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.

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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2 Unpublished work from this laboratory.

dition is irreversible. For this reason curative tests could not be used in the present study. The method of preventing the characteristic changes in the testes was used as the criterion for the absorption of vitamin E in bile fistula rats.

Three hundred fifty male rats, after weaning, were reared on a diet which was deficient in vitamin E and high in its content of fat.[3] At various intervals of time most of the animals were unilaterally castrated. The testis of each animal was weighed, sectioned, and studied histologically. Breeding tests were also carried out on these rats. Female rats of known fertility were employed in the breeding tests. In this manner the physiological state of the testis was correlated with the histological picture. The testis which was removed first served as a control. At the end of the experiment the second testis was removed and the histological pictures of the 2 were compared.

The weights of the first testes increased rapidly for a period of 50 to 70 days after the animals were placed on the diet. This was followed by a sudden and marked drop in the weights. This condition continued until about the hundred and twentieth day. The histological sections of these testes showed a progressive sloughing of the germ elements. In the earliest cases the condition manifested itself about the fortieth day and, in all cases, it was in evidence by the ninetieth day. There was a close parallelism between the weight of the testis and the degree of sloughing. The animals which did not receive supplements of vitamin E became sterile shortly after the hundredth day. The breeding data indicate that the animals reach sexual maturity about 30 to 60 days after being placed on the vitamin E free diets. After 60 days, increasing numbers of the animals gave histological evidence in the first testis of vitamin E deficiency. The condition was irreversible even though large amounts of vitamin E were administered. Most of the animals used in these experiments were castrated unilaterally shortly after the fortieth day, and only those which showed a normal picture on histological examination of the testis were used in the absorption studies.

A group of 82 animals served as controls. After unilateral castration they were placed on a low fat, vitamin E deficient diet.†

[3] Hawk, P. B., and Bergeim, O., *Practical Physiological Chemistry*, 10th ed., p. 680.

† The diet consisted of alcohol-extracted casein 18%, cane sugar 69%, Osborne and Mendel salt mixture 4%, dried yeast 7%. A concentrate of vitamins A and D, in amounts equivalent to a level of 1% of cod liver oil, was injected into the bile fistula rats and added to the diet of the others. Methyl linolate from corn oil (0.4 cc. per week) was administered likewise.

Groups of these rats were subjected either to various degrees of inanition or to laparotomy. Another group received vitamin E. This was done in order to rule out the possible effect of these factors on the histological picture of the remaining testis. Administration of vitamin E markedly delays the sloughing process. However, once the process has been initiated, it is irreversible, and sterility ultimately results. The effect of inanition and laparotomy on the testis is rapidly and completely reversible.

The rest of the experimental animals were likewise castrated and placed on a vitamin E free low fat diet. Bile fistulas were established soon after the first testis was removed. These rats were then fed vitamin E concentrates, with or without deoxycholic acid, for extended periods of time. Twenty-five to 40 days after the bile fistulas were established many of the animals showed hemorrhages into the gastro-intestinal tract, the thoracic cavity, and even the cranial vault, At times the rats would bleed profusely as a result of minor injuries. The coagulation time of the blood was prolonged. By administering 1 to 2 cc. of beef bile orally the condition could be controlled for a period of about 10 days. It was then necessary to administer bile again. The procedure of administering vitamin E 4 to 5 times per week and a dose of bile in the interval was adopted in the absorption studies.

Comparison of the first and second testis of the bile fistula rats which received vitamin E‡ for periods of 2 to 6 months shows that the course of the testicular degeneration was delayed by the administration of vitamin E. This indicates that small amounts of this vitamin were absorbed. It is possible that the feeding of small quantities of bile weekly may have been responsible for the absorption of some vitamin E. A number of the animals which received vitamin E plus deoxycholic acid orally for periods as long as 420 days showed a normal histology of the testis, indicating that feeding deoxycholic acid definitely increases the availability of vitamin E in the bile fistula rat.

Summary. 1. Experiments have been carried out to determine the influence of deoxycholic acid on the absorption of vitamin E in the rat. 2. The prevention of degeneration in the testis was used as a criterion for the absorption of vitamin E. 3. The evidence indicates that administration of deoxycholic acid increases the availability of vitamin E, when given orally, in the bile fistula rat.

‡ Each rat received 1 unit (Evans) of vitamin E in the form of the non-saponifiable fraction of wheat germ oil 4 to 5 times weekly.