

TABLE IV.
ATP-ase of the Uterus Through the Estrus Cycle.
 μg Inorganic P Liberated in 15 min per mg Dry Tissue.

Stage	No. of trials	Skeletal muscle		Uterus	
		Avg	Range	Avg	Range
Estrus	2	35.5	(35-36)	125.0	(120-130)
Metestrus	9	33.7	(25-56)	93.9	(62-122)
Diestrus	3	50.0	(32-52)	107.3	(63-143)
Proestrus	2	35.5	(17-54)	106.0	(88-140)

effect. Variation in the ATP-ase activity of the uterus could not be correlated with cyclical estrus change. With the possible excep-

tion of testosterone, hormones did not appear to affect the *in vitro* systems.

16128

Production of Essential Fatty Acid Deficiency Symptoms in the Mature Rat.*

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In the course of studies on the effect of vitamin imbalance under various conditions, mature rats were maintained on restricted caloric intake of fat free diets until they lost about 50% of their original weight. In the period of recovery, during which time they were fed the same diets *ad libitum*, various symptoms including those typical of the Burr and Burr syndrome¹ were observed.

Since the original report by Burr and Burr,² several workers^{3,4,5} have observed the

typical skin symptoms of the essential fatty acid deficiency, but in all cases young animals were used. So far as we know, in no case was this deficiency syndrome reported to have been produced in adult rats.

Experimental and results. Mature male rats of the Sprague-Dawley strain averaging about 225 g have been used in these experiments. The rats were placed in individual metal cages with raised screen bottoms at the beginning of the depletion period on the fat free diets. They were weighed and examined weekly or in later experiments twice weekly.

In the first experiment 4 groups of 4 rats each were placed on the 4 diets given in Table I. In addition they all received one drop of haliver oil weekly.

For a period of 2 months each rat received 6 g of food daily. At the end of this period the amount was decreased to 5 g per day. On the 91st day one rat died in each of the groups I, II, and IV (none in the "B + supplement" group). The rats which died weighed 94, 93 and 95 g respectively. On this day all the rats were allowed their respective diets *ad libitum*. The only symptom observed by this time besides the severe emaciation

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¹ Burr, George O., and Burr, M. M., *J. Biol. Chem.*, 1930, **86**, 587.

² Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345.

³ Graham, C. E., and Griffith, W. H., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 756.

⁴ Turpeinen, O., *J. Nutrition*, 1938, **15**, 351.

⁵ Hume, E. M., Nunn, L. C. A., Smedley-Maclean, I., and Smith, H. H., *Biochem. J.*, 1938, **32**, 2162.

TABLE I.
Composition of Diets.

Dietary component	I	II	III	IV
	Diet A	Diet B	Diet B + supplement	Diet C
Casein (fat free)*	30.0	30.0	30.0	30.0
Sucrose	66.0	66.0	66.0	56.0
Salt mixture IV†	4.0	4.0	4.0	4.0
Dried brewer's yeast	—	—	—	10.0
	per 100 g of diet			
Thiamine HCl	.20 mg	20.00 mg	20.00 mg	—
Riboflavin	.40	40.00	40.00	—
Pyridoxine HCl	.20	2.00	2.00	—
Calcium pantothenate	.30	3.00	3.00	—
Choline chloride	120.00	120.00	120.00	120.00
3-Methyl-naphthaquinone	.50	.50	.50	.50
Biotin	—	—	.02	—
Folic acid	—	—	.20	—
Inositol	—	—	50.00	—
p-Aminobenzoic acid	—	—	15.00	—
Niacin	—	—	.60	—
Corn oil	—	—	500.00	—

* Extracted for three 2-hour periods with boiling alcohol.

† Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, 1935, **109**, 657.

was slight rustiness of fur about the shoulders and neck.

On the 107th day (16 days after the start of the *ad libitum* feeding) various skin symptoms were noticed in the rats of the first 2 groups. Among these, scaly hind paws were almost uniformly present. Other symptoms which appeared among the rats, though not uniformly, were: scaly and/or annular tail; necrosis and loss of tip of tail; sloughing off of skin near base of tail and/or around the mouth; loss of hair around the mouth, eyes, over fore paws, at the base of tail and around the scrotum; thinness of hair coat over back, chest and abdomen; some edema of fore and/or hind paws and prolaps penis. The animals receiving the diet containing yeast developed scaly paws and other skin symptoms a few days later than the first 2 groups, while the group on the purified diet which was supplemented, as mentioned above (Table I), with both additional vitamins of the B complex and with 0.5% corn oil showed practically no symptoms at any time.

When these symptoms were evident, the animals in Groups I and IV were supplemented with 0.5% corn oil and those in Group II with the extra 5 vitamins included in diet III in order to see which of these factors was involved in the prevention of

the symptoms. The results of this experiment are summarized in Table II. Those animals which received additional vitamins (no corn oil) still showed scaly paws 7 weeks later, the time at which they were killed. All the rats which received the corn oil after having demonstrated the symptoms, started to show signs of recovery in 2 weeks and were completely cured within 5-6 weeks.

Along with the caloric restricted rats there was an equal number of rats on *ad libitum* regimen from the beginning of the experiment. None of these showed any symptoms at any time.

In the second experiment Diet A was used as the basal diet, and all the rats were again supplemented with one drop haliver oil weekly. The depletion period lasted 39 days, during which time the average weight dropped from 225 to 120 g. The rats were divided into two groups at this time and one group was continued on Diet A, while the other received Diet A supplemented with 0.5% corn oil. The results are summarized in Table III.

The effectiveness of corn oil in preventing development of the symptoms is obvious. The only symptoms which appeared in 5 rats of the supplemented group were slight and transient. Again the appearance of the

TABLE II.
Condition of animals at Different Periods.

	Beginning of exper.	End of depletion	Appearance of scaly paws		Beginning of disappearance of scaly paws due to $\frac{1}{2}\%$ corn oil	Complete clearing before exper. terminated
I—Diet A						
Days	0	91	107		120	154
Total No. rats	4	3	3		3	3
No. rats affected			3		3	3
%			100		100	100
Weight	223	118	153		200	334
II—Diet B						
Days	0	91	107		120	154
Total No. rats	4	3	3			3
No. rats affected			3			0
%			100			0
Weight	223	118	188		241	286
III—Diet B + supplement						
Days	0	91	107		120	154
Total No. rats	4	4	4		4	
No. rats affected			0			
%			0			
Weight	223	130	229		285	338
IV—Diet C						
Days	0	91	107	112	120	154
Total No. rats	4	3	3	3	3	3
No. of rats affected			0	3	3	3
%			0	100	100	100
Weight	223	125	209	238	266	317

other skin symptoms was observed in a large number of the rats on the unsupplemented diet.

Five of the rats from the unsupplemented group were given 2 drops of corn oil daily after they demonstrated the marked symptoms. Some improvement was noticed in all animals within 2 weeks, and they were all completely clear of symptoms within 3-5 weeks.

Ten rats were kept on the unsupplemented Diet A for 71 days. At the end of this period it was noticed that the scaliness of the paws had completely disappeared.

In the third experiment the animals were placed for 3 weeks on restricted amounts of our stock ration containing: ground wheat 25.00, soybean oil meal 10.00, linseed oil meal 10.00, ground yellow corn 29.00, powdered skim milk 12.00, alfalfa meal 8.00, butter 5.0, NaCl (iodized) 1.0, CaCO_3 0.5. This was followed by 4 weeks on restricted amounts (5 g/day/rat) of fat free Diet A, supplemented by one drop of haliver oil weekly. At the time when *ad libitum* feeding was started the rats were divided into 4 groups

receiving Diet A with supplements listed in Table IV, plus one drop of haliver oil/week/-rat. To guard against the possibility that the ultimate disappearance of the scaly paws of the unsupplemented animals was due to very small amounts of essential fatty acids in the haliver oil fed, in this and the following experiments carotene, calciferol (D_2) and alpha tocopherol suspended in propylene glycol or glyceryl (mono) laurate or methyl oleate were substituted for the haliver oil at the time when the scaliness of the paws appeared.

All of the 9 animals which received no essential fatty acid showed development of scaly paws within 2 weeks after the initiation of the *ad libitum* feeding. The 10 rats which received either ethyl-linoleate or corn oil did not develop the scaly paws except for a slight scaliness in 3 animals, but this disappeared in a few days. The average weights of the animals at the commencement of *ad libitum* feeding and at subsequent periods are recorded in Table IV.

It should be noted that in the 9 animals

TABLE III.
Average Weight and Number of Rats.

Supplement	Starting <i>ad lib.</i> feeding	Developing scaly paws within 17 days	Developing scaly paws within 28 days	Clearing in 1-2 weeks	Kept for 71 days	Cleared up spontaneously within 71 days
None	31 (126)*	26 (166)	31 (230)	0	10	10
0.5% corn oil	11 (129)	5 (slight) (182)	— (230)	5	—	—

* The numbers in parentheses indicate average weight.

TABLE IV.
Average Weight and Number of Rats.

Supplements	In a group starting <i>ad lib.</i>	Developing scaly paws within 2 weeks and lasting longer than 1 week	Showing scaly paws after 6 weeks	Showing complete clearance at 66 days
None	4 (114)*	4 (166)	4 (224)	4
2 drops oleic acid daily	5 (116)	5 (187)	5 (251)	5
2 drops ethyl linoleate daily	5 (117)	0 (178)	0 (268)	—
2 drops corn oil daily	5 (117)	0 (178)	0 (274)	—

* The numbers in parentheses indicate average weight.

which received no source of linoleic acid scalliness of the paws disappeared spontaneously, and within 66 days from the start of *ad libitum* feeding these animals, all of which had shown this symptom, were completely cured. The other skin lesions, when present, cleared much earlier.

This spontaneous disappearance of skin lesions within several weeks and paw scalliness within 66 days was again observed in a similar experiment carried out later with approximately the same number of animals on fat free ration A.

Discussion. Burr and Burr¹ stress the importance of scalliness of the feet as the most sensitive test of the essential fatty acid deficiency condition. Hume, Nunn, Smedley-Maclean and Smith⁵ observed dryness occurring invariably on the dorsal surface of the hind feet and front of the ankles, and therefore they finally adopted it as the most satisfactory criterion, rather than relying entirely upon weight increase which is a non-specific response. The objection to the use of skin symptoms as criterion because of their irregular appearance⁶ which is due to variation in relative humidity⁷ is obviated

in our work by the constant 50% relative humidity kept in our animal room.

Although the rats in the first experiment were maintained for a long period (3 months) on a restricted intake of fat free diets, none of the symptoms described above appeared before about 2 weeks after *ad libitum* feeding commenced, a period during which there was rapid regain of weight. In later experiments, the depletion periods on the fat free diet were considerably shorter (4-6 weeks), yet the symptoms again appeared within 2-3 weeks after the beginning of the *ad libitum* regimen.

The typical symptoms of essential fatty acid deficiency were produced heretofore only when young rats were used and generally after very long periods of fat deprivation,^{8,9} yet in our work the mature rats demonstrated the symptoms after a total period of 6-7 weeks

⁶ Evans, H. M., and Lepkovsky, S., *J. Biol. Chem.*, 1932, **96**, 587.

⁷ Brown, W. R., and Burr, G. O., *Proc. Am. Soc. Biol. Chem.*, 1936, **8**, XVI.

⁸ Hume, E. M., and Smith, H. H., *Biochem. J.*, 1931, **25**, 300.

⁹ Gregory, E., and Drumund, J. C. Z., *Vitaminforschung*, 1932, **1**, 257.

on a fat free diet. Therefore, the technique seems to suggest itself for other types of experiments where a rapid "growth" (weight gain) or some "strain" on body functions is desired.

The ultimate disappearance of the scaly paws in these mature rats may be explained on the basis of: (1) lower demand for essential fatty acids when the rate of tissue building reaches normal maintenance level, (2) synthesis of at least a limited amount of an "essential" fatty acid within the animal itself, or (3) the combined effect of 1 and 2 in these mature animals.

Smedley-Maclean and Hume¹⁰ found that the polyunsaturated acid content of the tissue of fat starved rats fell to a very low level during the first 6 months of fat starvation. In our rats the total amounts of essential fatty acids in the emaciated bodies at the commencement of *ad libitum* feeding is probably very low. Smedley-Maclean and Nunn¹¹ concluded that in the process of growth comparatively large quantities of arachidonic acid are utilized and disappear. In the light of these findings and the observed weight gains in our rats with the beginning of the *ad libitum* feeding of the fat free diet it seems likely that they further depleted their supply of essential fatty acids when the scaliness of the paws appeared. After this

there was further weight gain in which essential fatty acids were further utilized, yet the scaly symptoms finally disappeared without any external source of these acids. Therefore, it seems likely that at least a limited amount of the essential fatty acids is synthesized in the bodies of these mature rats. This appears to be in agreement with the work of Harris¹² who used the deuterium technique and found that under appropriate conditions the rat can synthesize the essential fatty acid arachidonic acid.

Summary. 1. Mature rats were maintained on a fat free diet for relatively long periods without showing any deficiency symptoms except emaciation.

2. Severe depletion followed by *ad libitum* feeding of the fat free diet precipitated in mature rats symptoms including those typical of essential fatty acid deficiency.

3. This method is suggested for other types of experiments where a "strain" on body functions is desired.

4. Either ethyl linoleate or corn oil prevented entirely or cured rapidly the symptoms of essential fatty acid deficiency when they appeared.

5. When the rats, after depletion, were maintained on the fat free diet *ad libitum* for sufficiently long periods, spontaneous disappearance of all the symptoms was observed, for which the explanation of some synthesis of essential fatty acid is suggested.

¹⁰ Smedley-Maclean, I., and Hume, E. M., *Biochem. J.*, 1941, **35**, 990.

¹¹ Smedley-Maclean, I., and Nunn, L. C. A., *Biochem. J.*, 1940, **34**, 884.

¹² Harris, R. S., 24th Annual Report of the National Live Stock and Meat Board, 1946-47.