A COMPARISON OF THE EFFECTIVENESS OF LINOLEIC, LINOLENIC AND ARACHIDONIC ACIDS IN ELIMINATING SCALINESS IN RATS

Thesis for the Degree of M. S.
MICHIGAN STATE COLLEGE

James L. Orbison

1939

A COMPARISON OF THE EFFECTIVENESS OF LINOLEIC, LINOLENIC AND ARACHIDONIC ACIDS IN ELIMINATING SCALINESS IN RATS.

by
James Lowell Orbison

A THESIS

Submitted to the Graduate School of Michigan State College of Agriculture and Applied Science in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

Department of Chemistry

1939

THESIS

ACKNOWLEDGMENT

The author wishes to express his indebtedness to Dr. C. A. Hoppert, Professor of Chemistry, for his assistance and advice in carrying out this project and in the preparation of this mamuscript.

It was a common observation of the early workers in the field of nutrition that the animal body could readily synthesize and store fat. As a result of this observation, it was concluded that the fat in the diet was only an accessory food factor which presented energy in a more concentrated form than the carbohydrate. However, the fats became of greater importance when it was discovered later that there were certain fat soluble vitamins which were necessary for the normal nutrition of the animal body. It was as a carrier for these new dietary factors that the fats gained their new importance.

In 1920 Osborne and Mendel (1) reinvestigated the subject of fat in nutrition and came to the conclusion that fat was needed only in exceedingly small amounts, if at all, provided the necessary vitamins were present in the diet.

It was not until 1927 that Evans and Burr (2) discovered what appeared to be a nutritional deficiency in rats which were fed on a highly purified diet. The work was being carried out on vitamin E, and in an attempt to remove all of this vitamin, the dietary constituents were extracted with solvents which removed the fats as well as the vitamin E. The result was that the animals fed on this diet showed a much slower rate of growth than the normal controls. This deficient growth rate was not attributable to insufficient vitamin E.

Evans and Burr (3) (4) continued their work on the new deficiency and found that the animals showed a definite growth plateau at the weight of 135 to 150 grams. Later, they found that

the females showed a failure of lactation when on the fat free diet and also that the ovulation became very irregular. With the addition of lard, butter, cocoanut oil and corn oil, normal growth, lactation and ovulation occurred. Separation of these added substances into their constituent parts showed that the effective factor was in the fatty acid fraction.

McAmis, Anderson and Mendel (5) later fed a fat free but otherwise complete diet and reported the appearance of necrotic tails, poor fur condition and late development of kidney lesions in the experimental animals. There was a tendency to priapism in the males; bloody urine and erythema of the eyes was generally present. It was also found that the addition of peanut oil was beneficial, but it was not definitely concluded that the fat was the responsible factor.

At the same time Burr and Burr (6) reported an experiment in which the nutritive ratio of the fat free diet was increased from 1:3 to 1:5 to 1:7 as the animals increased in weight. It was found that the animals showed cessation of growth at about the seventieth to the ninetieth day (25% underweight) and that they began to lose weight in the fifth month. The loss of weight continued and unless fat was given the rats died within three or four months. The outward symptoms of the syndrome were given as a scaliness of the skin, tail and feet; the scaliness developed into inflammation later and at times into necrosis. It was found that, although the syndrome had some of the symptoms of the vitamin A or vitamin B deficiencies, a diet rich in these vitamins

showed no curative power, whereas the addition of oils effected a cure.

An attempt to relate the vitamin B factors with the fatty acid factor has persisted, and although it is not yet established, there does seem to be a possibility of relationship between the two. Evans and Lepkovsky (7) (8) noted that the glyceride of oleic acid had a sparing action upon the vitamin B requirement in the diet, and by giving the vitamin B parenterally, they found a previous interaction in the intestinal tract was not necessary for the sparing action to be noticeable. Oleson, Bird, Elvehjem and Hart (9), on the other hand, found that the diseases which were formerly thought to be due to undiscovered vitamin B fractions could be cured by the use of the unsaturated fats. The diet used contained only specially purified vitamins. Birch (10) considered the acrodynia-like dermatitis to be due to the absence of one or both of two factors: the unsaturated fatty acids and/or vitamin B_6. He also concluded that the vitamin B_6 was concerned in the utilization of the unsaturated fats. The similarity of the symptoms of the two deficiencies is as yet, however, the most noticeable relationship between them.

The symptoms which Burr and Burr (11) reported as the most readily utilizable for the determination of cure as well as the detection of the deficiency was the scaliness of the feet. Upon testing several fatty acids for curative value, it was found that the saturated acids showed no curative effect; olive oil, lard, corn oil, poppy-seed oil, linseed oil and lecithin all showed curative effects, as did methyl linoleate. The methyl oleate was

considered of doubtful value.

Burr, Burr and Miller (12) in an attempt to fix the curative factors, found that methyl linoleate and methyl linolenate were both effective in correcting the growth deficiency, while methyl oleate and methyl -eleostearate were ineffective. The arachidonate seemed to have a depressing influence on the effectiveness of the methyl linoleate, but no work was done on this ester alone. Evans and Lepkovsky (13) (14) confirmed the work on the curative power of methyl linoleate.

Turpeinen (15) in 1938 published a report in which he found oleic, (Δ 12:13), erucic, recinoleic, chaulmoogric and -eleostearic acids to be ineffective. To the linoleic and linolenic which had already been reported as effective, he added linoleyl alcohol and arachidonic acid. The arachidonic acid was reported as being about three times as potent as linoleic. In the same year Burr, Cass, Brown, and Frankel (16) published an abstract in which they reported the finding of arachidonic acid as a very effective cure.

Evans, Lepkovsky and Murphy published a series of articles (17), (18), (19) on the effect of a fat free diet on reproduction. They found that a successful gestation on a fat free diet was impossible and that lactation was always subnormal. The males were found to be sterile due to an epithelial degeneration in the testicular tubule. The symptoms of both the male and female reproductive organs disappeared upon the additon of a little linoleic acid to the diet. Maeder (20) in 1938 corroborated the work of Evans and Lepkovsky and reported that the failure to become pregnant was directly due to the underdevelopment of the uterine

mucosa; thus, the ova failed to implant in the uterine wall. Unsuccessful gestation, however, was due to inflammation of the placenta and uterine wall.

Wesson in 1927 (21) reported a respiratory quotient greater than one for rats on a fat free diet. He also found that the only constituent of the normal diet of rolled oats, casein, calcium carbonate, sodium chloride and lard which would overcome this high respiratory quotient was the lard. In 1930 Wesson and Burr (22) related the work to the dietary deficiency and found that the rats with the deficiency disease had a respiratory quotient greater than one. This indicated that a conversion of carbohydrate to fat was occurring. Since no cessation of the deficiency symptoms was found, they concluded that no essential fatty acid was synthesized. Burr and Beber (23), (24), (25) found that the high respiratory quotient of diseased rats fell to a fat burning level when food was withheld, but that a permanent return to normal was found only when rats were cured by feeding essential fat. They concluded that although fat is synthesized, the fats having curative properties were not so synthesized. Wesson (26) eliminated the possibility of the high respiratory quotient being due to vitamin deficiency or to differences in exercise taken. Evans and Lepkovsky (27) also attempted to show lack of synthesis of the essential fatty acids when they reported that carcass fat as methyl esters of both rats on stock ration and rats newly weaned showed curative value for the deficiency, whereas that of rats on fat free diet had no such value. Ellis and Isbell (28) (29) found that a very definite correlation existed between dietary fat and resulting body fat of

swine. They found a change in both physical and chemical constants and in percentage of unsaturated and saturated fatty acids with change in diet. The most noticeable change was in the linoleic acid fraction. This acid changed from 1.9% on a brewer's rice diet to 30.9% on a soy bean diet, thus showing a definite correlation between diet and type of body fat in at least these cases.

The similarity of symptoms between rats on a fat free diet and infants showing eczema suggested to Hansen that the two diseases might have the same etiology. Working on this basis (30) (31) he found that the Iodine number of the blood sera of eczematous infants averaged 82, while the normal averaged 114. By feeding unsaturated oils, it was possible to get a clearing of the symptoms. Hansen and Burr (32), (33), (34) found that the highest values of the Iodine number of blood sera of fat free diseased rats was lower than the lowest value of the normal controls, showing the possible etiological relationship of the fat deficiency disease of rats to the eczema of infants. Cornbleet and Pace (35) obtained excellent results with an unsaturated fat treatment of 87 eczematous patients over a period of four years. Traub and Zakon (36), however, were unable to find any favorable results upon feeding linseed oil to patients suffering from eczema. In an attempt to produce symptoms similar to those of eczema, Brown, Hansen, McQuarrie and Burr (37) reported that an adult kept on a low fat diet for six months showed no ill effects, and, in fact, had a decrease of high blood pressure, less fatigue and a cessation of migrain headaches from which he had been suffering. This is not considered by the authors as proof that essential

fatty acids are not necessary for the human adult, for it is impossible to determine how much reserve the body may have stored.

Several authors have substantiated a large part of the work of the above researches. Among these Borland and Jackson (38) have reported the kidney lesions as consisting of calcification of the cortical tubules and necrosis of the apical medulla, and the presence of fatty and albuminous casts. Tange (39) (40) reported the effectiveness of linoleic and linolenic acids as cures and the relative ineffectiveness of oleic, elaidic, culpudanoic, and stearic acids. Evans and Lepkovsky (14) noted that a diet in which the energy was largely supplied by saturated fatty acids was decidedly deficient. They found, however, that the addition of linoleic acid greatly improved the nutritive value of the diet.

Several authors have published papers which challenged the possibility of a fat deficiency disease. Among these authors were Hume and Smith (41); Funk, Casmer, Caspe and Caspe (42); Roche and Roche (43); Parsons (44); Gregory and Drummond (45). Burr and Brown (46) discussed the above papers. The work of Parsons in which diets containing large amounts of egg white were used and that of Funk, Casmer, Caspe and Caspe in which no cure was effected with the unsaturated fat treatment does not necessarily apply, since the symptoms of the fat deficiency syndrome are by no means specific for this particular condition and may be produced under other experimental conditions as well. The diets of Funk, Casmer, Caspe and Caspe, and of Hume and Smith were both deficient in vitamin B, the absence of which could easily explain the symptoms which were not curable by the unsaturated fat supplement. Roche

and Roche must have had a diet deficient in factors other than fat, for the animals weighed only 80 to 100 grams at three months of age. Gregory and Drummond used a yeast extract in their diet which would undoubtedly have been low in one or more of the water soluble vitamins.

Mackenzie, Mackenzie and McCollum (47) have given evidence of fat in the so-called fat free diets prepared by other investigators. They prepared an exceptionally low fat diet, and from the results obtained by its use concluded that there are no fat soluble factors necessary for growth and reproduction other than the essential unsaturated fatty acids.

Since most of the criticisms and irregular results have been effectively answered, it would seem that the existence of a fat deficiency syndrome has been well established.

In a thesis presented at Michigan State College in 1938, Shannon (48) made use of the marginal curative value of fats on the symptoms of scaliness of feet and tail to assay oils for essential fat content. This method has the advantage of being shorter than the growth method, since symptoms appeared in 3 to 4 weeks and the length of treatment was taken as a six weeks' period. A marginal curative dose was set as that quantity of fat or fatty acid in grams per 100 grams of body weight of the rat, per week of time, which would cure the symptoms of scaliness upon feeding it for a six weeks' period after the animal had acquired the deficiency symptoms. By use of this method it was possible to assay oils accurately enough to compare with the values given for linoleic acid determined

by chemical methods. The percentage of linoleic acid in an oil was calculated upon the basis of linoleic acid equaling 100% when fed as a supplement. In following this method, indication was found that methyl linolenate was less effective than the methyl linoleate.

Hume, Nunn, Smedley-Maclean and Smith (49) in the same year published an article in which they used both weight increase and cure of scaliness as criteria for cure of the deficiency. In this paper they reported that linolenic acid was six times less effective as a cure than was linoleic. Oxidation products of linoleic acid were found to be effective, as were lard, linseed oil and raisin seed oil, but methyl docohexanoic and chaulmoogric acids and methyl arachidate were all ineffective.

In an attempt to check the work of Hume, Nunn, Smedley-Maclean and Smith and as a continuation of the work of Shannon, the following project was carried out. Since there had been indication that the linolenic acid was less effective for cure of the skin lesions than it had been reported for growth deficiency, it was felt that the other fatty acid reported effective (arachidonic) should be checked in comparison with the other two by this new method. The work was done to compare the effectiveness of linoleic, linolenic and arachidonic acids as cures for the skin lesions produced with a fat deficient diet.

EXPERIMENTAL PROCEDURE

Preparation of Materials Used

The methyl linoleate was prepared from soybean oil. The oil was first saponified, and the fatty acids liberated with acid and extracted with petroleum ether. The saturated fatty acids were removed by the fractional crystallization method of Brown and Stoner (50). The resulting solution of unsaturated fatty acids was treated according to the method of Rollet (51), in which the solution is brominated and the resulting bromides purified by recrystallization from boiling petroleum ether until the m.p. is 113-114°C. The fatty acid is then regenerated and methylated simultaneously. The resulting methyl esters are distilled under vacuum. b.p. 177° at 3-4 mm. pressure. Iodine no. 177, calculated, 173.

The methyl linolenate was prepared from linseed oil. The fatty acids were obtained by the usual method. The ethyl ether solution was brominated according to the method of Rollet (52) and the resulting bromides purified by washing with ether until the m.p. was 180-181°C. The acid was regenerated and simultaneously methylated, and the resulting methyl esters distilled under vacuum. The b.p. 170°C. at 1 mm. pressure. The Iodine no. 258, calculated, 261.

The liver lipids used in the preparation of the methyl arachidonate were very kindly presented by Dr. David Klein of the Wilson Company, Chicago, Illinois. The methyl arachidonate was prepared according to the method of Brown (53). Briefly, the method was as follows: the fatty acids were obtained by the usual method and extracted with ethyl ether. The ether was removed under vacuum, and

the fatty acids were refluxed with acidified methyl alcohol. The methyl esters were extracted with ether and distilled under reduced pressure. The crude methyl esters so obtained were brominated and washed with ether until the m.p. became 129-130°C. The methyl esters were regenerated by refluxing with Zn and methyl alcohol, and the regenerated esters distilled under reduced pressure. The b.p. 165-170°C. at 1 mm. pressure. The Iodine no. 310.

A little finely powdered hydroquinone was added to all of the esters to prevent oxidation, and all were stored under carbon dioxide or nitrogen in the refrigerator until needed.

Preparation of the Basal Diet.

Casein, edible	18%
Sucrose	68
Yeast	4
Yeast, irradiated	1
Alfalfa leaf meal	5
Salt mixture, Steenbock 40b	4
	100%

Shannon (48) discusses this diet and states that it is complete in all respects with the possible exception that it may be low in vitamin E, but that some of this vitamin is obtained from alfalfa leaf meal. He also states that it contains 0.22% ether extractable material having an Iodine no. of 107.9.

Preparation of Animals

The animals used in this experiment were albino rats 20 to 22 days old and weighing 40 to 50 grams. They were placed in pairs in wire cages with screen bottoms and fed the above basal diet until the symptoms of the deficiency appeared. The symptoms usually appeared at the end of the third week, although it sometimes took a longer period, if the humidity happened to be high.

Method of Carrying out the Experiment

After the rats had been kept on a fat free diet until the symptoms of the deficiency developed, they were placed in individual wire cages with screen bottoms and allowed all of the basal diet that they would eat.

The rats were arranged, usually in groups of four for each level of supplement. They were selected in such a way as to give as nearly equal distribution of sex and litter mates as was possible. This was done as a precautionary measure, although Shannon (48) found no noticeable difference in the reaction of males and females to the curative treatment.

Each animal was weighed and the symptoms scored each week. From the weight obtained, the amount of supplement was calculated. Since this calculation depended upon the weight of the animal, the supplement was in terms of grams of supplement per 100 grams of body weight. All of the supplements were oils; therefore, the specific gravity was determined and the supplement measured by means of a pipette. The oil was necessarily kept at a constant temperature during the measuring, 25°C.

The supplement was fed once a week except in the cases of the higher levels. The higher levels were divided into two portions per week in order to use smaller quantities, thereby lessening the time of consumption and decreasing the possibility of decomposition. Feedings of the supplement were made in open, porcelain dishes which were attached to the cage in such a manner that they could not be overturned. The supplement was usually consumed within 24 hours. In case some supplement was left, it was mixed with a little of the basal diet, and left in the basal mixture until consumed.

The feeding of the supplement was continued each week for six weeks. At the end of the sixth week, the lowest level of supplement which had cured the tail and feet symptoms was considered as the minimum effective level.

The scoring of the symptoms was as follows:

Tail

less than 1, usually questionable..... ±

definite scales from ½ to 1 inch on tip of tail..... +

scales on tip of tail from 1 to 2 inches..... + +

lower half of tail ridged and scaled..... + + +

most of tail ridged and scaled, occasionally small hemoorhages occuring..... + + + +

Feet

less than 1, usually questionable..... ±

scales between toes..... +

scales between the toes and on the
dorsal surface of the toes..... + +

scales between the toes, on the dorsal
surface of toes, and some scales extending
up dorsal surface of leg..... + + +

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

In accord with the work of Shannon (48) it was found that the scaliness of the feet was the most sensitive of the symptoms noted.

DATA

The following tables give a summary of the data obtained while feeding methyl linoleate, methyl linolenate, and methyl arachidonate to rats for a six weeks' supplementary period.

TABLE I

Supplement	Sex	Weight at beginning.	Weight at end	Symptoms at end of experiment	
				Tail	Feet
Methyl linoleate .06 gm. per 100 gm. body weight per week.	M	118	313	±	-
	M	107	238	+	++
	F	108	216	+	±
	F	117	212	+	±
.07	M	117	306	-	-
	M	143	307	-	-
	M	104	253	-	-
	M	78	244	-	+
.08	M	116	241	-	-
	M	141	320	+	-
	F	100	184	-	-
	F	132	213	-	-
0	F	124	218	+	+++
	F	97	176	+	++++
	F	86	200	++	++++
	F	102	186	+	++++

TABLE II

Supplement	Sex	Weight at beginning	Weight at end	Symptoms at end of experiment	
				Tail	Feet
Methyl linolenate .20	M	107	282	+++	++
	M	147	244	++	+
	F	154	266	±	++
	F	149	233	+	+++
.25	F	127	215	+++	++
	F	101	201	+	±
	M	122	270	++	++
	M	116	292	+++	++
.30	F	66	150	+++	++
	F	117	144	++	++
	M	129	324	+	+
	M	117	287	+	++
.40	F	123	214	++	+
	F	123	216	+	++
0	F	124	218	+	+++
	F	97	176	+	++++
	F	86	200	++	++++
	F	102	186	+	++++

TABLE III

Supplement	Sex	Weight at beginning	Weight at end	Symptoms at the end of experiment	
				Tail	Feet
Methyl linolenate .42	F	121	188	+	++++
	M	168	328	++	+++
	F	127	218	+	+++
	F	124	201	++	++
0	F	147	215	+	++
	F	139	205	++	+++
.49	F	103	219	+	++
	F	86	193	+	++
0	F	114	188	+	+++
	F	106	200	-	++

TABLE IV

Supplement	Sex	Weight at beginning	Weight at end	Symptoms at end of experiment	
				Tail	Feet
Methyl Arachidonate .0765	F	100	210	-	±
	F	122	208	-	-
.153	M	90	270	-	-
	F	107	214	±	-
.306	M	100	276	±	-
	F	96	200	±	-
.46	M	94	230	+	-
	F	98	216	±	-
0	F	114	194	+	+++ ++
	F	106	200	-	

DISCUSSION

It was found that a diet composed of crude edible casein, plain brewer's yeast, irradiated brewer's yeast, alfalfa leaf meal and a complete salt mixture was sufficiently free of essential fat to produce symptoms on young animals in 3 to 4 weeks. There was present in the diet 0.22% of ether extractable material, but this evidently contained too small a fraction of essential fatty acids to cause any interference.

The diet seemed to be complete in all factors except the fat, and possibly vitamin E. Carotene was supplied by the alfalfa leaf meal, and it quite likely that some vitamin E was also present in this material. The vitamin B complex was obtained from the dried brewer's yeast, and the irradiated yeast supplied sufficient vitamin D. The vitamin E factor, if deficient, would have no effect upon the experiment great enough to cover the fat deficiency symptoms.

It was found by Shannon (48) that animals kept longer than 20 to 22 days on the stock ration were more resistant to the fat deficiency. He also found that humidity showed a decided influence upon the rapidity of the development of the symptoms as well as their severity. As a result of this finding, the rats were started on the basal diet at 20 to 22 days of age, and most of the work was done in the winter and early spring to avoid as much as possible, any high humidity.

The first symptom to appear was a slight scaliness between the toes. This condition continued to develop until the dorsal

surface of the toes, then the foot, and finally the leg became covered with scales. Soon after the appearance of scales between the toes, small scales appeared on the tip of the tail. The scales became more and more pronounced on the tail until it was scaly over most of its entire length. In addition to the scales, the tail often showed heavy transverse ridges of keratinous material. No other symptoms were noted, except in the case of animals that were left on the basal diet for a few months after completing a supplemental period. In these animals, the scales gave way to an erythemic condition of the tail and feet, and occasional small hemorrhages appeared.

In Tables I and II are shown the results obtained by supplementing methyl linoleate and methyl linolenate, respectively, to the basal diet. The two experiments were carried out simultaneously, thus overcoming any possibility of differences due to changes in environmental conditions. From the data in Table I it can be readily noted that the supplement of 0.07 gms. of methyl linolenate is the minimum curative level with respect to the symptoms of scaliness. The data in Table II gives evidence that the effective level of methyl linolenate is above the highest level used in the experiment, that is, above 0.40 gms.

Hume, Nunn, Smedley-Maclean and Smith (49) published an article giving the effective level of methyl linolenate as six times that of methyl linoleate. As a result of this publication,

the approach was changed slightly in the subsequent experiments. The results of these experiments are shown in Table III. An attempt was made to relate the effectiveness of linolenate as a multiple of the effectiveness of linoleate. Hence a value was used in feeding the supplement which was 6 times that of the effective level of methyl linoleate (0.42) in the first experiment, and upon finding this level ineffective, another experiment was carried out using 7 times the effective dose of methyl linoleate (0.49 gms.) This high level was also ineffective, and since all experiments included negative controls, the chance of error was relatively small..These values indicated that the effective level of linolenate was greater than 0.49 grams, and thus greater than 7 times the effective value of linoleate.

In Table IV are found the results of anexperiment in which methyl arachidonate was used as a supplement to the basal diet. The values used in this experiment are not exact multiples of the effective linoleate values in terms of grams, but have been corrected for the difference in molecular weight between methyl arachidonate and the methyl linoleate. Since the arachidonate has a somewhat higher molecular weight than the linoleate, the values used are slightly larger than the exact multiples. This correction was not necessary in the case of the linoleate, for the difference of two less hydrogen atoms in the molecule made so slight a difference in the molecular weight as to be well within the experimental error of the experiment.

Since discrepencies had been found in the reported linolenate value, it was decided to perform a preliminary test to give an idea of the range within which the curative level would lie. As a result, the levels were set up as shown in Table IV. It was found, however, that all of the levels were too high, for the animals were cured at the end of three weeks time on the supplement instead of the usual six weeks. Two exceptions to the above statement were noted. The two rats on the highest level still showed some scaliness on the tail, but by the end of the fourth week this had largely disappeared. The effective value for methyl arachidonate was determined to be less than 0.0765 grams, or less than the value of the effective linoleate level.

An experiment previous to the one given in Table IV was started, but had to be discontinued, due to trouble in obtaining methyl arachidonate. The method of Brown (53) was used in the preparation and worked quite well down to the point of reducing the octabromide. Here the prescribed method gave only a poor yield of relatively impure product. It was only by increasing the time of refluxing from 24 to 72 hours and adding acidified methyl alcohol to increase the hydrogen production that sufficient product was obtained. Even this method was not efficient, and work still needs to be done to perfect the procedure. However, the material obtained by the revised method gave a fairly pure product after fractional distillation under vacuum. The Iodine no. was 310; calculated 319.2.

One can see from the Iodine no. that the methyl arachidonate used was not pure. The contaminating substances would, however, have occurred in amounts relatively slight to give an iodine number so

near the theoretical value. The only contaminant that could have any effect great enough to throw off the results of the experiment would be methyl linoleate. This is ruled out by the results of the experiment. The effective level of methyl linoleate is only 0.0765 grams, and since this arachidonate cured at this level in three weeks, it would be impossible for the linoleate to be the effective material present, even though it were the contaminant.

Another experiment is now under way in which doses of methyl arachidonate ranging downward from 0.0765 grams are being used. This should give a value showing the effective doses of methyl arachidonate in relation to that of linoleate.

CONCLUSIONS

1. A method for determining the comparative effectiveness of methyl linoleate, methyl linolenate, and methyl arachidonate in eliminating scaliness in rats is given.

2. The effective level of methyl linoleate was 0.07 grams per 100 grams of body weight per week.

3. The effective level of methyl linolenate was greater than 0.49 grams per 100 grams of body weight per week.

4. The effective level of methyl arachidonate was less than 0.0765 grams per 100 grams of body weight per week.

5. The effectiveness of methyl linolenate and methyl arachidonate in comparison with methyl linoleate is: methyl linolenate less than 1/7 methyl arachidonate greater than 1.

BIBLIOGRAPHY

1. Osborne, T. B., and L. B. Mendel.

Growth on diets poor in true fat.

J. Biol. Chem. 45: 145, 1920.

2. Evans, H. M., and G. O. Burr.

A new dietary deficiency with highly purified diets.

Proc. Soc. Exper. Biol. and Med. 24: 740, 1926-27.

3. Evans, H. M., and G. O. Burr.

A new dietary deficiency with highly purified diets.

Proc. Soc. Exper. Biol. and Med. 25: 41, 1927.

4. Evans, H. M., and G. O. Burr.

A new dietary deficiency with highly purified diets. III. The beneficial effect of fat in the diet.

Proc. Soc. Exper. Biol. and Med. 25: 390, 1927.

5. McAmis, A. J., W. E. Anderson and L. B. Mendel.

Growth of rats on "Fat Free" diet.

J. Biol. Chem. 82: 247, 1929.

6. Burr, G. O., and M. M. Burr.

A new dietary disease produced by the rigid exclusion of fat from the diet.

J. Biol. Chem. 82: 345, 1929.

7. Evans, H. M., and S. Lepkovsky.

The sparing action of fat on vitamin B. IV. Is it necessary for fat to interact with vitamin B in the alimentary canal to exert its sparing effect?

J. Biol. Chem. 99: 235, 1933.

8. Evans, H. M., and S. Lepkovsky.

The sparing action of fat on vitamin B. V. The role of glycerides of oleic acid.

J. Biol. Chem. 99: 237, 1933.

9. Oleson, J. J., H. R. Bird, C. A. Elvehjem, and E. B. Hart.

Additional nutritional factors required by the rat.

J. Biol. Chem. 127: 23, 1939.

10. Birch, T. W.

The relation between vitamin B_6 and the unsaturated fatty acid factor.

J. Biol. Chem. 124: 775, 1938.

11. Burr, G. O., and M. M. Burr.

On the nature and role of the fatty acids essential in nutrition. Part II.

J. Biol. Chem. 86: 587, 1930.

12. Burr, G. O., M. M. Burr and E. S. Miller.

On the fatty acids essential in nutrition. Part III.

J. Biol. Chem. 97: 1, 1932

13. Evans, H. M., and S. Lepkovsky.

Vital need of the body for certain unsaturated fatty acids. I. Experiment on fat free diets in which sucrose furnishes the sole source of energy.

J. Biol. Chem. 96: 143, 1932.

14. Evans, J. M., and S. Lepkovsky.

Vital need of the body for certain unsaturated fatty acids. II. Experiments with high fat diets in which saturated fatty acids furnish the sole source of energy.

J. Biol. Chem. 96: 157, 1932.

15. Turpeinen, O.

Further studies on the unsaturated fatty acids essential in nutrition.

J. Nutrition 15: 351, 1938.

16. Burr, G. O., J. P. Cass, J. B. Brown and J. Frankel.

On the fatty acids essential in nutrition.

J. Nutrition 15: Supp', 15, 1938.

17. Evans, H. M., S. Lepkovsky, and E. Murphy.

Vital need of the body for certain unsaturated fatty acids. IV. Reproduction and lactation upon fat free diets.

J. Biol. Chem. 106: 431, 1934.

18. Evans, H. M., S. Lepkovsky, and E. Murphy.

Vital need of the body for certain unsaturated fatty acids. V. Reproduction and lactation upon diets containing saturated fatty acids as the sole source of energy.

J. Biol. Chem. 106: 441, 1934.

19. Evans, H. M., S. Lepkovsky, and E. Murphy.

Vital need of the body for certain unsaturated fatty acids. VI. Male sterility on fat free diets.

J. Biol. Chem. 106: 445, 1934.

20. Maeder, E. C.

Effect of fat in simplified diets on the reproductive organs of the female rats during gestation.

Anat. Rec. 70: 73, 1938.

21. Wesson, L. G.

A fat formation under abnormal conditions from carbohydrate by the rat, and its relationship to a possible new dietary factor.

J. Biol. Chem. 73: 507, 1927.

22. Wesson, L. G., and G. O. Burr.

The metabolic rate and respiratory quotient of rats on a fat deficient diet.

J. Biol. Chem. 91: 525, 1931.

23. Burr, G. O., and A. J. Beber.

A study of the gas exchange of rats suffering from a deficiency of unsaturated fatty acids.

J. Biol. Chem. 97: xxxvi, 1932.

24. Burr, G. O., and A. J. Beber.

Metabolism studies with rats suffering from fat deficiency.

Proc. Soc. Exper. Biol. and Med. 31: 911, 1934.

25. Burr, G. O., and A. J. Beber.

Metabolism studies with rats suffering from fat deficiency.

J. Nutrition 14: 553, 1937.

26. Wesson, L. G.

The effect of protracted exercise, intestinal fermentation, and modification of diet on the attainment of abnormal respiratory quotients by rats on a fat deficient diet.

J. Biol. Chem. 100: 365, 1933.

27. Evans, H. M., and S. Lepkovsky.

The vital need of the body for certain unsaturated fatty acids. III. Inability of the rat to synthesize the essential unsaturated fatty acids.

J. Biol. Chem. 99: 231, 1932.

28. Ellis, N. R., and H. S. Isbell.

The effect of food fat upon body fat. As shown by the separation of the individual fatty acids of the body fat.

J. Biol. Chem. 69: 239, 1926.

29. Ellis, N. R., and H. S. Isbell.

The influence of the character of the ration upon the composition of the body fat of hogs.

J. Biol. Chem. 69: 219, 1926.

30. Hansen, A. E.

Study of iodine numbers of serum fatty acids in infantile eczema.

Proc. Soc. Exper. Biol. and Med. 30: 1198, 1933.

31. Hansen, A. E.

Serum lipid changes and therapeutic effects of various oils in infantile eczema.

Proc. Soc. Exper. Biol. and Med. 31: 160, 1933.

32. Hansen, A. E., and G. O. Burr.

Iodine numbers of serum lipids in rats fed on fat free diet.

Proc. Soc. Exper. Biol. and Med. 30: 1201, 1933.

33. Hansen, A. E., and G. O. Burr.

Studies on iodine absorption of serum in rats fed on fat free diets.

Proc. Soc. Exper. Biol. and Med. 30: 1200, 1933.

34. Brown, W. R., and A. E. Hansen.

Arachidonic and linoleic acid of the serum of normal and eczematous human subjects.

Proc. Soc. Exper. Biol. and Med. 36: 113, 1937.

35. Cornbleet, T., and E. R. Pace.

Use of maise oil (unsaturated fatty acids) in the treatment of eczema.

Arch. of Dermatology and Syphilology 31: 224, 1935.

36. Traub, S. J., and S. J. Zakon.

The use of unsaturated fat acids in the treatment of eczema.

J. Am. Med. Ass. 105: 1675, 1933.

37. Brown, W. R., A. E. Hansen, McQuqrrie, and G. O. Burr.

Observation on human subject subsisting six months on a diet extremely low in fat.

Proc. Soc. Exper. Biol. and Med. 36: 281, 1937.

38. Borland, V. G., and C. M. Jackson.

Effects of a fat free diet on the structure of the kidney in rats.

Arch. Path. 11: 687, 1931.

39. Tange, U.

Dietary deficiencies of fat free diet for rats.

Proc. Im p. Acad. (Toyko) 8: 190, 1932.

Chem. Abst. 26: 5131, 1932.

40. Tange, U.

Effect of fatty acids on nutrition. II. Experiment with diets composed of rice, oil, and lipid containing linoleic and linolenic acids.

Sci. Papers Inst. Phys. Chem. Research (Tokyo) 20: 13, 1932.

Chem. Abst. 27: 1664, 1932.

41. Hume, E. M., and H. H. Smith.

Relation of fat free diet to the scaly tail condition in rats described by Burr and Burr.

Biochem. J. 25: 300, 1931.

42. Funk, Casmer, S. Caspe, and H. Caspe.

A new pathological condition of probable dietetic origin in rats.

Proc. Soc. Exper. Biol. and Med. 28: 816, 1931.

43. Roche, A., and J. Roche.

Sur les accidents curanes attributes a la carence en lipide chez le rat.

Comptes Rendu de la Soc. Biol. 109: 463, 1932.

44. Parsons, H. T.

The physiological effects of diets rich in egg white.

J. Biol. Chem. 90: 351, 1931.

45. Gregory, E., and J. C. Drummond.

A study of fat metabolism with special reference to nutrition in diets devoid of fats.

Ztschr. Vitaminforschung 1: 257, 1932.

46. Brown, W. R., and G. O. Burr.

Some recent studies in fat deficiency.

J. Biol. Chem. 114: xvi, 1936.

47. Mackenzie, C. G., J. B. Mackenzie, and E. V. McCollum.

Growth and reproduction on a low fat diet.

Biochem. J. 33: 935, 1939.

48. Shannon, A. M.

A biological method for the determination of essential unsaturated fatty acids and its application to various animal and vegetable fats.

Thesis for the degree of Ph.D.: Michigan State College, 1938.

49. Hume, E. M., L. C. A. Nunn, I. Smedley-Maclean, and H. H. Smith.

Studies of the essential unsaturated fatty acids in their relation to the fat deficiency disease of rats.

Biochem. J. 32: 2162, 1938.

50. Brown, J. B., and G. G. Stoner.

Studies on the chemistry of the fatty acids.

J. Am. Chem. Soc. 59: 3, 1937.

51. Rollet, A.

Zur kenntnis der linolsaure.

Z. Physiol. Chem., Bd 62: 410, 1909.

52. Rollet, A.

Zur kenntnis der linolensaure und des leinols.

Z. Physiol. Chem., Bd 62: 422, 1909.

53. Brown, J. B.

The highly unsaturated fatty acids of liver lipids. The preparation of arachidonic acid.

J. Biol. Chem. 80: 455, 1928.

[redacted]

[redacted]

Aug 28 43

Oct 1 [illegible]