A BIOLOGICAL METHOD FOR THE DETERMINATION OF ESSENTIAL UNSATURATED FATTY ACIDS AND ITS APPLICATION TO VARIOUS ANIMAL AND VEGETABLE FATS

THESIS

Submitted to the Faculty of the Graduate School of
Michigan State College
in Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy

by

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December 1938

ACKNOWLEDGMENT

The author wishes to express his indebtedness and gratitude to Dr. C. A. Hoppert, Professor of Chemistry for his advice and assistance during the course of this study and in the preparation of this manuscript.

Since early workers had given ample proof of the ability of an animal to synthesize fat, it was a matter of indifference whether fat was present in the diet or not, after the provision for the proper amount of protein. In fact the value of fat in the diet was usually associated with the accessory food substances carried by them.

In 1920 Osborne and Mendel (1) conducted experiments on the value of fat in the diet of the healthy mammal, and concluded that if both the water soluble and fat soluble vitamins were present, the amount of fat necessary for optimal growth must be very small. In 1927 Evans and Burr (2), (3) found that diets freed from fat, but containing adequate amounts of vitamins A, B, D and E were not capable of maintaining normal growth and lactation. Ovulation was also irregular. By including fats such as lard, butter, coconut oil and corn oil in the diet, normal growth and lactation resulted. The response was, however, negative when stearic acid was fed. Separation of the above lipids into non-saponifiable matter, glycerol and fatty acids indicated that the active principle was present in the fatty acid fraction.

Subsequently McAmis, Anderson and Mendel (4) fed a fat free, but otherwise well balanced diet and observed comparatively good, although not optimal growth, a poor condition of the fur, bloody urine and also a peculiar eye condition resembling the beginning stages of Xerophthalmia. Addition of fat in the form of one drop of peanut oil per day produced better growth. The authors concluded that the question whether the beneficial effects of a small amount of fat were due to its content of vitamin A or other vitamins, or to its action as a vehicle for fat soluble vitamins, or whether fat itself was essential had not been settled.

Simultaneously Burr and Burr (5) presented a possible new role for certain fatty acids in the animal organism. They fed fat free diets and changed their nutritive ratio from 1 : 3 to 1 : 5 to 1 : 7 as the animals increased in weight. Between the seventieth and ninetieth day the fat deficiency syndrome appeared. First there developed an abnormal condition of the skin, later the tip of the tail became swollen and inflamed, and soon the whole tail was heavily ridged and scaled. Hemorraghic spots frequently appeared in the skin throughout the entire length of the tail. The swelling at the tip was often gradually replaced by a true necrosis resulting in the loss of one to three centimeters of the tail. The hind feet became red and somewhat swollen, and in some cases large scales developed over the dorsal surface. The hair on the back of the body became filled with dandruff and there was a tendency to lose hair around the face, back and throat. Sores often appeared on the skin. The early outward signs of an unhealthy condition were followed by a decline in weight at about the fifth month and unless fat was given to the animal it died within three or four months. The urinary tract and kidneys were extensively involved and this undoubtedly was an important factor in the death of the animal.

Although the fat deficiency syndrome had some features in common with those produced in pellagra and vitamin A and B deficiencies, the former condition had a different and distinct etiology because it yielded to the addition of fat to the diet.

In another paper the same authors (6) claimed that the most sensitive test of the fat deficiency disease was the scaliness of the

feet, followed by dandruff in the region of the neck and back. The kidneys were grossly abnormal, and showed a degeneration different from that due to vitamin A deficiency. The kidney disorder was neither cured nor prevented by the addition of vitamins A and D prepared from cod liver oil, but was cured or prevented by the addition of a mixture of fatty acids or the methyl ester of linoleic acid.

Disturbance of the pituitary was evidenced by the abnormally high consumption of water. Irregular ovulation and infertility were not corrected by the addition of vitamin E, although certain oils and fats were curative. It was found that saturated fatty acids including stearic, palmitic, myristic, lauric or lower members of the saturated series were ineffective. However, five drops per day of methyl linolate, and five drops per day of the following oils, corn, raw linseed, olive, poppyseed, and a hundred milligrams of egg lecithin, all of which contain linoleic acid were curative. This evidence pointed to the indispensability of linoleic acid for nutrition.

Previous studies had left the value of oleic acid uncertain, but in 1932 Burr, Burr and Miller (7) proved this acid to be ineffective in the curing of the fat deficiency disease. In addition linolenic acid and linoleic acid were shown to be equally effective whereas eleostearic acid, an isomer of linolenic acid, and arachidonic acid were apparently ineffective.

Turpeinen (44) later found, however, that arachidonic acid was a powerful curative agent, about three times as effective as methyl linolate. Linoleyl alcohol also showed some curative properties, but

was less effective than linoleic acid, whereas erucic, ricinoleic, $\Delta^{12,13}$ oleic, and chaulmoogric acid proved to be ineffective.

In 1938 Burr, Kass, Brown and Frankel (53) stated that arachidonic acid was of high curative value, that corn oil was superior to linseed oil, and that α linoleic acid was a better curative agent than the β linoleic acid.

Evans and Lepkovsky (8) partly confirmed Burr and Burr's work and found that the specific symptoms of the fat deficiency disease were cessation of growth and hematuria, but not dermatitis and tail necrosis. Liquid acids of coconut oil and linoleic acid were curative, but oleic acid was not. Even when the carbohydrate of the diet was replaced with glyceryl laurate (9), the rats showed deficiency symptoms, and not until linoleic acid was added did the rats return to a normal condition.

Evans and Lepkovsky (10) showed further that the presence of linoleic acid in the body fat of rats was dependent upon its inclusion in the dietary fat.

Evans, Lepkovsky and Murphy (11), (12), (13) made interesting studies upon the effect of a fat deficiency upon reproduction and lactation. Successful gestation and lactation was impossible, and sterility invariably occurred in males on the fat free diets, even when large amounts of vitamins A, D and E were supplied. The addition to the diet of the effective or so called essential fatty acids not only prevented but also corrected the above conditions.

Maeder (14) confirmed these investigations and observed that the reproductive process was impaired in the early stages of a fat deficiency,

but that ovulation was affected later. Both responded promptly to fatty acid therapy.

Numerous other workers have presented evidence to confirm Burr and Burr's work. Borland and Jackson (15) made a study of the kidney lesions; Sinclair (16) substantiated the results on growth and general symptoms; Tange (17), (18) reported that linolenic acid was as effective as linoleic, whereas oleic acid was ineffective; Becker (19) and Sahashi (20) emphasized the essential nature of linoleic acid; and Green and Hilditch (21) from a study of data on hog and rat body fats as well as on milk fats, concluded that linoleic acid when present in the glycerides of land animals was a product of assimilation and not of synthesis by the animal. Spadola and Ellis (22) found that the presence of linoleic acid could not be demonstrated in the adipose fat of rats fed a low fat diet. However, when as little as 0.2 percent of the acid was present in the diet, it could be readily detected in the storage fat.

The general conception that linoleic acid is essential has been challenged by a number of workers, Hume and Smith (23), Funk and Caspe (24), Roche and Roche (25) and Gregory and Drummond (26). Burr and Brown (27) commented upon the above investigators findings. Hume and Smith admit that the supply of vitamins B_1 and B_2 was inadequate for good growth, nor did the animals always receive sufficient vitamin A because bladderstones developed in some cases. The experimental diet used by Funk and his associates was somewhat deficient in vitamin B, and that used by Roche and Roche must also have been

deficient because the rats weighed only from 80 to 100 grams at twelve weeks. Gregory and Drummond used a yeast extract which was apparently deficient in one or more of the water soluble vitamins. Burr and Brown (28) have observed furthermore that the scaly condition of the feet and tails of rats was decreased by increasing humidity, and it is quite probable that this might have been the reason why some workers failed to produce the skin symptoms with fat deficient diets. It would appear, therefore, that the lack of agreement among various investigators has been satisfactorily accounted for.

An abnormal respiratory quotient above one, which is indicative of the transformation of carbohydrate to fat was noted by Wesson (29) in rats ingesting a fat deficient diet. However, in spite of this fat formation, the symptoms of the fat deficiency disease were not relieved, indicating that the curative linoleic and linolenic acids are not formed. This was confirmed by Wesson and Burr (30), Burr and Beber (31), (32), (33) and Wesson (34). Burr and Beber also report a higher basal metabolic rate and a higher specific dynamic action of food for rats on a fat deficient diet.

In the early studies of Burr and Burr (6) evidence was presented to show that butterfat was not an effective curative agent for the fat deficiency condition. This work indicated then that butterfat was deficient in certain unsaturated fatty acids. Hilditch and Sleightholme (35) found that some butterfats contained as much as 4.25 percent of linoleic acid, whereas Holland and co-workers (36)

and Bosworth and Brown (37) failed to verify its presence. Eckstein (38) working with Michigan butter reported approximately 0.2 to 0.5 percent of linoleic acid and an average of 0.13 percent of linolenic acid. Eckstein showed that the linoleic acid content of butter was related to the amount of this acid ingested by the cow. Recently Hilditch and Thompson (39) reported as much as 6 percent of linoleic acid in butter when linseed oil was consumed by the cow.

Since the evidence favors the essential nature of linoleic acid, consideration has been given to the occurrence of this fatty acid. Burr and Burr (6) indicated roughly the linoleic acid content of the oils and fats used in their experiments. According to the analysis recorded by Jamieson (40) linoleic acid is present in practically all vegetable oils. Hard lard contains roughly 1 to 7 percent whereas oily lard may contain as much as 36 percent of the acid (41). It is also present in egg fat to the extent of 15 to 19 percent (42). According to Hilditch (43) hen fat contains 22 percent, beef tallow 3 percent and mutton tallow 4 percent of linoleic acid.

The observations of Burr and Burr (6) that skin changes develop in rats fed diets lacking in certain unsaturated fatty acids suggested to Hansen (45) that infantile eczema might be partly dependent upon this type of a dietary deficiency, since he found that the serum fatty acids of eczematous infants contained less unsaturates than are present in the blood of normal healthy infants. Hansen (46), therefore fed corn oil and linseed oil to these eczematous children and observed improvement in their condition. Cornbleet and Pace (47) also obtained

favorable results when refined corn oil was administered to adolescent patients for a long time. Taub and Zakon (48), however, did not find any beneficial action when linseed oil was given to eczematous patients.

Boissevain (49) and Platonov (50) claimed that the unsaturated fatty acids including linoleic and linolenic acids rendered the tubercle bacillus less virulent. Platonov suggested that the unsaturated fatty acids deserve important consideration in the dietetic therapy for tuberculosis.

Studies on different animal species including man, with a variety of dietary fats have presented ample proof that food fat is readily digestible. Langworthy (51) has shown that the common fats are utilized from 93 to 98 percent, and Levine and Smith (52) report that rats fed fat which furnished 86 percent of the total energy intake, utilized 98 to 99 per cent of the ingested fat.

In the literature cited above the necessity for the essential unsaturated fatty acids has been demonstrated. However, with the exception of a recent report by Burr (53) little or no effort has been made to biologically assay the common fats and oils for their content of the essential fatty acids. It was felt that the method previously used for the testing of the various fats and oils was too cumbersome in that the animals had to be kept upon the basal diet until a growth plateau was reached. This required on the average five months. Subsequently the fat to be tested was fed and the gain in weight noted during a period of forty days or more. The time required for such a test was consequently at least six months.

It seemed highly desirable therefore to find a shorter, biological method of analysis and to assay the common fats and oils for their total essential unsaturated fatty acid content.

Experimental Procedure

Preparation of the materials tested.

In this study linoleic and linolenic acid were prepared from soybean oil and linseed oil respectively.

The fatty acids were fed in the form of the methyl esters since Lepkovsky and Evans (54) have shown that these esters are utilized by the rat even at comparatively high levels with almost the same ease as glycerides.

Methyl linolate was prepared from soybean oil by the following method. The fatty acids were obtained by acidifying the saponified oil, and the saturated acids were removed by the crystallization method of Brown and Stoner (55). The unsaturated fatty acids were then brominated by the method of Rollet (56). The resulting tetrabromostearic acid was recrystallized several times from boiling petroleum ether until a preparation melting sharply at 113.5–114° C was obtained. The debromination of this compound was carried out according to the method of Rollet, and the methyl ester distilled under reduced pressure in an atmosphere of nitrogen. A small amount of an antioxidant, hydroquinone, was added to the ester and it was stored in an atmosphere of nitrogen in a refrigerator. The final product had an iodine number (Hanus) of 177.0, calculated 173.0, Rollet (56) 171.6.

Methyl linolenate was prepared from linseed oil according to the following method. The fatty acids were obtained by the usual method, dissolved in anhydrous ether and brominated according to the method of Rollet. The resulting hexabromostearic acid was washed

several times with ether until a sharp melting point of 181-181.5° C was obtained. After debromination the methyl ester was distilled under reduced pressure in an atmosphere of nitrogen. The final product was stored in the same way as above. It had an iodine number (Hanus) of 256.4, calculated 261, Rollet (56) 257.5.

Basal low fat diet

Casein, edible #453	18 per cent
Sucrose	68 per cent
Salt Mixture	4 per cent
Yeast	4 per cent
Yeast, Irradiated	1 per cent
Alfalfa leaf meal	5 per cent

In the preliminary work vitamin free casein was used, but it was found that the fat deficiency symptoms could be produced just as quickly with the less expensive casein. In this connection it was of interest to know the lipid content of the basal diet. Accordingly a sample was extracted with ether and it was found to contain 0.22 per cent of ether extractable material which had an iodine number (Hanus) of 107.9.

Symptoms of a fat deficiency

In preliminary experiments it was observed that albino rats fed the above low fat diet developed a scaliness of the tail, appearing first at the tip, and then advancing gradually toward the base. Eventually the lower half of the tail became heavily ridged, the tip often becoming inflamed. In a few instances sores appeared along the surface of the tail. Scales also developed on the feet,

appearing first between the toes, usually of the hind feet and later along the dorsal surface. Gradually the leg became heavily scaled. The initial symptoms developed in a few cases as early as a week after weaning, although usually two to three weeks were necessary. All the animals showed definite symptoms at the end of four weeks. In the course of this work it was found that the humidity was an important factor in determining the time necessary for the appearance of the scales. In the summer when the humidity ranged from fifty to eighty per cent, three to four weeks were required whereas at other seasons when the humidity was quite low, two weeks were sufficient to develop the initial symptoms. The degree of scaliness varied somewhat among the different animals and litters. No appreciable difference was observed between males and females with respect to the development of the characteristic symptoms or their disappearance when curative measures were applied.

In the preliminary experiment various levels of butterfat and Wesson oil (refined cottonseed oil) were incorporated into the basal diet. 1, 2 and 4 per cent levels of butterfat were used whereas the levels of Wesson oil were 0.25, 0.50 and 1 per cent. Animals fed the butterfat produced the deficiency symptoms at all levels, whereas those fed Wesson oil did not, including even the lowest level. This difference in response was undoubtedly due to the difference of the essential unsaturated fatty acids and suggested a method for the biological determination of these acids.

Study of the maternal diet on the production of scaliness in the offspring.

In view of the fact that it is well recognized that the character of the maternal diet plays an important role in the appearance of the deficiency symptoms in the case of the fat soluble vitamins, it was considered necessary to study the influence of variations in the unsaturated fatty acid content of the maternal diet on the appearance of scaliness. Accordingly the stock ration and the same ration supplemented with 2.5 per cent and 5 per cent Wesson oil was fed to three groups of virgin females which were mated at the time the special feeding was begun. The offspring from the various groups were placed on the basal low fat ration at twenty days and kept under observation for the appearance of scaliness.

The results of these tests showed that the level of unsaturated fatty acids in the maternal diet had a pronounced effect on the time required for the appearance of scaliness. In the case of the stock ration, the symptoms appeared in two to three weeks; on the 2.5 per cent Wesson oil supplemented stock ration the time required was four to five weeks and at the 5 per cent Wesson oil level, seven to eight weeks. It is obvious, therefore, that the amount of essential unsaturated fatty acids in the stock diet has an important bearing on the production of scaliness.

Preparation of animals

Albino rats twenty or twenty-one days old, weighing at least thirty-eight grams were used for the assays. In a preliminary trial it had been found that when only slightly older rats were selected the deficiency symptoms were slow to appear.

The animals were then placed in pairs on the low fat diet and kept in cages provided with raised wire screen bottoms. They were weighed weekly and the condition of the feet and tail observed.

Conduct of the tests

After the rats were fed the low fat diet for three to four weeks and all showed the fat deficiency symptoms they were placed in single cages. The animals were arranged in groups of four with equal division of sexes and litter distribution wherever possible. The test materials were fed in separate dishes once a week and were usually quickly consumed. The dishes were fastened to the cage to prevent the animal from upsetting them.

Two techniques were used in this study. In both cases, the test material was fed at three or more levels. The first technique adopted, consisted of feeding the same amount of supplement per week throughout the experimental period of six to seven weeks. The rats were weighed weekly and the condition of the tail and feet noted. At the end of six weeks, the lowest level of fat or oil, which caused the complete disappearance of the scaliness of the feet and tail was considered as the effective level of test material.

It was found in some cases that the scaliness almost disappeared and then as the animal became larger, again increased. This was undoubtedly due to the fact that the requirements of an animal for a certain factor depends on the body weight. Consequently a certain level might give indications of eliminating the symptoms at a given stage and later when the animal had gained in size there would be a tendency for the symptoms to recur and become progressively more severe.

To overcome this difficulty a second technique was used. This method differed from the first in that the test material was fed weekly on the basis of grams of fat or oil per 100 grams of body weight.

In the course of these experiments it was observed that the scales on the feet were a more sensitive indication than the scales on the tail. Usually the scales on the feet appeared a little after those on the tail and disappeared sooner. In all cases negative controls were included and in these the scaliness on the tail and feet became progressively more pronounced. The negative controls did not weigh as much at the end of the experiment as the animals receiving fat.

Throughout the experiment the condition of the tail and feet were recorded by means of the following symbols.

Feet

± doubtful scales between the toes

\+ definite scales between the toes

++ scales between and on dorsal surface of toes

+++ scales between the toes, on dorsal surface and a few extending up the leg

++++ dorsal surface of feet and leg heavily scaled

Tail

±	doubtful scale at tip of tail
+	definite scales at tip of tail
++	scales extending about two inches from tip of tail
+++	lower half of tail heavily scaled and ridged slightly
++++	most of the tail heavily scaled and ridged

The following materials were assayed: Wesson oil, corn oil, olive oil, raw linseed oil, soybean oil, cod liver oil, halibut liver oil, wheat germ oil, linolic acid C.P., methyl linolate methyl linolenate, butterfat, oleomargarine, lard, crisco, duck fat, goose fat, tallow and egg yolk.

Results

The following data was compiled using the first technique. The test materials were: Wesson oil (a purified cottonseed oil), a vegetable oleomargarine, lard, tallow, cod liver oil, halibut liver oil, wheat germ oil, butterfat (winter), oat oil (Avenol) and goose fat.

TABLE 1

Supplement	Sex	Weight of animal at end of experiment	Condition at end of Exp. tail	feet
Wesson oil				
.05 gm per week	M	284 grams	++	++
	M	291	++	++
	F	220	++	++
	F	215	++	+
.10 gm	M	281	+	++
	M	234	++	++
	M	234	++	++
	M	246	++	++
.15 gm	M	315	++	+
	M	314	+	+
	F	212	+	±
	F	209	+	+
.20 gm	M	332	±	±
	M	287	±	±
	F	235	+	±
	F	217	+	±
.25 gm	M	285	±	-
	M	332	-	-
	F	220	-	-
	F	218	-	-
.30 gm	M	293	-	-
	M	282	-	-
	F	207	-	-
	F	227	-	-

TABLE 2

Supplement	Sex	Weight of animal at end of experiment	Condition at end of Exp. tail	feet
Oleomargarine				
1 gm per week	M	272 grams	+++	++
	M	272	+++	++
	F	164	+++	+++
	F	188	+++	++
2 gm	M	233	+++	+++
	M	212	++	+
	F	162	++	++
	F	176	++	++
4 gm	M	233	+	-
	M	249	+	+
	F	154	+	±
	F	189	+	±

TABLE 3

Lard				
1.0 gm per week	M	262	++	++
	M	222	+	+
	F	171	++	++
	F	164	++	+
2.0 gm	M	225	+	+
	M	259	++	+
	F	134	+	+
	F	191	+	±
4.0 gm per week	M	249	-	-
	M	222	-	-
	F	190	±	-
	F	190	-	-

TABLE 4

Supplement	Sex	Weight of animal at end of experiment	Condition at end of Exp. tail	feet
Tallow				
1.0 gm per week	M	218 grams	++++	++++
	F	150	++++	++++
	F	185	++++	++++
2.0 gm	M	166	++++	+++
	M	185	++++	++++
	F	170	++++	+++
	F	119	++++	+++
4.0 gm	M	251	+++	+++
	M	130	+++	+++
	F	154	++	++
	F	150	+++	+++

TABLE 5

Cod Liver Oil				
.5 gm per week	M	308 grams	++++	+++
	M	359	+++	++
	F	236	+++	+++
	F	228	+++	+++
1.0 gm	M	342	+++	++
	M	350	+++	++
	F	202	+++	+
	F	234	++++	+++
2.0 gm	M	327	++	+
	M	302	+++	+++
	F	256	++	+++
	F	254	++	+

TABLE 6

Halibut Liver Oil				
.25 gm per week	M	292 grams	++++	++++
	F	224	++++	++++
	F	217	+++	++++
	F	210	+++	++++
.5 gm	M	300	++	++++
	M	299	+++	++++
	F	220	++	++++
	F	210	+	+++
1.0 gm	M	306	++	+++
	M	268	++	++++
	F	213	++	++++
	F	218	+	+++

TABLE 7

Supplement	Sex	Weight of animal at end of experiment	Condition at end of Exp. tail	feet
Wheat Germ Oil				
.10 gm per week	M	302 grams	±	+
	F	214	±	±
	F	207	+	±
	F	200	+	±
.25 gm	M	323	-	-
	M	285	-	-
	F	222	-	-
	F	211	-	-
.50 gm	M	337	-	-
	M	342	-	-
	F	248	-	-
	F	234	-	-

TABLE 8

Butterfat (Winter)				
2.0 gm per week	M	286 grams	++	++
	M	293	++	++
	F	166	++	++
	F	183	++	++
4.0 gm	M	307	+	-
	M	276	±	-
	F	190	+	±
	F	193	±	±
6.0 gm	M	271	-	-
	M	297	-	-
	F	184	-	-
	F	171	-	-

TABLE 9

Oat Oil (Avenol)				
2.0 gm	M	293 grams	++	++
	M	304	++	+
	F	220	+++	+++
	F	213	+++	+++
3.0 gm	M	268	++	++
	M	295	++	+
	F	211	++	++
	F	207	±	+
4.0 gm	M	286	±	±
	M	299	-	-
	F	223	-	-
	F	214	±	±

TABLE 10

Supplement	Sex	Weight of animal at end of experiment	Condition at end of Exp. tail	feet
Goose Fat				
	M	310 grams	++	+
1 gm	M	296	-	-
per week	F	225	±	-
1.5 gm	M	290	-	-
	F	218	-	-
	F	210	-	±
2.0 gm	M	285	-	-
	M	300	±	-
	F	217	-	-
3.0 gm	F	220	-	-
	F	222	-	-
	F	218	-	-

TABLE 11

Negative Controls				
	F	180 grams	+++++	+++++
	F	185	++++	+++++
	F	199	+++++	+++++
	M	233	+++++	+++++
	M	235	+++++	+++++

The following materials were tested according to the second technique: Wesson oil, corn oil, duck fat, egg yolk, butterfat (summer), crisco, linolic acid C.p.*, raw linseed oil, soybean oil, methyl linolate and methyl linolenate. The fat was fed weekly on the basis of grams per hundred grams of body weight. Table 12 shows the course of an experiment.

* A product obtained from Eimer and Amend, New York, which contained at least 16 per cent α linolenic acid.

TABLE 12

Wesson Oil		Beginning of Exp.				1st week				2nd week				3rd week				4th week				5th week				6th week		
Supplement	Sex	wt.	T	F	wt. oil.	wt.	T	F	wt. oil.	wt.	T	F	wt. oil.	wt.	T	F	wt. oil.	wt.	T	F	wt. oil.	wt.	T	F	wt. oil.	wt.	T	F
.10 gm per	M	136	+	++	.14	176	+	3+	.18	214	+	++	.21	247	+	++	.25	269	+	+	.27	284	+	+	.29	314	+	+
100gm body	M	139	++	++	.14	182	2+	2+	.18	223	++	++	.22	250	+	++	.25	276	++	+	.28	303	++	+	.30	326	++	+
wt. / week	F	86	+	+	.09	104	+	++	.10	127	+	+	.13	135	+	+	.14	145	+	±	.15	159	+	±	.16	171	+	±
.125 gm per	F	114	+	++	.14	140	+	3+	.18	152	++	++	.19	162	+	+	.20	171	-	±	.21	178	-	-	.23	186	-	-
100gm body	M	115	+	+	.15	155	+	3+	.20	190	++	++	.24	209	+	++	.26	228	-	±	.29	253	-	±	.31	258	-	-
wt. / week	M	150	+	++	.19	189	++	3+	.24	235	++	3+	.30	258	+	++	.33	286	+	+	.36	307	+	±	.39	334	±	-
	F	115	+	++	.18	139	++	3+	.21	158	+	++	.24	170	+	±	.26	183	-	±	.27	200	-	-	.30	210	-	-
.15 gm	F	86	++	+	.14	123	++	++	.18	151	3+		.23	165	3+	++	.26	173	++	+	.26	186	+	-	.29	200	±	-
	M	120	+	++	.18	159	++	3+	.24	198	++	++	.30	216	++	++	.33	241	+	±	.36	265	±	±	.41	293	-	-
	M	141	3+	++	.28	159	3+	4+	.32	197	3+	3+	.40	215	3+	3+	.44	235	++	+	.42	256	+	±	.52	283	-	-
.20 gm	F	114	+	++	.22	132	+	++	.26	150	+	+	.30	160	+	±	.32	169	+	-	.40	180	+	-	.36	192	-	-
	F	113	+	++	.22	137	+	3+	.28	160	++	++	.32	181	+	++	.36	195	-	±	.36	211	-	-	.42	223	-	-

TABLE 13

Supplement	Sex	Weight of animal at end of experiment	Condition at end of Exp. tail	feet
Corn Oil				
.10 gm per 100 gm body wt. per week	F	199 grams	++	+
	F	167	+++	++
	M	311	++	+
.125gm	M	252	-	±
	M	278	-	-
	F	196	-	-
.15 gm	M	304	-	-
	F	185	-	-
	F	186	±	-
.20 gm	M	225	-	-
	M	217	-	-
	F	196	-	-

TABLE 14

Olive Oil				
.75 gm per 100 gm body wt.per week	M	300	+	+
	F	180	+	+
	F	238	-	-
	F	208	+	+
1.0 gm	M	309	++	±
	F	261	±	-
	F	227	+	-
	F	208	+	-
1.25 gm	F	207	-	-
	F	201	±	-
	M	289	-	-
	F	234	-	-

TABLE 15

Supplement	Sex	Weight of animal at end of experiment	Condition at end of Exp. tail	feet
Duck Fat				
.75 gm per 100 gm body wt.per week	M	225 grams	++	+
	F	185	++	+
1.0 gm per 100 gm body wt.per week	M	326	-	-
	F	231	-	-
	F	181	±	-
1.25 gm	M	334	-	-
	M	318	-	-
	F	178	-	-
1.5 gm	F	207	±	-
	M	325	-	-
	F	189	-	-
1.75 gm	M	310	-	-
	F	201	-	-
	F	206	-	-

TABLE 16

Egg Yolk 1 : 1 dilution with water				
1 ml per 100 gm body wt.per week	F	223	+	+
	M	268	++	++
	F	174	+	+
2 ml	M	281	++	+
	F	208	+	+
	F	208	++	+
3 ml	M	301	+	-
	F	187	±	±
	M	311	±	±
4 ml	M	339	±	-
	F	189	±	-
	M	345	-	-

TABLE 17

Supplement	Sex	Weight of animal at end of experiment	Condition at end of Exp. tail	feet
Butterfat (Summer)				
1.25 gm per 100 gm body wt.per week	M	272 grams	+	+
	F	212	+	+
	F	224	+	+
	M	257	+	++
1.5 gm	F	208	++	++
	F	219	+	+
	M	260	+	+
	M	328	+	+
1.75 gm	F	202	-	±
	M	315	±	±
	F	202	-	-
	F	190	±	±

TABLE 18

Crisco				
1 gm per 100 gm body wt.per week	M	300 grams	+	+
	F	208	+	±
	F	214	+	-
	F	182	+	+
1.25 gm	M	241	+	+
	F	216	+	±
	F	225	±	-
	F	224	-	-
1.50 gm	M	315	±	-
	F	232	-	-
	M	311	-	-
	F	193	-	-

TABLE 19

Supplement	Sex	Weight of animal at end of experiment	Condition at end of Exp. tail	feet
Linolic Acid Impure				
	M	275 grams	++	++
.05 gm per	M	271	+++	+
100 gm body	F	195	++	++
wt.per week	F	198	+	+
	M	258	++	+
.075 gm	M	277	+++	++
	F	188	-	-
	F	192	+	+
	M	264	-	-
.100 gm	M	260	-	-
	F	169	+++*	+++ *
	F	213	-	-
	M	275	-	-
.125 gm	M	280	-	-
	M	273	-	-
	M	270	-	-

*Animal refused to eat supplement

TABLE 20

Linseed Oil				
	F	206 grams	+	+
.075 gm per	M	240	+	+
100 gm body	F	195	+	+
wt.per week	M	288	+	+
	M	327	+	±
.100 gm	M	343	±	-
	F	218	+	+
	M	334	++	++
	M	319	-	±
.125 gm	F	215	+	+
	M	247	+	+
	F	222	±	±

TABLE 21

Supplement	Sex	Weight of animal at end of experiment	Condition at end of Exp. tail	feet
Soybean Oil				
	M	294 grams	+	+
.10 gm per	F	187	+	±
100 gm body	M	307	++	+
wt.per week	M	294	+	±
	M	231	±	-
.11 gm	F	212	+	±
	F	217	-	-
	M	297	+	-
	F	181	-	-
.125 gm	M	259	±	-
	F	238	-	-
	M	320	-	-

TABLE 22

Methyl Linolate				
.05gm	F	197 grams	+	+
	F	217	±	-
.06 gm per	F	203	±	±
100 gm body	M	332	+	+
wt.per week	M	241	+	±
	M	320	-	-
.07 gm	M	284	±	-
	F	210	-	-
	F	207	-	-
	F	220	-	-
.08 gm	M	314	-	±
	F	212	-	-
	F	219	-	-

TABLE 23

Methyl Linolenate				
	M	245 grams	++	+
.06 gm per	M	309	+++	+++
100 gm body	F	205	+	+
wt.per week	F	187	+++	+++
	M	278	+++	+++
.07 gm	M	286	+++	+++
	F	210	++	++
	F	195	+++	++
	M	304	++	+
.08 gm	M	238	++	++
	F	182	++	+
	F	186	++	++

TABLE 24

Test material	Effective level	Calculated % indispensable unsaturated fatty acid based on linoleic acid = 100 %	% linoleic acid by Chemical analysis	
Wesson oil	.125 gm	53.6	42.8	(40)
Corn oil	.125	53.6	39.1	(40)
Olive oil	1.25	5.36	3.9 - 6.9	(40)
Duck fat	1.00	6.7		
Egg yolk	2.00	3.35		
Butterfat (Summer)	1.75 ±	sl.less than 3.8	2.3 - 4.25	(35)
Crisco	1.50	4.46		
Linolic acid C.p.	.10	67.00		
Linseed oil (Raw)	.125 +	less than 53.6	48.5 linoleic 34.1 linolenic	(40)
Soybean oil	.125	53.6	55-57 linoleic 2-4 linolenic	(40)
Methyl linolate	.07	95.7		
Linoleic Acid	.067(Cal)	100.0		
Methyl linolenate	ineffective at .08 gm	less than 100.0		

1st Technique

Wesson oil	.25 gm	53.6 *		
Oleomargarine	4.00 +	less than 3.35		
Lard	4.00	3.35	1 - 7	(41)
Wheat germ oil	.25 −	more than 53.6		
Butterfat (Winter)	4.00 ±	sl.less than 3.35	2.3 - 4.25	(35)
Oat oil	.40	33.5	31.3	(40)
Goose fat	1.50	8.9		
Tallow	ineffective		3.0	(43)
Codliver oil	"			
Halibut liver oil	"			

*This value was determined by comparison with methyl linolate subsequent to its use as a reference material for the earlier assays.

Discussion of Results

In the experiments carried out it was found that when a low fat diet composed of edible crude casein, sucrose, complete salt mixture, yeast (partly irradiated) and alfalfa leaf meal, was fed to young rats, a scaliness of the feet and tail could be produced with regularity in approximately three weeks. This scaliness could be made to disappear by the feeding of fats and oils, the level required depending on the content of the so-called essential unsaturated fatty acids. The diet used proved to be quite satisfactory although no attempt was made to remove the fat from the natural food components which it contained. It is more than likely that the small amount of ether extractable lipid fraction which amounted to 0.22 per cent contained a negligible amount of the essential unsaturated fatty acids.

The partly irradiated yeast was a convenient source of vitamin D as well as of the vitamin B complex. Carotene which is convertible to vitamin A was supplied by the alfalfa leaf meal. The diet appeared to be adequate in all the vitamins, with possibly the exception of vitamin E, although alfalfa leaf meal is known to contain some of it.

The production of scaliness is dependent on several factors and these must be kept under control if uniform results are to be obtained. It is very important that animals be selected at an early age, twenty to twenty-one days and that they come from stock which has not been fed too large amounts of the essential unsaturated fatty acids. When the above precautions are taken and if the humidity is

not too high, little difficulty is experienced in producing the fat deficiency symptoms in three to four weeks.

In the course of these experiments it was observed that the feeding of the test material on the basis of body weight was more satisfactory since all the animals did not weigh the same at the time the supplement was fed and also grew at variable rates after the supplementary feeding was begun.

Since it is desirable in the biological assay of any material to have a standard for reference, Wesson oil was used in the early stages of the work although it was decided to make comparisons with pure preparations of the esters of linoleic and linolenic acids in as much as these had been used by other investigators. A source of the corresponding ester of arachidonic acid was not available in time to include it in these studies. Time and lack of materials also did not permit the determination of the effective level in the case of the methyl ester of linolenic acid although the results indicate that it was appreciably less effective than the linolate in causing the disappearance of scaliness. The effective level of the latter was, however, determined and serves as the basis for comparison for most of the materials assayed.

Assuming that the methyl linolate was relatively pure, the calculated effective level in terms of the free acid was 0.067 as indicated in Table 24. The effective levels of the various test materials were then determined and on the basis of the assumption that all effective levels contained an equivalent amount of the

essential unsaturated fatty acids, the percentage of the latter in each of the test materials was calculated. The values so calculated show on the whole excellent agreement with those found in the literature. This close agreement in values is due to the reliability of the biological method used and to the happy circumstance that most edible fats and oils contain little or no linolenic acid.

Tables 2, 7, 8, 17, 20 and 23 indicate that additional levels of oleomargarine, wheat germ oil, winter and summer butter, linseed oil and methyl linolenate, would be necessary to determine the exact effective level for each. Time did not permit to finish this portion of the work so that only close approximations are possible. In the cases in which approximate values are given + and − signs prefix the numerical value.

The summarized data shown in Table 24 indicates excellent agreement with the values obtained by chemical methods in the case of olive oil, butterfat, soybean oil and oat oil. The values for Wesson oil and corn oil are appreciably higher. This may be due in part at least to the fact that the refined edible products were assayed and the values used for comparison apply to the crude oils.

The most effective natural occurring oil tested appeared to be wheat germ oil. The effective level was between .10 and .25 gram per week. Since the .10 gram level showed slight evidence of scaliness, the .25 gram level was used, and the essential unsaturated fatty acid was caculated to be 53.6 per cent. Undoubtedly the effective level was somewhere between .10 and .25 gram, which would make the percentage of unsaturated acid above 53.6.

It is interesting to note that linseed oil with a linoleic acid content of 48.5 per cent and a linolenic acid content of 34.1 per cent, a total of 82.6 per cent unsaturated acid gave a lower essential unsaturated fatty acid by the biological method than corn oil, which by chemical analysis contains only 39.1 per cent linoleic acid. It may be that the digestibility of linseed oil is of a lower order than that of the edible oils. At any rate a similar observation of the superiority of corn oil over linseed oil was made by Burr et al (53).

Methyl linolenate prepared from linseed oil and judged by its iodine number and the melting point of its hexabromide to be relatively pure, failed to relieve the fat deficiency syndrome at any of the levels fed, whereas methyl linolate eliminated the symptoms at a level of .07 gram per hundred grams of body weight. Both experiments were conducted simultaneously and the animals were from the same litters, thus eliminating any influences due to heredity and humidity. This justifies the conclusion that linolenic acid is not as effective as linoleic acid. Inasmuch as the effective level of methyl linolenate was not determined, a strictly quantitative comparison between the two acids cannot be made at this time.

Butter was found to contain from 3.3 to 3.8 per cent essential unsaturated fatty acid which agrees with values given by Hilditch although not with those of Eckstein. The latter found Michigan butter to contain 0.2 to 0.5 per cent of linoleic acid and

from 0.07 to 0.17 per cent linolenic acid. On this basis the analysis of Eckstein appears to be much too low.

Beef tallow was found to be ineffective at all levels, 4.0 grams per week had only the slightest effect and it is doubtful if the animal could consume enough of the fat to cure the scaliness. The fowl fats tested, goose and duck, proved to be fairly effective, goose fat being the better.

The results with cod liver oil and halibut liver oil were negative showing that both of these oils are low in essential unsaturated fatty acids, although they are rich in other highly unsaturated fatty acids. These results are in accord with those of Sinclair (16) in which cod liver oil did not protect against scaliness even when fed at a level of 10 per cent by weight of the entire diet.

Conclusions and Summary

1. A low fat diet, consisting of edible crude casein, sucrose, salt mixture, yeast (partly irradiated) and alfalfa leaf meal produced the fat deficiency symptoms in three to four weeks.

2. The essential unsaturated fatty acid content of fats and oils can be determined by using the disappearance of scaliness of the tail and feet of the rat as a criterion.

3. The results obtained are in close agreement with the existing chemical data for the linoleic acid content of the fats and oils tested.

4. Wesson oil and corn oil were more effective in curing the fat deficiency syndrome than raw linseed oil, although by chemical analysis the latter has a higher value for linoleic and linolenic acids.

5. Linoleic acid was more effective than linolenic acid when they were fed as the methyl esters.

6. Vegetable oils with the exception of olive oil were found to be by far the richest sources of the essential unsaturated fatty acids. Fowl fats ranked next followed by olive oil, crisco, summer butterfat, lard, egg yolk, oleomargarine and winter butterfat. Cod liver oil, halibut liver oil and beef tallow were ineffective at all levels fed.

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