STUDIES ON THE PRODUCTION OF SCALINESS IN RATS AND THE EFFECTIVENESS OF ARACHIDONIC ACID IN ELIMINATING THIS CONDITION

Thesis for the Degree of M. S.
MICHIGAN STATE COLLEGE
Louis J. Barton
1940

STUDIES ON THE PRODUCTION OF SCALINESS IN RATS AND THE EFFECTIVENESS OF ARACHIDONIC ACID IN ELIMINATING THIS CONDITION

by

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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

1940

ACKNOWLEDGEMENT

The writer wishes to express his deep appreciation and gratitude to Dr. C. A. Hoppert for his many helpful suggestions, his never tireing interest and his invaluable guidance throughout the duration of this project. It is through such associations as enjoyed at our great institutions of higher learning that one is made to realize the advantages offered to the youth of this great democracy....advantages and associations enjoyed by the youth of but few if any of the other countries of the world today.

And so....to Dr. Hoppert and the others who so generously gave advice and counsel, the writer wishes to extend his sincere thanks.

INTRODUCTION

Purpose of the work:

The purpose of this project was as follows:

1. To determine the ability of three "fat free" diets containing 5, 10 and 15 percent of yeast to produce scaliness in rats.

2. To determine the effectiveness of arachidonic acid in eliminating the scaliness in rats produced by a "fat free" diet.

Nature of the work:

The work involved:

1. The preparation and feeding of three "fat free" diets with 5, 10 and 15 percent levels of yeast and noting the time of the appearance and the severity of scaliness.

2. The preparation and feeding of methyl arachidonate to rats which have developed scaliness on a "fat free" diet and noting the smallest quantity of methyl arachidonate necessary to cure this scaliness.

HISTORICAL

It was Prout (1) in 1834 who was one of the first to recognize the importance of fat in the diet when he wrote, "a diet to be complete, must contain more or less of all of the three staminal principles," i.e., carbohydrate, fat and protein. Starling (2) in 1918 stated that the human alimentary tract was such that the diet should contain 20 to 25 percent of the energy in the form of fat and that when the intestine was overloaded with carbohydrates, excessive fermentation resulted. Premature hunger and lack of staying power was also exhibited by individuals on a diet low in fat. McClendon (3) noted a fermentative dyspepsia among the Japanese who subsisted on a low fat diet. The importance of fats as carriers of the fat soluble vitamins should also be cited.

The importance of fat in the diet became more evident when Burr and Burr (4, 5) presented work which was the first to indicate that there were certain fatty acids which seemed to be essential for the health of the rats. They noted subnormal growth, scaliness of the skin and an abnormal condition of the kidneys of rats on "fat free" diets. They found linoleic acid to be an important prophylactic and curative agent while saturated acids were ineffective. They concluded that warm blooded animals could not synthesize unsaturated acids. The inability of human subjects (55) to synthesize unsaturated fatty acids has also been shown.

However, Lawes and Gilbert (6) and Jordan, Jenter (7) and others (56, 71, 72, 73, 74, 75) have shown definitely that an animal can synthesize fat.

Evans et al (8, 9, 10) found that it was necessary to include linoleic acid in the diet before it could be found in body fat. They noted that certain unsaturated fatty acids were necessary for gestation and lactation, also that the males invariably developed sterility on "fat free" diets. Burr, Burr and Miller (11) had previously stated that oleic, arachidonic and l-eleostearic acids were ineffective while linoleic and linolenic acids were equally effective. Recent work by Shannon (59), Turpeinin (54), Orbison (60), and Martin (76) indicates arachidonic acid to be very effective. Hume, Nunn, Smedley-Maclean and Smith (62) found methyl linoleate about six times as effective as methyl linolenate. They also made a study (79) of the fatty acids stored in the body on a "fat free" diet and in rats on a "fat free" diet plus methyl linoleate.

Tange (12), Sahashi (13) and Becker (14) have observed the essential nature of linoleic acid. Spadola and Ellis (15) and Green and Hilditch (16) have shown that linoleic acid found in the body must come from the diet. Eckstein (17) obtained evidence that the curative action of butter due to its linoleic acid content was relative to the linoleic acid content of the feed injested. Hilditch and Thompson (18) agree with Eckstein's work. Hilditch and Sleightholme (19), Bosworth and Brown (20) and Holland et al (21) give linoleic acid contents of milk varying from 0.2% to 4.5%. No comment was made in the last three mentioned works about the linoleic acid content of the feed injested. There is considerable controversy over the curative action of butter.

Wesson and Murrel (22) noted that there was a substance in lard other than linoleic acid which had a specific metabolic action in curing fat deficiency symptoms. Wesson (23) had previously noted an abnormal respiratory quotient in rats on a "fat free" diet. This was later confirmed by Wesson and Burr (24) and Burr and Beber (25).

Hansen (26, 27), Brown and Hansen (57) and Cornbleet and Pace (28) observed favorable effects in the treatment of infantile eczema and similar conditions in adolescents. Taub and Zakon (29) in similar experiments did not find linseed oil effective in eczema. Brown, Hansen, Barr and McQuarrier (80) after a study of humans on an extremely low fat diet concluded that the body could not synthesize the highly unsaturated fatty acids. Rats fed the same diet showed the typical fat-deficiency syndrome. In the human subject on a "fat free" diet the linoleic acid content of the blood dropped from 5.7 to 3.2 and the arachidonic acid dropped from 3.2 to 1.8. Hume and Smith (30) and Gregory and Drummond (31) challenge the essential nature of linoleic acid and believe the symptoms are due to a deficiency of some member of the vitamin B complex. It has also been shown by Hume and Smith (32), Drummond (33) and Schoenheimer and Rittenberg (34) that the body can probably desaturate fat to a greater extent than oleic acid.

Burr and Burr (5) in their early work noted a kidney disturbance. Borland and Jackson (35) made a study of kidney lesions supposedly resulting from fat deficiency.

In 1928 Evans and Lepkovsky (36, 37, 38, 39) advanced the theory that the symptoms attributed to fat deficiency were closely associated with vitamin B_1. They found that lard in the ration decreased the amount of vitamin B_1 necessary for growing rats. They showed that the ease with which the fat was absorbed was related to its "vitamin B_1 sparing action". They also stated that this sparing action was not due to an interaction in the alimentary tract (40). Kemmerer and Steenbock

(41) were unable to confirm these results using rats, chickens, and pigs. Drummond (42), Gregory and Drummond (31), and Sure (43) were likewise unable to demonstrate any vitamin B_1 sparing action by the fat. However, Guha (44) found that lard seemed to have some sparing action while no other fat did, indicating that the sparing action was due to something carried specifically by the lard. There is also the theory advanced by Westerbrink (45, 46) and Elvehjem et al (68, 69) that fat might have some sparing action in that the diet contains less carbohydrates and hence less B_1 would be needed for its metabolism. This is further brought out by Evans and Lepkovsky (9) who found that oleic acid had a sparing action on thiamin equal to other fats. This indicates that the sparing action of fat is that of furnishing energy and thereby decreasing the energy derived from carbohydrates. As a result of a lesser carbohydrate utilization for energy, there is a lessened need for thiamin. This gives further proof that it is not specifically the essential fatty acids which have a sparing action on thiamin but just fat in general decreasing the amount of energy derived from carbohydrates.

Oleson, Bird, Elvehjem and Hart (58) claim that the presence of fat is important when liver is used as a source of the B complex. McKibbin, Oleson, Elvehjem and Hart (78) found that it was necessary to have fat in the diet to obtain the maximum response from the vitamin B complex.

Melnick and Field (70) have also shown that the decreased requirements of thiamin on diets high in fat was not concerned with the presence of traces of vitamin in the lard. Evidence has been obtained by Whipple and Church (71, 72), McHenry and Gavin (73, 74) and Engel and

Phillips (75) that thiamin is concerned in the synthesis of fat from carbohydrate, probably through pyruvic acid.

Work of McCollum et al (77) indicates that there is no fat soluble vitamin other than vitamin E which is necessary for reproduction.

In later work Evans et al (47, 48, 49) arrived at the conclusion that the sparing action of fat was more complex than previously thought and that it probably involves B_2 (G) also. As a result of their work they arranged the fats in the order of their sparing action on vitamin B_1. In a subsequent publication Evans et al (50) found that fat had no sparing action on vitamin B_2 (G).

According to Cowgill (51), the sparing action of fat on vitamin B_1 may be due to the B_1 forming a compound in the intestine which is fat soluble and thereby making it more easily absorbed. However, Burr and Brown (52) believe that the many variations in this work are due to variations in technique.

Present Work: (Part I)

Reference has been made (31, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50) to a considerable quantity of work that has been done concerning the sparing action of fat on the vitamin B_1 and B_2. It was thought desirable to try to elucidate further any possible relation between vitamin B_1 and essential fatty acids.

The procedure was as follows:

Twelve young rats, 20 days old, were selected, half males and half females. This made possible three groups of animals, four animals to a group. These were placed in separate cages on the "fat free" diet used by Shannon (59) and Orbison (60). Shannon (59) discussed this diet and stated that the alfalfa meal has only a small amount of ether extractable material, a large part of which is non saponifiable and hence any fat from this source in negligible. There is some ether extractable matter in yeast but Brown and Burr (61) found yeast oil ineffective. The diet was varied only so as to give yeast at a level of 5, 10, and 15 percent. The diets used were as follows:

	Diet I	Diet II	Diet III
Sucrose	180 g.	180 g.	180 g.
Casein (vitamin free)	680	680	680
Alfalfa meal	50	50	50
Salt Mixture	40	40	40
Yeast *	50	105.6	167.7
	1000 g.	1055.6 g.	1117.7 g.

The results are shown in Table I.

To check these results the experiment was repeated. The diet and conditions were exactly the same except for the kind of yeast. This time 5, 10 and 15 percent of irradiated yeast** was used with the inten-

* The yeast was a mixture of "Nyco" (98%) and irradiated yeast (2%).

** Claimed to have a potency of 7200 units of vitamin D per gram.

tion of securing the additional information of the effect that large quantities of vitamin D might have on the fat deficiency symptoms. Also it was thought desirous to find out if these large quantities of irradiated yeast might have any toxic action on the rat. The results of this experiment are seen in Table II.

When the rats had developed a severe scaly condition, they were fed a weekly supplement of .25 g. of cottonseed oil per 100 g. of body weight, (a quantity of oil found by Shannon (59) sufficient to bring about a rapid disappearance of scaliness). This was done to note if the time of disappearance of the scaly condition might be influenced by the level of yeast in the diet.

It was found by several workers (59, 60, 62) that one of the most sensitive symptoms of the fat deficiency was a scaly condition which appears first between the toes and spreads up the legs and onto the body. There is also an early scaly condition of the tail beginning at the tip and working up the tail to the body. The tail becomes ridged and annulated with appearance of widespread petechia and in severe cases necrosis of the tip of the tail results. Hume, Nunn, Smedley-Maclean and Smith (62) found that the tail was not a good criterion of healing as it responds too slowly. Petechia also appears on the feet in a severe condition. Eventually the entire body becomes scaly and the rat reaches a growth plateau and starts to decline. Death results unless fat is fed at this point.

The scoring of this condition as worked out by Shannon (59) and later used by Orbison (60) is as follows:

For Feet

Scales between toes....................................	+1
Scales between toes and on dorsal surface of foot.....	+2
Scales between toes and some on dorsal surface of the leg.....	+3
Dorsal surface of feet and legs heavily scaled........	+4

For Tail

Definite scales ½" to 1" up the tail..................	+1
Scales from 1" to 2" up the tail......................	+2
Lower half of tail ridged and scaled..................	+3
Most of tail ridged and scaled........................	+4

A condition of 4+ is used by the writer to denote any condition in excess of +4.

TABLE I

Animal No.	Sex	Diet No.	Days necessary for appearance of symptoms		Condition* when fat supplement started		Condition** at end of experiment		Weight at start	Weight of rat when fat started	Weight at end of Experiment
			Feet	Tail	Feet	Tail	Feet	Tail	g.	g.	g.
1	0		16	16	4+	4+	0	3+	48	320	358
2	0	I	16	16	4+	4+	0	4+	36	196	220
3	0		16	16	4+	4+	0	+3	40	202	244
4	0		16	16	4+	4+	0	4+	44	206	230
5	0		13	16	4+	4+	0	4+	38	175	224
6	0	II	13	16	4+	4+	0	+3	44	340	388
7	0		12	16	4+	4+	0	+4	40	224	248
8	0		13	16	4+	4+	3	4+	36	196	234
9	0		10	18	4+	4+	0	4+	33	270	350
10	0	III	11	18	4+	4+	0	+3	43	250	298
11	0		11	15	4+	4+	0	4+	36	224	250
12	0		10	14	4+	4+	1	4+	38	236	260

* The rats were on "fat free" diet for ten weeks.
** The rats were on supplement for seven weeks.

TABLE II

Animal No.	Sex	Diet No.	Days necessary for appearance of symptoms		Condition* when fat supplement started		Condition** at end of experiment		Weight at start	Weight of rat when fat started	Weight at end of Experiment
			Feet	Tail	Feet	Tail	Feet	Tail	g.	g.	g.
1	0	Irrad.	18	14	4+	4+	+1	+1	43	120	224
2	0	Yeast	18	19	4+	4+	0	+3	44	192	194
3	0	I	20	19	4+	4+	0	4+	42	200	330
4	0		21	22	4+	4+	+1	4+	41	158	270
5	0	Irrad.	11	16	4+	4+	0	+1	46	190	222
6	0	Yeast	11	16	4+	4+	0	4+	39	160	210
7	0	II	14	16	4+	4+	0	+1	42	190	290
8	0		14	14	4+	4+	0	4+	46	240	305
9	0	Irrad.	10	12	4+	4+	+1	+3	39	178	282
10	0	Yeast	9	14	4+	4+	+1	+3	39	175	251
11	0	III	9	16	4+	4+	0	+1	40	128	192
12	0		9	16	4+	4+	+1	+4	42	140	196

* The rats were on "fat free" diet for eight weeks.
** The rats were on supplement for seven weeks.

Discussion:

The most interesting information in these results is the earlier appearance of scaliness on the rats fed diets with the 10 and 15 percent yeast levels. This is shown clearly in Table I and Table II. The earlier appearance of scaliness on these rats would seem to indicate a more rapid metabolism of the essential fatty acids in these animals. It was thought that possibly the increased metabolism of these essential fatty acids might be a result of increased growth at the higher yeast levels, so the average weight increase was figured for the animals in Table I over the first ten weeks. The results were as follows:

Diet with		Diet I	Diet II	Diet III
"Nyco" Yeast	av. wt. increase for 10 wk. period	189 g.	194 g.	207 g.
Irradiated Yeast	av. wt. increase for 8 wk. period	125 g.	152 g.	113 g.

It is true that there was some difference in growth on the three diets. However, in a matter of ten to nineteen days (the time necessary for appearance of first symptoms) there was not enough growth difference to warrant the claim that growth had anything to do with the earlier appearance of symptoms in the higher yeast levels. In fact, the animals on the 5 percent yeast weighed more when symptoms appeared than the rats on the 15 percent level weighed when their first symptoms appeared. The average weight of the rats on the three levels of yeast was also calculated at the time of the first appearance of symptoms. The results were as follows:

Diet with		Diet I	Diet II	Diet III
"Nyco" Yeast	av. wt. increase at first symptoms	54 g.	49 g.	42 g.
Irradiated Yeast	av. wt. increase at first symptoms	19 g.	46 g.	22 g.

It may be that at the higher yeast levels the destruction of essential fatty acids was increased and as a result scaliness appeared at an earlier date.

As has been stated previously, there is an impaired kidney function on "fat free" diets. The fact that the diets with 10 and 15 percent yeast levels contained a higher percentage of protein must not be overlooked. It may be that the higher levels of protein in the 10 and 15 percent yeast diets were the cause of the earlier appearance of symptoms in the animals on these diets. This possibility is in close agreement with work of Burr and Burr (4, 5) and Eckstein (63). This affect on the kidney is not so strange when we remember that the kidney is one of the most active of the semi-permeable membranes of the body and that membranes contain many intimately associated lipids. Phospho-lipids are rich in unsaturated fatty acids and furnish one of the best sources for the preparation of arachidonic acid.

In the irradiated yeast diets it can be noted that there was a distinct difference in growth between Diets I and II for the eight weeks. However, the average weight of the animals on Diet III was considerably lower than either Diet I or II. Also it was noted that these animals developed a severe diarrhea when first placed on the diets. This was particularly noticeable in the animals on Diet III. The feces was black and very watery indicating an intestinal disturbance probably due to the large amount of irradiated yeast in the diet. The failure of the animals on Diet III to grow well indicates a probable toxic condition of a diet of this high irradiated yeast content. None of the animals on the diet containing irradiated yeast grew as well as the animals on the "Nyco" yeast. The interesting thing is that the appearance of scaliness of the rats on the irradiated closely paralleled the rats on similar diets using the plain yeast. This second experiment served as a check on the previous one and proved that a higher yeast content in the diet caused an earlier appearance of scaliness. It had no affect on the disappearance of the symptoms, however, nor did it seem to affect the severity of the symptoms. In fact, the only apparent effect that a higher yeast content in the diet had is one of producing an earlier appearance of scaliness.

An extremely high water intake was noted in animals on the "fat free" diet. This is in agreement with the work of Burr and Burr (4) and Borland and Jackson (35) who found a kidney involvement on "fat free" diets. Also Burr and Burr (4) found that even though the animals consumed twice as much water, they voided no more urine than usual. This indicates a disruption of the normal skin function which is probably closely correlated with the scaliness recognized as among the symptoms of essential fatty acid deficiency. The appearance of scaliness obviously signifies an abnormal condition of the skin and, as previously mentioned, this may not be so strange since the structure of the skin, and the walls of the cells making up the skin, have an appreciable phospholipid content. Linoleic acid is probably one of the normal fatty acids in the oil of the skin and if there is not enough supplied in the diet, the production of oil will diminish to the point at which excessive drying of the skin results. Also, the maintaining of a normal oiliness is perhaps of secondary importance to the animal, so scaliness appears before there is serious involvement of more important tissues. It is logical that if unsaturated fatty acids are not supplied to replenish similar fatty acids in the body that are being metabolized, that the lack of these essential fatty acids will manifest itself in some manner or other. Also, the logical place for such a deficiency to manifest itself is where those fatty acids play an important role in the body, as for example the skin and the kidneys.

Present Work: (Part II)

Reference has been made to the essential nature of arachidonic acid (54, 59, 60, 76). The claim has also been made that arachidonic acid is not essential (11). However, the evidence at the present time seems to indicate that arachidonic acid is one of the essential fatty acids.

The second part of this work consisted in the preparation of methyl arachidonate and feeding it at different levels so as to determine the critical level necessary per 100 g. of rat per week to eliminate the symptoms of fat deficiency (scaliness). The supplement was fed after the symptoms had developed on the "fat free" diet. The "fat free" diet No. II given previously containing 10 percent yeast (Nyco) was selected for these tests.

The method of Brown (64) for the preparation of methyl arachidonate gave some trouble so that several methods (65, 66, 67) were studied and parts of each combined to give a complete and satisfactory procedure. This procedure, with variations and adaptations, is given as follows:

Preparation of Methyl Arachidonate

Saponification:

600 g. of suprarenal lipids were placed in a 5 L. 3 necked flask equipped with a reflux condenser and a tube for the introduction of nitrogen which leads to the bottom of the flask. The air was flushed from the flask and the lipids melted. A hot solution of 400 g. KOH in 400 ml. water was added slowly with thorough mixing. 400 ml. of methyl alcohol was added and again mixed thoroughly. Saponification was complete in 10 minutes. The nitrogen was introduced in a slow steady stream to insure agitation of the solution. After saponification, two liters of hot water were added to dissolve the soaps and the fatty acids were liberated by slowly adding concentrated HCl. The contents were heated on the steam bath with agitation until

a clear definite layer developed. The contents were then cooled, 300 ml. of ethyl ether added, mixed thoroughly and the fatty acid layer separated quickly by means of a separatory funnel. The fatty acid layer was saved, returned to the flask under N. and 500 ml. of ethyl ether added to it. The first addition of 300 ml. of ether reduced the viscosity of the fatty acids and also reduced the amount of oxidation that could have taken place during separation.

Bromination:

The flask containing fatty acids was then immersed in ice and the temperature reduced nearly to 0°C. The bromination was carried out by passing nitrogen through an erlenmeyer flask containing the bromine* and then through to the bottom of the ether solution of fatty acids. The flask containing the bromine was immersed in a container of water which could be heated with steam. In this way the rate of bromination was controlled by the temperature of the container. Bromination, when carried out in this way, reduced to a minimum the possibility of the experimenter coming in contact with bromine and it also gave a smoother, more uniform bromination and at the same time considerable agitation. It proved to be far better than bromination with a separatory funnel.

Separation of the octabromoarachidic acid:

Any water in the bottom of the flask was removed by a capillary tube connected to an aspirator. The octabromide settled out as a brownish grey precipitate. The ether containing dark brown impurities was decanted off and the octabromide was then washed repeatedly with ether in a centrifuge until the washings were free from color. It was then washed similarly sev-

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* It was absolutely essential that glass tubing be used from the bromine flask to the flask containing the fatty acids. Rubber connections are rapidly destroyed when in contact with bromine.

eral times with benzene. According to Cartland and Hart (67) this removes contaminating hexabromides and leaves nearly a pure octabromide. The precipitate was then washed with ether to remove the benzene, centrifuged, dried and powdered. 120 g. was obtained.

Debromination:

In the same 3 necked flask with condenser, and nitrogen agitator, there was placed 120 g. of the octabromide and 200 g. of copper-coated zinc (64). The cooper-coated zinc was prepared as follows:

> 200 g. of zinc powder was placed in a flask and treated with 1% HCl for a minute or two to remove the oxide. The HCl was poured off and the zinc was treated with successive portions 1.0% $CuSO_4$ solution until the zinc was covered with a black coating. The zinc was washed several times with anhydrous methyl alcohol. It was then ready for use.

The octabromide and copper-coated zinc was then covered with 1 liter of absolute methyl alcohol containing dry HCl. This was then heated to boiling on a steam bath and agitated. Mechanical agitation was necessary to keep the Zn from settling. Dry HCl gas was introduced to the bottom of the flask at a moderate uniform rate during the entire debromination. At the end of the two hours the supernatant was decanted off and covered again with anhydrous methyl alcohol containing dry HCl and the process repeated. The nearly pure methyl arachidonate was obtained from the supernatant by vacuum distillation in the cold to remove the methyl alcohol.

Purification:

The successive portions of crude methyl arachidonate prepared above were combined. The purified methyl arachidonate was prepared by distillation under reduced pressure (200° C. - 210 C. at 7 mm. pressure). The crude methyl esters were heated in an oil bath to 200° C. previous to reducing the pressure. The pump was then started and the vacuum drawn until foaming started (best regulated by a stopcock between the pump and the distillation flasks). When foaming became excessive, the stopcock was closed until the foaming subsided. This was repeated until full vacuum could be drawn. When carried out in this way, there is much less foaming than when the vacuum distillation is started in the conventional manner of drawing the vacuum first and heating secondly. The high temperature decreases the tendency to foam.

The esters were distilled and placed in a tube. A small quantity of hydroquinone was added and the methyl arachidonate was stored under nitrogen in a sealed container. 9 g. were obtained. Iodine number 304 (30 min.) --theoretical 319.2.

Feeding Tests:

Five groups of animals were set up, two males and two females to a group, making a total of 20 animals. The groups were as follows:

Group I	0.025 g. of ester/100 g. body wt./week.
Group II	0.050 g. of ester/100 g. body wt./ week.
Group III	0.075 g. of ester/100 g. body wt./ week.
Group IV	Negative control
Group V	Positive control. 0.205 g. Wesson Oil/100 g. body wt./ week.

The animals were placed on the "fat free" diet for a period of five weeks, a time which allowed quite severe symptoms to develop. They were then given the weekly weighed supplements designated above. The supplement was fed weekly for a period of seven weeks and the results taken at the end of that time. The results are shown in Table III.

TABLE III

	Animal No.	Sex	Supplement	Condition when rat supplement started		Condition at End of Experiment		Weight at Start	Weight when Supplement Started	Wt. at end of Experiment
				Feet	Tail	Feet	Tail			
	1	0	0.025 g.	+4	+4	+4	4+	52	216	333
Group I	2	0	per 100 g.	4+	+3	4+	4+	45	172	268
	3	0	of rat per	+4	+4	4+	4+	44	151	202
	4	0	week	+4	+3	4+	4+	41	160	208
	5	0	0.050 g.	4+	4+	+2	4+	42	186	313
Group II	6	0	per 100 g.	+4	+1	+2	+1	58	227	327
	7	0	of rat per	+2	+1	+1	+1	62	166	214
	8	0	week	+4	+2	0	+1	40	140	188
	9	0	0.075 g.	4+	4+	0	+4	39	149	200
Group III	10	0	per 100 g.	+4	+3	0	+3	40	180	278
	11	0	of rat per	+4	+1	0	0	48	145	192
	12	0	week	+4	+3	0	+3	40	154	214
	13	0		+3	+1	0	+1	61	220	316
Group IV	14	0	Positive	+4	+3	0	+3	38	152	252
	15	0	Control	+4	+2	0	0	46	171	238
	16	0		+1	+1	0	0	62	172	226
	17	0		4+	4+	4+	4+	46	187	241
Group V	18	0	Negative	4+	4+	4+	4+	39	160	229
	19	0	Control	+4	+4	4+	4+	42	159	180
	20	0		+4	4+	4+	4+	42	157	183

Discussion:

It has been noted by several workers (54, 59, 60, 76) that arachidonic acid is a potent curative agent for scaliness due to essential fatty acid deficiency. The work presented here bears out this fact. It can be seen from Table III that the quantity of methyl arachidonate necessary per week to cure scaliness was between 50 and 75 mg. At the 75 mg. level there was a complete cure of the symptoms on the feet but due to the severity of the condition of the tail, it did not show as marked an improvement as did the feet. The tail is not a good criterion of healing because the process is too slow. This is in agreement with the findings of Hume, Nunn, Smedley-MacLean and Smith (53). Turpeinin (54) has shown that 33 mg. of this ester daily gave maximum growth in rats which had previously reached a growth plateau. This corresponds to 231 mg. per week which is approximately three times the quantity used here. This is in fair agreement as Turpeinin fed to give maximum growth while in this experiment it was fed solely to eliminate scaliness. This would require less than the amount necessary to give maximum growth. Also the amount of ester that Turpeinin fed was the total amount fed to the rat while the amount noted in these experiments was fed per 100 g. of rat. Thus these values are in much closer agreement. Also it will be noted in Table III that 50 mg. per 100 g. of rat per week gave a noticeable curative action in the period of seven weeks. This indicates that 50 mg. is a curative does but its action is much slower than the 75 mg.

It was noticed that during the spring while the humidity was high that it took longer for the appearance of scaliness than it did during the winter. This coincides with a similar observation by Brown and Burr (61) which prompted them to control the humidity in their experiments and as a result they obtained more uniform results.

The 0.250 g. of Wesson Oil fed per week in the control resulted in a slightly faster clearing of the symptoms than did the 0.075 g. of methyl arachidonate. The rats on the Wesson Oil supplement showed complete disappearance of scaliness on the feet about one week before the rats on the 0.075 g. of methyl arachidonate. This indicates that this 0.075 g. of methyl arachidonate per week is not quite as potent a cure as 0.250 g. of Wesson Oil per week.

Summary

1. By raising the percentage of yeast in the "fat free" diet, the time necessary for the appearance of scaliness may be shortened. The results were duplicated using irradiated yeast in place of the plain yeast although growth was somewhat retarded.

2. Humidity has a noticeable affect on the time of appearance and severity of scaliness. The higher the humidity, the longer it takes for its appearance and the symptoms are not as severe in a given lenth of time.

3. A modified procedure for the preparation of methyl arachidonate is given which greatly increases the ease and efficiency of the preparation of this compound.

4. Methyl arachidonate is an effective curative agent for scaliness in rats maintained on a "fat free" diet. 0.075 g. or less per 100 g. of rat per week was found to effect a cure of quite severe conditions within seven weeks.

5. In agreement with the experience of others, the feet were found to be the best suited for observing the developement and disappearance of scaliness.

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