the excretion of female sex hormone in patients with cortical tumors. Quantitatively negative results were reported by Corey and Britton[7] in ovariectomized rats and by Corey[8] in hypophysectomized rats in attempts to produce estrus with adrenal cortical extracts.

Immature female rats were spayed when 22-23 days old. Injections of A-C-T (prepared and assayed by the method previously published[9]) were given intraperitoneally beginning on the day following the operation. The vaginæ of the injected spayed rats opened after the administration of 30-42 units of A-C-T. The vaginæ of untreated spayed rats did not open.

TABLE I.

Rats	Opening of Vagina Days of Treatment	Daily Dose of A-C-T Units	Total Dose of A-C-T Units
W 43	24	1.5	36.0
W 45	24	1.5	36.0
BH 48	25	1.5	37.5
W 18	16	1.9	30.4
BH 28	18	1.9	34.2
B 05	22	1.9	41.8

Vaginal smears of the treated spayed rats showed the presence of cornified cells, a few nucleated epithelial cells and leucocytes. Animals W 18 and B 05 showed, 2 days after the opening of the vagina, vaginal smears consisting predominantly of leucocytes.

At autopsy the adrenals of the injected rats were found to be greatly hypertrophied.

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Effectiveness of Arachidonic Acid in Curing "Fat Deficiency" Disease.*

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The earlier work by Evans and Burr,[1] by Burr and collabora-

7 Corey, E. L., and Britton, S. W., *Am. J. Physiol.*, 1934, **107**, 207.

8 Corey, E. L., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 41.

9 Moon, H. D., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 649.

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† Holder of a Finnish State Fellowship.

1 Evans, H. M., and Burr, G. O., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 740.

tors,[2, 3] and by Evans and Lepkovsky[4] has definitely shown that the rat cannot maintain growth and well-being on diets rigidly devoid of fat, but invariably develops a characteristic deficiency disease. The vital need of the animal is not, however, as the same investigators have demonstrated, for fat in general but for certain unsaturated fatty acids. Of these, only linoleic and linolenic acids have so far proved effective in curing this specific deficiency, whereas oleic and alpha-eleostearic acids have been found inactive. Now, whether the 2 first-mentioned acids are the only ones capable of satisfying the need of the body for unsaturated fatty acids or whether other fatty acids with the same curative effect exist is naturally a question of great interest and of considerable theoretical significance.

In the following, the results of some experiments with arachidonic acid are reported. This acid has been tested once before by Burr, Burr and Miller,[3] who found that an addition of methyl arachidonate as 10% to a mixture of equal parts of methyl linoleate and methyl linolenate slightly decreased the curative effect of the original mixture. This result did not, however, seem quite plausible, even to the above mentioned investigators, and it was felt by us that further tests with arachidonic acid were urgently needed.

Young female rats immediately after weaning were placed on a fat-low, casein-sucrose-salt diet supplemented with yeast and the non-saponifiable fractions from cod liver oil and wheat germ oil. When growth had ceased and the animals had reached a "plateau", they were deemed ready for tests. They were not used, however, before they had shown a constant or slightly declining weight over a period of at least 30 days. The test substances were fed together with the vitamin supplements daily (except Sundays). Arachidonic acid was given in the form of methyl arachidonate‡ at 2 levels, *viz.*, 33 and 100 mg. of the ester daily. The curative effect was measured mainly by the intensity of the renewed growth, for this was easiest to record quantitatively, although as a rule, close parallelism existed between the resumption of growth and the disappearance of other deficiency insignia (irregularity of ovulation, renal hemorrhage, etc.).

Results and Conclusions. The results are shown in the curves in

[2] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82** 345; 1930, **86**, 587.

[3] Burr, G. O., Burr, M. M., and Miller, E. S., *J. Biol. Chem.*, 1932, **97**, 1.

[4] Evans, H. M., and Lepkovsky, S., *J. Biol. Chem.*, 1932, **96**, 143, 157.

‡ We wish to acknowledge the gift of this substance from Professor J. B. Brown of the Ohio State University, and Mr. George Y. Shinowara associated with him. Without it our work would have been impossible.

Fig. 1, each of which represents the average growth of a group of 3 rats. A curve demonstrating the growth response produced by 100 mg. of methyl linoleate daily is included for comparison. As the graph clearly indicates, growth is very similar in all 3 cases. The lag in the beginning of the 33 mg. curve is probably not significant because of the striking agreement of the curves in the later phases. These results leave no doubt as to the efficacy of arachidonic acid as a powerful curative agent in the "fat deficiency" disease. It is even more potent than linoleic acid. Some recent experiments, which will be described in detail elsewhere, have shown that about 100 mg. of methyl linoleate daily is needed to produce maximal growth in plateaued "fat-deficient" rats. The same effect can be obtained with 33 mg. of methyl arachidonic. Whether even smaller doses of arachidonic acid would give maximal growth response must be left undecided through lack of trials, but the tests reported here already justify the conclusion that arachidonic acid is at least approximately

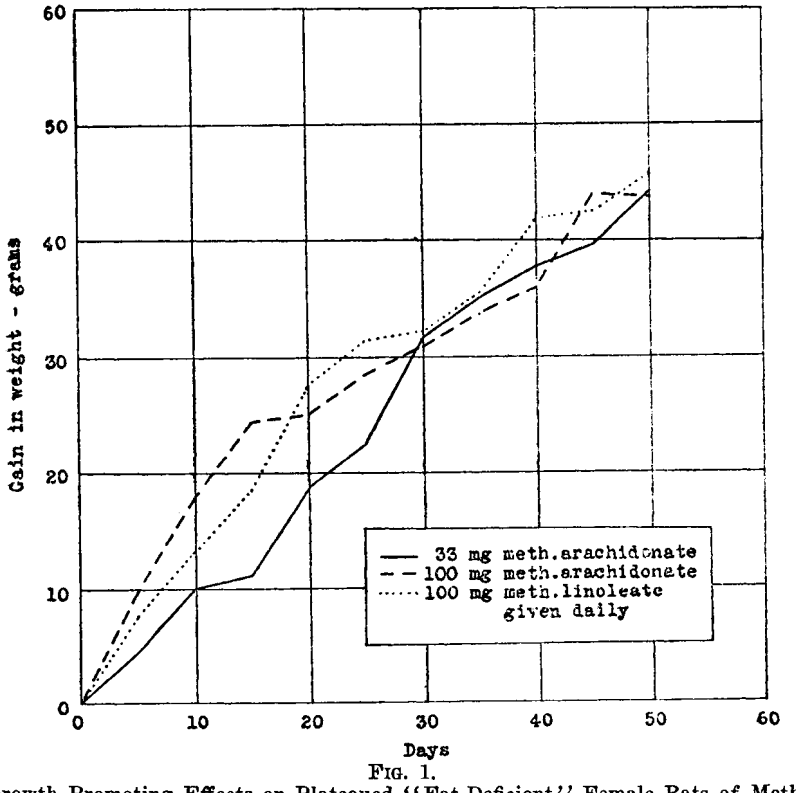

FIG. 1.
Growth Promoting Effects on Plateaued "Fat-Deficient" Female Rats of Methyl Arachidonate as Compared with Methyl Linoleate.

3 times as effective as linoleic acid in curing the "fat deficiency" disease.

The effectiveness of arachidonic acid as a curative agent might seem to be in contradiction to the finding by Spadola and Ellis[5] that the rat is able to synthesize this substance. The controversy can be eliminated, however, by assuming that this synthesis can take place only from (or in the presence of) linoleic (or linolenic) acid, which assumption seems to be in full accordance with the experimental data of the above mentioned workers. The superiority of arachidonic to linoleic acid as a curative substance would suggest the tentative hypothesis that the need of the animal organism might be primarily for the former acid and that linoleic (and linolenic) acid could be beneficial solely on account of its conversion into arachidonic acid in the body.

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Relation of Bile to Absorption of Vitamin E in the Rat.

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The present study is an extension of the previous work reported from this laboratory on the relation of bile to the absorption of the fat soluble vitamins.[1]

In order to determine whether or not vitamin E is absorbed by the bile fistula rat, female rats cannot be employed since female rats with bile fistulas fail to breed, and the usual criterion, *viz.*, the prevention of resorption of the fetuses by administration of vitamin E to the mother before the fifth day of pregnancy, cannot be used. Furthermore, if the bile fistula is established after breeding and before the eleventh day of pregnancy, resorption of the litter takes place.[2] Therefore the male rat was used in these experiments. In vitamin E deficiency, characteristic changes in the testes take place. The con-

5 Spadola, J. M., and Ellis, N. R., *J. Biol. Chem.*, 1936, **113**, 205.

* E. R. Squibb and Sons Fellow. Technical assistance was supplied by the Works Progress Administration.

1 Greaves, J. D., and Schmidt, C. L. A., *J. Biol. Chem.*, 1933, **102**, 101; *Univ. of Calif. Pub. Physiol.*, 1934, **8**, 43; *Am. J. Physiol.*, 1935, **111**, 492, 502; 1936, **116**, 456; PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 434.

2 Unpublished work from this laboratory.