of the whole gastrocnemic muscle (TChE) in rats was found to be reduced by approximately one-third after denervation and in nutritional muscular dystrophy. It was slightly lowered after repeated injections of high doses of prostigmine and after adrenalectomy. Single injections of high doses of prostigmine had no such effect.

Thymectomy, thyroidectomy, parathyroidectomy, and castration had no effect on the TChE of the rat gastrocnemius.

The TChE of the musc. obliqu. inf. oculi in a patient who died from myasthenia gravis was found to be only half as high as that of the lowest in a series of 12 controls. It was not possible to decide whether or not all of this reduction of the enzyme activity was an effect of prostigmine treatment. The ChE activity in the muscle of a patient dying from hyperthyroidism was within the range of the controls.

We are greatly indebted to Dr. D. Nachmansohn for valuable suggestions and technical advice.

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Effect of Soybean Phosphatides on Utilization of the Fat-soluble Vitamins.

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We reported[1] that the utilization of vitamin A and carotene in the rat is influenced by an as yet unidentified factor present in soy bean lecithin. Patrick and Morgan[2] confirmed our findings in regard to the chick and found that the same factor influences vitamin E utilization as well as vitamin A metabolism. They also showed that yeast, extracted with certain fat solvents, was lacking in this specific factor. Earlier, Polskin[3] reported that lecithin plays an important role in the conservation of the hepatic vitamin A reserves of the chick.

Jensen, Hickman, and Harris[4] have questioned our findings on the grounds that soy bean lecithin contains a certain amount of vitamin E and they express the belief that the observed effects were due to the increased vitamin E intake provided by soybean lecithin in our diet. They could obtain no marked difference from addition of soybean lecithin to the diet of the rats in their experiments. Apparently they overlooked our suggestion that the unknown factor is contained in yeast, which was proved by Patrick and Morgan. Inasmuch as the basic diet of Jensen, Hickman, and Harris contained 8% yeast, their observations do not bear upon the problem under consideration.

To exclude the possibility that the effects of the unknown factor may be attributed to vitamin E, the following experiments were carried out in which the amount of vitamin E contained in soy bean lecithin was taken into consideration. The vitamin E content of soy bean lecithin* was determined according to the method of Emmerie and Engel, and Scudi and Buhs, as 1.3 mg per g.

Experimental. Female albino rats, Sherman strain from our laboratory colony, 28 to 30 days old, weighing approximately 52 to 60 g were kept in individual metal cages with wire mesh floors. The basal diet fed had the following percentage composition: casein, Labco, 20; cerelose, 72; salts, Sure's No. 1, 4; cottonseed oil, 4. The diet was supplemented with thiamine, 2 mg; riboflavin, 4 mg; pyri-

[1] Slanetz, C. A., and Scharf, A., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 17.

[2] Patrick, H., and Morgan, C. L., *Science*, 1943, **98**, 434.

[3] Polskin, L. J., A thesis submitted to Rutgers University, New Brunswick, N.J., May, 1940.

[4] Jensen, J. L., Hickman, K. C. D., and Harris, P. L., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 294.

* This assay was made by Food Research Laboratories, Inc., Long Island City.

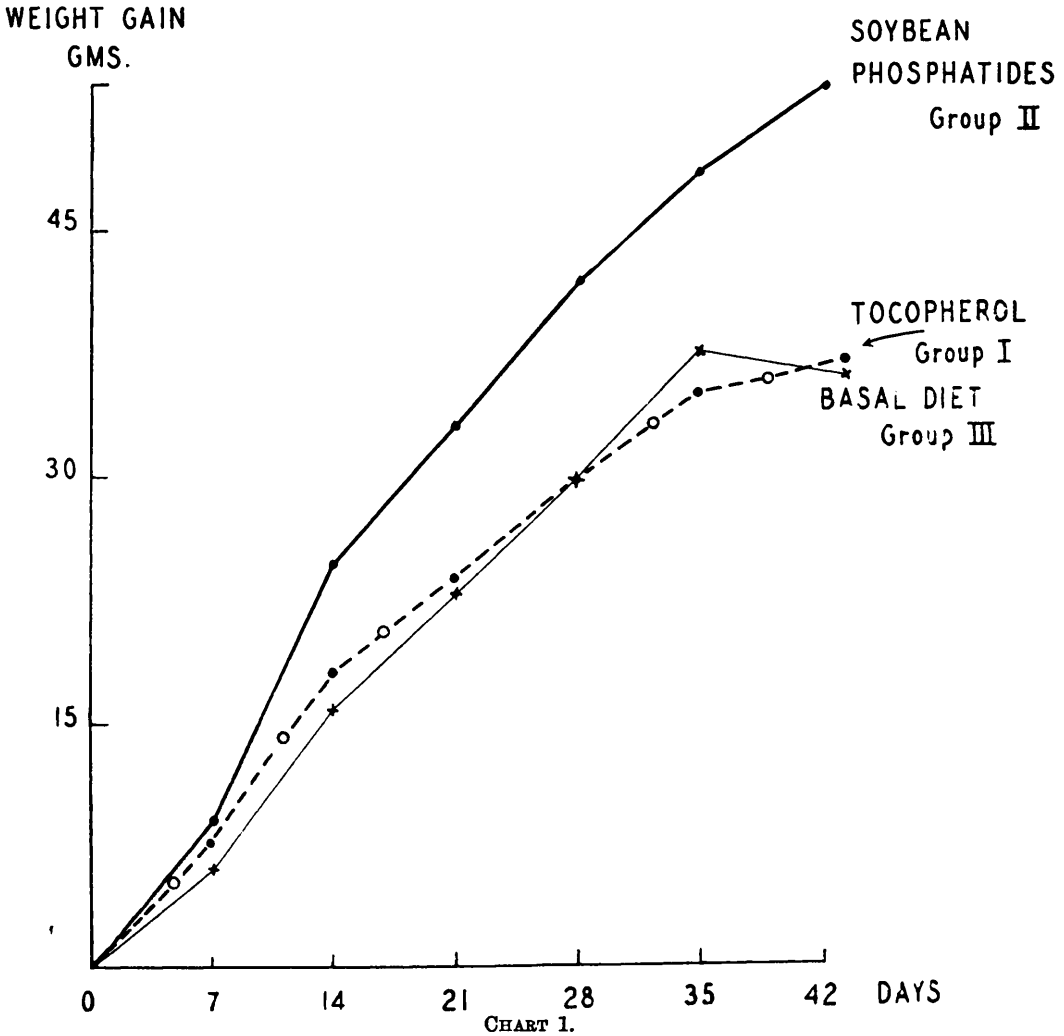

CHART 1.
Growth Curves of Rats.
Diet of Group I contained added alpha-tocopherol, of Group II soybean phosphatides of equivalent vitamin E content.

doxine, 4 mg; nicotinic acid, 1 mg; choline chloride, 500 mg; and 200 mg Viosterol per kg of diet. 100 μg of calcium pantothenate was given orally daily. After depletion of vitamin A, 3 groups of 10 rats each were fed the following diets for a period of 6 weeks in addition to 1.2 μg of carotene daily. Group I received the basal diet supplemented with 13 mg alpha-tocopherol per kg of diet. Group II was given the basal diet supplemented with 1% soy bean phosphatides;† Group III was fed the basal diet alone. The rats were weighed weekly during the test period. Chart I represents the average gain in weight for each group.

At the end of the test period the rats were continued on their respective diets and fed 6 μg of carotene daily. After 10 days some of each group were mated and examined for sperm. On the sixteenth day of pregnancy the rats were sacrificed and the results in regard to vitamin E activity were evaluated in part according to the formula outlined by Mason.[5] The rats receiving 1% soybean lecithin showed a normal gestation with no

† Soybean lecithin furnished by the American Lecithin Co., Elmhurst, N.Y.

[5] Mason, K. E., *J. Nutrition*, 1942, **23**, 59.

resorptions and uterine index above 3.00. The animals fed the basal diet with added tocopherol gave a negative response, with resorptions and a low uterine index.

The remaining rats were kept an additional 10 days, then sacrificed and pooled samples of liver tissue and blood were obtained for vitamin A determinations. Blood from the soy bean lecithin-fed group of rats yielded vitamin A values more than 3 times as great as those of the other groups.

Discussion. The results shown in Chart I indicate that vitamin E is not the determining factor for the differences in growth. The animals fed tocopherol grew no better than those on the basal diet alone. Furthermore, the growth of both of these groups was inferior to that of rats given soybean lecithin. That the vitamin A metabolism is primarily involved is not only concluded from the experimental conditions but also by the appearance of the animals, showing a well defined vitamin A deficiency of the non-lecithin-fed controls regardless of the vitamin E intake. The rats fed lecithin showed no signs of vitamin A deficiency. The blood vitamin A findings are further confirmation of the observed differences in vitamin A metabolism.

The evaluation of vitamin E deficiency by Mason's formula shows that the unknown factor is essential for vitamin E metabolism also and that the rats, even after receiving adequate amounts of vitamin E, develop vitamin E deficiency if the unknown factor is absent. These results are in agreement with the findings of Patrick and Morgan.

Summary. Our earlier findings, that soy bean lecithin contains an unknown factor essential for the utilization of vitamin A, have been confirmed. It has been found that in the rat, as in the chick, vitamin E metabolism is also influenced by this factor.

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Simple, Standardized Culture Medium for Physiological Studies on *Entamoeba histolytica.**

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Since the successful cultivation by Boeck and Drbohlav[1] of *Entamoeba histolytica* in the presence of a bacterial flora, a valuable method has been available for the study *in vitro* of this important protozoan pathogen, especially in regard to diagnostic procedures. Other culture methods also have been developed.[2,3,4,etc.] These have in common the use of a solid nutrient slant and a fluid overlay containing an isotonic salt solution plus serum, or egg albumin, or other protein substances. There are several disadvantages in these procedures: (1) It is tedious to prepare culture tubes, especially in the case of egg-slants; (2) the fluid overlays must be sterilized by filtration owing to their coagulability upon heating; (3) the media become turbid owing to active growth of the bacterial flora, resulting in poorer visibility and limited life of the cultures; (4) the amebæ adhere to the slants and render quantitative counting difficult.

More recently Reardon and Rees[5] reported in a brief note that it was possible to omit the serum from the method of Boeck and

* Aided by a research grant from the Graduate School of Northwestern University.

1 Boeck, W., and Drbohlav, J., *Am. J. Hyg.*, 1925, **5**, 371.

2 Dobell, C., and Laidlaw, P., *Parasitol.*, 1926, **18**, 283.

3 Tanabe, N., and Chiba, E., *Acta med., Keijo*, 1928, **11**, 221.

4 Cleveland, L., and Collier, J., *Am. J. Hyg.*, 1930, **12**, 606.

5 Reardon, L., and Rees, C., *J. Parasitol.*, 1939, **25** (Suppl.), 1.