diet.

TABLE 7. THE AVERAGE CONCENTRATION OF SUGAR IN THE BLOOD OF VIRGIN RATS

Diet of rats	Number of rats in group	Average age of the rat	Average body wt. in gm. after starvation	Food fed after starvation	Average mg. glucose per 100 cc. blood
Steenbock V	13	143	184.2	Steenbock V	129.2
Pork I	13	129	184.0	Pork I	144.1

The virgin rats fed the control ration were about 143 days old when the determination was made, the virgin rats given the

experimental diet, 129 days old. The average body weight of both groups of rats was the same. The concentration of sugar in the blood of the pork-fed rats appears to be higher than in the control animals. However, analysis of variance (table 8) shows that the difference is not significant.

TABLE 8. ANALYSIS OF VARIANCE OF MEAN CONCENTRATION OF SUGAR IN BLOOD IN EXPERIMENTAL AND CONTROL VIRGIN RATS

Sources of variation	Degrees of freedom	Sum of squares	Mean square	F^1
Total	25	15612.62		
Between groups	1	1444.56	1444.56	
Within groups	24	14168.06	590.34	2.45

Variance within the groups was greater than that observed between the groups. Apparently then, consumption of the diet containing meat does not produce any changes in the sugar content of the blood in virgin rats.

1. When no asterisk appears on the F value, it is not significant; however, one asterisk (*) denotes the F value is significant, and two asterisks (**) that it is highly significant. This plan will be followed throughout the thesis.

Virgin vs. Pregnant Rats

Control Group

The data showing the concentration of sugar in the blood of pregnant rats fed the Steenbock V control diet are shown in table III in the appendix. To determine whether pregnancy affects the amount of glucose found in the blood, these data were compared with the blood sugar values in the blood of virgin rats fed the same diet. These data are summarized in table 9 below.

TABLE 9. THE AVERAGE CONCENTRATION OF SUGAR IN BLOOD OF VIRGIN AND GRAVID RATS FED THE STEENBOCK V DIET

Status of rat	Number of rats in group	Food fed after starvation	Average mg. glucose per 100 cc. blood
Virgin	13	Steenbock V	129.2
Gravid	11	Steenbock V	92.7

Analysis of the data (table 10) shows that the quantity of blood sugar is reduced in normal pregnancy.

TABLE 10. ANALYSIS OF VARIANCE OF MEAN CONCENTRATIONS OF SUGAR IN BLOOD OF VIRGIN AND PREGNANT RATS IN CONTROL GROUPS

Sources of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	23	17006.30		
Between groups	1	10247.80	10247.80	33.36**
Within groups	22	6758.50	307.2	

This undoubtedly is due to the fact that the feti demand a share

of the blood sugar for their metabolic needs. This idea is in accord with a statement of Rowe, McManus, and Plummer who wrote in 1936 that pregnancy produces a "low normal blood sugar."

Experimental Group

The effect of pregnancy in the pork-fed groups of rats was also determined. The individual blood sugar values in pregnancy for this group are recorded in table IV in the appendix and the comparative summary data pertaining to the virgin and gravid groups in table 11.

TABLE 11. THE AVERAGE CONCENTRATION OF SUGAR IN BLOOD OF VIRGIN AND GRAVID RATS FED THE PORK I DIET

Status of rat	Number of rats in group	Food fed after starvation	Average mg. glucose per 100 cc. blood
Virgin	13	Pork I	144.1
Gravid	11	Pork I	97.4

The same difference in sugar concentration in the virgin and gravid control rats appears in this group also. Again, the difference is significant (table 12).

TABLE 12. ANALYSIS OF VARIANCE OF MEAN CONCENTRATION OF SUGAR IN BLOOD OF VIRGIN AND PREGNANT RATS IN EXPERIMENTAL GROUPS

Sources of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	23	21148.86		
Between groups	1	13093.56	13093.56	35.76**
Within groups	22	8055.30	366.15	

TABLE 13. CONCENTRATION OF GLUCOSE IN BLOOD OF PREGNANT RATS FED THE STEENBOCK V DIET WITH SOME DATA PERTAINING TO FETAL DEVELOPMENT

Rat number	Wt. of pregnant rat after starvation	Blood sugar	Wt. of intact uterus	Wt. of placenta plus feti	Live feti	Resorbing feti
	gm.	mg.%	gm.	gm.	no.	no.
25436	261	93.7	37.7	33.1	11	1
25577	290	100.1	57.2	54.2	11	1
25491	261	101.3	42.9	40.2	9	4
25757	243	98.6	49.7	47.0	10	1
25553	285	82.4	60.5	57.0	13	-
25662	273	83.2	56.3	52.4	11	-
25807	251	82.7	54.3	50.7	10	-
25737	291	87.0	61.2	57.2	11	-
25898	286	78.2	58.3	55.8	12	2
26027	260	97.1	48.3	45.0	9	2
26110	242	115.6	44.7	41.8	9	1
Average	267.5	92.7	51.9	48.6	10.5	1.1

TABLE 14. CONCENTRATION OF GLUCOSE IN BLOOD OF PREGNANT RATS FED THE PORK I DIET WITH SOME DATA PERTAINING TO FETAL DEVELOPMENT

Rat number	Wt. of pregnant rat after starvation	Blood sugar	Wt. of intact uterus	Wt. of placenta plus feti	Live feti	Resorbing feti
	gm.	mg.%	gm.	gm.	no.	no.
25578	239	92.7	40.5	38.4	9	1
25554	225	101.9	24.6	22.4	5	5
25490	282	100.1	45.1	42.0	10	2
25663	224	94.4	34.2	31.4	9	2
25783	245	92.0	37.5	35.1	8	5
25738	292	82.4	55.4	51.9	11	-
25899	251	132.8	32.3	29.3	6	4
26111	267	93.2	52.1	49.2	11	1
26028	248	63.5	45.6	40.2	9	2
27996	325	99.3	34.6	31.7	7	4
28052	252	99.0	36.1	32.3	7	-
Average	259.1	97.4	39.6	36.7	8.2	2.2

Pork I Pregnant vs. Steenbock V Pregnant Rats

Interest in the present study has been centered on the concentration of sugar in the blood of pregnant rats. The physiologic status of pregnancy itself introduces many factors that might influence blood sugar value. They must be taken into consideration in evaluating the data. Therefore, several important associated phenomena have been tabulated with the blood sugar data in tables 13 and 14.

While the average concentration of glucose in the blood of the rats reared on the pork diet was higher (97.4 mg. per cent vs. 92.7 mg. per cent) than in the control group, variance within the groups was greater than that between them (table 15).

TABLE 15. ANALYSIS OF VARIANCE OF MEAN CONCENTRATIONS OF SUGAR IN BLOOD IN EXPERIMENTAL AND CONTROL PREGNANT RATS

Sources of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	21	3150.44		
Between groups	1	120.09	120.09	1.26
Within groups	20	3030.35	151.52	

Comparison of the data pertaining to the development of the feti in Tables 13 and 14 shows that on the whole, gestation had progressed more favorably in the control group than in the pork group. For instance, the uteri of the Steenbock V rats contained on the average 10.5 live feti; those of the pork rats, 8.2. Further, the average number of resorptions per rat noted among the latter group was higher than in the control group, i.e., 2.2 and

1.1 respectively.

It seemed possible that differences in maternal blood sugar in the two groups of rats might be obscured by these differences in fetal development. To determine the effect of fetal development on maternal blood sugar, pairs of sister mates were assembled from the two sets of data in Tables 13 and 14. Data relating to mg. per cent of blood sugar, number of live feti in the uterus, and the number of resorptions were recorded for each pair of rats as shown in Table 16. It may be noted that in six out of the eight pairs, the difference in the number of live feti present never exceeds two. In these instances, the blood sugar concentrations are similar. However, in the other two pairs, the litter is larger in the Steenbock rat than in the pork rat. In these cases, the differences in blood sugar are marked, i.e., 19 mg. per cent and 54 mg. per cent (see pairs 2 and 6). Thus, while these data indicate that the blood sugar level in the maternal rat may bear an inverse relation to the number of feti developing, the rats in the two groups are too uniform to establish this point. However, this very uniformity gives further weight to the conclusion that the concentration of sugar in blood of pregnant rats fed the pork containing diet is normal.

TABLE 16. BLOOD SUGAR CONCENTRATION, NUMBER OF LIVE FETI AND NUMBER OF RESORBING FETI
IN SISTER MATE PAIRS OF RATS FED THE STEENBOCK V AND PORK I DIETS

Pair number	Rat number	Diet of the rat	Blood sugar in mg. per cent	No. of live feti	No. of resorbing feti
1	25577	Steenbock V	100.1	11	1
	25578	Pork I	92.7	9	1
2	25553	Steenbock V	82.4	13	-
	25554	Pork I	101.9	5	5
3	25491	Steenbock V	101.3	9	4
	25490	Pork I	100.1	10	2
4	25662	Steenbock V	83.2	11	-
	25663	Pork I	94.4	9	2
5	25737	Steenbock V	87.0	11	-
	25738	Pork I	82.4	11	-
6	25898	Steenbock V	78.2	12	2
	25899	Pork I	132.6	6	4
7	26027	Steenbock V	97.1	9	2
	26028	Pork I	83.5	9	2
8	26110	Steenbock V	115.6	9	1
	26111	Pork I	93.2	11	1

ABSORPTION OF GLUCOSE IN CONTROL
AND EXPERIMENTAL GROUPS

It was thought that results obtained in an experiment like the one reported in Series I might be invalidated by differences brought about by variations in the digestibility and absorption of the food fed in the post starvation period. Therefore, as it may be recalled, a second experiment was planned in which the rats were fed a specified amount of a standard solution of pure glucose. In the present section, data are presented to show that results obtained in the glucose-feeding experiment to be described in the next section are not vitiated by differences in absorption.

Reference has been made regarding the method used for quantitative determination of sugar absorption. The amount of sugar fed minus the amount of sugar recovered from the gastro-intestinal tract represents the amount of sugar absorbed. In the present experiment, 2.5 cc. of a 50 per cent glucose solution, was given by stomach tube. Absorption was allowed to continue for four hours. The average quantity of glucose fed and the average per cent of glucose absorbed by the rats reared on the Steenbock V stock ration and the rats fed the Pork I experimental diet are shown in Table 17.

TABLE 17. THE PERCENTAGE ABSORPTION OF GLUCOSE BY RATS FED TWO DIETS

Diet of rat	Rats in the group	Average weight of rat	Glucose fed	Glucose absorbed	Glucose absorbed
	no.	gm.	mg.	mg.	%
Steenbock V	10	267.4	861.4	861.7	100.04
Pork I	10	234.1	865.1	862.7	99.71

It may be noted that the absorption of the glucose at the end of four hours was 100 per cent completed in the animals reared on both the control and experimental diets. Cori in 1925 showed that about two-thirds of a feeding of 1.25 gm. of glucose is absorbed at the end of three hours.

The data in Table 17 also show that the average quantity of glucose entering the blood stream was approximately the same in each group. Therefore, the rats are on a common basis in this respect.

CONCENTRATION OF SUGAR IN BLOOD OF RATS IN EXPERIMENTAL SERIES II

Starved Groups

The concentrations of glucose observed in the blood of rats in the control group after a 13-hour starvation period are recorded in Table 18, together with data on the weight of the rat, the weight of the uterus, the number of live feti, and the number of resorptions. Similar data pertaining to the pork-fed group appear in Table 19. See also Tables V and VI in the appendix. The mean

TABLE 18. CONCENTRATION OF GLUCOSE IN BLOOD OF PREGNANT RATS REARED ON STEENBOCK V DIET
WITH SOME DATA PERTAINING TO FETAL DEVELOPMENT, AFTER STARVING THE RAT FOR 13 HOURS

Rat number	Blood sugar	Wt. of pregnant rat after starvation	Wt. of intact uterus	Wt. of placenta and feti	Live feti	Resorbing feti
	mg.%	gm.	gm.	gm.	no.	no.
28145	56.5	232	41.3	37.8	8	3
28197	42.3	248	56.3	53.2	11	1
28444	85.1	265	64.4	60.6	13	--
28298	83.7	267	51.9	48.3	10	1
28389	46.3	257	58.4	54.6	12	--
28391	50.9	256	52.9	49.7	11	--
28436	54.6	283	76.6	72.9	13	--
28554	48.9	300	61.4	56.9	12	2
28425	57.7	279	60.1	54.3	10	4
Average	58.4	265.2	58.1	54.2	11.1	1.2

TABLE 19. CONCENTRATION OF GLUCOSE IN BLOOD OF PREGNANT RATS REARED ON PORK I DIET WITH SOME DATA PERTAINING TO FETAL DEVELOPMENT, AFTER STARVING THE RAT FOR 13 HOURS

Rat number	Blood sugar	Wt. of pregnant rat after starvation	Wt. of intact uterus	Wt. of placenta and feti	Live feti	Resorbing feti
	mg.%	gm.	gm.	gm.	no.	no.
27926	73.5	241	35.3	31.1	7	3
28144	57.9	229	39.4	36.2	8	4
28194	70.1	208	28.5	25.3	5	5
28196	50.2	231	47.7	44.2	10	2
28297	77.0	223	25.2	22.2	5	4
28390	68.4	210	7.7	4.8	1	4
28443	115.6	203	2.1	0.5	--	8
28552	78.8	251	61.4	57.7	10	1
Average	73.9	224.5	30.9	27.8	5.8	3.9

concentration of sugar in the blood of the first group was 58.4 mg. per cent; in the second, 73.9 mg. per cent. Upon analysis of variance, the difference in the average values proved to be non-significant (Table 20).

TABLE 20. ANALYSIS OF VARIANCE OF MEAN CONCENTRATION OF SUGAR IN BLOOD IN EXPERIMENTAL AND CONTROL-PREGNANT RATS, STARVED FOR 13 HOURS

Sources of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	16	5575.29		
Between groups	1	1016.62	1016.62	
Within groups	15	4558.67	303.91	3.35

The animals composing the two groups were next re-sorted into sister-mate pairs, and the data relating to blood sugar concentration, number of feti produced, and number of resorbing feti for the individual pairs tabulated as in Table 21. A study of the data presented there shows that part of variability observed in the mean values for each group was due to differences in the number of live and resorbing feti found in the uterus. In nearly every pair, where there was[a] difference in the number of living feti, the blood sugar value was lower in the rat with the larger number of feti.

Glucose-Fed Groups

The mean concentration of glucose in the blood of control pregnant rats, four hours after the feeding of 2.5 cc. of 50 per cent glucose solution is 56.2 mg. per cent; that in the blood of rats reared on the pork-containing diet, 75.3 mg. per cent (Tables

TABLE 21. BLOOD CONCENTRATION, NUMBER OF LIVE FETI AND NUMBER OF RESORBING FETI IN SISTER MATE PAIRS OF RATS REARED ON THE STEENBOCK V AND PORK I DIETS, AFTER STARVING THE RATS FOR 13 HOURS

Pair number	Rat number	Diet of the rat	Blood sugar in mg. per cent	No. of live feti	No. of resorbing feti
1	28145	Steenbock V	56.5	8	3
	28144	Pork I	57.9	8	4
2	28197	Steenbock V	42.3	11	1
	28194	Pork I	70.1	5	5
3	28298	Steenbock V	83.7	10	1
	28297	Pork I	77.0	5	4
4	28389	Steenbock V	46.3	12	--
	28390	Pork I	68.4	1	4
5	28444	Steenbock V	85.1	13	--
	28443	Pork I	78.8	10	1

TABLE 22. CONCENTRATION OF GLUCOSE IN BLOOD OF PREGNANT RATS REARED ON THE STEENBOCK V DIET WITH SOME DATA PERTAINING TO FETAL DEVELOPMENT, AFTER THE FORCED FEEDING OF 2.5 CC. OF 50 PER CENT GLUCOSE SOLUTION

Rat number	Blood sugar	Wt. of pregnant rat after starvation	Wt. of intact uterus	Wt. of placenta and feti	Live feti	Resorbing feti
	mg.%	gm.	gm.	gm.	no.	no.
28195	64.0	235	41.6	38.0	8	---
28143	68.2	272	61.1	56.8	12	---
28363	54.4	263	51.6	47.2	11	---
28300	53.2	269	50.5	47.0	10	---
28490	40.2	274	60.4	55.9	12	---
28552	54.8	267	50.4	47.4	10	1
28434	54.4	269	61.0	57.1	12	1
28355	51.6	297	72.2	68.4	14	---
28442	57.4	270	63.7	58.4	12	---
28488	63.8	258	22.9	20.1	4	4
Average	56.2	267.4	53.4	49.6	11.5	0.6

TABLE 23. CONCENTRATION OF GLUCOSE IN BLOOD OF PREGNANT RATS REARED ON THE PORK I DIET WITH SOME DATA PERTAINING TO FETAL DEVELOPMENT, AFTER THE FORCED FEEDING OF 2.5 CC. OF 50 PER CENT GLUCOSE SOLUTION

Rat number	Blood sugar	Wt. of pregnant rat after starvation	Wt. of intact uterus	Wt. of placenta and feti	Live feti	Resorbing feti
	mg.%	gm.	gm.	gm.	no.	no.
28142	109.6	200	7.2	3.0	1	10
28388	89.2	210	14.7	13.3	1	9
28362	86.1	236	30.2	26.9	6	3
28424	84.6	214	11.0	8.7	2	9
28354	56.9	243	43.9	40.6	9	---
28441	55.4	229	43.8	40.8	9	---
28299	76.6	232	35.1	31.7	7	4
28433	88.2	265	32.1	29.1	7	1
28551	52.4	275	40.3	37.4	9	2
28651	53.7	243	49.3	46.8	8	3
Average	75.3	234.7	30.8	27.8	5.9	4.1

22 and 23 and Tables VII and VIII in the appendix). Analysis of variance shows that the difference in the means is significant (Table 24). In fact, the F value approaches the 1% point.

TABLE 24. ANALYSIS OF VARIANCE OF MEAN CONCENTRATION OF SUGAR IN BLOOD IN EXPERIMENTAL AND CONTROL PREGNANT RATS, 2.5 CC. OF A 50 PER CENT GLUCOSE HAS BEEN FED

Sources of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	19	5843.43		
Between groups	1	1818.33	1818.33	
Within groups	18	4025.10	223.62	8.13*

This is the first instance that a difference in the blood sugar values of the experimental and control groups has proved significantly different.

It was necessary, therefore, to determine whether this difference was actually due to an effect on carbohydrate metabolism produced by feeding the pork diet or whether some other physiological factors were operating to produce this result. Again, we studied the possible influence of the size of the uterine contents. From Tables 22 and 23, it may be seen that only an average of 5.9 feti are found in the uterus of the pork-fed group while 11.5 are present in the uterus of the control rats. Also, approximately seven times as many resorbing feti are recorded per pork rat as per control rat. It seems likely, therefore, that the metabolic requirements of the feti in the rats in the control series may be great enough to produce the low level of maternal blood sugar observed.

This idea was tested. First, in order to rule out some

variation, the blood sugar values observed in litter mate pairs of

control and experimental rats were again compared in relation to

the number of living feti found in the uterus. This analysis is

found in table 25. From the data therein presented regarding 7

pairs of rats, it may be seen that the number of living feti pro-

duced by the Steenbock rat always exceeded the number produced by

the pork rat. This difference is marked as in pairs 1, 2, 3, 5, 6,

and 7. In five of these pairs, the blood sugar concentration is

definitely lower in the Steenbock rats. In the one case (Pair 4),

where the number of living feti in the uterus of each rat are

similar, the blood sugar values are nearly alike, 54.8 vs. 52.4 mg.

per cent.

Again we re-sorted the data, this time choosing rats from both

experimental and control groups that had approximately the same

number of live feti. Several rats in each group had either 9, 10 or

11 feti. The blood sugar values in mg. per cent for each group are

enumerated below:

Steenbock V rats	Pork I rats
54.4 (11)	52.4 (9)
53.2 (10)	56.4 (9)
54.8 (10)	56.9 (9)

The blood sugar levels are comparable in these rats.

Thus, again it has been demonstrated that the feeding of the

pork diet during pregnancy to the rat does not cause any change in

the concentration of sugar in the blood. Such differences as have

TABLE 25. BLOOD CONCENTRATION, NUMBER OF LIVE FETI AND NUMBER OF RESORBING FETI IN SISTER MATE PAIRS OF RATS REARED ON THE STEENBOCK V AND PORK I DIETS, AFTER THE FORCED FEEDING OF 2.5 CC. OF 50 PER CENT GLUCOSE SOLUTION

Pair number	Rat number	Diet of the rat	Blood sugar in mg. per cent	No. of live feti	No. of resorbing feti
1	28143	Steenbock V	68.2	12	--
	28142	Pork I	109.6	1	10
2	28363	Steenbock V	54.4	11	--
	28362	Pork I	86.1	6	3
3	28300	Steenbock V	53.2	10	--
	28299	Pork I	76.6	7	4
4	28552	Steenbock V	54.8	10	1
	28551	Pork I	52.4	9	2
5	28434	Steenbock V	54.4	12	1
	28433	Pork I	86.2	7	1
6	28355	Steenbock V	51.6	14	--
	28354	Pork I	56.9	9	--
7	28442	Steenbock V	57.4	12	--
	28441	Pork I	55.4	9	--

been observed are due to the large number of resorptions occurring
in each litter of the rats fed the pork diet. The pork diet seems
directly responsible for the number of resorptions. It is not be-
lieved at the present time that this phenomenon is due to a dietary
deficiency of vitamin E. Armstrong (1939) has shown that the Pork
I diet contains ample amounts of the vitamin to produce successful
littering in rats previously depleted of their stores of vitamin E.
Dyar (1935) also has shown that the addition of vitamin E in the
form of wheat germ oil to the ration does not improve reproduction
in rats fed the Pork I diet.

Dyar also demonstrated that the diet is rich in vitamin A. So
although Mason (1935) has shown that resorptions occur in rats fed
a vitamin A-deficient diet, avitaminosis A is probably not a factor
in producing resorptions in the present experiment.

What causes them is unknown at present. The occurrence seems
to be more or less seasonal. At times very few resorptions are ob-
served (as in Series I); at other times, every rat in the laboratory
seems affected. The rats in series II were studied at a time when
all workers were observing the abnormality. The fact that the
difference in the blood sugar levels of the two groups in series I
were not statistically different while it was in series II can cer-
tainly be explained on the basis of the number of living feti found
in the uteri of the four groups. In series I, the rats were com-
parable in this respect; in series II, they were not.

Results obtained with this series are not conclusive because the concentration of blood sugar approached that of the starvation level. The quantity of glucose fed and the length of the absorption period were based on recommendations of Cori (1925). These studies, however, did not consider the needs of pregnancy. Therefore, the present experiment should be repeated. Larger quantities of glucose than were used this time should be fed, and the concentration of blood sugar determined at several intervals. A glucose tolerance curve thus obtained might divulge facts the present experiment does not.

When the experiment described herein was first planned a study of the concentration of sugar in the blood of the toxic rats was also included. However, many things interfered, so that no data were collected from such rats. For one thing, toxic pregnancies always come later than the twenty-first day of gestation. We attempted therefore to study rats on the twenty-second day, thinking toxicity might then appear. But there was no way of estimating the exact time the litter would be born. Therefore, when we attempted to standardize the animals to permit the analyses to be made on the twenty-second day, in many cases, the litter were born before the post starvation absorption period had elapsed. Vaginal bleeding and consumption of placenta then upset the routine of standardization. These rats were, therefore, discarded. On the other hand, when analyses were attempted on "unstandardized" toxic rats, the stomachs were found full of food and the data therefore were not

comparable with any others. Finally in certain rats that were

successfully standardized, the phenomena of resorption appeared.

An "epidemic" occurred in the laboratory, all rats seeming to be

affected. Analysis of data already obtained showed how this re-

sorption effected the blood sugar, so attempts to obtain glucose

determinations on the blood of these rats were abandoned.

More studies dealing with the concentration of blood sugar at

the final stages of gestation and in rats developing pregnancy

disease will be conducted in the laboratory at a later time.

DISCUSSION

The Source and the Fate of Sugar in the Blood

The principal sources of sugar in blood needs to be considered.

Duke (1937) writes that sugar may be added to the blood (1) by ab-

sorption from the alimentary canal, (2) by hydrolysis of the glyco-

gen of the liver (glycogenolysis), and (3) by transformation of

glucose from non-carbohydrate substances such as amino acids, and

possibly fat (gluconeogenesis).

The increase in blood sugar, which is usually associated with

carbohydrate absorption promptly stimulates the secretion of in-

sulin (Bodansky, 1934), which, in turn, promotes the storage of

glycogen in the liver. As a result, the blood sugar returns to its

normal level. Therefore, glycogenesis may be considered as one of

the factors causing the disappearance of sugar from the blood when

the concentration is elevated above normal. Glucose also disappears from the blood through its conversion into muscle and tissue glycogen. It is continually used up in the tissues. Oxidation, however, produces no pronounced depletion of the sugar in the blood, for even a slight fall in the sugar level causes a discharge of epinephrin from the adrenal glands which in turn accelerates liver glycogenolysis. Thus, hormones play a very important role in the regulation of the concentration of sugar in the blood (Bodansky, 1934).

The workers in the Nutrition Laboratory at Iowa State College have shown that fatty infiltration was marked in the livers of virgin rats fed a diet containing pork as its main source of protein. These abnormalities were more pronounced in pregnancy. Fatty infiltration was noted, in addition, in cases of pregnancy disease. It is believed that fatty livers may interfere with either glycogenesis or glycogenolysis and thus influence the concentration of sugar in the blood. Therefore, a discussion regarding the relationship between fatty livers, toxemic pregnancy, and blood sugar levels may be helpful in interpreting the findings of the present investigation.

Disturbances in Blood Sugar Concentration
Observed in the Clinic

Toxemia of pregnancy is a common complication of pregnancy (Hurwitz, 1933). Changes take place in the processes of metabolism

which manifest themselves by means of certain symptoms and signs (McIlroy, 1936). These disturbances for the most part occur during the latter part of the gestation period at a time of rapid fetal growth when increased quantities of glucose are needed to maintain the nutrition of fetus (Roderick and Hershfield, 1932).

Titus and Dodds (1928) believe that toxemia is a disturbance of carbohydrate metabolism. Twenty-five out of 40 pregnant women showed blood sugar values below 80 mg. per cent, and only 3 cases had higher values than the average range (80-100 mg.). It seemed that these patients had a tendency toward the lower rather than the upper limits of the average range. To the authors, this tendency indicates a glycogen depletion in hyperemesis as previously postulated from clinical observation (Titus et al, 1928). The lowest values indicative of the most profound glycogen depletion of the tissues were seen in the sickest patients. In 1930, Titus indicates again that hypoglycemia is much more predominant and is an even more constant feature of preeclampsia, than it is after the sugar values have been disturbed by convulsions.

Stevens (1933) believes that a sudden drop in blood sugar indicates the onset of convulsions. After the attack, the blood sugar rises as the convulsion stimulates the liver. Siegel and Wylie (1933) also state that there is some disturbance in carbohydrate metabolism in eclampsia and preeclampsia. Eclamptic convulsions are generally preceded by a fall in blood sugar, which is often one of "relative hypoglycemia". Usually there is a temporary

rise in blood sugar following a convulsion. After each succeeding convulsion, there is an increasing tendency toward the establishment of lower blood sugar values. In 19 cases of preeclampsia, low normal or subnormal blood sugar (60 to 85 mg. per 100 cc. of blood) were characteristic. As the patient improves under treatment or by delivery, the blood sugar assumes more normal levels (110-114 mg. per 100 cc. of blood). Siegel and Wylie state that hyperglycemia is relatively infrequent in preeclampsia.

Stander (1929) writes that liver damage is one of the most constant findings in human eclampsia. The disorder involves a peripheral necrosis of the liver lobule. Stander, Duncan, and Sisson (1925) state that the most significant changes noted in the toxemias of pregnancy are those occurring in the increased amount of sugar in the blood with simultaneously increased inorganic phosphorus values. All of his cases showed a definite and in some cases, quite a marked hyperglycemia. A patient may have eclamptic convulsions with the blood sugar at different levels, and these levels are not greatly disturbed by convulsions. But in 1934, Stander and Cadden found that the concentration of sugar in blood in 692 blood samples of preeclamptic patients was not greatly disturbed. Occasionally, a definite hyperglycemia followed an eclamptic convulsion, due perhaps to muscular activity.

Williams and Wills (1929) investigated the alterations in the blood sugar curves in pregnancy in 640 unselected cases in the

Royal Free Hospital. They followed these patients from the early pregnancy to the puerperium. Non-pregnant cases were carried as controls. The blood sugar curves of some normal pregnant women in all periods of pregnancy and puerperium fell well within the range of the curves of normal non-pregnant women. Those of others tended to drop as pregnancy progressed, then to rise in early puerperium with a return to normal in a few weeks. They report that the fasting blood sugars of these patients were either normal or only very moderately raised.

Rowe, McManus, and Plummer (1936) state that the pregnancy produces a "low normal" concentration of sugar in the blood. They found that toxemia in the pregnant women produced no change in blood sugar levels which would differentiate the several states from that of normal gestation. However, the tolerance test with both galactose and levulose showed that there was an unmistakable downward trend from the levels of normal pregnancy to those reported in the toxemias.

Mays and McCord (1935) demonstrated that neither hypo- nor hyperglycemia is characteristic of eclampsia. The blood sugar concentration probably depends upon the patient's nutritional state and the degree of emotional stability and muscular activity of the patient immediately preceding the taking of the specimen. The absolute blood sugar concentration has no effect whatsoever on the incidence of convulsions. Convulsion occurs independently during

a rapid rise in blood sugar concentration as well as during a rapid decline.

A few cases have been reported in the clinical literature that deal with the concentration of blood sugar associated with fatty livers. Bodansky and Bodansky (1940)* have described two cases as follows:

"Fatty metamorphosis of the liver may be accompanied by hypoglycemia. Judd, Kepler, and Rynearson (1934) reported a case in which a diabetic woman patient developed intractable glycosuria and hyperglycemia with a very marked resistance to insulin. Associated with the resistance to insulin were periodic severe hypoglycemic attacks. The hypoglycemic symptoms were difficult to control even though enormous amounts of glucose were administered. After several months on a weight reducing diet during which insulin was not given, the patient developed spontaneous attacks of hypoglycemia which became progressively worse. The blood glucose at times fell as low as 30 mg. per 100 cc. An exploratory laparotomy showed a small cirrhotic liver. The microscopic diagnosis was fatty metamorphosis with slight portal cirrhosis.

"Kramer, Grayzel, and Solomon (1934) have reported the case of a six-months-old infant in which symptoms of weakness, convulsion, etc., were related to hypoglycemia. The blood sugar was frequently

---

* Bodansky, M., and Bodansky, O., 1940, Biochemistry of disease, The Macmillan Co., New York, pp. 244-246

below 30 mg. Epinephrine had no effect in raising the blood sugar. Glucose caused a marked rise. Levulose was practically without effect. The infant died of pneumonia. Microscopic study of the liver disclosed extensive fat replacement throughout the entire section. There was no evidence of glycogen, which may account for the negative effect with epinephrine.

"Krakower (1936) found that glycogen storage disease is accompanied by disturbance of fat metabolism as shown by the presence of ketonuria. It is significant that the livers of the patients with glycogen disease often show a considerable amount of fatty infiltration, resembling in many respects the condition of fatty metamorphosis. In both disorders, hypoglycemia has frequently dominated the clinical picture."

Disturbances in Blood Sugar Concentration Observed Experimentally

Roderick and Harshfield (1932) stated that the symptoms of toxemia of pregnancy of animals are very similar to those of the human condition. The occurrence of the disease seems to be world-wide wherever animals are raised. Roderick and Harshfield have produced the disease experimentally in sheep by varying certain factors in the care and management of the animals. On autopsy, the liver often presents a striking appearance. There is a marked alteration in the color from the reddish brown of a normal liver to a tawny yellow, clay, or putty color. The liver is very friable,

and its cooked appearance is evidence of an extreme degree of

parenchymatous injury. It is interesting to note that the injury

is quite similar to the acute yellow atrophy of the liver encoun-

tered in human pregnancy and that also it resembles the livers of

the gravid rats fed the pork diet in the Nutrition laboratory at

Iowa State College. The microscopic examination of the liver

sections from sheep showed a remarkable fatty degeneration and in-

filtration. Roderick and Harshfield believe that the injured

livers in the advanced stages of the disease show few if any cells

which might be capable of normal function. The blood sugar findings

(below 30 mg. per 100 cc. of blood) are lower than those for normal

sheep (46.1 mg. per 100 cc. of blood). This coincides with some of

the clinical observations of hypoglycemia in human eclampsia. A

few cases observed among the sheep show higher than normal blood

values (65-75 mg. per cent). Roderick and Harshfield explain this

observation on the basis that since carbohydrate intake has not yet

diminished, the injured liver can not store the excess.

Sampson and Hayden (1935) state that there seems to be no

evidence to indicate that the power to oxidize carbohydrate is im-

paired in pregnancy disease of ewes. Peters and Van Slyke (1931)

write that either an insufficient exogenous supply of carbohydrate

or carbohydrate-forming material in the diet, or a deficient

endogenous carbohydrate store in relation to the amount of fat

burned in the body must be responsible for the ketosis found in

toxemia of pregnancy. A depletion of the carbohydrate in the body

may be induced by the demand for glucose by the rapidly developing fetus or feti in the pregnant ewe. The significant factor in the disorder, then, would be the lack of a sufficient amount of glucose in the metabolic mixture for the adequate burning of the fat.

The blood of the ewe with the pregnant disease also showed marked low glucose values, i.e., 35-54 mg. per cent (Sampson, Gonzaga, and Hayden, 1935). After treatment with alfalfa supplemented with one pint of grain per day, the concentration of sugar of the blood attained a higher level, 79.68 mg. per 100 cc. of blood.

Roderick, Harshfield, and Hawn in 1937 believe that pregnancy diseases are quite definitely related to carbohydrate metabolism. With an inadequate carbohydrate intake, the glycogen is withdrawn from the liver to maintain the blood sugar level in metabolism. Then fat takes its place in the liver.

Greene (1937) observed that among 650 female rabbits fed a diet consisting of hay, oats, and a standard commercial ration, 72 fatal cases of toxemic pregnancy appeared. The concentration of sugar in the blood of the rabbits that developed toxemia of pregnancy was compared with that in the blood of resting females and in pregnant females of the same genetic origin. The concentration of sugar in the blood of apparently healthy resting animals ranged from 94 to 153 mg. per cent; in the healthy pregnant rabbits, 118 to 159 mg. per cent. In the rabbits in advanced stages of the

fatal toxemia, three distinct groups were noted. One group showed a marked hypoglycemia (35 to 96 mg. per cent); another, a marked hyperglycemia (304 to 425 mg. per cent); and a third, approximately normal values (146 to 167 mg. per cent).

On autopsy, the liver alteration in the great majority of cases consisted of widespread fatty infiltration and degeneration and showed complete absence of glycogen. But in other instances, fatty changes were not marked and focal areas of necrosis were the pre-dominating lesions. The final opinion of the author was that while the concentration of sugar in the blood of the rabbits with fatal toxemia of pregnancy may be normal, it is often increased, and even in some cases decreased.

In conclusion, data that were obtained both from the clinical and experimental fields that bear on the concentration of sugar in the blood in toxic pregnancy and conditions where fatty livers occur, show that no one condition exists predominantly. The individual may be normal, hyperglycemic, or hypoglycemic. The data presented in the present investigation indicate that blood sugar values in the albino rat suffering from pregnancy disease are essentially normal. However, these findings do not mean that the carbohydrate metabolism is undisturbed in these rats. Farrenkop (1941) has presented evidence to show that the concentration of glycogen in the livers of the animals is significantly reduced even in rats that show no sign of the disease. The normal blood sugar

values, therefore, might represent a break in glycogenesis. That
is, the sugar remains in the blood following its absorption, be-
cause the ability of the liver to synthesize glycogen has slackened.
On the other hand, the data may show that glycogenolysis is normal
and that an effort is made by the organism to keep the blood
sugar levels adequate for its metabolic needs. In this case, the
lower liver glycogen must indicate some inefficiency in the
glycogenetic mechanism. The latter thesis is perhaps the more
logical. The influence of the number of living feti found in the
uterus on maternal blood sugar levels supports this view.

SUMMARY AND CONCLUSIONS

Gestational failures have been consistently produced in albino rats by workers in the Nutrition Laboratory of the Foods and Nutrition Department at the Iowa State College by feeding a semi-synthetic diet known as Pork I. This ration contained 25 per cent dried autoclaved pork muscle as its main source of protein. The mortality of both mothers and young was very high as compared with that observed in control rats reared on the stock ration (Steenbock V). The characteristic syndrome noted in the reproductive disturbance in the rats fed the pork diet is similar to that of human eclampsia.

On autopsy, the livers of the animals receiving the Pork I diet showed definite changes. The liver increased in size, was yellow in color, and friable and spongy in consistency. Microscopic examination of sections of liver showed a fatty infiltration that became more prominent when the disorder appeared among the gravid animals (Armstrong, 1939). In this case, fatty degeneration developed, also. The chemical analysis of the organ also revealed a high fat content. The infiltration of fat in the hepatic cells may injure the functions of either glycogenesis or glycogeneolysis which in turn may cause fluctuation in the concentration of glucose in the blood. Therefore, it seemed that a study of the blood sugar values of the pregnant rats fed the pork ration should yield some information regarding carbohydrate metabolism.

The purpose of the present investigation was to determine the concentration of sugar in the blood of pregnant rats fed the Pork I ration and to compare it with that in rats receiving the control stock ration.

In the experiment, 85 female rats were used. They were divided into two experimental series.

The pregnant groups in the first experimental series consisted of a control group fed the Steenbock V ration. The other experimental group received the Pork I ration. In order to determine whether pregnancy itself exerted an effect upon blood glucose levels, pregnant and virgin females were studied in the control and experimental groups. The pregnant animals were killed on the 21.5 day of the second gestation. They were standardized by means of starvation for 13 hours; 4 gm. of their own diet was fed at the end of this period; and then the animals were stunned four hours after the feeding. The blood was drawn immediately for chemical analysis. The virgin females were killed when they were as old on the average as the pregnant animals in that group. They were also standardized before autopsy.

Since the control and experimental diets contained different ingredients, their respective digestibility and absorbability might be different. In order to minimize possible variations from these sources, a second series of experiments was planned. In this experiment, the quantity and quality of food fed after starvation was kept constant by administering 2.5 cc. of 50 per cent glucose

solution to each rat instead of giving it its own ration.

In the second series of the experiment, both pregnant starved and pregnant glucose-fed groups of rats were studied. Each group was further subdivided on the basis of the control and experimental diets used in rearing the rats. All the animals were treated in the same way as they were in the first series of the experiment except that no food was fed to the starved group. These animals were stunned right at the end of the starvation period. The blood and gastro-intestinal tract were analyzed for glucose and reducing substances, respectively. The difference between the amount of glucose fed and the amount of glucose present in the gastro-intestinal tract was then the amount of glucose absorbed.

A modification of the method described by Cori (1925) was used for the quantitative study of intestinal absorption. Somogyi's methods for deproteinization of the blood and analysis of glucose were adopted. A glucose recovery test proved that the method was satisfactory.

A glucose-thiosulphate linear regression was established for each new copper reagent used by analyzing four to five series of chemically pure standard glucose solutions. The regression equation was used to translate the analytical data into their glucose equivalents.

Results in Series I showed that the average concentration of sugar in the blood of virgin rats fed the Pork I experimental diet was 144.1 mg. per cent in contrast to 123.2 mg. per cent in the

blood of the virgin animals receiving the Steenbock V control ration. It seemed that the experimental rats had a higher concentration of blood sugar than the control rats, but analysis of variance showed that the difference was not significant.

To determine whether pregnancy per se affected the concentration of sugar in the blood, virgin and pregnant rats fed the same diet were compared under the same standard conditions. The blood sugar values in the control group averaged 123.2 and 92.7 mg. per cent, respectively; in the experimental group, 144.1 and 97.4 mg. per cent, respectively. Thus, the quantity of blood sugar appeared to be lower in gravid rats fed both diets than it was in virgin animals. The differences noted in the level of blood glucose in pregnant and non-pregnant rats proved significant upon analysis of variance. Undoubtedly, the metabolic needs of the feti were responsible for the low blood sugar values observed in the pregnant group.

When the average quantity of sugar in the blood of the pregnant rats fed the Pork I diet (97.4 mg. per cent) was compared with that in the blood of pregnant rats fed the Steenbock V diet (92.7 mg. per cent), no marked difference was noted, and analyses showed that the variance within the groups was greater than that between them.

In the second series of the experiment, a specified quantity of glucose was fed by stomach tube after the starvation period. Analysis of the reducing substances in the intestinal tract of the rats in the control and experimental groups showed that the percentage of glucose absorbed was 100.04 and 99.71, respectively. This observation

indicated that the consumption of the pork-containing diet had not affected the absorbing function of the gastro-intestinal tract.

In order to determine the basal levels of the sugar concentration in the blood, both the control and experimental groups were starved for 13 hours. The blood sugar values at the end of this period averaged 58.4 and 73.9 mg. per cent, respectively. Statistical analysis showed that the difference was not significant.

Of the rats that were fed the glucose solution, the blood sugar concentration in the pregnant control group was 56.2 mg. per cent, and in the rats reared on the pork diet, 75.7 mg. per cent. Analysis of the variance indicated the difference was significant. It seemed strange that a significant difference should exist in this instance, when no such difference occurred in the levels of sugar observed in the blood of the two pregnant groups studied in the first series.

In the first series, fetal development was comparable in the experimental and control groups. The number of living feti found in the uteri of the experimental group was 8.2; in the control group, 10.5. However, in the second series of glucose-fed rats, there were only 5.9 feti alive in the uteri of the pork rats in contrast to 11.5 in the Steenbock V rats. Before it could be said that the blood of the pork rats was hyperglycemic, it was necessary to determine whether or not differences in fetal metabolism were affecting the maternal blood values. By a re-sorting of the data that made it possible to examine the concentration of glucose in the bloods of paired sister mates reared on the two diets, it was found that the

larger the number of feti present, the lower was the concentration
of blood sugar. If the number of feti produced by each member of the
pair happened to be the same, the glucose concentration was also
approximately the same. Therefore, the difference originally observed,
while real, was not one that pointed to a hyperglycemia in the pork
rats.

From the data presented above, the following conclusions seem
warranted:

1. Pregnancy per se lowers the concentration of sugar in the
   blood;

2. The greater the number of living feti present in the uterus,
   the lower is the concentration of sugar in the maternal
   blood;

3. The level of blood sugar remains normal in rats fed the
   pork-containing diet until the 21.5 day of gestation, even
   though the concentration of glycogen is known to be sub-
   normal; and

4. Whether this statement holds true when acute toxemia
   develops remains to be demonstrated.

ACKNOWLEDGMENTS

The author wishes to express her sincere appreciation to Dr. Pearl P. Swanson who proposed the problem, for her kind advice and constructive criticism throughout the investigation, and for her stimulating counsel in the preparation of the manuscript.

The author is also indebted to Dr. P. Mable Nelson for the generous encouragement given in this study, to Professor G. W. Snedecor for helpful advice on the statistical analysis, and to Miss Ethelwyn B. Wilcox for her valuable assistance in the laboratory work.

LITERATURE CITED

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 Ph. D. Thesis, Library, Iowa State College, Ames, Iowa.

Atkinson, H. V., and Ets, H. N.
1922. Chemical changes of the blood under the influence of drugs
J. Biol. Chem., 52, 5-10.

Best, C. H., and Taylor, N. B.
1937. The physiological basis of medical practice
William Wood and Company, Baltimore.

Bodansky, Meyer
1934. Introduction to physiological chemistry, 3rd edition
John Wiley and Sons, Inc., New York

Campbell, Gwendolyn
1940. Meat in nutrition. XVI. Effect of supplementary feeding of fresh pancreas on the reproductive performance of the albino rat fed a diet containing pork muscle
Unpublished M. S. Thesis, Library, Iowa State College, Ames, Iowa.

Cori, C. F.
1925. The fate of sugar in the animal body. I. The rate of absorption of hexoses and pentoses from the intestinal tract
J. Biol. Chem., 66, 691-715.

Cori, C. F., and Cori, G. T.
1926. The fate of sugar in the animal body. II. The relation between sugar oxidation and glycogen formation in normal and insulinized rats during the absorption of glucose
J. Biol. Chem., 70, 557-576.

Cori, C. F.
1926. The fate of sugar in animal body. III. The rate of glycogen formation in the liver of normal and insulinized rats during the absorption of glucose, fructose and galactose
J. Biol. Chem., 70, 577-585.

Duke, H. H.
1937. The physiology of domestic animals, 4th edition
Comstock Publishing Company, Inc., Ithaca, N. Y.

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

Epstein, A. A., and Baehr, G.
1914. Certain new principles concerning the mechanism of hyper-
glycemia and glycosuria
J. Biol. Chem., 18, 21-27.

Epstein, A. A., and Aschner, P. W.
1916. The effect of surgical procedures on the blood sugar content
J. Biol. Chem., 25, 151-162.

Farrankop, Helen
1941. Meat in nutrition. XVIII. Glycogen in maternal and fetal
livers of rats fed a diet containing dried autoclaved pork
muscle
Unpublished Ph. D. Thesis, Library, Iowa State College,
Ames, Iowa.

Graham, A. M.
1939. Stock colony IV. Factors affecting concentration of hemo-
globin in the blood of the albino rats, Wister stock, strain
A
Unpublished M. S. Thesis, Library, Iowa State College, Ames,
Iowa.

Greene, H. S. N.
1937. Toxemia of pregnancy in the rabbits
J. Exp. Med., 65, 809-832.

Greenwood, M. L.
1940. Stock colony studies. III. The reproductive and lactating
behavior of the female albino rat, Wistar stock, strain A
Unpublished Ph. D. Thesis, Library, Iowa State College,
Ames, Iowa

Harris, R. E., and Ingle, D. J.
1937. The influence of destruction of the adrenal medulla on
emotional hyperglycemia in rats
Amer. J. Physiol., 120, 420-422.

Hurwitz, D.
1933. Toxaemias of pregnancy; a critical review of recent liter-
       ature
       New England J. Med., 209, 1281-1289.

Judd, E. S., Kepler, E. J., and Rynearson, E. H.
1934. Spontaneous hypoglycemia. Reports of two cases associated
       with fatty metamorphosis of the liver
       Amer. J. Surg., 24, 345-363.

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

Krakower, C.
1936. Lipoid factor in glycogen storage disease
       J. Ped., 9, 728-743.

Kramer, B., Grayzel, H. G., and Solomon, C. I.
1934. Chronic hypoglycemia in childhood
       J. Pediatrics, 5, 299-321.

de Langen, C. D., and Schut, H.
1918. Mededeelingen van den Burg. Geneesk. Dienst Nederl. Indië
       No. 3, 1
       Cited by Feinblatt, H. M., J. Lab. Clin. Med., 8, 300; 1923.

Letonoff, T. V.
1934. A zinc hydroxide powder for the preparation of protein-free
       filtrates of blood
       J. Biol. Chem., 106, 693-699.

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

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

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

McIlroy, D. L.
1936. The toxaemias of Pregnancy
     William Wood and Company, Baltimore.

Myers, V. C.
1924. Practical chemical analysis of blood, 2nd edition
     C. V. Mosby Company, St. Louis.

Peters, J. P., and Van Slyke, D. D.
1931. Quantitative clinical chemistry, I, Interpretations
     The Williams and Wilkins Company, Baltimore.

Peters, J. P., and Van Slyke, D. D.
1932. Quantitative clinical chemistry, II, Methods
     The Williams and Wilkins Company, Baltimore.

Reinecke, R. M., Ball, H. A., and Samuels, L. T.
1939. High fat and high carbohydrate diets that can be fed
     to rats
     Proc. Soc. Exp. Biol. Med., 41, 44-46.

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

Roderick, L. M., Harshfield, G. S., and Hawn, M. C.
1937. The pathogenesis of ketosis: Pregnancy disease of sheep
     J. Amer. Vet. Med. Ass., 90, 41-49.

Ross, E. L.
1918. Blood dextrose as affected by morphine and morphine with
     ether anesthesia
     J. Biol. Chem., 34, 335-342.

Roudin, I. S., Vars, H. M., Goldsmidt, S., and Klengensmith, L. E.
1938. Anesthesia and liver damage. II. Effect of anesthesia on
     blood sugar, liver glycogen, and liver fat
     J. Pharm. Exp. Therap., 64, 119-129.

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

Sampson, J., Gonzaga, A. C., and Hayden, C. E.
1933. The ketones of the blood and urine of the cow and ewe in
     health and disease
     Cornell Vet., 23, 185-208.

Sampson, J., and Hayden, C. E.
1935. The acid-base balance in cows and ewes during and after
       pregnancy with special reference to milk fever and
       acetonemia
       J. Amer. Vet. Med. Ass., 86, 13-23.

Shaffer, P. A., and Somogyi, M.
1933. Copper-iodometric reagents for sugar determination
       J. Biol. Chem., 100, 695-713.

Siegel, I. A., and Wylie, H. B.
1933. Blood sugar findings in eclampsia and preeclampsia
       Amer. J. Obstet. Gynec., 26, 29-37.

Snedecor, G. W.
1940. Statistical methods, 3rd edition
       Collegiate Press, Inc., Ames, Iowa.

Somogyi, M.
1927. Reducing non-sugars and true sugar in human blood
       J. Biol. Chem., 75, 33-43.

Somogyi, M.
1930. A method for the preparation of blood filtrates
       for the determination of sugar
       J. Biol. Chem., 86, 655-663.

Somogyi, M.
1937. A reagent for the copper-iodometric determination
       of very small amounts of sugar
       J. Biol. Chem., 117, 771-776.

Stander, H. J., Duncan, E. E., and Sisson, W. E.
1925. Chemical studies on the toxemias of pregnancy
       Bul., Johns Hopkins Hospital, 36, 411-427.

Stander, H. J., and Radelet, A. H.
1926. Blood studies in general anesthesia
       Science, 63, 642.

Stander, H. J.
1929. The toxemias of pregnancy
       Medicine Monographs, 15,
       William and Wilkins, Baltimore.

Stander, H. J., and Harrison, P. H.
1929. Carbohydrate metabolism in eclampsia
       Amer. J. Obstet. Gynec., 18, 17-27.

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

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

Stevens, W. J.
1933. The toxaemias of pregnancy
        Canad. Med. Assoc. J., 28, 513.

Stinchfield, A. J.
1937. Blood sugar determinations valueless unless drawn and
        examined with special precaution
        Maine Med. J., 28, 71.

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

Tatum, A. L.
1920. Alkaline reserve capacity of whole blood and carbohydrate
        metabolism as affected by hemorrhage
        J. Biol. Chem., 41, 59-73.

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

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

Titus, P., Willetts, E. W., and Lightbody, H. D.
1930. Fluctuations in blood sugar during eclampsia
        Amer. J. Obstet. Gynec., 19, 16-25.

Underhill, F. P., and Sprunt, D. H.
1927. The influence of amytal upon blood sugar content
        Proc. Soc. Exp. Biol. Med., 25, 137-138.

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

Wilcox, E. B.
1937. Meat in nutrition. XII. Effect of supplementary feed-
        ing of lecithin and lipocaic on the reproductive per-
        formance of the albino rat fed a diet containing pork
        muscle.
        Unpublished M. S. Thesis, Library, Iowa State College,
        Ames, Iowa.

Williams, E. C., Pillman, and Wills, L.
1929. Studies in blood and urinary chemistry during
        pregnancy
        Quart. J. Med., 22, 493-501.

APPENDIX

FORM I. CONDITION OF FEMALE RATS AT AUTOPSY (EXTERNAL CONDITION)

Rat no. _____
Age rat (in days) _____
Pregnancy no. _____
Day of gestation period _____
Wt. before starving _____
Wt. after starving _____

Diet no. _____
Autopsy date _____
Hour _____
Period of starvation _____
Hour initiated _____
Hour terminated _____

Description of diet _____
Post-starvation period _____
Food _____
Quantity _____
Length of period _____

Physical condition[1]
General _____ Alert _____ Paws pinkish _____
Fat _____ Gaunt _____ Eyes pink _____

Eyelids _____ Inflamed _____ Infected _____

Muscle tone
General _____ Abdominal _____

Respiration
Sniffy _____ Wheezy _____
Palpitations _____

Gait
Dragging _____ Elevated _____
Sprawling _____ Awkward _____

Exudates[2]
Nasal _____ Anal _____
Oral _____ Vaginal _____

Hair
Clean _____ Smooth _____
Creamy _____ Thick _____
Fine _____

Hematuria _____

Tail
Clean _____ Smooth _____
Discolored _____ Sores _____

Remarks _____

[1]In recording the degree to which any condition is present use a scale ranging
from minus (-) to four plusses (++++).
[2]Indicate character of exudate.

FORM II. CONDITION OF FEMALE RATS AT AUTOPSY (INTERNAL CONDITION)

Rat no. _____ Diet no. _____ Description of diet _____

Fat depots[1]
Subcutaneous _____ Perirenal _____
Peritoneal _____ Genital _____
Omental _____ Intermuscular _____

Stomach ulcers
Number _____ Severity _____

Condition of the lungs:

	Infection	Atelectasis	Emphysema
Lobe 1.	_____	_____	_____
2.	_____	_____	_____
3.	_____	_____	_____
4.	_____	_____	_____
5.	_____	_____	_____

Liver
Yellow _____ Mottled _____
Friable _____ Spongy _____

Pus pockets:
Ovary _____
Placental sites _____
Ear _____
Base of the tongue _____

Kidneys
Cortex, color _____ friable _____
Medulla, color _____ friable _____
Pelvis, color _____ friable _____

Teeth
Straight _____ Orange _____

Pancreas, any gross abnormalities: _____

Wt. of uterus plus feti _____

Corpora lutea
No. in left ovary _____ right _____
Color _____

Wt. of uterus _____

Remarks _____

Fetal sites, no. of _____

Live feti, no. of _____

Resorptions, no. of _____

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

TABLE I. CONCENTRATION OF SUGAR IN BLOOD OF VIRGIN RATS FED THE STEENBOCK V DIET 4 HOURS
AFTER THE FEEDING OF 4 GM. OF THEIR OWN DIET

Rat number	Dilutions of blood	Normality of $Na_2S_2O_3$	Cc. of $Na_2S_2O_3$ used for blank	Cc. of $Na_2S_2O_3$ used for titration	Difference			Mg. of glucose per 100 cc. blood	Average mg. of glucose per 100 cc. blood
					In terms of cc. of $Na_2S_2O_3$	In terms of cc. of 0.005n $Na_2S_2O_3$	In terms of mg. of glucose in 5 cc. aliquot		
26146*	1:20	0.004926	6.00	4.10	1.90	1.872	0.210	84.0	93.2
				4.09	1.91	1.882	0.211	84.4	
				3.70	2.30	2.266	0.257	102.8	
				3.73	2.27	2.236	0.254	101.6	
26350	1:20	0.005000	5.60	3.26	2.34	2.340	0.284	113.6	114.5
				3.24	2.36	2.360	0.287	114.8	
				3.25	2.35	2.350	0.286	114.4	
				3.23	2.37	2.370	0.288	115.2	
26357	1:20	0.005000	5.60	3.10	2.50	2.500	0.304	121.6	122.1
				3.08	2.52	2.520	0.307	122.8	
26560	1:20	0.005000	5.58	2.71	2.87	2.870	0.351	140.4	141.1
				2.69	2.89	2.890	0.353	141.2	
				2.67	2.91	2.910	0.356	142.4	
				2.71	2.87	2.870	0.351	140.4	
26621	1:20	0.005000	5.58	2.94	2.64	2.640	0.322	128.8	121.4
				2.98	2.60	2.600	0.317	126.8	
				3.21	2.37	2.370	0.288	115.2	
				3.22	2.36	2.360	0.287	114.8	

TABLE I (cont.) CONCENTRATION OF SUGAR IN BLOOD OF VIRGIN RATS FED THE STEENBOCK V DIET
4 HOURS AFTER THE FEEDING OF 4 GM. OF THEIR OWN DIET

26633	1:20	0.005000	5.58	2.97 2.97 3.17 2.17	2.61 2.61 2.41 2.41	2.610 2.610 2.410 2.410	0.318 0.318 0.293 0.293	127.2 127.2 117.2 117.2	122.2
26655	1:20	0.005000	5.49	2.27 2.29 2.17 2.20	3.22 3.20 3.32 3.29	3.220 3.200 3.380 3.290	0.395 0.392 0.408 0.404	158.0 156.8 163.2 161.6	159.9
24885	1:20	0.004990	5.86	3.18 3.20	2.68 2.66	2.675 2.655	0.326 0.324	130.4 129.6	130.0
24930	1:20	0.004990	5.86	3.30 3.30	2.56 2.56	2.555 2.555	0.311 0.311	124.4 124.4	124.4
25959	1:20	0.004990	5.87	4.14 4.16	1.73 1.71	1.726 1.706	0.207 0.205	82.8 82.0	82.4
25007	1:20	0.004990	5.88	3.09 3.09 3.08 3.09	2.79 2.79 2.80 2.79	2.784 2.784 2.794 2.784	0.340 0.340 0.341 0.340	136.0 136.0 136.4 136.0	136.1
25009	1:20	0.004980	5.81	2.53 2.52 2.49 2.50	3.28 3.29 3.32 3.31	3.267 3.277 3.307 3.297	0.401 0.402 0.406 0.405	160.4 160.8 162.4 162.0	161.4

TABLE I (cont.) CONCENTRATION OF SUGAR IN BLOOD OF VIRGIN RATS FED THE STEENBOCK V DIET
4 HOURS AFTER THE FEEDING OF 4 GM. OF THEIR OWN DIET

25010	1:20	0.004980	5.81	2.30 2.32	3.51 3.49	3.494 3.476	0.429 0.427	171.6 170.8	171.2
Average									129.2

* The quantity of glucose was calculated from the 1940 regression equation.

TABLE II. CONCENTRATION OF SUGAR IN BLOOD OF VIRGIN RATS FED THE PORK I DIET 4 HOURS AFTER THE FEEDING OF 4 GM. OF THEIR OWN DIET.

Rat number	Dilutions of blood	Normality of $Na_2S_2O_3$	Cc. of $Na_2S_2O_3$ used for blank	Cc. of $Na_2S_2O_3$ used for titration	Difference			Mg. of glucose per 100 cc. blood	Average mg. of glucose per 100 cc. blood
					In terms of cc. of $Na_2S_2O_3$	In terms of cc. of 0.005n $Na_2S_2O_3$	In terms of mg. of glucose in 5 cc. aliquot		
27778*	1:20	0.005002	5.81	2.55	3.26	3.261	0.377	150.8	151.1
				2.54	3.27	3.271	0.378	151.2	
				2.53	3.28	3.281	0.379	151.6	
				2.55	3.26	3.261	0.377	150.8	
28198*	1:20	0.004919	6.00	2.75	3.25	3.197	0.369	147.6	144.7
				2.76	3.24	3.188	0.368	147.2	
				2.87	3.13	3.079	0.355	142.0	
				2.87	3.13	3.079	0.355	142.0	
28254*	1:20	0.004919	6.00	3.54	2.46	2.420	0.276	110.4	109.4
				3.55	2.45	2.410	0.274	109.6	
				3.56	2.42	2.380	0.271	108.4	
				3.56	2.44	2.400	0.273	109.2	
28263*	1:20	0.004919	6.00	3.60	2.40	2.361	0.269	107.6	107.6
				3.60	2.40	2.361	0.269	107.6	
28296*	1:20	0.004926	6.00	3.38	2.62	2.581	0.295	116.0	117.4
				3.41	2.59	2.552	0.292	116.8	
26278	1:20	0.004990	5.78	2.11	3.67	3.663	0.450	180.0	180.2
				2.10	3.68	3.673	0.451	180.4	

TABLE II (cont.) CONCENTRATION OF SUGAR IN BLOOD OF VIRGIN RATS FED THE PORK I DIET 4 HOURS AFTER THE FEEDING OF 4 GM. OF THEIR OWN DIET.

26358	1:20	0.004950	5.69	2.09	3.60	3.564	0.438	175.2	174.8
				2.10	3.59	3.554	0.437	174.8	
				2.10	3.59	3.554	0.437	174.8	
				2.11	3.58	3.544	0.436	174.4	
26398	1:20	0.004950	5.54	2.29	3.25	3.218	0.395	158.0	158.0
				2.29	3.25	3.218	0.395	158.0	
26571	1:20	0.005000	5.33	2.28	3.05	3.050	0.374	149.6	149.6
				2.25	3.08	3.050	0.374	149.6	
26622	1:20	0.005000	5.52	2.35	3.17	3.170	0.389	155.6	155.0
				2.37	3.15	3.150	0.386	154.4	
				2.35	3.17	3.170	0.389	155.6	
				2.35	3.17	3.150	0.386	154.4	
26634	1:20	0.005000	5.52	2.36	3.16	3.160	0.387	154.8	154.8
				2.36	3.16	3.160	0.387	154.8	
26656	1:20	0.005000	5.54	2.57	2.97	2.970	0.364	145.6	145.6
				2.57	2.97	2.970	0.364	145.6	
26693	1:20	0.005000	5.54	3.01	2.53	2.530	0.308	123.8	125.5
				2.93	2.61	2.610	0.318	127.2	
Average									144.1

* The quantity of glucose was calculated from the 1940 regression equation.

TABLE III. CONCENTRATION OF SUGAR IN BLOOD OF PREGNANT RATS FED STEENBOCK V DIET 4 HOURS
AFTER THE FEEDING OF 4 GM. OF THEIR OWN DIET

Rat number	Dilutions of blood	Normality of $Na_2S_2O_3$	Cc. of $Na_2S_2O_3$ used for blank	Cc. of $Na_2S_2O_3$ used for titration	Difference			Mg. of glucose per 100 cc. blood	Average mg. of glucose per 100 cc. blood
					In terms of cc. of $Na_2S_2O_3$	In terms of cc. of 0.005n $Na_2S_2O_3$	In terms of mg. of glucose in 5 cc. aliquot		
25436	1:20	0.004995	5.79	3.86	1.93	1.928	0.233	93.2	93.7
				3.86	1.93	1.928	0.233	93.2	
				3.80	1.99	1.988	0.240	96.0	
				3.82	1.97	1.968	0.238	95.2	
				3.87	1.92	1.918	0.231	92.4	
				3.88	1.91	1.908	0.230	92.0	
25577	1:20	0.004990	5.84	3.76	2.08	2.076	0.251	100.4	100.1
				3.78	2.06	2.056	0.249	99.6	
				3.77	2.07	2.066	0.250	100.0	
				3.76	2.08	2.076	0.251	100.4	
25491	1:20	0.004990	5.47	3.35	2.12	2.116	0.256	102.4	101.3
				3.36	2.12	2.116	0.256	102.4	
				3.40	2.07	2.066	0.250	100.0	
				3.39	2.08	2.076	0.251	100.4	
25757	1:20	0.004988	5.94	3.91	2.03	2.026	0.245	98.0	98.6
				3.88	2.06	2.055	0.249	99.6	
				3.90	2.04	2.035	0.246	98.4	
				3.90	2.04	2.035	0.246	98.4	

TABLE III (cont.) CONCENTRATION OF SUGAR IN BLOOD OF PREGNANT RATS FED STEENBOCK V DIET 4 HOURS AFTER THE FEEDING OF 4 GM. OF THEIR OWN DIET.

25553	1:20	0.004995	5.81	4.10	1.71	1.708	0.205	82.0	
				4.10	1.71	1.708	0.205	82.0	
				4.08	1.73	1.728	0.208	85.2	
				4.09	1.72	1.718	0.206	82.4	82.4
26662	1:20	0.004993	5.81	4.10	1.71	1.708	0.205	82.0	
				4.10	1.71	1.708	0.205	82.0	
				4.00	1.81	1.807	0.217	86.8	
				4.10	1.71	1.708	0.205	82.0	85.2
26607	1:20	0.004990	5.58	3.86	1.72	1.716	0.206	82.4	
				3.85	1.73	1.726	0.207	82.8	
				3.85	1.73	1.726	0.207	82.8	
				3.85	1.75	1.726	0.207	82.8	82.7
25737	1:20	0.004970	5.75	3.90	1.83	1.819	0.219	87.6	
				3.95	1.78	1.769	0.213	85.2	
				3.90	1.85	1.819	0.219	87.6	
				3.90	1.83	1.819	0.219	87.6	87.0
25898	1:20	0.004990	5.51	3.88	1.63	1.627	0.195	78.0	
				3.87	1.64	1.637	0.196	78.4	
				3.87	1.64	1.637	0.196	78.4	
				3.88	1.63	1.627	0.195	78.0	78.2

TABLE III (cont.)   CONCENTRATION OF SUGAR IN BLOOD OF PREGNANT RATS FED STEENBOCK V DIET
4 HOURS AFTER THE FEEDING OF 4 GM. OF THEIR OWN DIET.

26027	1:20	0.004990	5.44	3.40 3.45 3.45 3.44	2.04 2.01 2.01 2.00	2.036 2.006 2.006 1.996	0.246 0.242 0.242 0.241	98.4 95.8 96.8 96.4	97.1
26110	1:20	0.004988	5.51	3.12 3.13 3.15 3.10	2.59 2.38 2.38 2.40	2.384 2.374 2.374 2.394	0.290 0.289 0.289 0.291	116.0 115.0 115.0 116.4	115.6
Average									92.7

TABLE IV.   CONCENTRATION OF SUGAR IN BLOOD OF PREGNANT RATS FED THE PORK I DIET 4 HOURS
AFTER THE FEEDING OF 4 GM. OF THEIR OWN DIET.

Rat number	Dilutions of blood	Normality of Na₂S₂O₃	Cc. of Na₂S₂O₃ used for blank	Cc. of Na₂S₂O₃ used for titration	Difference			Mg. of glucose per 100 cc. blood	Average mg. of glucose per 100 cc. blood
					In terms of cc. of Na₂S₂O₃	In terms of cc. of 0.005n Na₂S₂O₃	In terms of mg. of glucose in 5 cc. aliquot		
25578	1:20	0.004995	5.74	3.78 3.80 3.85 3.84	1.96 1.94 1.89 1.90	1.958 1.938 1.888 1.898	0.236 0.234 0.228 0.229	94.4 93.6 91.2 91.6	92.7
25554	1:20	0.004998	5.71	3.61 3.61 3.60 3.59	2.10 2.10 2.11 2.12	2.099 2.099 2.105 2.115	0.254 0.254 0.255 0.256	101.6 101.6 102.0 102.4	101.9
25490	1:20	0.004998	5.71	3.66 3.67 3.61 3.61	2.05 2.04 2.10 2.10	2.045 2.035 2.099 2.099	0.247 0.246 0.254 0.254	98.8 98.4 101.6 101.6	100.1
25663	1:20	0.004998	5.85	3.88 3.89 3.90 3.89	1.97 1.96 1.95 1.96	1.965 1.955 1.945 1.955	0.237 0.236 0.235 0.236	94.8 94.4 94.0 94.4	94.4

TABLE IV (cont.) CONCENTRATION OF SUGAR IN BLOOD OF PREGNANT RATS FED THE PORK I DIET 4 HOURS AFTER THE FEEDING OF 4 GM. OF THEIR OWN DIET.

ID									
25783	1:20	0.004993	5.74	3.81 / 3.82 / 3.83 / 3.85	1.93 / 1.92 / 1.91 / 1.89	1.927 / 1.917 / 1.907 / 1.887	0.232 / 0.231 / 0.230 / 0.227	92.8 / 92.4 / 92.0 / 90.8	92.0
25738	1:20	0.004993	5.55	3.88 / 3.87 / 3.78 / 3.79	1.67 / 1.68 / 1.77 / 1.76	1.668 / 1.678 / 1.768 / 1.758	0.200 / 0.201 / 0.212 / 0.211	80.0 / 80.4 / 84.8 / 84.4	82.4
25899	1:20	0.005002	5.71	2.99 / 2.99 / 2.98 / 3.00	2.72 / 2.72 / 2.73 / 2.70	2.721 / 2.721 / 2.731 / 2.701	0.332 / 0.332 / 0.334 / 0.330	132.8 / 132.8 / 133.6 / 132.0	132.8
26111	1:20	0.004990	5.59	3.68 / 3.62 / 3.68 / 3.69	1.96 / 1.97 / 1.91 / 1.90	1.956 / 1.966 / 1.906 / 1.896	0.236 / 0.237 / 0.230 / 0.229	94.4 / 94.8 / 92.0 / 91.6	93.2
26028	1:20	0.004990	5.56	3.80 / 3.80 / 3.82 / 3.85	1.76 / 1.76 / 1.74 / 1.71	1.756 / 1.756 / 1.736 / 1.706	0.211 / 0.211 / 0.208 / 0.205	84.4 / 84.4 / 85.2 / 82.0	83.5

TABLE IV (cont.)   CONCENTRATION OF SUGAR IN BLOOD OF PREGNANT RATS FED THE PORK I DIET 4 HOURS
AFTER THE FEEDING OF 4 GM. OF THEIR OWN DIET.

27996*	1:20	0.004838	5.99	3.75	2.24	2.190	0.248	99.2	99.3
				3.74	2.25	2.200	0.249	39.6	
				3.75	2.25	2.180	0.247	98.8	
				3.74	2.25	2.200	0.249	99.6	
28052*	1:20	0.005028	5.78	3.62	2.16	2.172	0.246	98.4	99.0
				3.60	2.18	2.192	0.248	99.2	
				3.60	2.18	2.192	0.248	99.2	
				3.60	2.18	2.192	0.248	99.2	
Average									97.4

* The quantity of glucose was calculated from the 1940 regression equation.

TABLE V. CONCENTRATION OF SUGAR IN BLOOD OF PREGNANT RATS REARED ON THE STEENBOCK V DIET, AFTER STARVING THE RAT FOR 13 HOURS.

Rat number	Dilutions of blood	Normal-ity of $Na_2S_2O_3$	Cc. of $Na_2S_2O_3$ used for blank	Cc. of $Na_2S_2O_3$ used for titra-tion	Difference			Mg. of glucose per 100 cc. blood	Average mg. of glucose per 100 cc. blood
					In terms of cc. of $Na_2S_2O_3$	In terms of cc. of 0.005n $Na_2S_2O_3$	In terms of mg. of glucose in 5 cc. aliquot		
28145	1:20	0.004919	5.98	4.66	1.32	1.299	0.141	56.4	56.5
				4.66	1.32	1.299	0.141	56.4	
				4.67	1.31	1.289	0.140	56.0	
				4.74	1.34	1.318	0.143	57.2	
28197	1:20	0.004919	5.98	4.96	1.02	1.003	0.105	42.0	42.3
				4.97	1.01	0.994	0.104	41.6	
				4.96	1.02	1.003	0.105	42.0	
				4.93	1.05	1.033	0.109	43.6	
28444	1:20	0.004931	6.00	4.06	1.94	1.913	0.215	86.0	85.1
				4.07	1.93	1.903	0.214	85.6	
				4.10	1.90	1.874	0.210	84.0	
				4.08	1.92	1.894	0.212	84.8	
28298	1:20	0.004933	6.00	4.09	1.91	1.892	0.212	84.8	83.7
				4.10	1.90	1.882	0.211	84.4	
				4.12	1.88	1.862	0.209	83.6	
				4.15	1.85	1.833	0.205	82.0	

TABLE V (cont.) CONCENTRATION OF SUGAR IN BLOOD OF PREGNANT RATS REARED ON THE STEENBOCK
V DIET, AFTER STARVING THE RAT FOR 13 HOURS.

28389	1:20	0.004946	5.95	4.92 4.88 4.80 4.80	1.03 1.07 1.15 1.15	1.019 1.058 1.138 1.138	0.107 0.112 0.122 0.122	42.8 44.8 48.8 48.8	46.3
28391	1:20	0.004946	5.99	4.78 4.80 4.79 4.80	1.21 1.19 1.20 1.19	1.197 1.177 1.187 1.177	0.129 0.126 0.128 0.126	51.6 50.4 51.2 50.4	50.9
28436	1:20	0.004926	5.96	4.68 4.67 4.68 4.70	1.28 1.29 1.28 1.26	1.261 1.271 1.261 1.241	0.136 0.138 0.136 0.134	54.4 55.2 55.4 53.6	54.6
28554	1:20	0.004924	5.96	4.80 4.80 4.80 4.79	1.16 1.16 1.16 1.17	1.142 1.142 1.142 1.152	0.122 0.122 0.122 0.123	48.8 48.8 48.8 49.2	48.9
28425	1:20	0.004924	5.96	4.63 4.64 4.59 4.59	1.33 1.32 1.37 1.37	1.310 1.300 1.349 1.349	0.142 0.141 0.147 0.147	56.8 56.4 58.8 58.8	57.7
Average									58.4

TABLE VI (cont.) CONCENTRATION OF SUGAR IN BLOOD OF PREGNANT RATS REARED ON THE PORK I DIET, AFTER STARVING THE RAT FOR 13 HOURS

28297	1:20	0.004919	6.00	4.26 4.27 4.22 4.25	1.74 1.73 1.78 1.77	1.712 1.702 1.751 1.741	0.191 0.190 0.195 0.194	76.4 76.0 78.0 77.6	77.0
26590	1:20	0.004931	5.95	4.40 4.38	1.55 1.57	1.529 1.548	0.169 0.173	57.6 69.2	68.4
28443	1:20	0.004953	6.00	3.43 3.46	2.57 2.54	2.546 2.516	0.291 0.287	116.4 114.8	115.6
28352	1:20	0.004953	6.00	4.21 4.21 4.23 4.20	1.79 1.79 1.77 1.80	1.773 1.773 1.753 1.783	0.198 0.198 0.196 0.199	79.2 79.2 78.4 78.4	78.8
Average									73.9

TABLE VII. CONCENTRATION OF SUGAR IN BLOOD OF PREGNANT RATS REARED ON THE STEENBOCK V DIET, AFTER THE FORCED FEEDING OF 2.5 CC. OF 50 PER CENT GLUCOSE SOLUTION.

Rat number	Dilutions of blood	Normality of $Na_2S_2O_3$	Cc. of $Na_2S_2O_3$ used for blank	Cc. of $Na_2S_2O_3$ used for titration	Difference			Mg. of glucose per 100 cc. blood	Average mg. of glucose per 100 cc. blood
					In terms of cc. of $Na_2S_2O_3$	In terms of cc. of 0.005n $Na_2S_2O_3$	In terms of mg. of glucose in 5 cc. aliquot		
28195	1:20	0.004931	5.95	4.50	1.45	1.430	0.157	62.8	64.0
				4.50	1.45	1.430	0.157	62.8	
				4.46	1.49	1.469	0.162	64.8	
				4.44	1.51	1.489	0.164	65.6	
28143	1:20	0.004931	5.94	4.37	1.57	1.548	0.171	68.4	68.2
				4.37	1.57	1.548	0.171	68.4	
				4.38	1.56	1.538	0.170	68.0	
				4.38	1.56	1.538	0.170	68.0	
28363	1:20	0.004953	5.97	4.25	1.42	1.407	0.154	61.6	54.4
				4.23	1.44	1.426	0.156	62.4	
				4.87	1.10	1.090	0.116	46.4	
				4.85	1.12	1.110	0.118	47.2	
28300	1:20	0.004946	6.00	4.76	1.24	1.227	0.132	52.8	53.2
				4.74	1.26	1.246	0.135	54.0	
				4.76	1.24	1.227	0.132	52.8	
				4.75	1.25	1.236	0.133	53.2	

TABLE VII. (cont.) CONCENTRATION OF SUGAR IN BLOOD OF PREGNANT RATS REARED ON THE STEENBOCK V DIET, AFTER THE FORCED FEEDING OF 2.5 CC. OF 50 PER CENT GLUCOSE SOLUTION.

28490	1:20	0.004946	6.00	5.02	0.98	0.969	0.101	40.4	40.2
				5.04	0.96	0.950	0.099	39.6	
				5.04	0.96	0.950	0.099	39.6	
				5.02	0.98	0.969	0.101	40.4	
28552	1:20	0.004946	5.97	4.71	1.28	1.266	0.137	54.8	54.8
				4.71	1.28	1.266	0.137	54.8	
28434	1:20	0.004946	5.97	4.66	1.31	1.296	0.141	56.4	54.4
				4.64	1.33	1.316	0.143	57.2	
				4.74	1.23	1.217	0.131	52.4	
				4.76	1.21	1.197	0.129	51.6	
28355	1:20	0.004926	5.94	4.72	1.22	1.202	0.129	51.6	51.6
				4.72	1.22	1.202	0.129	51.6	
				4.70	1.24	1.222	0.129	51.6	
				4.70	1.24	1.222	0.129	51.6	
28442	1:20	0.004926	6.00	4.67	1.33	1.310	0.142	56.8	57.4
				4.65	1.35	1.330	0.145	58.0	
28488	1:20	0.004926	5.96	4.48	1.48	1.458	0.160	64.0	63.8
				4.50	1.46	1.438	0.158	63.2	
				4.48	1.48	1.458	0.160	64.0	
				4.48	1.48	1.458	0.160	64.0	
Average									56.2

TABLE VIII. CONCENTRATION OF SUGAR IN BLOOD OF PREGNANT RATS REARED ON THE PORK I DIET, AFTER THE FORCED FEEDING OF 2.5 CC. OF 50 PER CENT GLUCOSE SOLUTION.

Rat number	Dilutions of blood	Normal-ity of $Na_2S_2O_3$	Cc. of $Na_2S_2O_3$ used for blank	Cc. of $Na_2S_2O_3$ used for titre-tion	Difference			Mg. of glucose per 100 cc. blood	Average mg. of glucose per 100 cc. blood
					In terms of cc. of $Na_2S_2O_3$	In terms of cc. of 0.005n $Na_2S_2O_3$	In terms of mg. of glucose in 5 cc. aliquot		
28145	1:20	0.004919	6.00	3.61 3.60 3.50 3.51	2.39 2.40 2.50 2.49	2.351 2.361 2.460 2.450	0.367 0.369 0.381 0.379	106.8 107.9 112.4 111.6	109.6
28236	1:20	0.004919	6.00	3.99 3.98	3.01 3.02	1.977 1.987	0.222 0.224	88.8 89.6	89.2
28362	1:20	0.004953	6.00	4.03 4.00 4.10 4.10	1.97 2.00 1.90 1.90	1.952 1.981 1.883 1.883	0.216 0.223 0.211 0.211	86.4 89.2 84.4 84.4	86.1
28424	1:20	0.004953	6.00	4.10 4.11 4.08 4.10	1.90 1.89 1.92 1.90	1.883 1.872 1.902 1.883	0.211 0.210 0.214 0.211	84.4 84.0 85.6 84.4	84.6
28394	1:20	0.004955	5.97	4.65 4.65 4.65 4.64	1.32 1.32 1.32 1.33	1.308 1.308 1.308 1.318	0.142 0.142 0.142 0.143	56.8 56.8 56.8 57.2	56.9

TABLE IX.   THE QUANTITY OF GLUCOSE GIVEN TO PREGNANT RATS FED THE STEENBOCK V DIET, AS DETERMINED IN VITRO.

Rat number	Total volume resulting from dilution (cc.)	Normality of $Na_2S_2O_3$	Cc. of $Na_2S_2O_3$ used for blank	Cc. of $Na_2S_2O_3$ used for titration	Difference			Mg. of glucose per total volume of solution fed	Average mg. of glucose per total volume of solution fed
					In terms of cc. of $Na_2S_2O_3$	In terms of cc. of 0.005n $Na_2S_2O_3$	In terms of mg. of glucose in 5 cc. aliquot		
28195	25,000	0.004931	5.95	3.73	2.22	2.189	0.248	1240.0	1260.0
				3.69	2.26	2.229	0.253	1265.0	
				3.67	2.28	2.248	0.255	1275.0	
28143	25,000	0.004931	5.94	3.74	2.20	2.170	0.246	1230.0	1253.3
				3.69	2.25	2.219	0.252	1260.0	
				3.67	2.27	2.239	0.254	1270.0	
28365	25,000	0.004953	5.97	3.73	2.24	2.219	0.252	1260.0	1218.3
				3.81	2.16	2.140	0.242	1210.0	
				3.85	2.12	2.100	0.237	1185.0	
28300	25,000	0.004946	6.00	3.67	2.33	2.305	0.262	1310.0	1308.3
				3.67	2.33	2.305	0.262	1310.0	
				3.68	2.32	2.295	0.261	1305.0	
28490	25,000	0.004946	6.00	3.67	2.33	2.305	0.262	1310.0	1308.3
				3.67	2.33	2.305	0.262	1310.0	
				3.68	2.32	2.295	0.261	1305.0	

TABLE IX (cont.) THE QUANTITY OF GLUCOSE GIVEN TO PREGNANT RATS FED THE STEENBOCK V DIET, AS DETERMINED IN VITRO.

28552	25,000	0.004946	5.97	3.66 3.65 3.66	2.31 2.32 2.31	2.285 2.295 2.285	0.260 0.261 0.260	1300.0 1305.0 1300.0	1301.7
28434	25,000	0.004946	5.97	3.66 3.67 3.66	2.31 2.30 2.31	2.285 2.275 2.285	0.260 0.258 0.260	1300.0 1290.0 1300.0	1296.7
28355	25,000	0.004926	5.94	3.64 3.62 3.63	2.30 2.32 2.31	2.266 2.286 2.276	0.257 0.260 0.258	1285.0 1300.0 1290.0	1291.7
28442	25,000	0.004926	6.00	3.70 3.70 3.70	2.30 2.30 2.30	2.266 2.266 2.266	0.257 0.257 0.257	1285.0 1285.0 1285.0	1285.0
28488	25,000	0.004926	5.96	3.64 3.61 3.60	2.32 2.35 2.36	2.286 2.315 2.325	0.260 0.263 0.264	1300.0 1315.0 1320.0	1311.7
Average									1283.5

TABLE X. THE QUANTITY OF GLUCOSE GIVEN TO PREGNANT RATS FED THE PORK I DIET, AS DETERMINED IN VITRO.

129-

Rat number	Total volume resulting from dilution (cc.)	Normality of Na S O	Cc. of Na S O used for blank	Cc. of Na S O used for titration	Difference			Mg. of glucose per total volume of solution fed	Average mg. of glucose per total volume of solution fed
					In terms of cc. of Na S O	In terms of cc. of 0.005n Na S O	In terms of mg. of glucose in 5 cc. aliquot		
28142	25,000	0.004919	6.00	3.80 3.70 3.76	2.20 2.30 2.24	2.174 2.263 2.204	0.246 0.257 0.250	1230.0 1285.0 1250.0	1255.0
28388	25,000	0.004919	6.00	3.72 3.92 3.78	2.28 2.08 2.22	2.243 2.026 2.184	0.254 0.228 0.247	1270.0 1140.0 1235.0	1215.0
28362	25,000	0.004953	6.00	3.90 3.89 3.87	2.10 2.11 2.13	2.080 2.090 2.110	0.235 0.236 0.238	1175.0 1180.0 1190.0	1181.7
28424	25,000	0.004953	6.00	3.86 3.86 3.86	2.14 2.14 2.14	2.120 2.120 2.120	0.240 0.240 0.240	1200.0 1200.0 1200.0	1200.0
28354	25,000	0.004953	5.97	3.59 3.60 3.62	2.38 2.37 2.35	2.358 2.348 2.328	0.268 0.267 0.265	1340.0 1335.0 1325.0	1333.3

TABLE X (cont.) THE QUANTITY OF GLUCOSE GIVEN TO PREGNANT RATS FED THE PORK I DIET, AS
DETERMINED IN VITRO.

28441	25,000	0.004946	5.37	3.66 3.65 3.66	2.31 2.32 2.31	2.285 2.295 2.285	0.260 0.261 0.260	1300.0 1305.0 1300.0	1301.7
28299	25,000	0.004946	5.99	3.67 3.67 3.68	2.32 2.32 2.31	2.295 2.295 2.285	0.261 0.261 0.260	1305.0 1305.0 1300.0	1303.3
28435	25,000	0.004926	5.94	3.64 3.62 3.63	2.30 2.32 2.31	2.266 2.286 2.276	0.257 0.260 0.258	1285.0 1300.0 1290.0	1291.7
28551	25,000	0.004926	5.96	3.62 3.60 3.58	2.34 2.36 2.38	2.305 2.325 2.345	0.262 0.264 0.267	1310.0 1320.0 1335.0	1321.7
28651	25,000	0.004926	5.96	3.62 3.60 3.58	2.34 2.36 2.38	2.305 2.325 2.345	0.262 0.264 0.267	1310.0 1320.0 1335.0	1321.7
Average									1272.5

TABLE XI.   QUANTITY OF GLUCOSE PRESENT IN THE SOLUTION OF SUGAR LEFT IN THE STOMACH TUBE
AFTER THE FORCED FEEDING OF GLUCOSE TO PREGNANT RATS FED THE STEENBOCK V DIET.

Rat number	Total volume of washings (cc.)	Normality of $Na_2S_2O_3$	Cc. of $Na_2S_2O_3$ used for blank	Cc. of $Na_2S_2O_3$ used for titration	Difference			Mg. of glucose per total volume of washings	Average mg. of glucose per total volume of washings
					In terms of cc. of $Na_2S_2O_3$	In terms of cc. of 0.005n $Na_2S_2O_3$	In terms of mg. of glucose in 5 cc. aliquot		
28195	10,000	0.004931	5.95	4.00	1.95	1.923	0.216	432.0	
				3.90	2.05	2.022	0.228	456.0	448.0
				3.90	2.05	2.022	0.228	456.0	
28143	10,000	0.004931	5.94	4.02	1.92	1.894	0.213	426.0	
				4.07	1.87	1.844	0.206	412.0	413.3
				4.12	1.82	1.795	0.201	402.0	
28363	10,000	0.004953	5.97	4.20	1.77	1.753	0.196	392.0	
				4.10	1.87	1.852	0.208	416.0	402.7
				4.16	1.81	1.793	0.200	400.0	
28300	10,000	0.004946	6.00	4.02	1.98	1.959	0.220	440.0	
				4.03	1.97	1.949	0.219	438.0	440.7
				4.01	1.99	1.969	0.222	444.0	
28490	10,000	0.004946	6.00	4.17	1.83	1.810	0.202	404.0	
				4.18	1.82	1.800	0.201	402.0	404.7
				4.16	1.84	1.820	0.204	408.0	

TABLE XII. QUANTITY OF GLUCOSE PRESENT IN THE SOLUTION OF SUGAR LEFT IN THE STOMACH TUBE AFTER THE FORCED FEEDING OF GLUCOSE TO PREGNANT RATS FED THE PORK I DIET.

Rat number	Total volume of washings (cc.)	Normality of $Na_2S_2O_3$	Cc. of $Na_2S_2O_3$ used for blank	Cc. of $Na_2S_2O_3$ used for titration	Difference			Mg. of glucose per total volume of washings	Average mg. of glucose per total volume of washings
					In terms of cc. of $Na_2S_2O_3$	In terms of cc. of 0.005n $Na_2S_2O_3$	In terms of mg. of glucose in 5 cc. aliquot		
28142	10,000	0.004919	6.00	3.92 3.94	2.08 2.06	2.046 2.027	0.231 0.228	462.0 456.0	459.0
28388	10,000	0.004919	6.00	4.12 4.14 4.00	1.88 1.86 2.00	1.850 1.830 1.968	0.207 0.205 0.221	414.0 410.0 442.0	423.0
28362	10,000	0.004953	6.00	4.10 4.09 4.12	1.90 1.91 1.88	1.882 1.892 1.862	0.211 0.212 0.209	422.0 424.0 418.0	421.0
28424	10,000	0.004953	6.00	4.11 4.10 4.09	1.89 1.90 1.91	1.872 1.882 1.892	0.210 0.211 0.212	420.0 422.0 424.0	422.0
28354	10,000	0.004953	5.97	3.90 3.89 3.90	2.07 2.08 2.07	2.050 2.060 2.050	0.231 0.232 0.231	462.0 464.0 462.0	462.7

TABLE XIII. CONCENTRATION OF REDUCING SUBSTANCES IN THE INTESTINAL TRACT OF PREGNANT
RATS FED STEENBOCK V DIET, AFTER FORCED FEEDING OF 2.5 CC. OF 50 PER CENT
GLUCOSE SOLUTION.

Rat number	Total intesti-nal content (cc.)	Normal-ity of $Na_2S_2O_3$	Cc. of $Na_2S_2O_3$ used for blank	Cc. of $Na_2S_2O_3$ used for titra-tion	Difference In terms of cc. of $Na_2S_2O_3$	Difference In terms of cc. of 0.005n $Na_2S_2O_3$	Difference In terms of mg. of glucose in 5 cc. of aliquot	Mg. of glucose per total intesti-nal content	Average mg. of glucose per to-tal vol-ume of intesti-nal content
28195	500	0.004931	5.95	4.98 4.98 5.00	0.97 0.97 0.95	0.957 0.957 0.937	0.100 0.100 0.097	10.0 10.0 9.7	9.9
28143	500	0.004931	5.94	5.10 5.10 5.10	0.84 0.84 0.84	0.828 0.828 0.828	0.084 0.084 0.084	8.4 8.4 8.4	8.4
28363	500	0.004953	5.97	5.10 5.04 5.08	0.87 0.93 0.89	0.862 0.931 0.882	0.088 0.096 0.091	8.8 9.6 9.1	9.2
28300	500	0.004946	6.00	4.92 4.93 4.90	1.08 1.07 1.10	1.068 1.058 1.088	0.113 0.112 0.116	11.3 11.2 11.6	11.4

TABLE XIII (cont.) CONCENTRATION OF REDUCING SUBSTANCES IN THE INTESTINAL TRACT OF PREGNANT RATS FED STEENBOCK V DIET, AFTER FORCED FEEDING OF 2.5 CC. OF 50 PER CENT GLUCOSE SOLUTION.

28490	500	0.004946	6.00	5.18 5.16 5.15	0.82 0.84 0.85	0.811 0.831 0.841	0.082 0.085 0.086	8.2 8.5 8.6	8.4
28552	500	0.004946	5.97	4.92 4.90 4.90	1.05 1.07 1.07	1.039 1.058 1.058	0.110 0.112 0.112	11.0 11.2 11.2	11.1
28434	500	0.004946	5.97	5.35 5.34 5.36	0.62 0.63 0.61	0.613 0.623 0.603	0.058 0.060 0.057	5.8 6.0 5.7	5.8
28355	500	0.004926	5.94	5.16 5.15 5.16	0.78 0.79 0.78	0.768 0.778 0.768	0.077 0.078 0.077	7.7 7.8 7.7	7.7
28442	500	0.004926	6.00	5.09 5.10 5.08	0.91 0.90 0.92	0.896 0.887 0.906	0.093 0.091 0.094	9.3 9.1 9.4	9.3
28468	500	0.004926	5.96	5.11 5.12 5.11	0.85 0.84 0.85	0.837 0.828 0.837	0.085 0.084 0.085	8.5 8.4 8.5	8.5
Average									9.0

TABLE XIV. CONCENTRATION OF REDUCING SUBSTANCE IN THE INTESTINAL TRACT OF PREGNANT
RATS FED FORK I DIET, AFTER FORCED FEEDING OF 2.5 CC. OF 50 PER CENT
GLUCOSE SOLUTION.

Rat number	Total intestinal content (cc.)	Normality of $Na_2S_2O_3$	Cc. of $Na_2S_2O_3$ used for blank	Cc. of $Na_2S_2O_3$ used for titration	Difference			Mg. of glucose per total intestinal content	Average mg. of glucose per total volume of intestinal content
					In terms of cc. of $Na_2S_2O_3$	In terms of cc. of 0.005N $Na_2S_2O_3$	In terms of mg. of glucose in 5 cc. aliquot		
28142	500	0.004919	6.00	4.99	1.01	0.994	0.104	10.4	10.5
				4.98	1.02	1.002	0.105	10.5	
				4.98	1.02	1.003	0.105	10.5	
28238	500	0.004919	6.00	4.67	1.33	1.308	0.142	14.2	14.1
				4.69	1.31	1.289	0.140	14.0	
				4.68	1.32	1.299	0.141	14.1	
28262	500	0.004953	6.00	4.78	1.22	1.208	0.130	13.0	12.9
				4.78	1.22	1.208	0.130	13.0	
				4.80	1.20	1.189	0.128	12.8	
28424	500	0.004953	6.00	4.96	1.04	1.030	0.109	10.9	10.9
				4.95	1.05	1.040	0.110	11.0	
				4.96	1.04	1.030	0.109	10.9	
28354	500	0.004953	5.97	5.25	0.72	0.713	0.071	7.1	7.2
				5.23	0.74	0.733	0.073	7.3	
				5.23	0.74	0.733	0.073	7.3	

TABLE XIV (cont.) CONCENTRATION OF REDUCING SUBSTANCE IN THE INTESTINAL TRACT OF PREGNANT RATS FED PORK I DIET, AFTER FORCED FEEDING OF 2.5 CC. OF 50 PER CENT GLUCOSE SOLUTION.

28441	500	0.004946	5.97	4.92 4.92 4.90	1.05 1.05 1.07	1.039 1.039 1.058	0.110 0.110 0.112	11.0 11.0 11.2	11.1
28299	500	0.004946	5.99	5.26 5.28 5.20	0.73 0.71 0.79	0.722 0.702 0.781	0.072 0.069 0.079	7.2 6.9 7.9	7.3
28433	500	0.004926	5.94	4.80 4.78 4.76	1.14 1.16 1.18	1.123 1.143 1.162	0.120 0.122 0.124	12.0 12.2 12.4	12.2
28551	500	0.004926	5.96	4.80 4.78 4.83	1.16 1.18 1.13	1.143 1.162 1.113	0.122 0.124 0.119	12.2 12.4 11.9	12.2
28551	500	0.004926	5.96	4.64 4.65 4.67	1.32 1.31 1.29	1.300 1.291 1.271	0.141 0.140 0.138	14.1 14.0 13.8	14.0
Average									11.2

TABLE XV. QUANTITY OF REDUCING SUBSTANCES IN THE INTESTINAL TRACT OF PREGNANT RATS FED STEENBOCK V DIET, AFTER STARVING THE RAT FOR 13 HOURS.

Rat number	Total volume of intestinal content	Normality of $Na_2S_2O_3$	Cc. of $Na_2S_2O_3$ used for blank	Cc. of $Na_2S_2O_3$ used for titration	Difference			Mg. of glucose per total volume of intestinal content	Average mg. of glucose per total volume of intestinal content
					In terms of cc. of $Na_2S_2O_3$	In terms of cc. of 0.005n $Na_2S_2O_3$	In terms of mg. of glucose in 5 cc. aliquot		
28145	500	0.004919	5.98	5.14	0.84	0.826	0.084	8.4	8.5
				5.13	0.85	0.836	0.085	8.5	
				5.12	0.86	0.846	0.087	8.7	
28197	500	0.004919	5.98	4.90	1.08	1.062	0.112	11.2	11.3
				4.90	1.08	1.061	0.112	11.2	
				4.88	1.10	1.082	0.115	11.5	
28444	500	0.004931	6.00	4.78	1.22	1.203	0.129	12.9	12.8
				4.80	1.20	1.183	0.127	12.7	
				4.80	1.20	1.183	0.127	12.7	
28298	500	0.004953	6.00	5.22	0.78	0.773	0.078	7.8	8.1
				5.16	0.84	0.832	0.085	8.5	
				5.20	0.80	0.792	0.080	8.0	
28389	500	0.004946	5.95	5.15	0.80	0.791	0.080	8.0	8.2
				5.15	0.80	0.791	0.080	8.0	
				5.10	0.85	0.841	0.086	8.6	

TABLE XV (cont.)   QUANTITY OF REDUCING SUBSTANCES IN THE INTESTINAL TRACT OF PREGNANT
RATS FED STEENBOCK V DIET, AFTER STARVING THE RAT FOR 13 HOURS.

28391	500	0.004946	5.99	5.20 5.18 5.16	0.79 0.81 0.83	0.781 0.801 0.821	0.079 0.081 0.084	7.9 8.1 8.4	8.1
28436	500	0.004926	5.96	5.30 5.31 5.32	0.66 0.65 0.64	0.650 0.640 0.630	0.063 0.062 0.061	6.3 6.2 6.1	6.2
28554	500	0.004924	5.96	4.90 4.88 4.90	1.06 1.08 1.06	1.044 1.064 1.044	0.110 0.113 0.110	11.0 11.3 11.0	11.1
28425	500	0.004924	5.96	5.02 4.99 5.02	0.94 0.97 0.94	0.926 0.955 0.926	0.096 0.100 0.096	9.6 10.0 9.6	9.7
Average									9.3

TABLE XVI. QUANTITY OF REDUCING SUBSTANCES IN THE INTESTINAL TRACT OF PREGNANT RATS FED PORK I DIET, AFTER STARVING THE RAT FOR 13 HOURS.

Rat number	Total volume of intestinal content	Normality of $Na_2S_2O_3$	Cc. of $Na_2S_2O_3$ used for blank	Cc. of $Na_2S_2O_3$ used for titration	Difference			Mg. of glucose per total volume of intestinal content	Average mg. of glucose per total volume of intestinal content
					In terms of cc. of $Na_2S_2O_3$	In terms of cc. of 0.005n $Na_2S_2O_3$	In terms of mg. of glucose in 5 cc. aliquot		
27926	500	0.005002	5.87	5.29 5.26 5.29	0.58 0.61 0.58	0.580 0.610 0.580	0.055 0.058 0.055	5.5 5.8 5.5	5.6
28144	500	0.004888	5.86	4.70 4.71 4.72	1.16 1.15 1.14	1.134 1.124 1.114	0.121 0.120 0.119	12.0 12.0 11.9	12.0
28194	500	0.004888	5.89	5.00 5.00 5.00	0.89 0.89 0.89	0.870 0.870 0.870	0.079 0.079 0.079	7.9 7.9 7.9	7.9
28196	500	0.004888	6.00	5.30 5.31 5.30	0.70 0.69 0.70	0.684 0.674 0.684	0.067 0.066 0.067	6.7 6.6 6.7	6.7
28297	500	0.004919	6.00	5.00 4.99	1.00 1.01	0.984 0.994	0.103 0.104	10.3 10.4	10.4

TABLE XVI (cont.) QUANTITY OF REDUCING SUBSTANCES IN THE INTESTINAL TRACT OF PREGNANT
FED PORK I DIET, AFTER STARVING THE RAT FOR 13 HOURS.

28390	500	0.004931	5.95	4.92 4.89 4.91	1.03 1.06 1.04	1.016 1.045 1.026	0.107 0.110 0.108	10.7 11.0 10.8	10.8
28443	500	0.004953	6.00	5.04 5.06 5.04	0.96 0.94 0.96	0.951 0.931 0.951	0.099 0.097 0.099	9.9 9.7 9.9	9.8
28352	500	0.004953	6.00	5.19 5.20 5.20	0.81 0.80 0.80	0.802 0.792 0.792	0.081 0.080 0.080	8.1 8.0 8.0	8.0
Average									8.9

TABLE XVII.   ABSORPTION OF GLUCOSE BY PREGNANT RATS REARED ON THE STEENBOCK V DIET

Rat number	The amount of glucose used for feeding	The amount of glucose left in the washings	The actual amount of glucose fed to each rat	Amount of glucose recovered from the intestinal tract after feeding	Average amount of glucose present in the intestinal tract after starving	Amount of glucose actually left in the intestinal tract	Difference	
							Amount of glucose absorbed	The percentage of glucose absorbed
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	per cent
28195	1260.0	448.0	812.0	9.9	9.3	0.6	811.4	99.93
28143	1253.3	413.3	840.0	8.4	9.3	-0.9	840.9	100.11
28363	1218.3	402.7	815.6	9.2	9.3	-0.1	815.7	100.01
28300	1308.3	440.7	867.6	11.4	9.3	2.1	865.5	99.76
28490	1308.3	404.7	903.6	8.4	9.3	-0.9	904.5	100.10
28552	1301.7	423.3	878.4	11.1	9.3	1.8	876.6	99.79
28434	1296.7	433.3	863.4	5.8	9.3	-3.5	866.9	100.40
28335	1291.7	415.3	876.4	7.7	9.3	-1.6	878.0	100.18
28442	1285.0	404.0	881.0	9.3	9.3	0.0	881.0	100.00
28488	1311.7	436.0	875.7	8.5	9.3	-0.8	876.5	100.09
Average	1283.5	422.1	861.4	9.0	9.3	-0.3	861.7	100.04

TABLE XVIII. ABSORPTION OF GLUCOSE BY PREGNANT RATS REARED ON THE PORK I DIET

Rat number	The amount of glucose used for feeding	The amount of glucose left in the washings	The actual amount of glucose fed to each rat	Amount of glucose recovered from the intestinal tract after feeding	Average amount of glucose present in the intestinal tract after starving	Amount of glucose actually left in the intestinal tract	Difference	
							Amount of glucose absorbed	The percentage of glucose absorbed
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	per cent
28142	1255.0	459.0	796.0	10.5	8.9	1.6	794.4	99.80
28388	1215.0	423.0	792.0	14.1	8.9	5.2	786.8	99.34
28362	1181.0	421.0	760.0	12.9	8.9	4.0	756.0	99.47
28424	1200.0	422.0	778.0	10.9	8.9	2.0	776.0	99.74
28354	1333.3	462.0	871.3	7.2	8.9	-0.7	872.0	100.08
28441	1301.7	404.0	897.7	11.1	8.9	2.2	895.5	99.75
28299	1303.3	402.0	901.3	7.3	8.9	-1.6	902.9	100.18
28433	1291.7	423.3	868.4	12.2	8.9	3.3	865.1	99.62
28551	1321.7	327.0	994.7	12.2	8.9	3.3	991.4	99.67
28651	1321.7	330.0	991.7	14.0	8.9	5.1	986.6	99.48
Average	1272.5	407.5	865.1	11.2	8.9	2.4	862.7	99.71

PREPARATION OF REAGENTS

Approximately Normal Sodium Hydroxide Solution

Even though of highest purity, sodium hydroxide as purchased contains considerable quantities of sodium carbonate which must be removed before the substance can be used for the preparation of a standard solution. In order to remove the carbonate, make a saturated solution of sodium hydroxide (110 gm. of NaOH in 100 cc. of CO_2-free water) in a beaker. The solution becomes very hot. Transfer to a stoppered paraffin-lined pyrex flask after it has been cooled and let stand until the solution becomes clear (at least 2 - 5 days). The sodium carbonate, being insoluble in this concentrated solution, settles to the bottom leaving a clear solution of sodium hydroxide nearly free of carbonate. Portions of the clear solution may be withdrawn from the sediment and diluted as 75 gm. of sodium hydroxide in 100 cc. of solution.

A normal solution of NaOH contains 40.01 gm. per liter of solution.

The saturated solution contains 75 gm. in 100 cc. of solution or 0.75 gm. per cc. solution.

$$0.75:1 = 40.0048:x$$

$$\therefore x = 1 \times \frac{40.0048}{0.75} = 53.34$$

Therefore 53.34 cc. of the saturated solution will furnish

40.0048 gm. of NaOH and 53.34 cc. diluted to a volume of one liter will give an approximately normal solution.

The solution of N NaOH is then made up to volume with carbon dioxide-free water, shaking the solution 50 times and transferred to a large bottle. After the required amount of solution has been prepared, the storage bottle may be shaken by turning the bottle on its side and rolling it back and forth. In order that the solution may be adequately mixed, the storage bottle should be given at least 500 rolls divided into six periods of shaking at least one hour apart. After the solution has been shaken, the bottle should be equipped with a siphon and a tube of soda lime.

Preparation of Carbon Dioxide-Free Water

In order to remove carbon dioxide or other dissolved gasses, distilled water must be boiled vigorously for at least 30 minutes. Cool, lightly stoppered. After the water has been cooled, the flask should be stoppered tightly. This water should be withdrawn by means of a siphon and the flask shaken or moved as little as possible. For certain determinations, the water must be reboiled after 48 hours, since gasses may again be dissolved in storage.

Standardization of Approximately Normal
Sodium Hydroxide Solution with
Potassium acid thalate

Clean the flasks and burette thoroughly by means of cleaning solution, rinse well with tap water followed by distilled water, and let stand in inverted position until dry.

A solution of NaOH is often standardized by titration with a weighed quantity of potassium acid thalate, a weak acid salt, which has been previously dried in the electric hot air oven at 110° C for one to two hours. About 3 gm. of thalate is weighed in a tared flask and 25 cc. of CO_2-free water is added to dissolve the salt. Avoid too vigorous shaking.

About 2 - 3 drops of phenolphthalein is added to the potassium acid thalate solution as an indicator, this substance being colorless in acid solution and pink in alkaline solution. A very slight excess of NaOh above that required to react with the thalate causes the solution to turn a pale pink. This is considered the end point of the titration. The equation for the reaction is as follows:

$$NaOH + KHC_8H_4O_4 \rightarrow KNaC_8H_4O_4 + H_2O$$
$$40.0048 \quad 204.14$$

Some results are summarized in table XIX.

Table XIX. Titration of Approximately Normal Sodium
Hydroxide Solution

Trial	Weight of potassium acid thalate	Quantity of sodium involved in titration		Concentration of NaOH	Normality of NaOH
	gm.	cc.	gm.	gm. per cc.	
1	3.0319	12.88	0.59423	0.04613	1.1529
2	3.0014	12.76	0.58825	0.04610	1.1522
3	3.0057	12.77	0.58899	0.04609	1.1519
4	3.0031	12.76	0.58823	0.04613	1.1529
Average					1.1525

Normal Sulphuric Acid Solution and Its Standardization with Standard Sodium Hydroxide Solution

The strength of the C. P. commercial concentrated sulphuric acid was on the label; the specific gravity being 1.84 and the solution containing 94 gm. of anhydrous H_2SO_4 per 100 gm. solution. From the specific gravity of water we know that 1 cc. of pure water weighs 1 gm. at 4° C. Therefore the specific gravity of water equals one. Here the specific gravity of sulphuric acid is 1.84 i.e., 1 cc. of sulphuric acid will weigh 1.84 gm. at 4° C. Therefore 1 cc. of this sulphuric acid actually contains 0.94 x 1.84 = 1.7296 gm. of H_2SO_4. A normal sulphuric acid solution contains 49 gm. of H_2SO_4 in one liter. Then how many cc. of this C. P. concentrated commercial sulphuric acid are needed to make a normal solution? The amount needed may be calculated as follows:

$$1:1.7296 = x:49$$

$$\therefore x = \frac{1 \times 49}{1.7296} = 28$$

Therefore, 28 cc. of the sulphuric acid are required to make one liter of a normal solution.

Fill the volumetric flask about one-half full of cold distilled water. Measure the concentrated sulphuric acid in a graduated cylinder. Then touch the lip of the cylinder to the mouth of the volumetric flask, and let the H_2SO_4 flow slowly down the side of the neck of the flask. Rinse the cylinder well and pour into the volumetric flask. Shake gently. Dilute the solution to volume and shake it very thoroughly as in the procedures described earlier in making NaOH.

Clean the flasks as mentioned before and measure 25, 20 and 15 cc. of H_2SO_4 solution, two of each in separate flasks. Two or three drops of methyl red are added as the indicator. Titrate with standard NaOH solution. The first appearance of an apricot color is the end point of titration.

Some results are shown in table XX.

Table XX. The Titration of Approximately Normal
Sulphuric Acid with 1.1525n NaOH SOLUTION

Trial	Aliquot of H₂SO₄	Quantity of standard NaOH required in titration	Normality of H₂SO₄
	cc.	cc.	
1	20	17.50	1.0084
2	20	17.48	1.0073
3	15	13.12	1.0080
4	15	13.10	1.0069
5	25	21.81	1.0054
6	25	21.80	1.0050
Average			1.0068

Standard Potassium Iodate Solutions

The pure form of potassium iodate can be used for the preparation of stable solutions for the standardization of sodium thiosulphate and also in the alkaline copper solution used for the determination of the sugar. Each molecule of iodate, when reacting with excess potassium iodide in the presence of acid, liberates six atoms of iodine by the reaction, $2KIO_3 + 10KI + 12 HCL \rightarrow 12 KCL + 6H_2O + 6I_2$. Therefore, a liter of normal solution of iodate contains one-sixth of a gram molecule. Of potassium iodate, KIO_3, with a molecular weight of 214.032, a liter of normal solution contains 214.032 ÷ 6 = 35.6720 gm. KIO_3.

For 0.1n solution = 3.5672 gm. KIO_3 per liter.

For 0.08n solution = 2.8538 gm. KIO_3 per liter.

For 0.12n solution = 4.2806 gm. KIO_3 per liter.

Weigh the portions indicated above separately on tared pieces of glazed black paper. (If no glazed paper can be secured, a watch glass can be used for this purpose.) Transfer quantitatively to a volumetric flask fitted with a funnel and a camel's hair brush. Wash the funnel slowly with a stream of water, rinse thoroughly, and dilute to the mark with CO_2-free water. Mix thoroughly and keep in a glass-stoppered bottle in a cool place. This solution is stable and will retain its strength indefinitely.

Approximate One-tenth Normal Sodium Thiosulphate Solution and its Standardization with Standard Potassium Iodate Solutions

The reaction of sodium thiosulphate and iodine is as follows:

$$2 Na_2S_2O_3 + I_2 \rightarrow 2 NaI + Na_2S_4O_6$$
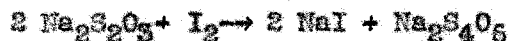
One iodine atom will react with one molecule of thiosulphate. Therefore, a one-tenth normal solution of thiosulphate in respect to I_2 may be expected when one-tenth of its molecular weight is dissolved in one liter of solution. The molecular weight of crystalline sodium thiosulphate $Na_2S_2O_3 \cdot 5H_2O$ is 248.192 gm. Therefore, a liter of one-tenth normal solution will contain 24.8192 gm. of this crystalline salt.

This thiosulphate solution will keep unchanged for several months (Somogyi). The addition of about 1 cc. of 1n NaOH per liter greatly increases its stability. The solution should be restandardized at intervals when the solution is diluted for use.

In a chemically clean titration flask, 25 cc. of standard potassium iodate, 10 cc. of 10 per cent solution of potassium iodide, and 20 cc. of 1N sulphuric acid are added. Sodium thiosulphate solution delivered from a U. S. Bureau of Standard burette is then titrated against this solution. When the iodine color has faded to a pale yellow, 1 cc. of the starch solution is added and the titration continued until the blue color of the starch-iodine compound has entirely disappeared.

The results of a titration are shown in table XXI.

When making the 0.005n thiosulphate solution, if two drops of 1n NaOH are added to 1 liter of the solution before it is diluted to volume, the solution will keep unchanged for at least 5 days.

Table XXI. The Titrations of Approximately One-tenth
Normal Sodium Thiosulphate with Various
Standard Potassium Iodate Solutions

Date	Normality of KIO$_3$	Quantity of KIO$_3$	Quantity of Na$_2$S$_2$O$_3$ required in titration	Normality of Na$_2$S$_2$O$_3$
		cc.	cc.	
1939	0.10	25.00 / 25.00	24.88 / 24.90	0.1004
	0.08	25.00 / 25.00	19.93 / 19.90	0.1004
	0.12	25.00 / 25.00	29.84 / 29.86	0.1005
Average				0.1004
1940	0.10	25.00 / 25.00	25.72 / 25.74	0.09716
	0.08	25.00 / 25.00	20.58 / 20.58	0.09718
	0.12	25.00 / 25.00	30.38 / 30.36	0.09715
Average				0.09716

Starch Solution

Triturate 2 gm. of soluble starch and 10 mg. of mercury iodide with a little water and add the suspension slowly to one liter of boiling water.

Continue boiling until the solution is clear. Cool and transfer to a glass stoppered bottle.

Use 1 cc. of this starch solution for each 5 cc. aliquot titration.