A NEW DEFICIENCY DISEASE PRODUCED BY THE RIGID EXCLUSION OF FAT FROM THE DIET.*

By GEORGE O. BURR and MILDRED M. BURR.

(*From the Department of Botany, University of Minnesota, Minneapolis.*)

(Received for publication, February 11, 1929.)

The present paper is the first of a series which the authors expect to offer on the more general subject of the effects of fat in the diet. This paper will be limited to the following topics: experimental methods; description of the new disease; comparative growth and ovulation with and without fat; curing the disease; discussion, and summary.

It is believed that the data presented here definitely settle the uncertainty as to the necessity for fats in the diet (of the rat), and prove not only that ingested fats have a beneficial effect upon the animal but that under the experimental conditions outlined in this paper they are *essential* constituents of the diet. When dietary fats are reduced below a certain minimum, the rat develops a characteristic disease and dies at an early age.

Experimental Methods.

The experimental colony is kept in a spacious north room in which the upper half of the wall is glass. The light conditions are the most constant possible since the north sky furnishes all the illumination throughout the year. The room is maintained at a constant temperature of 24.5° ±1°.

Individual circular cages, 8 inches in diameter by 6 inches high, made of galvanized wire 3 meshes per inch, except for the bottom which is 2 meshes per inch, are provided for each animal. The

* This work was supported largely through a grant from the Medical Research Fund of the University of Minnesota. These funds have been generously augmented by a grant from the Graduate School of the University of Minnesota.

food cup and water bottle are attached to opposite sides of the cage and are so made that there is very little spilling of food or water and practically no contamination of either with urine and feces. The cage stands on 3 inch legs.

The experimental animals are always taken from the normal stock colony on the 21st day of life, unless otherwise indicated. A minimum weaning weight of 35 gm. is allowed. The stock colony is maintained on McCollum's Diet I (whole ground wheat 67.5, casein 15, whole milk powder 10, butter fat 5.2, calcium carbonate 1.5, sodium chloride 0.8).

The experimental diets used in this work are unique in their simplicity and purity. They are slight modifications of Diet 519 previously introduced by one of us (1). First, the sucrose is no longer purified by recrystallization from 80 per cent alcohol since we have been unable to detect any effect upon the rat, due to this additional purification. Second, the casein is not made from fresh milk according to the directions of Van Slyke and Baker (2) because this involves the handling of such a large bulk of material. The procedure for the production of casein has been so modified as to meet the two demands: (1) high purity and (2) quantity production.

The following is the process adopted. 110 gm. lots of high grade curd casein are put in 3 liter Pyrex Erlenmeyer flasks and washed twice (for 2 days) with distilled water. This removes much soluble material. After draining the second wash water for several minutes 1300 cc. of 0.085 N NaOH are added along with 1 cc. of toluene and the flasks shaken during the next 24 hours. This gives a solution with about 7 per cent casein and relatively little other material. Six of these batches are strained into a tall 12 liter battery jar. Before the casein solution is added the stirrer and two acid-carrying tubes are set up so that the bent tubes feed a slow fine stream of acid into the most violently stirred liquid a few mm. from the stirrer. A 1 : 1 mixture of normal hydrochloric acid and normal acetic acid is used. The stirrer runs at 2000 R.P.M. Since about 700 cc. of the acid must be added for complete precipitation, the time of precipitation has been decreased by increasing the rate of addition of acid somewhat over that advised by Van Slyke and Baker. Acid flows from each burette at the rate of about 3 cc. per minute until the solution becomes white and begins

to increase in viscosity. The acid flow is then cut in half and continued until the dilution test shows almost complete precipitation. It is then reduced to a very slight dribble and the stirring continued for another half hour, when the dilution test will show complete precipitation. The total time of precipitation is almost 2 hours.

The mixture is now transferred to a 5 gallon bottle and washed with distilled water daily until the chloride test is negative or extremely faint. This washing requires about a week if there is thorough shaking with each washing. Alcohol is then added to the settled casein to bring it to about 50 per cent alcohol. This is settled and after the supernatant liquid has been siphoned off, the casein is transferred to three large closed filter funnels and drained. It is then transferred to tall settling jars and washed with alcohol twice or until the supernatant liquid is 90 to 92 per cent alcohol by volume.

Two such batches of casein are prepared at a time and these are transferred to a strong cloth bag which fits in a large glass Soxhlet extractor (7″ diameter × 21″ tall); the last of the alcohol is drained off for 24 hours, and ether extraction begun. The ether washes out the alcohol, returns it to the 12 liter boiling flask where a fractionating column separates the ether from the dilute alcohol, and after 1 week of extraction the casein is ready to spread in pans to dry at 35°. This prolonged ether extraction is very important in producing a casein free from fat.

The casein thus prepared has not been injured by exposure to alkali (3) or high temperature (maximum temperature is the boiling point of ether). No known vitamins are present in detectable quantity. By producing the double batch weekly (1320 gm. of crude casein yielding about 1000 gm. of the pure air-dried product) a colony of 100 healthy rats can be maintained.

The salts are commercial products of C.P. or U.S.P. quality. McCollum's Salt Mixture 185[1] has been used. A trace of potassium iodide is added to the water.

The vitamin B (complex) is furnished by Northwestern pure dehydrated yeast which has been extracted for 48 hours with ether in a Soxhlet apparatus. About 1.5 per cent fat is removed by

[1] McCollum, E. V., and Simmonds, N., *J. Biol. Chem.*, **33**, 63 (1918).

this extraction, and the product is designated as Yeast 5. This is fed separately in Syracuse dishes.

Vitamins A and D are furnished by the non-saponifiable matter from high grade cod liver oil.[2] The non-saponifiable matter from 0.5 gm. of the oil is fed to each animal in a week. Experiments have proved that larger amounts are not beneficial. The rate of destruction of vitamin A in solution or in dry form when sealed in a partial vacuum is uncertain and probably varies greatly with small changes in condition. For this reason the storage of this material is not attempted but a new lot is made each week and fed during the following 7 days. For a group of forty animals the following procedure is employed: Alcoholic potassium hydroxide is prepared by dissolving 8 gm. of high grade KOH in 5.4 cc. of water and this solution is mixed with 40 cc. of purified 96 per cent alcohol. This product gives no color on boiling for 1 hour. The potassium hydroxide is decanted from the insoluble carbonate into a 200 cc. Erlenmeyer flask containing the 20 gm. of cod liver oil. After being refluxed for 1 hour on a steam bath, the whole is transferred to a 1000 cc. separatory funnel with 160 cc. of water and extracted once with 200 cc. of ether (U.S.P. for anesthesia).[3] The extract is then washed free from alkali and concentrated to a few cc. so that about 2 drops carry the daily dose. The bottle is stored in the dark and the product retains its potency throughout the week. The dose is evaporated on the yeast, which is then mixed with distilled water and fed at once. The product is designated as Fraction AD.

A basal diet of the greatest simplicity was desired and everything was left out which was not definitely known to be necessary for the production of healthy, vigorous animals. Vitamin E was therefore omitted from the diet (which, accordingly, always produces sterile animals, readily curable). Some animals are now being fed vitamin E to determine whether this vitamin plays any rôle in the deficiency described here.

Distilled water has been given throughout all experiments.

In the planning of the diets some of the principles advocated by

[2] Patch cod liver oil, with a guaranteed vitamin A value, has been used throughout these experiments.

[3] Merck's ether put up in the ½ pound can has been used, so that it is perfectly fresh each week. No purification is necessary.

feeders of farm animals, but largely ignored by nutrition workers using the smaller animals like the rat, have been applied. Henry and Morrison (4) advise the following schedule for pigs: from weaning time until 100 pounds in weight, a nutritive ratio of about 1 : 4, from 100 to 200 pounds in weight a nutritive ratio of 1 : 5 to 6, and from this time on a nutritive ratio of 1 : 7. A similar schedule is followed in this laboratory for many experiments. At weaning date the animals are given a diet of nutritive ratio 1 : 3; when about 100 gm. in weight they are changed to a ratio of 1 : 5; when 175 gm. in weight (or when the growth curve is definitely flattened) they are changed to a ratio of 1 : 7. A diet with a ratio of 1 : 7 is considered good for maintenance of mature rats.

TABLE I.

Composition of Diets.

Diet No.	Pure casein.	Sucrose.	Salt Mixture 185.	Lard.	Nutritive ratio.	Salts per 100 calories.	Fat calories.	Calories per gm. of diet.
	per cent	*per cent*	*per cent*	*per cent*		*gm.*	*per cent of total*	
550	24.0	72.1	3.9	0	1:3	1.0	0	3.85
550 A	16.0	80.1	3.9	0	1:5	1.0	0	3.85
550 B	12.0	84.1	3.9	0	1:7	1.0	0	3.85
560	30.1	45.1	4.8	20	1:3	1.0	37.4	4.81
560 A	20.0	55.1	4.8	20	1:5	1.0	37.4	4.81
560 B	15.0	60.1	4.8	20	1:7	1.0	37.4	4.81

The advantages of this gradual change in nutritive ratio are three: (1) Adequate protein for early growth is assured without taxing the kidneys in later life. (2) Even carefully purified casein is the chief source of organic impurities in the basal ration and this factor is greatly reduced by partial replacement of casein by sucrose as the animal grows older. (3) The cost of the purified casein is so great that it should not be used as energy food except for special experiments.

The quantity of salt mixture in the diet is also given a constant value, based on calories. In the absence of reliable data on optimum values, the arbitrary standard of 1 gm. of salt mixture per 100 calories of diet has been adopted.

In Table I the data are compiled for the diets used in this

investigation. The approximate physiological fuel values of 4, 4, and 9 are used for casein, sucrose, and lard[4] respectively.

Description of the New Disease.

When the rat (either the Wistar or Long-Evans strain) is reared on Diet 550 (Diets 550 A and 550 B) supplemented by 0.65 gm. of ether-extracted yeast and the non-saponifiable matter (our Fraction AD) from 70 mg. of cod liver oil daily, an abnormal, scaly condition of the skin is observed between the 70th and the 90th day of life. Later the tip of the tail may become inflamed and swollen, and the whole tail soon is heavily scaled and ridged. Hemor-

TABLE II.

Summarizing Observations Made on Group of Animals Kept on Diet 550 + Fraction AD + Yeast 5 without Any Fat Supplement.

Rat No.	Maximum weight.	Weight at death.	Age at first marked abnormality of feet or tail.	Age at onset of decline in weight.	Age at death.
	gm.	*gm.*	*days*	*days*	*days*
2,897 ♀	150	108	77	147	188
28,110 ♀	160	115	84	133	239
28,104 ♀	138	105	70	126	253
28,108 ♀	160	110	77	168	253
2,899 ♂	182	108	77	140	190
28.103 ♂	197	115	70	140	225
28,111 ♂	163	Less than 137	84	112	145
28,109 ♂	178	115	70	140	244

rhagic spots may arise in the skin throughout the entire length of the tail. The swelling of the tip may gradually be replaced by a true necrosis, resulting in the loss of 1 to 3 cm. of the tail. The hind feet become red and somewhat swollen at times, in some cases with large scales over the dorsal surfaces. The hair on the back of the body becomes filled with dandruff. There is a tendency to lose the hair, especially about the face, back, and throat. Sores often appear on the skin.

[4] Swift's Silverleaf brand of lard has been used throughout these experiments.

The skin of the face especially seems to become sore at times and the irritation causes the animal to rub the face continually with his fore feet.

TABLE III.

Summarizing Some Observations Made on Animals of Table II.

Rat No.	Before death.	At autopsy.
2,897 ♀	Ovulating normally at onset of decline.	Feet, tail, and skin very scaly. No visceral fat. Kidney normal in appearance.
28,110 ♀	Ovulating normally at onset of decline. Blood in urine.	Feet, tail, and skin very scaly. No fat in body. Kidneys mottled.
28,104 ♀	Ovulating poorly (long cycles). Much blood in urine.	Tail and feet very heavily scaled. Loss of hair at several spots. No body fat.
28,108 ♀	Ovulating poorly at onset of decline. Much blood in urine. Tail black and dead for 3 cm. from tip.	Kidney spotted. Tail, feet, and skin heavily scaled. No body fat.
2,899 ♂	Prolapsed penis. Very much blood in urine. Loss of hair on back.	Bloody urine in bladder. Kidneys abnormal. Tail, feet, and skin scaly. No body fat.
28,103 ♂	Prolapsed penis. Loss of hair on back. Blood in urine.	Tail, feet, and skin scaly. No body fat. Kidneys abnormal, white. Hard concretion in bladder.
28,111 ♂	Bloody urine.	Tail congested and hemorrhagic in places. Yellow concretion in bladder.
28,109 ♂	Prolapsed penis. Much blood in urine. Loss of hair on back.	Feet and back scaly. Tail heavily ridged with hemorrhagic spots. Kidneys abnormal. Concretion in urethra. Loss of hair about eyes and nose.

Often the progress of the caudal necrosis is not unlike that described by Smith and Bogin (5). These workers produced experimental gangrene by partial fasting (underfeeding), but observed no marked changes (aside from those of the cartilage) in microscopic sections. In their animals the advance of the process was checked

when the same diet (containing 22 per cent of lard) was fed *ad libitum*.

In the present experiments, the early outward signs of an unhealthy condition of the animal are soon followed by a cessation of growth when the animal is about 25 per cent underweight in comparison with the controls receiving fat. A plateau in the weight curve is maintained over a period of a few weeks to several months, when the animal begins to decline and invariably dies, unless given a curative dose of fat. Table II gives some observations on animals which were allowed to die. It is seen from these data that a decline in weight begins about the 5th month of life and the animal dies within 3 or 4 months.

Further observations and notes are summarized in Table III. Excepting the skin and tail lesions, the most marked and uniform pathology is observed in the urinary tract and the kidney. There seems little doubt that this is an important factor in the death of the animal. Some animals have died without the development of visible lesions in the gross external appearance of the kidney and without the appearance of blood or protein in the urine. But it seems likely that the renal disorder may prove to be an important and fairly constant result of the dietary fat deficiency (a good experimental method for producing renal disorders). Prolapse of the penis was frequently noted in the late stages of the disease, and vesical concretions occurred in a few cases. Histological studies of the various organs will be made by colleagues of the authors and it is hoped that biochemical studies will give a more exact knowledge of the nature of the metabolic derangement.

The lesions seem to be essentially different from those characteristic of low vitamin A or low vitamin B. There is never a xerophthalmia and neither low vitamin A nor low vitamin B has been shown to produce the caudal necrosis and scaly skin found in the present experiments. In the next section of the paper it will be shown further that increases in the dose of vitamin A and vitamin B (complex) do not effect cures.

The loss of hair and the skin lesions remind us of the pellagra symptoms in the rat described by Goldberger and Lillie (6). The following description of the development of the pellagra-like condition in the rat is given by these authors (p. 1028):

"After a variable period following the arrest of growth already mentioned, there has been observed in many of the animals so fed a tendency for the lids of one or both eyes to adhere together, with, in some instances, an accumulation of dried secretion on the margins of the lids. At about the time or shortly after the appearance of this ophthalmia there has developed in nearly, if not quite, every one of the animals on the indicated diets, some loss of fur. This fur loss has in some begun in irregularly distributed patches. More commonly it has been observed to begin either at the side or over the top of the head, the sides or front of the neck, or in the

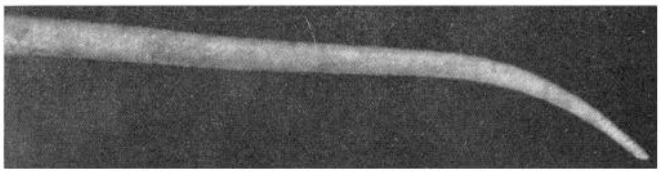

FIG. 1. Tail of Rat 28113 ♀. Diet 550 + 10 drops of lard daily. Entirely normal in appearance.

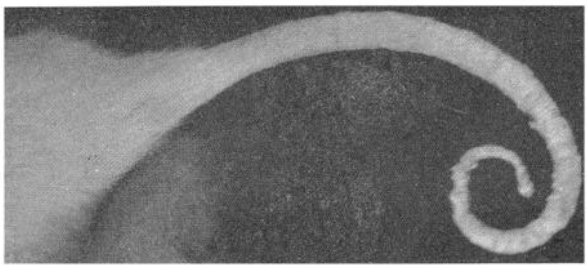

FIG. 2. Tail of Rat 28114 ♀. Diet 550 + 5 drops of glycerol daily. Glycerol gives no protection.

region of the shoulders. From these initial sites the depilation has extended and in some of the animals has led to almost complete denudation of the head, neck, and trunk. . . .

"With or without such loss of fur some of the animals have developed a dermatitis at one or more of the following sites: ears, front of neck and upper part of chest, forearms, backs of forepaws, shins, and the backs of the hind paws. This dermatitis, particularly as it has affected the paws, forearms, neck, and ears, has been sharply outlined and bilaterally symmetrical. To the eye it has differed somewhat with the site affected. The ears seemed definitely reddened and thickened with what appeared to be a

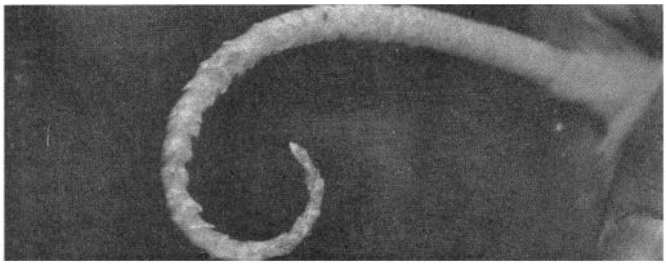

FIG. 3. Tail of Rat 2895 ♀. Diet 550 + the non-saponifiable matter from 10 drops of lard daily. There is no protection from the disease.

FIG. 4. Throat and thorax of Rat 27121 ♀. Diet 550. Age 62 weeks. The animal was rapidly declining and died a week later.

yellowish incrustation of dried serum. In healing, desquamation took place leaving the skin of the pinna with a polished, glistening, somewhat parchmentlike appearance. . . . In a few of the cases so far observed, the affected animals have presented a linear fissuring or ulceration at the angles of the

mouth. In a somewhat larger number there has been a lesion at the tip of the tongue, which first appeared as a small roughly circular grayish opacity or bleb, or as an ulceration which, in some went on to the formation of a localized yellowish slough."

Unlike pellagra the new disease affects the posterior portions of the body first. Necrosis of the tail has not been mentioned by Goldberger and his coworkers, while it is the most marked symptom of the new disease. Sores and ulcers seem common in the pellagra rat while they occur rarely in animals afflicted with the disease caused by low fat diets.

The most convincing evidence against the identity of the two diseases is the method of producing them. The pellagra diet contains 2 per cent of cod liver oil and 3 per cent of Crisco and must be deficient in the P-P factor, a normal constituent of whole yeast. With the addition of 9 per cent autoclaved yeast the pellagra-like condition disappears. But for the production of the new disease, all fat must be excluded from the diet, while 0.7 gm. daily of whole yeast powder does not protect the animal.

Figs. 2 to 4 show some of the lesions characteristic of the disease. Fig. 1 is a photograph of a tail kept normal by the addition of 10 drops of lard daily to the fat-free diet. Fig. 2 shows the swollen tip with a constriction about 2 cm. from the tip. The tail was finally severed at this point, while the 2 cm. end blackened before dropping off. Another conspicuous tail change is pictured in Fig. 3. Very heavy scales and ridges occur along almost the entire length of the tail, with hemorrhagic spots scattered over the surface. The loss of hair on the ventral aspect of the throat and thorax is shown in Fig. 4. The very dark spots are sores. This condition is not so common as the tail lesions and has been observed in those animals only which are in very bad condition and have but a few weeks to live.

In two of the older and more severe cases all four feet have become swollen and in one case the toes of the hind feet have developed sores and ring lesions apparently similar to those of the tail.

Comparative Growth and Ovulation with and without Fat.

When the young rat is reared on Diet 560 + 0.65 gm. of Yeast 5 + Fraction AD, growth is not greatly retarded during the first 60 days, but the animal is always somewhat subnormal in size and

reaches a plateau earlier than animals receiving fat. In most of the graphs presented here curves for averages of groups of an mals have been given. This is permissible because the groups are quite uniform in weight. This method of presentation avoids the confusion of many intersecting curves.

There is an apparent sex difference in the results. Chart 1 shows that the curves for the males plateau when they have at-

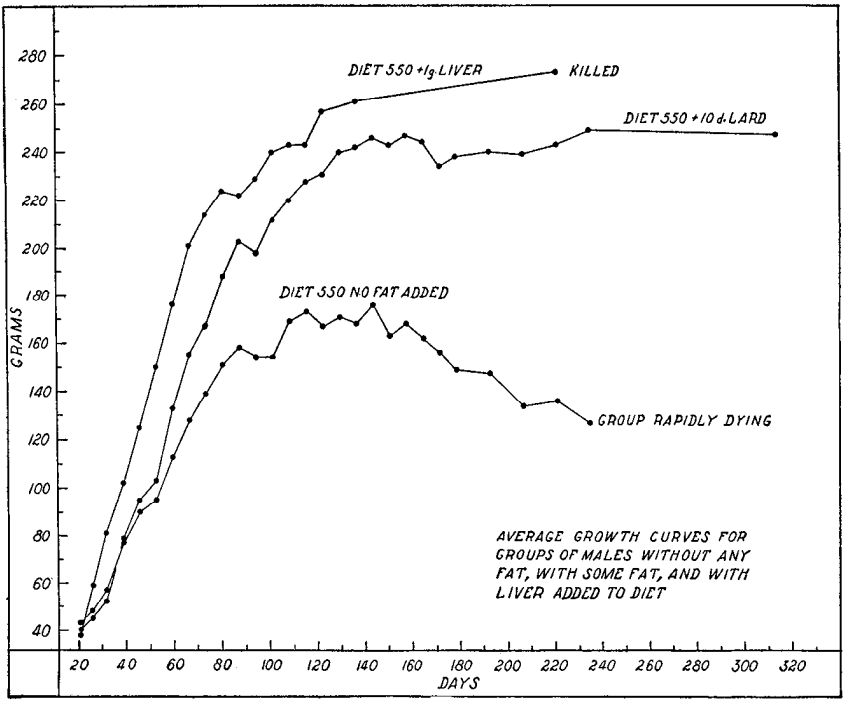

CHART 1.

tained only about 70 per cent of the weight of their controls receiving lard. Chart 2 shows that the curves for the females plateau when their weight is about 80 per cent of the maximum reached by animals receiving lard. Furthermore, when the males begin to decline their loss in weight is much more rapid than that of the females. But in the case of second generation animals (Chart 3) the females are greatly retarded, never quite weighing 70 per cent

as much as their controls at maximum weight. Liver seems always to improve the diet more than the addition of fats. An effort is being made to explain the exceptional value of liver as a supplement to these diets.

These data are in agreement with the findings of Evans and Burr (1) that diets containing 5 to 20 per cent of lard gave materially better growth than did diets containing no fat except 3 drops of cod liver oil.

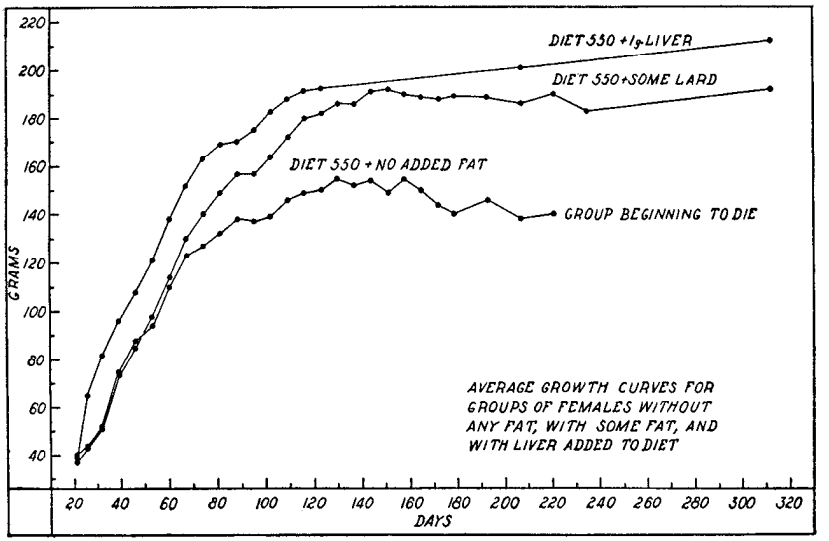

CHART 2.

The next step was to find out about what quantity of lard the rat must consume daily to be greatly benefited in growth and health. Chart 4 gives the average growth curves for females on Diet 560 (20 per cent lard) and Diet 550 + 10 drops of lard. 10 drops of melted lard weigh about 200 mg. and are therefore about 2 per cent of the total food consumed. It is evident that 2 per cent of lard is just as beneficial as 20 per cent. The animals receiving the smaller amount are entirely free from skin lesions or tail trouble.

Other groups of animals have been reared with 3 drops of cod liver oil as a source of vitamins A and D instead of Fraction

AD. This amount of fat in the diet is sufficient to prevent the decline and early death of animals. They will have some scales on the tail and feet but otherwise will be healthy for a period of a year. Two groups of animals receiving cod liver oil were changed to Fraction AD. One group had received no other fat besides the cod liver oil, while the other group consumed some lard in the diet. When the cod liver oil was stopped, the

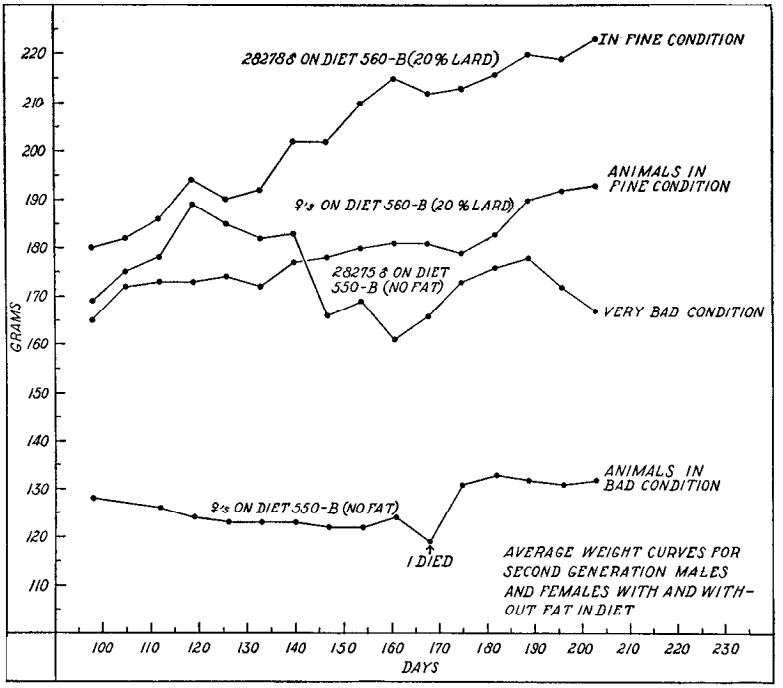

CHART 3.

former animals were on a fat-free diet. The gradual decline of the animals deprived of fat is shown in Chart 5. The male lost weight at a remarkable rate. The animals receiving lard were not adversely affected by the change from cod liver oil to Fraction AD.

Next, it was necessary to determine what part of the lard was responsible for the good nutritive effects observed. A standard procedure for saponification was followed. 100 gm. of fresh leaf lard were saponified for 1 hour on a steam bath in alcoholic potash

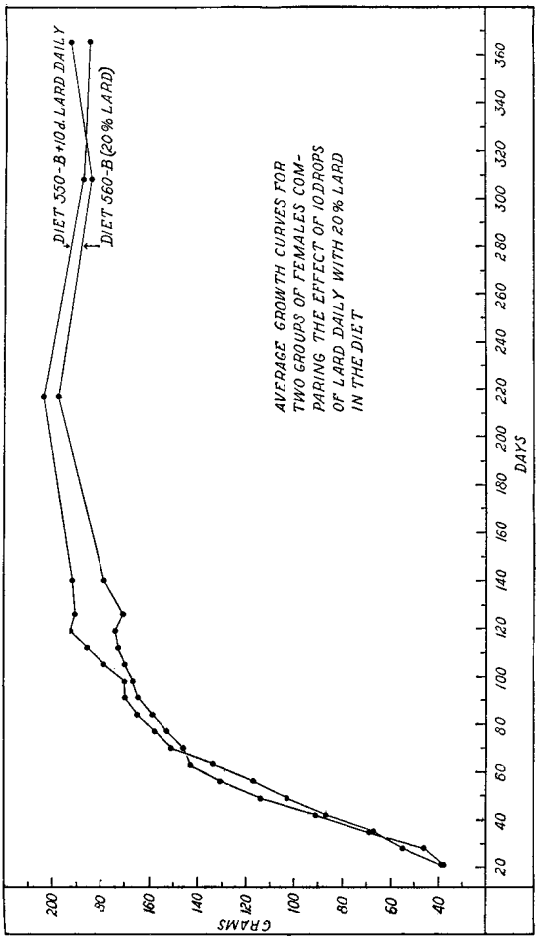
AVERAGE GROWTH CURVES FOR TWO GROUPS OF FEMALES COMPARING THE EFFECT OF 10 DROPS OF LARD DAILY WITH 20% LARD IN THE DIET
DIET 550-B+10d. LARD DAILY
DIET 560-B (20% LARD)
GRAMS
DAYS

CHART 4.

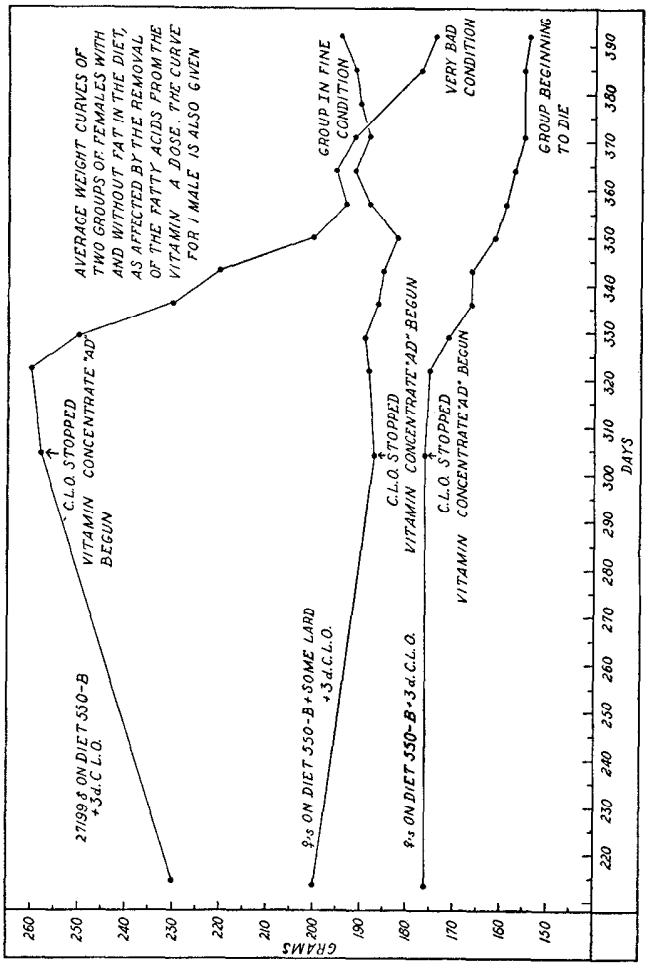
AVERAGE WEIGHT CURVES OF TWO GROUPS OF FEMALES WITH AND WITHOUT FAT IN THE DIET, AS AFFECTED BY THE REMOVAL OF THE FATTY ACIDS FROM THE VITAMIN A DOSE. THE CURVE FOR 1 MALE IS ALSO GIVEN
27 99 ♀s ON DIET 550-B +3d. C.L.O.
C.L.O. STOPPED
VITAMIN CONCENTRATE "AD" BEGUN
GROUP IN FINE CONDITION
♀s ON DIET 550-B + SOME LARD +3d. C.L.O.
C.L.O. STOPPED
VITAMIN CONCENTRATE "AD" BEGUN
VERY BAD CONDITION
♀s ON DIET 550-B+3d. C.L.O.
C.L.O. STOPPED
VITAMIN CONCENTRATE "AD" BEGUN
GROUP BEGINNING TO DIE
GRAMS
DAYS

CHART 5.

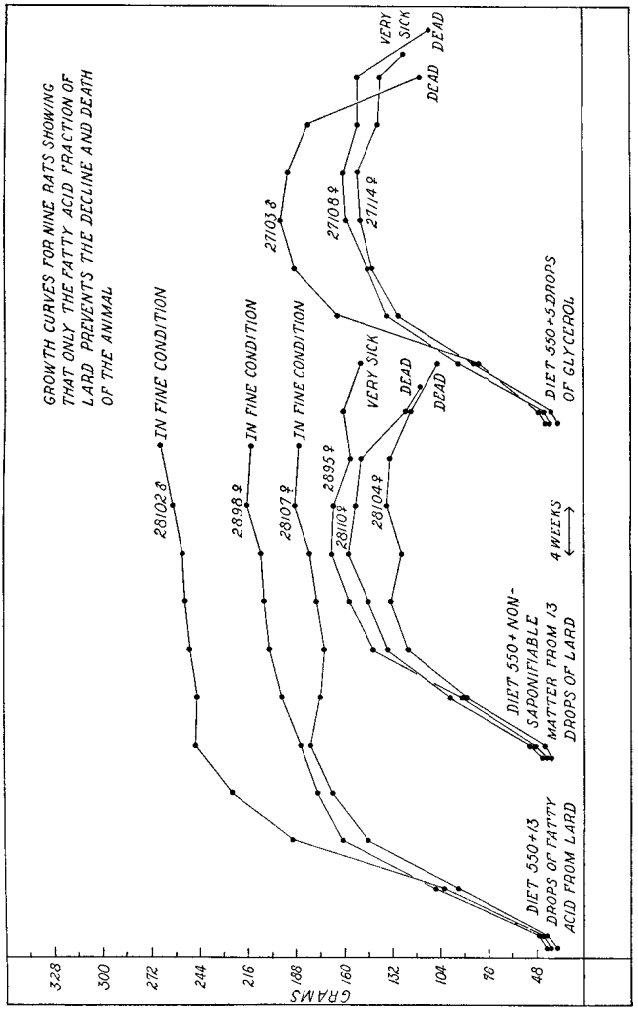
GROWTH CURVES FOR NINE RATS SHOWING THAT ONLY THE FATTY ACID FRACTION OF LARD PREVENTS THE DECLINE AND DEATH OF THE ANIMAL
GRAMS
328
300
272
244
216
188
160
132
104
76
48
28102 ♂
IN FINE CONDITION
28108 ♀
IN FINE CONDITION
28107 ♀
IN FINE CONDITION
28110 ♀
28105 ♀
VERY SICK
28104 ♀
DEAD
DEAD
27103 ♂
27108 ♀
27114 ♀
VERY SICK
DEAD
DEAD
DIET 550+13 DROPS OF FATTY ACID FROM LARD
DIET 550+ NON-SAPONIFIABLE MATTER FROM 13 DROPS OF LARD
4 WEEKS
DIET 550+5 DROPS OF GLYCEROL

CHART 6.

(200 cc. of purified alcohol, 40 gm. of pure potassium hydroxide dissolved in 27 cc. of water). The hydrolysate was diluted with 1000 cc. of water and extracted three times with 700 cc. of U.S.P. ether. The fatty acids were liberated with an excess of hydrochloric acid, separated, and washed thoroughly. They were then freed from solvents and water by prolonged treatment *in vacuo* at 60° and were stored at 0°.

The non-saponifiable matter was washed free from alkali, concentrated, and then dried on the water bath at 60° *in vacuo*. The material was dissolved in ether for daily feeding.

Pure glycerol was used as the third fraction of lard and was fed in daily doses of 5 drops.

13 drops of melted fatty acids were fed daily to one group and the non-saponifiable matter from 13 drops of lard was fed to another group. The results are summarized in Chart 6. The fatty acids gave complete protection, while the glycerol and non-saponifiable matter did not affect the animals in any measurable way. They soon developed the characteristic skin and tail trouble and later declined and died unless a curative dose of fat was fed.

It should be noted that ovulation was recorded for many of the animals, and that the results lack uniformity. As a group, the fat-fed animals ovulate more regularly (4 day cycles) than those animals receiving no fat. But in many cases the animals will have ceased growing and will have developed serious tail and skin trouble before ovulation is affected. The ovulation histories are therefore omitted here because the appearance of the skin and the growth curves are more reliable measures of the condition of the animals.

Curing the Disease.

In one of the earlier experiments six females, which had developed tail lesions, and had ceased growing, were changed from Diet 550 B to Diet 560 B. Renewed growth was observed at once and the tail and feet of every animal became smooth and glossy. The emaciated animals soon were well covered with fat. It was thought that much of the gain in weight was due simply to storage of ingested fat.

A new experiment was then tried with the much smaller dose of lard, 10 drops daily. Four animals were chosen which were in

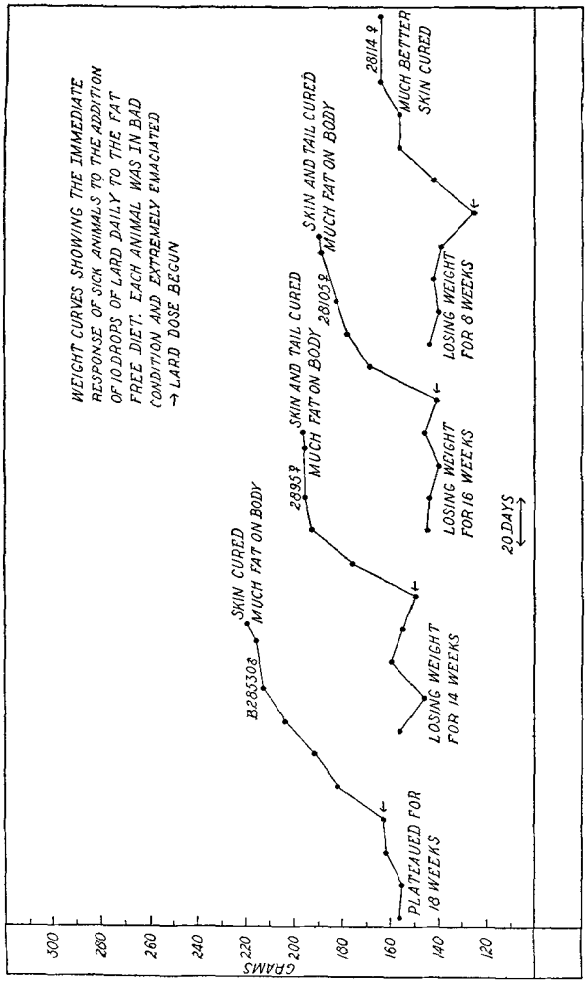
WEIGHT CURVES SHOWING THE IMMEDIATE RESPONSE OF SICK ANIMALS TO THE ADDITION OF 10 DROPS OF LARD DAILY TO THE FAT FREE DIET. EACH ANIMAL WAS IN BAD CONDITION AND EXTREMELY EMACIATED
→ LARD DOSE BEGUN
GRAMS
300
280
260
240
220
200
180
160
140
120
B285308
SKIN CURED
MUCH FAT ON BODY
PLATEAUED FOR 18 WEEKS
LOSING WEIGHT FOR 14 WEEKS
2695 ♀
SKIN AND TAIL CURED
MUCH FAT ON BODY
LOSING WEIGHT FOR 16 WEEKS
20 DAYS
281058
SKIN AND TAIL CURED
MUCH FAT ON BODY
LOSING WEIGHT FOR 8 WEEKS
28114 ♀
MUCH BETTER
SKIN CURED

CHART 7.

very bad condition and could not have lived much longer. Their mates were already dead and these were the survivors from a total of 16 animals. The remarkable effect of this small addition of fat is shown in Chart 7. An immediate change in metabolism is indicated. These emaciated animals, which are from 40 to 60 gm. underweight, begin to grow and for a period of several weeks show a gain of about 2 gm. per day. The skin and tail become smooth and after 10 weeks complete cures are realized. The animals become normally fat and are in general good health. The cure is as spectacular as those cures produced by the well known vitamins. Similar cures have recently been made with even smaller doses of fat. These will be reported in a forthcoming paper.

Although it seemed improbable that there was an insufficiency of vitamins A and D in the dose of Fraction AD fed, two tests were made to prove the adequacy of the fraction. Three young animals were taken from stock mothers and put on Diet 550 +0.65 gm. of ether-extracted yeast +10 drops of lard daily. No Fraction AD was fed. These animals reached maximum weights of 73, 78, and 90 gm. After being on the diet 7 weeks all had xerophthalmia and on the 8th week one died, while the other two were killed because their eyes were closed and they were not eating. Their controls, which were receiving a daily dose of Fraction AD were growing normally and have shown no signs of deficiency at 10 months of age.

Then an attempt was made to cure sick animals by doubling the dose of Fraction AD. Four animals were used. All continued to decline in weight and become worse. Two died and the other two were saved by being returned to the normal dose of Fraction AD and fed a few drops of fat daily.

Similarly, two animals which were declining in weight and were badly diseased, were given a 30 per cent increase of yeast. They were then receiving about 0.88 gm. of dried yeast daily instead of 0.65 gm., the usual dose. There was no gain in weight and the feet and tails continued to grow worse. These animals would have died if a curative dose of fat had not been given.

DISCUSSION.

In 1920 Osborne and Mendel (7) took up the question of the requirement of animals for fat in the diet and concluded that "if

true fats are essential for nutrition during growth the minimum necessary must be exceedingly small." But their diets were only relatively low in fat since anhydrous ether extracted 0.24 per cent of solids and it is certain that meat residue, alfalfa leaves, and starch contain much fatty material which is non-extractable. In fact, it is impossible to conduct a fat-free experiment if corn-starch is used as a source of energy since it carries about 0.6 per cent of fatty substance which is within the granule and is non-extractable (8).

Since Osborne and Mendel (7) review the literature and the arguments on both sides of the question, this will not be repeated here. It is sufficient to say that these earlier experiments were not critical since either they were of very short duration or the diets contained appreciable amounts of fat.

About 3 years ago one of us began working with highly purified basal rations consisting of carefully prepared casein, sucrose, and salts. The basal ration might be said to be almost fat-free but the experiments were again made less critical for this specific problem by the addition of 3 drops daily of cod liver oil to supply vitamins A and D and the 700 mg. of yeast was not ether-extracted. Together, these two supplements added to the diet of each rat at least 70 mg. daily of ether extract, besides some non-extractable fatty substances in the yeast cell. Even with the ingestion of this much fat the animals were markedly inferior to their controls receiving 5 to 20 per cent of some well known fat (1). Fat was thus shown to be beneficial to the animal but not essential to long life and comparatively good health.

In a comprehensive paper, Krogh and Lindhard (9) demonstrate that there is a certain fat minimum as well as a carbohydrate minimum for the best utilization of foods. They postulate that when the respiratory quotient is above 0.9 there is a transformation of carbohydrates to fat and this gives rise to an extra expenditure of energy during rest. This effect would reach a maximum with a respiratory quotient of 1.0.

By the present work the authors have shown that if the young animal is subjected to a complete fat starvation over a period of several months, it develops a disease and soon dies. Whether this effect is caused by the strain of long continued fat synthesis suggested by Krogh and Lindhard or whether a special type of fatty

acid is required by the animal which it is unable to synthesize from the diet consumed, are unanswered questions. There is the further possibility that the ingestion of 200 mg. of fatty acids favorably affects the alimentary canal and improves the general well being of the animal. These problems are being studied now.

The fatty acid fraction of lard is fairly well known. Some workers have almost quantitatively accounted for all acids present with about the following distribution: stearic acid, 15 per cent; palmitic acid 25 per cent; oleic acid, 50 per cent; and linoleic acid, 10 per cent. Traces of arachidonic, linolenic, lauric, and myristic acids have been reported. Ellis and Isbell (10) show that the diet has a marked effect upon the distribution of the fatty acids in lard. If these well known fatty acids are responsible for the cures described (Chart 7), then we must assign to them a function far more subtle than the production of nine calories of energy per gm. burned. By their presence they have changed the entire economy of the animal, causing an increase in body weight equal to 10 times the weight of the acids consumed. The increase in weight is always accompanied by a return to normal health.

If the effect is not due to the ordinary fatty acids, then we must look for a new substance of the nature of an ether-soluble organic acid which must be present in exceedingly small amounts. This acid would be classed as a vitamin until its isolation permitted the assigning of a definite chemical formula and name. No conclusion can be drawn from the data at hand and for the present we will speak of this dietary deficiency as due to the absence from the diet of the acids present in fats.

SUMMARY.

1. New diets of high purity and extremely low in fat have been devised.

2. A new deficiency disease involving caudal necrosis in the rat has been produced by the careful exclusion of fats from the diet.

3. This disease is readily prevented or cured by the addition of 2 per cent of fatty acids to the diet.

4. The non-saponifiable fraction of fats and glycerol are ineffective for the curing of the disease.

5. As little as 3 drops of fat fed daily has a measurable effect upon the animal.

6. With fat-free basal rations, storage fat can be almost entirely eliminated and it seems probable that the amount of body fat can be controlled over a wide range by the addition of minute quantities of fat to the diet.

The authors wish to express their thanks to Professor J. Arthur Harris and Professor C. M. Jackson for their interest and for their assistance in the preparation of the paper.

BIBLIOGRAPHY.

1. Evans, H. M., and Burr, G. O., *Proc. Soc. Exp. Biol. and Med.*, **25**, 390 (1928).
2. Van Slyke, L. L., and Baker, J. C., *New York Agric. Exp. Station, Techn. Bull. 65* (1918).
3. Pertzoff, V., and Cohn, E. J., *J. Gen. Physiol.*, **11**, 239 (1928).
4. Henry, W. A., and Morrison, F. B., Feeds and feeding, Madison, 18th edition 746 (1923).
5. Smith, A. H., and Bogin, M., *Am. J. Path.*, **3**, 67 (1927).
6. Goldberger, J., and Lillie, R. D., *Pub. Health Rep., U. S. P. H. S.*, **41**, 1025 (1926).
7. Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, **45**, 145 (1920–21).
8. Taylor, T. C., and Iddles, H. A., *Ind. and Eng. Chem.*, **18**, 713 (1926).
9. Krogh, A., and Lindhard, J., *Biochem. J.*, **14**, 290 (1920).
10. Ellis, N. R., and Isbell, H. S., *J. Biol. Chem.*, **69**, 239 (1926).