ON THE NATURE AND RÔLE OF THE FATTY ACIDS ESSENTIAL IN NUTRITION.*

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In our introductory paper on this subject (1) it was shown that when rats were reared on a fat-free diet a deficiency disease developed which had not been previously described. This disease is rather specific since the scaly condition of the skin develops while growth is continuing at an approximately normal rate. Later the tail often becomes necrotic and the kidneys degenerate, allowing the passage of blood into the urine. The animals always die at an early age unless fed a curative dose of fat. The fatty acid fraction is the only part of the fat effective in curing or preventing the disease. When a curative dose is fed, the extremely emaciated animals begin to grow and store depot fat.

In the present communication the authors will discuss the following topics: further observation on the effects of the disease; effect of protein intake on the severity of the disease; effect of fat exclusion on water exchange; effect of fat exclusion on ovulation and reproduction; nature of the essential fatty acids.

Further Observations on Effects of the Disease.

In our earlier paper the necrosis of the tail was greatly emphasized as a condition due to the fat deficiency. During the past year much of the work has involved the testing of substances by the cure method already described. For this work animals which have been on the fat-free diet only 4 to 6 months after weaning can be used since they have already reached a growth plateau or are declining in weight. But at this age many animals have not developed severe lesions of the tail. And when they have done

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so these lesions are often so severe that they require many weeks to heal. The most sensitive test of the disease is the scaliness of the feet, especially the hind feet. This always appears within a few weeks after the young animal is put on the fat-free diet and will disappear completely in 3 or 4 weeks after a good oil is fed. Another reliable indication of the condition of the animal is dandruff on the back. The feet and back are always carefully described in the data for cure work.

The condition of the kidneys should be emphasized much more than was done in the earlier paper. In fact, most of the animals on the fat-free diet which have been killed during the past year have shown grossly abnormal kidneys. Even the breaking down of the kidneys to such a degree that blood appears in the urine is very common and this observation may be used as a measure of the severity of the disease. It now seems probable that in most cases the immediate cause of death of the animal is kidney degeneration. This kidney degeneration must not be confused with that due to lack of vitamin A. It has been shown that increased doses of Fraction AD do not improve the animal, while the addition of vitamin A-free fatty acids[1] or oils does. That Fraction AD is readily absorbed in the absence of fat was shown by the very early decline and death of animals from which Fraction AD was withheld ((1) p. 364). The animals receiving no fat grow to twice the size of those receiving fat but no Fraction AD and live several times as long. Furthermore, the animals receiving the low fat diet show no signs of xerophthalmia throughout life.

We may, therefore, consider growth, scaliness of the skin, and condition of the kidneys as measures of the effect of various fats in the diet, all other known factors being excluded or constant. Other measures will be discussed in following sections.[2]

Effect of Protein Intake on Severity of the Disease.

While convenient quantitative measures of the severity of the disease due to low fat diet were being sought, the question of ideal

[1] Fraction AD designates the non-saponifiable matter of cod liver oil prepared and fed as described in our earlier paper. Fraction E designates the concentrated vitamin E prepared as described below.

[2] The histopathology of the kidneys will soon be reported by Professor C. M. Jackson.

diet for producing the disease arose. There were only the two constituents of the diet to be varied, casein and sucrose, the salts being kept constant except for the ash of the casein. Since the disease involves the kidneys, it seemed likely that merely increasing the per cent of protein in the diet would increase the severity

TABLE I.

Effect of High Protein Diet and Low Protein Diet on Rats.

Male rats were used throughout.

Group No. and diet.	Rat No.	Maximum weight.	Condition of skin (scale and dandruff).	Condition of urine.
		gm.		
Group 58. Diet 550 + 0.65 gm. yeast + Fraction AD + Fraction E.	2913	214	Medium.	Bloody.
	BH29536	210	Much.	No blood.
	W29531	178	Great deal.	Much "
	GH29533	160	" "	Bloody.
	2908	242	" "	Great deal of blood.
	2920	205	Extreme.	No blood.
Average		201		
Group 59. Diet 550 B + 0.65 gm. yeast + Fraction AD + Fraction E.	BH29532	196	Medium.	No blood.
	W29530	185	"	" "
	2909	245	Great deal.	" "
	2915	211	Extreme.	Much "
	W29540	208	Great deal.	No "
	W29534	187	" "	" "
Average		205		
Controls. 4 rats on Diet 550. 4 on Diet 550 B + 0.65 gm. yeast + Fraction AD + Fraction E + 10 drops lard.		273	Normal.	Normal.

of the disease and hasten the decline of the animal. There has been lack of agreement generally as to whether high protein diets caused the lesions of the kidneys known as nephritis and nephrosis, but recent workers (2–4) have agreed that exceedingly high protein diets do not produce kidney lesions.

To furnish further facts concerning the breakdown of the kidney in the present work as well as to check the above work the following experiment was performed. Twelve young male rats were divided into two groups (Nos. 58 and 59). Group 58 received Diet 550[3] + 0.65 gm. of yeast + Fraction AD + Fraction E. Group 59 received Diet 550 B + 0.65 gm. of yeast + Fraction AD + Fraction E. The two groups were therefore receiving identical diets except that one diet contains twice as much casein as the other. The results are summarized in Table I. The maximum weights may be considered equivalent. The condition of the skin is so bad that no general difference between the two groups could be detected. But when the numbers of cases with bloody urine are compared, there is evidence that the high protein diet has increased the injury to the kidneys. Four of the six in Group 58 showed bloody urine before autopsy (8 months old), while only one of the six in Group 59, low protein, showed bloody urine. These groups are small and the evidence cannot be considered as final. But if these results can be repeated (or made even more marked by using greater differences in the protein contents of the diets), then it must be concluded that high protein intake does aid in the breakdown of the kidney when the kidney is already weakened by the absence of essential fatty acids. From the histological studies of Professor Jackson we will know whether even the kidneys of those animals in which there was no blood in the urine differed in degree of degeneration. No kidney degeneration was detected in the controls (Table I).

Effect of Fat Exclusion on Water Exchange.

Early in the course of this work it was noticed that those animals on the fat-free diets consumed more water than their controls receiving some fat. Water consumption was soon put on a quantitative basis and recorded with the same regularity as food consumption. Both values have been known throughout this work. All experimental animals are kept in the individual round cages described in the earlier paper. The food cup is of a type which

[3] For these diets see Burr and Burr (1). Diet 550 contains casein 24 per cent, sucrose 72.1 per cent, salts (*cf.* (1)) 3.9 per cent, nutritive ratio 1:3. Diet 550 B contains casein 12 per cent, sucrose 84.1 per cent, salts 3.9 per cent, nutritive ratio 1:7.

permits almost no spilling, and these records can be kept with considerable accuracy. The water is kept in 250 cc. bottles filled to the 250 cc. mark on the neck. They are stoppered with No. 6 rubber stoppers carrying a bent glass tip made of 10 mm. tubing. These tips are blown with especially high lips about the opening which is on top of the nearly horizontal arm. Almost no detectable spilling takes place. The bottles do not vary more than 5 cc. in volume. The chief cause of spilling from these bottles when first used was found to be the expansion and contraction of the air in the bottle when the room temperature fluctuated greatly. But for nearly 2 years now the room has been kept at a constant temperature with a maximum daily fluctuation of $\pm 1°$. The amount of spilling has been found to be extremely little in those cages which have been tested by placing them over funnels. The difference in urine excretions when the animal is watered outside the cage and when watered by the bottle is not appreciable. Any spilling of water would have increased the apparent urine volume.

It should be pointed out here that water consumption differs greatly among individuals of the same size and on the same diet so that individual cages must be used to get any true picture of the variations. The same statement applies to food consumption.

Table II gives a comparison of actual volumes of water consumed, expressed in cc. per day for individuals with and without fat in the diet. Each water consumption period is from 7 to 12 days in length, depending upon the rate the bottles are emptied. Daily measurements are not made. The bottles are filled, left until nearly empty, the residual water measured, and the total consumption divided by the length of the period in days. Only a few of the groups and a few periods for these groups can be given here because of lack of space. The data given here are typical and will illustrate the variation among members of the same group and the variation of the same individual from week to week. It should be noted that these values represent the water consumed from the bottles only. The daily yeast dose is fed with 4.5 cc. of water. Therefore, for total water consumed by each animal add 4.5 cc. to the amount given in Table II.

Although there is considerable variation among the members of the same group, it is evident from Table II that this variation is

TABLE II.

Typical Water Consumption Records for Animals with and without Fat in Diet.

Female rats were used except in the cases noted.

Group No. and diet.	Amount of fat in diet.	Rat No.	Weight of rats Nov. 17.	Water consumption per day for period.					
				Oct. 22–30.	Oct. 30–Nov. 7.	Nov. 7–14.	Nov. 14–23.	Nov. 23–Dec. 1.	Dec. 1–10.
			gm.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
Group 3. Diet 550 B + Y* + Fraction AD.	None.	27139	144	23.8	24.4	23.6	25.5	26.9	28.3
		27278	171	21.2	20.0	20.7	21.7	24.4	24.4
		27123	162	15.0	16.3	17.1	20.0	22.5	26.1
Group 4. Same + lard.	10 drops lard daily.	27132	214	10.0	11.3	10.0	9.4	10.0	10.6
		27261	156	16.2	13.1	11.4	15.6	14.4	13.9
		27122	180	10.6	11.3	10.0	9.4	9.4	10.0
Group 19. Diet 560 B + Y + Fraction AD.	20% lard in diet.	27202	191	9.4	9.4	10.0	11.1	11.9	11.7
		27186	201	10.0	10.0	9.3	8.3	10.6	9.4
		27190	185	11.9	11.9	12.9	12.2	12.5	12.2
Group 56. Same as for Group 3.	None.	28276	123	20.0	20.0	19.3	18.9	19.4	21.1
		28282	131	20.0	18.1	19.3	18.3	21.3	21.7
		28281	112	20.0	19.4	21.4	20.6	20.6	Died.
		28275♂	166	18.8	18.1	18.6	18.9	18.8	19.4
Group 57. Same as for Group 19.	20% lard in diet.	28277	160	8.8	8.8	7.1	9.4	8.8	8.9
		28280	184	10.6	11.9	10.7	9.4	11.3	12.2
		28279	190	8.1	8.3	8.3	7.8	9.4	9.4
		28278♂	202	9.4	10.7	10.7	10.6	11.3	11.1
Average for no fat groups			144	19.8	19.5	20.0	20.6	22.0	23.5
" " 10 drops lard group			183	12.3	11.9	10.5	11.5	11.3	11.5
" " 20 per cent lard groups			188	9.7	10.1	9.9	9.8	10.8	10.7

Average daily water consumption.

Fat-free	20.9 cc.
10 drops lard	11.5 "
20 per cent lard	10.2 "

* Y designates the daily dose of 0.65 gm. of ether-extracted yeast, as described in the earlier paper.

small as compared with the average difference between groups with and without fat. It is also evident that 10 drops of lard (2 per cent of total diet) cut down water consumption just as effectively as 20 per cent lard in the diet. Only a few cases have been given here but these results apply uniformly to all rats reared during the past 2 years. On examination of all records it is found that among those animals receiving 10 drops of an oil there is occasionally an exceptional rat whose water consumption is high. Two of these exceptions were checked up and the rats found to have a very large urine volume to account for the excess water consumed.

TABLE III.

Average Urine Output for 2 Successive Days (October 18 to 19) by Groups 56 and 57.

Female rats were used except in the cases noted.

Group No.	Fat in diet.	Rat No.	Daily urine output.	Average for group.
			cc.	cc.
56	None.	28276	3.6	
		28282	3.3	
		28281	2.1	
		28275♂	3.4	3.1
57	20 per cent lard.	28277	2.2	
		28280	4.5	
		28279	3.8	
		28278♂	4.9	3.9

To account for this difference in water consumption two values were checked: urine volume and food consumption. The measurement of urine volume was made by placing the cages over funnels and collecting the urine under toluene in 12 mm. tubes. Evaporation from the tubes was negligible but of course considerable evaporation took place on the walls of the funnels. Therefore the volumes recorded have no absolute value but should be good for comparisons. The authors were surprized to find that the average urine excretion of the animals on the fat-free diet (high water consumption) was actually less than that of the animals receiving fat. A single example of the measurements taken for 2 successive days on Groups 56 and 57 is given in Table III. Evi-

TABLE IV.

Food Consumption of Animals in Table II.

The food consumption records are taken every 4 days but the values given here are the average gm. consumed per day during each water consumption period. Female rats were used except in the cases noted.

Group No. and diet.	Animal No.	Food consumption per day.											
		Oct. 22–30.		Oct. 30–Nov. 7.		Nov. 7–14.		Nov. 14–23.		Nov. 23–Dec. 1.		Dec. 1–10.	
		gm.	*cal.**	*gm.*	*cal.*	*gm.*	*cal.*	*gm.*	*cal.*	*gm.*	*cal.*	*gm.*	*cal.*
Group 3. Diet 550 B + Y† + Fraction AD (no fat).	27139	9.95	40.4	10.10	41.0	10.75	43.5	9.75	39.6	10.55	42.7	10.75	43.5
	27278	11.70	47.1	10.40	42.1	14.65	58.5	11.25	45.4	11.80	47.5	12.25	49.3
	27123	8.50	34.8	10.10	41.0	10.10	41.0	10.30	41.8	9.90	40.2	10.15	41.2
Group 4. Same + 10 drops lard.	27132	12.40	51.8	10.65	45.2	14.40	59.6	12.15	51.0	10.85	46.0	11.50	48.5
	27261	8.20	35.8	7.55	33.3	9.90	42.3	9.55	41.0	9.60	41.2	7.15	31.7
	27122	9.05	39.0	10.30	43.9	10.75	45.6	8.75	37.9	8.95	38.7	8.15	35.6
Group 19. Diet 560 B + Y + Fraction AD (20 per cent lard).	27202	9.05	45.6	9.55	48.0	10.60	53.1	8.30	42.0	9.15	46.1	9.90	49.7
	27186	9.90	49.7	9.90	49.7	13.05	64.9	10.55	52.8	10.35	51.9	10.40	52.1
	27190	6.95	35.5	8.20	41.5	8.15	41.3	8.30	42.0	8.20	41.5	8.40	42.5
Group 56. Same as for Group 3.	28276	9.05	36.9	9.90	40.2	10.75	43.5	9.00	36.7	9.95	40.4	9.40	38.3
	28282	9.10	37.1	9.90	40.2	11.80	47.5	9.80	39.8	9.65	39.3	10.50	42.5
	28281	9.60	39.1	9.45	38.5	10.25	41.6	10.00	40.6	10.50	42.5	Died.	
	28275♂	11.40	46.0	9.95	40.4	10.30	41.8	10.15	41.2	9.45	38.5	11.15	45.0
Group 57. Same as for Group 19.	28277	6.60	32.8	6.10	31.4	7.50	38.2	6.65	34.1	6.25	32.2	7.00	35.8
	28280	8.35	42.3	7.55	38.4	9.00	45.4	8.15	41.3	8.10	41.1	8.05	40.8
	28279	8.60	43.5	8.75	44.2	8.85	44.7	8.65	43.7	8.80	44.4	8.65	43.7
	28278♂	8.45	42.7	8.80	44.4	9.00	45.4	8.30	42.0	8.50	43.0	7.40	37.7

Average.												
Fat-free.	9.90	40.2	9.97	40.5	11.23	45.3	10.04	40.7	10.26	41.6	10.70	43.3
10 drops lard.	9.88	42.2	9.50	40.8	11.68	49.2	10.15	43.3	9.80	42.0	8.93	38.6
20 per cent lard.	8.27	41.7	8.41	42.5	9.45	47.6	8.41	42.6	8.48	42.9	8.54	43.2

Average for entire period.	Fat-free	10.35 gm.	41.9 cal.
	10 drops lard	9.99 "	42.7 "
	20 per cent lard	8.59 "	43.4 "

* Total calories = calories from diet + calories from supplement. Yeast has about 80 per cent digestible protein and carbohydrate; hence 0.65 gm. of yeast = 2.08 calories, 10 drops of lard = 230 mg. = 2.07 calories.

† Y designates the daily dose of 0.65 gm. of ether-extracted yeast.

dently the small animals in Group 56 (fat-free) consume twice as much water but excrete no more urine than Group 57 (20 per cent fat).

In order to determine the total water intake, the food consumption must be known for calculation of the water of metabolism. Food consumption is given in gm. and calories in Table IV. The average figures show that the calorie intake is practically identical for all groups, the difference between the fat-free group and those animals receiving some fat being only 1 calorie in 42, or less than 2.5 per cent. This probably is not significant. Since the water value of a calorie of these diets is only about 0.14 gm., the lesser requirement for water by the normal animals cannot be accounted for by greater food consumption.

Summarizing, we find that animals receiving no fat consume almost twice as much water as their controls receiving fat and that they do not excrete this excess water as urine. The feces are very dry and are very small in amount. Although they weigh only 80 per cent as much as their controls, the animals on the fat-free diet consume the same amount of food. They are not hyperactive. The excess water used (10 gm. daily) must be lost by evaporation from the lungs and skin. This alone amounts to nearly 6 calories, or 14 per cent of the total calorie consumption. Further, it has been pointed out by Du Bois (5) that thin people may produce 50 per cent more heat per kilo of body weight than fat people. The rats on the fat-free diet are very emaciated and it seems that the larger water loss and lack of body fat may readily account for the large food consumption of such small animals.

This indicates that the careful collection of food consumption data may be almost worthless unless the water loss and amount of fat on the animal are taken into consideration.

The water loss by evaporation reflects in some way the condition of the skin or lungs or both. It is not known which is involved but it is quite clear that the lack of dietary fat has so injured the tissues that they are no longer the normal membranes separating the interior of the animal from its relatively dry air environment.

Effect of Fat Exclusion on Ovulation and Reproduction.

As was pointed out in our earlier paper it was thought desirable to exclude all materials not known to be essential to the well being

of the animals. It has been known for some years that both males and females thrive on a diet deficient in vitamin E. Accordingly this vitamin was not included in the diet of our first groups of animals. But it has been recently pointed out by Evans (6) that rats attain a greater size when vitamin E is included in the diet. In order to exclude the vitamin E factor from the new disease and to demonstrate that none of the effects noted on low fat diets could be ascribed to the lack of vitamin E (*i.e.* poor growth, diseased skin and kidneys, increased water consumption) a concen-

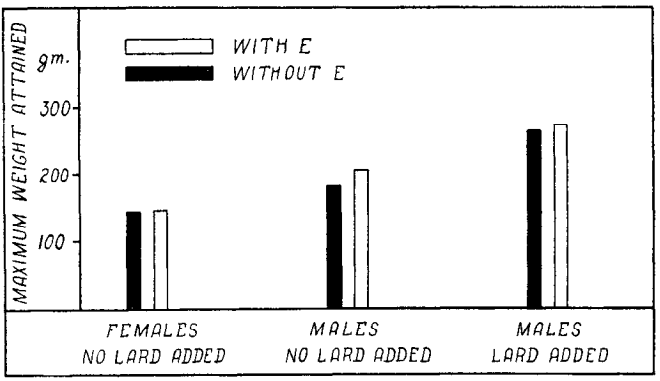

CHART 1. Average maximum weights attained by male and female rats without the addition of Fraction E to fat-free diets and to diets supplemented by 10 drops of lard. Total number of males, 22; total number of females 22.

trated, fat-free preparation called Fraction E has been added to the daily yeast dose of all animals started during the past year.

The preparation of Fraction E is identical with that of Fraction AD (1). Well cleaned wheat germs from the Washburn Crosby Milling Company are extracted with purified U.S.P. ether in a large Soxhlet apparatus (1 gallon size). The last of the ether is removed from the oil *in vacuo* at 60°. The oil is stored at 0° in a dark bottle.

For a group of 60 rats, 30 gm. of wheat germ oil are saponified with 20 per cent alcoholic KOH, and the non-saponifiable matter is extracted with 300 cc. of U.S.P. ether (for anesthesia) and washed free from alkali. The ether is concentrated to 20 cc. and a drop or two evaporated on the yeast dose daily. This quantity is fed

every 2 weeks. Therefore each animal receives the non-saponifiable matter from 250 mg. of wheat germ oil each week. It is known that 250 mg. of a good wheat germ oil will cure a sterile female, so that this amount fed each week was expected to be entirely adequate. Our fertility tests have proved this to be true.

We have made no effort to determine finally whether vitamin E has any appreciable effect upon the growth of animals on a fat-free diet. When the effects are small and the variations are large, more animals are required than we have been able to give to prove this one point. From Chart 1 it is evident however that in these two groups of twenty-two females (total) there is no appreciable effect due to vitamin E. These animals were selected litter mate sisters, reared especially for this comparison.

No comparable experiment has been performed with males. Since the lack of vitamin E causes a degeneration of the seminiferous epithelium of the testis, it might be suspected that tissues which affect the growth of the male would also be somewhat affected. We have recently reared a group of seventeen males with Fraction E. For comparison with these there are only four males reared a year earlier. Groups so widely separated in time should not ordinarily be compared. But the great uniformity of the diet, colony temperature, and cage conditions may justify the present comparison.

The average maximum weight attained by the seventeen males receiving Fraction E is compared with the average maximum weight of the four males without Fraction E in Chart 1. On the same chart are plotted average maximum weights of males receiving 10 drops of lard without Fraction E and 10 drops of lard with Fraction E. This limited evidence indicates that the males receiving no fat were helped more by the addition of Fraction E than those receiving fat. This might be interpreted as meaning that the non-saponifiable matter fed with Fraction E is helpful and this same non-saponifiable matter is fed with the vitamin E-free lard. But in no case is the effect of vitamin E large as compared with the effect of a trace of fat in the diet. Furthermore, all animals receiving vitamin E develop the scaly condition and kidney trouble as early as those animals without vitamin E. We feel justified in concluding that vitamin E offers no protection from the new disease.

Ovulation of the twenty-two females compared in Chart 1 may serve as another check on the effect of vitamin E on the general well being of females receiving a fat-free diet. They may be divided into the groups: (1) those ovulating regularly (every 4 to 6 days), and (2) those ovulating very irregularly (less than every 9 days) or not at all. Comparative data for the two groups are as follows:

Total No. of rats.	Addition to Diet 550 + Fraction AD + yeast.	No. ovulating regularly.	No. not ovulating regularly.
11	+ Vitamin E	4	7
11	− " "	5	6

It is evident that vitamin E does not affect the well being of the females on the fat-free diet sufficiently to improve ovulation.

It should be pointed out here that in the study of the effects of the well known vitamins carried in cod liver oil and wheat germ oil the results may be confused by the use of the whole oil rather than just the non-saponifiable fraction. 3 drops daily of cod liver oil or of wheat germ oil will cure badly diseased animals when these animals are suffering from a lack of essential fatty acids. Many of the ordinary diets used in nutrition may be deficient in this respect.

Although Fraction E has no effect on ovulation, curative oils cause an immediate resumption of ovulation in those females in which ovulation has ceased. The figures cited above show that in less than 50 per cent of that special group was ovulation regular. About half of these animals had not ovulated at all. The animals which are not ovulating at all provide fine material for testing the effect of fats on ovulation. Four examples are given in Table V. Those animals which received 5 drops daily of corn oil, olive oil, or linseed oil ovulated within 5 days after the dose was begun; *i.e.*, vaginal smear changes were resumed as quickly as though they were castrated females receiving a daily injection of ovarian hormone. On the other hand, the coconut oil, which is not a curative oil, did not cause the resumption of ovulation.

It is an almost invariable rule that every animal which is receiving a curative oil and has resumed growth also ovulates normally (a cycle every 4 to 6 days). We need not interpret this as meaning

TABLE V.

Immediate Resumption of Normal Ovulation by Females, in Which Ovulation Had Ceased, on Low Fat Diet, When Fed 5 Drops Daily of Curative Oil.

Date.	Ovulation histories.			
	Rat BH29537.	Rat B29543.	Rat W29544.	Rat W29535.
1929				
July 1	LE	LE	LE	LE
" 2	"	"	"	"
" 3	"	"	"	"
" 4	"	"	"	"
" 5	"	"	"	"
" 6	"	"	"	"
" 7	"	"	"	"
" 8	"	"	"	"
" 9	"	"	"	"
" 10	"	"	"	"
" 11	"	"	Linseed oil begun.	"
" 12	"	"	LE	"
" 13	Corn oil begun.	Olive oil begun.	"	Coconut oil begun.*
" 14	LE	LE	"	LE
" 15	"	"	"	"
" 16	"	"	"	"
" 17	"	" ?	0–1	"
" 18	0–1	Cornif.	Cornif.	"
" 19	Cornif.	All three.	All three.	"
" 20	"	LE and C.	LE and C.	"
" 21	All three.	0	" ?	"
" 22	LE and C.	Cornif.	0–1	"
" 23	"	All three.	Cornif.	"
" 24	0–1	" "	All three.	"
" 25	Cornif. and leuc.	0–1	LE and C.	"
" 26	All three.	Cornif.	"	"
" 27	LE and C.	Cornif. and leuc.	0–1	"
" 28	" " "	LE and C.	Cornif.	"
" 29	0–1	"	LE and C.	"
" 30	Cornif.	" ?	" " "	"
" 31	All three.	Cornif.	"	"
Aug. 1	LE and C.	All three.	Cornif.	"

The stages in the estrous cycle are indicated as follows: 0 = epithelial cells only, in heat toward end; 0–1 = epithelial and cornified cells, in heat; cornif. = cornified cells only, possibly still in heat; cornif. and leuc., all three, and LE and C = varying mixtures of cornified cells, leucocytes, and epithelial cells, not in heat; LE = diestrus with varying amounts of leucocytes, epithelial cells, and mucus.

* Coconut oil does not cure readily.

that these oils contain a fatty acid specific for ovulation. Ovulation may be considered as dependent upon the general well being of the animal, a function which suffers when the animal is in poor condition or its metabolism is low. The added oils so improve the animal as a whole that normal ovulation is resumed.

But much work indicates that the ovarian hormone is a lipoid and certainly its method of extraction shows it to be closely associated with the cell lipoids. Furthermore, the resumption of ovulation is so rapid that growth has hardly begun. Because of these facts, as well as the effects of fat upon the male (see Table VII), we are inclined to the view that the synthesis of ovarian hormone ceases when fatty acids are eliminated from the diet because the fatty acids are closely associated with the hormone.

Fertility.

As a test of the effectiveness of our vitamin E preparation three females which were on the fat-free diet were bred to normal males. These females were the only ones of the group which were ovulating. They were bred in wire bottom cages, with access to no food except their usual pure diet. They were then returned to their individual wire bottom cages for gestation. Vitamin B was not increased. It was hoped that the young would fall through the large wire mesh and not be destroyed. Data for the actual gestations are given in detail (Table VI) so that the gain in weight, prolonged presence of red blood cells, and delayed littering may be clear. Two of these three very emaciated animals actually produced litters, proving the presence of sufficient vitamin E in their diets. Although they had been on a growth plateau for over 6 weeks, presumably due to the limitations of their diet, they gained 12 to 17 gm. during gestation. None of the young lived more than a few hours after birth.

The tremendous effect of the fatty acid fraction of cod liver oil and of wheat germ oil may be best illustrated by gestation studies. Six females on Diet 550 B were changed from the non-saponifiable matter, Fraction AD, to the 2 drops of cod liver oil daily. In 4 weeks their weight had increased markedly (an average of about 35 gm. per animal). All were ovulating and were mated positively. On the day of positive mating 250 mg. of wheat germ were added to the daily supplement. One of the six animals (Rat 27169)

TABLE VI.

Gestation Histories of Three Females (from Group 62) on Fat-Free Diet 550 B + Fraction AD + Fraction E.

Date.	Rat BH29545.	Weight.	Rat BH29538.	Weight.	Rat BH29539.	Weight.
1929		*gm.*		*gm.*		*gm.*
June 26	0–1 mated with ♂ W29526.	143				
" 27	Cornif., plug, sperm.					
" 28	LE and C.					
" 29	LE	144			0 mated with ♂ 29509.	168
" 30	"				Cornif., plug, sperm.	
July 1	"		0–1 mated with ♂ 29526.	137	" and leuc.	
" 2	"		Cornif., plug, sperm.		LE and C.	
" 3	"		All three.		LE	
" 4	"		LE		"	
" 5	"		"		"	
" 6	"	150	"	139	"	170
" 7	"		"		"	
" 8	"		"		"	
" 9	"		"		"	
" 10	R.B.C. (light).		"		"	
" 11	" (very heavy).		"		R.B.C. (faint).	
" 12	Vagina full of dark red blood; not normal.		"		" "	

July 13	R.B.C. (very heavy).		LE	R.B.C.	175
" 14	" " "	155	"	142 " (heavy).	
" 15	" " "		R.B.C. (very heavy).	141 " (faint).	
" 16	"		" " "	" (heavy).	
" 17	LE, isolated.	153	" " "	LE	
" 18	R.B.C. (faint).		"	R.B.C.	185
" 19	No litter.	153	" (faint).	" (faint), isolated.	
" 20	" "	152	" "	149 No litter. R.B.C.	181
" 21	" "	153	" " isolated.	" " "	183
" 22	" "	149	No litter.	149 " " " (heavy). Large blood spot under cage.	
" 23	" "	149	" " R.B.C. (faint).	R.B.C. (heavy). Litter. 1 ♀, 3.4 gm.	
" 24	" "	151	" "	153 Littering continued. 1 ♀, 4.5 gm., partly eaten; 1 ♀, 4.5 " " " ; 1 ♀, 4.5 " still alive.	150
" 25	Cornif.	149	" " R.B.C. (faint).		
" 26	LE and C.		" " " "		
" 27			Litter, 1 dead, partly eaten; 1 dead, body eaten.	149	

0 = epithelial cells only, in heat toward end; 0–1 = epithelial and cornified cells, in heat; cornif. = cornified cells only; cornif. and leuc. = cornified cells and leucocytes; LE = diestrus; R.B.C. = red blood cells which appear about the 14th day of a normal gestation and disappear within 2 or 3 days. The presence of red blood cells proves that positive fertilization and implantation have taken place.

became wheezy and sick and failed to gain any weight. Resorption occurred and the animal died 2 weeks later. The other five animals (Rats 27181, 27259, 27176, 27182, 27154) had an average gain of 55 gm. during gestation as compared with 12 to 17 gm. for the three animals receiving Fraction AD + Fraction E instead of 2 drops cod liver oil + 250 mg. of wheat germ. Rat 27181 had a litter of only two (females), weighing 5 gm. each. These were eaten the 2nd day. The other four rats produced four litters, totaling twenty-five young, with an average weight of 5.3 gm. The young were all lactated successfully with no loss of weight by the mothers. The weaning weights were small (average weight = 29 gm. on 21st day) but the young were in good condition.

Although this vitamin E preparation keeps the healthy animals which receive fat fertile, in order to prove that the vitamin E was actually retained by the animals on low fat diet the above test was necessary. It appears that we are justified in concluding that the vitamin E was absorbed in the absence of the dietary fat and that the very poor gestations were due to the poor condition of the females.

It seemed that males might be more useful than females for testing this point. Their tests simply show whether or not they are capable of mating and fertilizing the eggs of normal stock females, without the uncertainty as to whether failure in gestation is due to a lack of vitamin E or to the general poor condition of the animal. Two groups of males have been thus tested (sixteen in one group and ten in another). Normal young stock females, showing the 0 or 0 to 1 stage of the ovulation cycle, were put with the males and examination was made the next morning for plug and sperm. If a positive mating took place the gestation was followed in the usual way, except that daily smears were taken. Each male in the group of sixteen animals was given four chances to mate, unless a positive mating resulted sooner. Only normal females were used. That is, if a female failed to show positive fertilization after a positive mating, the animal was tested with a normal stock male. Each male of the group of ten was given six chances to mate unless positive mating occurred sooner. The results are summarized in Table VII. The difference between those males receiving 10 drops of lard and those receiving no fat is remarkable. A new and uniform cause of sterility is shown. The

normal sex responses have been lost in most cases, while in vitamin E sterility the sex responses are retained long after the loss of the

TABLE VII.

Comparison of Mating Histories of Male Rats Which Received 10 drops of Lard with Those of Males on Complete Fat-Free Diet 550 B + 0.65 gm. Yeast + Fraction AD + Fraction E.

Total attempted matings = the total number of females in estrus put with the males of each group. Total positive matings = total number of females which showed plug and sperm (or just sperm) the day after mating. Total R.B.C. = total number of positive fertilizations.

Group No. and diet.	Total No. of rats.	Age.	Average weight.	Total attempted matings.	Total positive matings.	Total R.B.C.	Total litters.	Per cent of males proved fertile.
		mos.	*gm.*					
Group 58. Diet 550 + Y* + Fraction AD + Fraction E.	5	5–6	202	17	7	0	0	0
Group 59. Diet 550 B +Y+ Fraction AD + Fraction E.	6	5–6	199	22	0	0	0	0
Group 65. Same as for Group 59.	5	4	147	30	0	0	0	0
Group 60. Same as for Group 58 + 10 drops lard.	2†	5–6	255	5	1	1	1	50
Group 61. Same as for Group 59 + 10 drops lard.	2	5–6	251	2	2	2	2	100
Group 63. Same as for Group 61.	5	4	226	10	6	5	5	100

* Y designates the daily dose of 0.65 gm. of ether-extracted yeast.

† Rat 29541 was a nervous, abnormal animal which refused to mate in four attempted matings. The other, Rat 29025, mated the first time.

seminiferous epithelium (6). The testis is small and watery but the histology is not yet known. The animals were small but their condition was fairly good at the time of the tests (4 to 6 months

old). Yet the effect on the animal is so profound that in only three cases were there positive matings and in no case was there a litter sired. Of the nine animals receiving 10 drops of lard, eight sired normal litters and the ninth animal was nervous and somewhat abnormal in general behavior.

It is impossible to know from this experiment whether vitamin E was absorbed by the animals on the fat-free diets, while it is certain that the animals receiving 10 drops of lard received a sufficient supply of vitamin E. Those three animals in the high protein group which showed a total of seven sterile matings may be sterile from a lack of vitamin E. Even if this proves to be true, the sterility must nevertheless be considered as primarily due to the lack of fat in the diet since the same quantity of vitamin E in the presence of 10 drops of lard is adequate. The restoration of the normal sex responses by the addition of lard to the diet is now being tried.

The testicular hormone, which is responsible for the development of secondary sex characters and possibly responsible for the normal sex responses, is also a lipoid if the solubility of the impure substance is a correct indication. Its purification can be effected by very much the same procedure as that used for the ovarian hormone (7). It is possible that the almost complete loss of sex response by the males on the low fat diet is due to the failure of this hormone to be synthesized in the absence of the essential fatty acids.

Nature of Essential Fatty Acids.

Since the new disease is caused by a lack of the fatty acids present in lard, the problem of the comparative value of the acids known to be in lard was next attacked. It is well known that the preparation of pure fatty acids is quite difficult. But a greater drawback to the use of individual fatty acids is found in the isomeric changes of the unsaturated acids when isolation is attempted. All workers recognize the fact that the acids isolated by the bromination method may not have exactly the same structure that they had in the natural oil. Therefore, a series of oils was chosen which would give a variety of fatty acid combinations, as shown by the best available analyses. On the basis of the results of Ellis and Isbell (8) it was assumed that the pig can readily synthesize palmitic, stearic, and oleic acids from carbohydrates but

that linolenic and linoleic acids may be entirely of dietary origin. Arachidonic acid did not show such a definite trend but the results indicate that it may be largely of dietary origin.

The best available analyses of a selected group of fats and oils used in curative work are assembled in Table VIII. Fortunately we are able to get two fats (coconut oil and butter) of high digesti-

TABLE VIII.

Approximate Fatty Acid Content of Oils and Fats Used for Cures.

Butter fat and lard probably vary most widely from these figures.

	Coconut oil (9).	Butter fat (10).	Olive oil (11).	Lard (8) (corn-fed hogs).	Corn oil (12).	Poppy-seed oil (13).	Linseed oil (14).
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Unsaturated acids.							
Oleic.	5.5	31.1	80.5	52.0	45.4	28.3	4.5
Linoleic.	1.3	0.0?	6.9	6.7	40.9	α29.5	α17.0
						β29.0	β41.8
Linolenic.	0.0	0.0	0.0	0.0	0.0	0.0	α20.1
							β 2.7
Arachidonic.	0.0	0.0	0.0	0.06	0.0	0.0	0.0
Saturated acids (total).	83.4	63.7	11.2	36.9	11.8	7.2	8.3
Lignoceric.					0.2		
Arachidic.			0.2	0.0	0.4		Trace (16).
Stearic.	3.0	11.4	1.4	12.2	3.5	Present (15).	Little.
Palmitic.	7.6	15.5	9.4	24.1	7.7	"	Present.
Myristic.	18.5	22.6	0.2	0.6		"	"
Lauric.	50.3	6.9				"	
Soluble (total).	4.0	7.3					

bility which contain little or no linoleic acid and no more unsaturated acids. Olive oil, lard, corn oil, and poppy-seed oil furnish increasing amounts of linoleic acid without any linolenic acid. Linseed oil is rich in linoleic and linolenic acids with very little oleic acid. Lard is peculiar in having arachidonic acid instead of arachidic acid which is present in most plant oils. The coconut oil, olive oil, lard, and corn oil were well known brands of refined

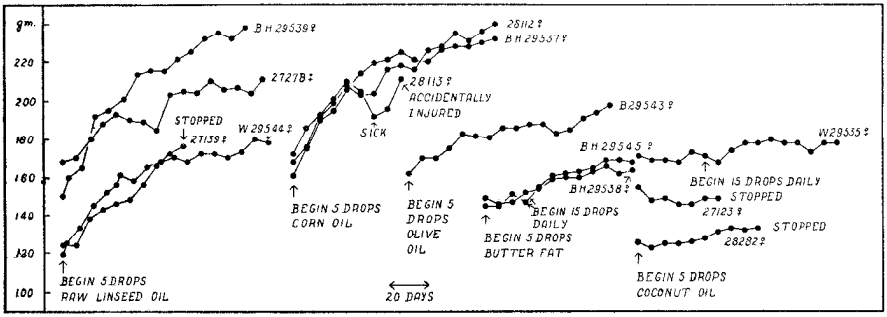

CHART 2. Weight curves showing the growth response of cures to the addition of five natural oils to the fat-free diet. Rats receiving linseed oil, corn oil, or olive oil were cured of scaly feet and dandruff on the back. Rats receiving butter fat or coconut oil showed no improvement of the skin.

products on the local market. Poppy-seed oil was purchased from Eimer and Amend. The raw linseed oil was obtained from a paint company. Butter fat was made by melting the highest grade butter in a moderately warm water bath.

As in previous work, the tests were made by the cure method. Young animals were put on the fat-free diet at weaning and after a few months growth was stationary, they had scaly feet and dandruff on the back, and were consuming more than normal quantities of water. Diet 550 B was used from the day of weaning so that growth would stop and the scaly condition supervene with as little degeneration of the kidneys as possible. The animals were then given the curative dose on yeast. The results of the first series of tests are shown in Chart 2. The older work with 10 drops of lard is not included (see (1) p. 363). The results are so clear cut that no extended discussion is necessary. Butter and coconut oil which contain very little linoleic acid and no more unsaturated acids allow little or no growth when fed at the 5 drop level and cause no clearing of the scale or dandruff. Olive oil takes an intermediate position, while corn oil and linseed oil are about equally good. The results with butter are of particular importance since the butter adds appreciable amounts of vitamins A and E to the diet without improving the animals' condition.

An examination of the composition of these oils in Table VIII leads to the conclusion that linoleic acid is the essential unsatu-

rated fatty acid, with the possibility that arachidic acid may also be involved. The failure of coconut oil might be attributed to its low content of C_{18} acids, which would not give the liver a chance for desaturation. This point was tested in the next group of experiments.

To measure again the relative value of saturated acids, oleic acid, and linoleic acid, it seemed desirable to have a readily digestible fat of complex composition but containing no unsaturated acids. Ozaki (17) has shown that the chief fatty acids of coconut oil, lauric and myristic, are equal to oleic acid in digestibility and growth-promoting power. Furthermore, coconut oil may be completely saturated by hydrogenation and still have a low melting point. This makes the hydrogenated oil an ideal basal fat. This product was very kindly furnished us by Dr. J. J. Vollertsen, Chief Chemist, Armour and Company. It was prepared under his direction especially for this work. The hydrogenated oil had the following constants: free fatty acids 0.04 per cent, iodine number 0.61. It melts gradually over a wide range and is completely liquid at 50°. Since all of the oleic acid and linoleic acid has been converted into stearic acid, the total stearic acid of the hydrogenated oil is 9.8 per cent.

Ozaki (17) has further shown that although the free fatty acids are generally poorly utilized the methyl and ethyl esters are well utilized. This is especially true of the saturated fatty acids. It was decided, therefore, to feed the individual fatty acids as their methyl esters. Methyl stearate (Eastman, MP 36–38°) was used as a source of stearic acid.

Methyl linolate was prepared by the method of Rollett (18) from the same sample of corn oil as was used for the earlier feeding experiment. Corn oil contains no more highly unsaturated acid so that an uncontaminated tetrabromide is obtained. The properties of the debrominated methyl ester agreed with those given in the literature. Rollett showed that this acid is not homogeneous but is a mixture of more or less definite proportions of α and β forms. There may be even more than two isomers present. But it was hoped that the linoleic acid thus prepared would have a considerable proportion of the acids as found in natural plant oils.

Two more animals were given 15 drops of butter fat daily to

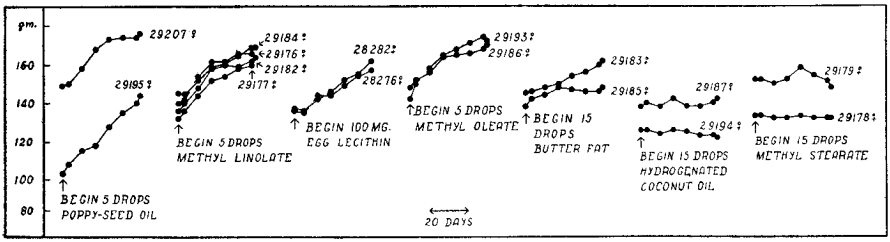

CHART 3. Weight curves showing the growth response of cures to some oils and special fatty substances. Poppy-seed oil and methyl linolate completely cured the scaly skins. Methyl oleate and egg lecithin partially cured the skin. Rats receiving butter fat, hydrogenated coconut oil, or methyl stearate showed no improvement of the skin.

check again the value of oleic acid. Since butter fat contains a minimum of 30 per cent oleic acid, the 15 drops (averaging 330 mg.) contributed 99 mg. of oleic acid to the daily diet.[4] This is about 1 per cent of the total food consumption. As a further test of oleic acid 5 drops daily of methyl oleate (Eastman, Practical, BP 189–191°/10 mm.) were fed to two other rats.

Another oil, poppy-seed oil, containing no acids more unsaturated than linoleic, was added to the list.

Another source of fatty acids which can be freed from most contamination is lecithin. The fatty acids of lecithin are mixed, possibly as badly mixed as they are in a whole oil, but the lecithin can be so purified as to dispel any illusion that the cure of the disease is due to "vitamins" or other indefinite substances mixed with the fatty acids. Egg yolk lecithin was prepared according to the method of Levene and Rolf (19). This lecithin is free of amino nitrogen and has the correct analysis except for a slightly high nitrogen content. Egg yolk lecithin contains the following acids: palmitic, stearic, oleic, linoleic, and arachidonic. The presence of linolenic acid is uncertain (20).

The results of feeding these substances are summarized in

[4] Medicine droppers of uniform tip have been used throughout this work. The following average values of the drops have been found: 10 drops lard = 232 mg.; 10 drops butter fat = 218 mg.; 10 drops hydrogenated coconut oil = 197 mg.; 10 drops methyl stearate = 185 mg.; 10 drops methyl linolate = 180 mg.

Chart 3. These tests confirm our earlier findings (Chart 2) that the curative effect of oils is due largely to acids more unsaturated than oleic acid. And the fairly good gains made on pure methyl linolate indicate that linoleic acid is highly important. Furthermore the rats receiving linoleic acid lost the scale and dandruff from their feet and backs. Failure to grow on 5 drops of butter followed by only slight growth on 15 drops upholds the view that the oleic acid in butter is not of great value as a cure for this disease. However, butter probably contains some linoleic or other unsaturated acid not precipitated by the ordinary analytical methods (21). If this is true, then the better growth of the rats on larger doses of butter fat is readily explained; otherwise we must assume that oleic acid is of some value. It should be pointed out, however, that even 15 drops of butter fat do not cure the scaliness of the feet and back. The butter causes a return to the condition found in the early life of the animal, slow growth taking place, while the feet and back are very scaly.

Since lecithin did not bring about a more rapid recovery than did the methyl esters of fatty acids, it seems quite clear that the animals do not need some special lipoids but only the fatty acids. It is true that the word lecithin covers a group of substances of variable composition and it is quite possible that other lecithins are more valuable than egg lecithin. Liver lecithin is now being investigated.

The high curative value of commercial methyl oleate is to be attributed to the presence of more unsaturated acids. Commercial oleic acid is made from olive oil which contains 7 per cent linoleic acid. In the preparation of oleic acid no effective methods are used to remove the linoleic acid present. A considerable amount of methyl linolate will distil with larger amounts of methyl oleate. In fact, on bromination in petroleum ether an appreciable precipitate was obtained and we know that the solubility of the tetrabromide in the presence of the dibromide is fairly high.

We must then conclude that this small amount of linoleic acid in the presence of oleic acid is almost as effective as a much larger amount of pure linoleic acid, while oleic acid alone is of much smaller value. Otherwise we would be forced to the conclusion either that the oleic acid of butter is different from that prepared from olive oil or that the other fatty acids of butter are actually

detrimental. These points are now being studied with larger numbers of animals.

Finally, the data show conclusively that the saturated acids of coconut oil and methyl stearate will not promote renewed growth or cure the skin, although they have high food value.

Chart 4 summarizes the average maximum gains made by the rats in 40 days after the curative doses were begun. All of the groups in Charts 2 and 3 are included. The average for the rats

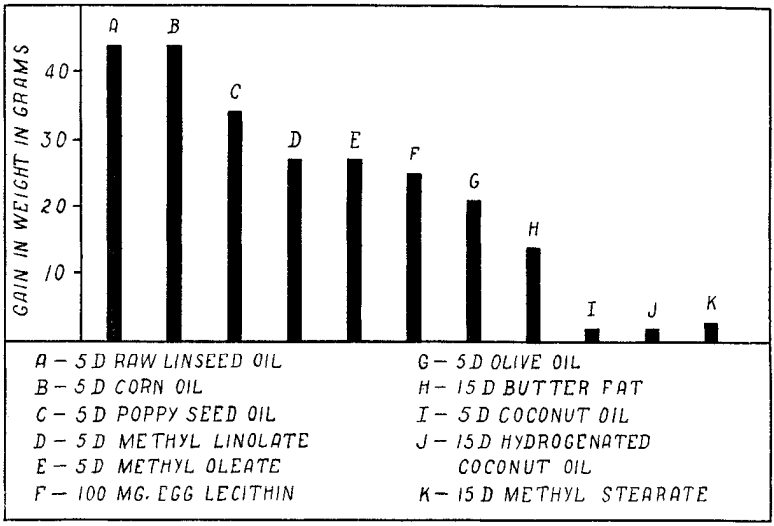

CHART 4. Mean maximum gains made in 40 days after feeding 5 to 15 drops of cure lipoids. Data from Charts 2 and 3.

receiving 15 drops of butter includes the data from both charts 2 and 3. It should be pointed out that the growth response to butter was due largely to the high level at which it was fed. When fed at the 5 drop level, the animals did not grow (Chart 2).

DISCUSSION.

Recently considerable emphasis has been placed on the fat requirements of the rat and mouse. Although the earlier work indicated that a fat-free diet was adequate in every respect (22, 23), the later work has not been in agreement with this point of

view. Hesse (24) found weight increases and increased oxygen consumption of mice on a bread diet after addition of fat and phosphatides. Wesson (25) found some interesting effects of the addition of certain fats to the diet. Jaffé (26) found that mice could not be supported on a diet of white bread, beef, and synthetic fat without the addition of a mixture of lipoids called Promonta. But the three works above were concerned especially with more complex lipoids rather than with fatty acids. Furthermore, the diets were not always balanced or the fat-soluble vitamins were not certainly present so that interpretation of the results cannot well be made. In 1928 Evans and Burr (27) showed a definitely subnormal weight and irregular ovulation on diets complete in every respect except for fats. Last year Burr and Burr (1) found that a definite deficiency disease resulted from the rigid exclusion of fats from the diet. At about the same time McAmis, Anderson, and Mendel (28) reported subnormal weights for animals on a low fat diet, thus supporting the view that fats are beneficial to the rat. Palmer and Kennedy (29) did not find fat beneficial but their control diets were not fat-free. 160 to 500 mg. of cod liver oil were fed daily and this amount is entirely adequate to protect against the low fat disease. Evans and Lepkovsky (30) have suggested that the beneficial effect of fat in the diet is apparent only when the antineuritic vitamin is low. But their work is not a critical check on the effects of small amounts of fat in the diet since they also fed 2 drops of cod liver oil daily along with several drops of wheat germ oil. The deficiency disease reported by Burr and Burr (1) occurs regularly when an excess of vitamin B is fed (0.65 gm. of dry whole yeast daily) and is not cured by an increase of 30 per cent in the yeast dose. The present authors showed in their earlier paper that the fatty acids were the essential part of the fat and that those acids in lard were adequate. In the present paper it has been shown that only the unsaturated acids will cure the sick animal and that pure linoleic acid is far better than the oleic acid of butter.

The finding that saturated fatty acids do not cure the low fat disease or promote renewed growth while certain unsaturated fatty acids do, leads to the conclusion that this work is not concerned with the fat minimum of Krogh and Lindhard (31). Their conclusions were based upon quantitative data, and all fats of respira-

tory quotient 0.71 and equal digestibility would be of equal value. But for the present work fats are not of equal value and the differences are due to the structure of the fatty acids. We have thus taken another step away from Rubner's law of isodynamic equivalence, and chemical structure in the fats has assumed some of the same importance as in the proteins (32). For the rat, a typical warm blooded animal, unsaturated fatty acids are essential constituents of the diet.

Evidence has been presented to show that oleic acid as found in butter and coconut oil is of little value, while linoleic acid, in oils and isolated, is of great value in the diet. Linolenic acid and arachidonic acid are now being studied in isolated forms. Ordinary arachidonic acid does not occur in the oils of highest curative value (corn oil and linseed oil) in our series. Thus it would seem to be not essential in the diet. But the importance of arachidonic acid as an intermediate in metabolism has been emphasized recently by Wesson (33). This importance has been hypothetical, based upon the indirect evidence of its occurrence in various tissues. Certainly the arachidonic acid content of active tissues such as liver, pancreas, kidney, suprarenal, and spleen is high (33, 34) and it is natural to assume some important rôle for this highly unsaturated, long chain acid.

In the absence of complete analytical data (which we are now collecting) no exact statement can be made as to the rate of fat synthesis in the presence of small amounts (100 mg. daily or less) of unsaturated fatty acids but this is of such interest that it should be mentioned here. The small emaciated animals on the fat-free diet consume as much food daily as their larger, fat-containing controls (Table IV), yet this food is burned and none is used in growth and fat synthesis. On the addition of the unsaturated fatty acids, growth is resumed and a normal amount of subcutaneous and visceral fat is found in the cured animal at autopsy. Therefore, fat synthesis is dependent upon the presence in the tissues or blood stream of a certain minimum quantity of the essential fatty acids. What percentage of the growth observed is due to storage of fat and what percentage is due to increase in other compounds (protein and carbohydrate) is not yet known.

This brings up the interesting question of desaturation by the liver. Since the work of Leathes (35) it has been generally ac-

cepted that the liver readily desaturated all the fatty acids necessary for the organism's needs. But the careful work of Ellis and Isbell (8) points to the view that the ability of the warm blooded animal to produce unsaturated fatty acids may be quite limited and that oleic acid represents the greatest degree of unsaturation attainable over long periods of time. In discussing these results Bloor stated, "the extent of the ability of the liver to desaturate is not known and it is possible that the ability of the animal body to synthesize fatty acids may be limited" (36). The results of Ellis and Isbell are of course concerned only with the rendered lard and do not give us values for all tissue fats. But other feeding tests indicate that most animal fats become more saturated the freer the diet is from unsaturated acids. McAmis, Anderson, and Mendel (37) fed rats a high sucrose, fat-free diet and rendered the fat of the entire animal. This fat had an iodine number of 64 to 71, a fairly normal value for lard. In a more extensive paper Eckstein (38) showed that the rat produced fat of about the same iodine number and saponification number from diets high in carbohydrate or high in protein. The iodine values ranged from 62 for the skin to 74 for the organs. It would seem, then, that the rat, like the pig, tends to synthesize a relatively constant body fat which contains very little of any acid more unsaturated than oleic. Eckstein's work shows this very clearly in the case of arachidonic and linoleic acids. Although the total fat of young rats (less brain and gastrointestinal tract) at weaning (40 gm. in weight) contains 1.06 per cent arachidonic acid and 3.21 per cent linoleic acid, after the test animals had been kept on a low fat diet for 8 weeks these values had fallen to 0.12 per cent and 0.31 per cent respectively. Linoleic acid thus reached a much lower value than that found for lard from pigs on brewers' rice (8).

Butter fat has been carefully studied by Holland and coworkers (10). Butter production represents a long continued, high rate fat synthesis from materials in the blood stream and Holland *et al.* have found that although the type of fat produced is not affected by the protein-carbohydrate ratio of the diet, the addition of oils to the diet has a very material effect. Their cows were kept on a hay-grain basal ration containing about 2.5 per cent extractable fat (composition unknown). Cows on this diet produced a butter fat of low iodine number (about 28) and low oleic acid content

(30.7 per cent). On adding 0.75 pound of coconut oil daily no appreciable change in iodine number or oleic acid content took place, but when like quantities of peanut oil, corn oil, or soy bean oil were fed, the iodine number rose and the oleic acid content increased to 42 to 46 per cent. Linoleic acid was not definitely determined to be present or absent. It seems quite definitely demonstrated that butter fat produced *de novo* by the cow from non-fats probably would contain a definite minimum of oleic acid (about 30 per cent) and no more unsaturated acids. Maynard and McCay (39) have recently given evidence supporting this view.

Finally, there is the much earlier work of McCollum, Halpin, and Drescher (40) which was concerned with the phospholipids of hen's eggs and is therefore of even more vital interest. The production of egg yolk by hens is another case of long continued and rapid lipoid synthesis. The above workers showed that when hens are maintained on a low fat ration the iodine number of the phospholipid falls from a normal of 63 to 34, while for the ordinary fats it falls from 64 to 52. The phospholipids are more seriously affected than the ordinary fats, and again it would seem that another warm blooded animal is definitely limited in its ability to synthesize highly unsaturated fatty acids.

But the degree of this limitation was hardly suspected until the results of the present paper were obtained. It has been definitely shown that when a rat has been reared on a fat-free diet its store of unsaturated acids has been so depleted that its health suffers severely. And the feeding of 15 drops (3 per cent of the total diet) of the mixed fatty acids of hydrogenated coconut oil or of a like amount of methyl stearate does not make possible the production of the required unsaturated acids. The actual minimum of linoleic acid required, as indicated by the response to lard, corn oil, methyl linolate, egg lecithin, and impure methyl oleate, is so small that it seems probable that the liver of these animals is unable to produce any linoleic acid. Our working hypothesis is, therefore, that warm blooded animals in general cannot synthesize appreciable quantities of linoleic acid and some of the other highly unsaturated acids. Furthermore, it is assumed that these unsaturated acids are normal constituents of essential cellular phospho-

lipids and that the more active the tissue the greater the requirement for the unsaturated phospholipids (36, 41).

This conclusion does not reject the work of Leathes. It must be remembered that he observed apparent desaturation of ingested oils by normal livers. The two results are harmonized by either of the following conditions: (1) desaturation of oleic acid does not produce linoleic and linolenic acids but produces other substances which absorb iodine; or (2) the presence of undetermined acids is essential to the desaturation process and animals long deprived of such acids lose this function of the liver. The first condition is illustrated by the production of hepatic oleic acid discovered by Hartley (42).

Whichever hypothesis is correct, the end result is the same. The supply of unsaturated acids in the animal is soon depleted and the tissues begin to suffer. Oils are reputed to have a beneficial effect upon the skin and we find that the skin soon becomes scaly in the absence of the unsaturated acids. The other organ which apparently suffers most is the kidney. This result is to be expected when we remember that the kidney is the most active of semipermeable, secretory membranes and that membrane structures contain intimately associated lipoids (43). As shown by McCollum, Halpin, and Drescher, even the phospholipids may become more and more saturated. Hydrogenated lecithin differs markedly from ordinary egg lecithin. The amount of lecithin might remain quite constant, while its value as a membrane constituent would be lost. Furthermore, oxidation activity has been ascribed to the kidney in addition to its membrane activity. Much work has been done to show that the highly unsaturated acids may play an important rôle in biological oxidations. We are led to the view, therefore, that the uniform failure of the kidney in these rats is due to a lack of unsaturated fatty acids essential to normal kidney tissues.

The results with moderately high and low protein diets indicate that high protein diets aid in the breakdown of the kidney. The most recent workers on this subject have come to the conclusion that exceedingly high protein diets cause the kidneys to hypertrophy but do not produce any recognizable nephritis (2–4). It would then seem probable that on a complete diet high protein intake will not break down the rat kidney in a period of a year but

if the diet is subnormal in other respects (low in unsaturated fatty acids) high protein intake may hasten the degeneration.

The authors are aware that great care should be exercised in drawing conclusions concerning human nutrition from results with rats. It seems worth while to point out, however, that the human diet is often exceedingly low in fats of any kind and that when fats are added they usually contain little of the acids more unsaturated than oleic. Butter and coconut oil are the chief table fats and beef fat is probably equally poor as a source of unsaturated acids. It is possible that our high carbohydrate and protein diets, carrying very little of the unsaturated oils, are contributing factors to poor health. The addition of egg yolk and cod liver oil to diets may often improve the patient because of the fatty acid rather than the vitamin content. For example, cures of anemia with cod liver oil have been reported and it has been shown that there is a relation between experimental anemia and the unsaturated fatty acids of the blood plasma (44, 45). The prevalence of dry skins and abnormal kidneys may be directly attributable to improper fat intake. The nerve tissue, kidneys and other organs contain several unsaturated acids. If the liver is limited in its ability to produce these acids, they should be plentifully supplied through the diet.

CONCLUSIONS.

1. Fat-free diets regularly produce kidney lesions in the rat, which have been observed in every case at autopsy. Kidney degeneration probably causes the death of the rat in most cases.

2. High protein diet seems to increase the severity of the kidney degeneration so that bloody urine appears more frequently.

3. The small emaciated animals on fat-free diets drink twice as much water as their controls and eat the same amount of food. The excess water is not lost through the urine.

4. Ovulation often is irregular or ceases entirely in fat-free animals. When a curative oil is fed, ovulation is resumed within a few days.

5. Vitamin E is not a controlling factor in the disease resulting from fat-free diet, but may affect the size of the males somewhat.

6. Female rats on the fat-free diet will mate when ovulation

occurs and will produce litters. The very poor litters are attributed to the general poor condition of the mother.

7. With few exceptions, males on a fat-free diet will not mate, while their controls, receiving 10 drops of lard, mate and sire normal litters. Those males fed fat-free diets, which do mate, cannot sire litters.

8. In this new type of sterility the normal sex responses are lost, while in the sterility resulting from lack of vitamin E the sex response is retained after the loss of the seminiferous epithelium.

9. Rats suffering from the low fat disease are not cured by the saturated fatty acids (stearic, palmitic, myristic, lauric, or lower acids).

10. These same rats are cured by linoleic acid (either isolated in pure state, or in olive oil, lard, corn oil, poppy-seed oil, linseed oil, or egg lecithin).

11. The phospholipid, egg lecithin, is of no more value than pure methyl linolate.

12. Oleic acid in butter and coconut oil is of doubtful value but certainly it is not equal to linoleic acid.

13. Complex, unsaturated oils (like corn oil, linseed oil, cod liver oil) appear to be more effective curative substances than a single fatty acid or phospholipid.

14. High grade butter fat (3 per cent of the total diet) does not cure the skin condition, supporting the view that vitamin A and vitamin E deficiency is not involved.

15. The hypothesis is put forward that warm blooded animals in general cannot synthesize appreciable quantities of linoleic acid. The synthesis of other unsaturated acids, including linolenic, is probably equally limited.

16. Linoleic acid (and possibly other acids) therefore is an essential fatty acid.

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