TABLE II.
Water Content of Tissues.

Group	Time, hr	Liver	Muscle
Control			
A	0	68.8	77.1
B	0	67.7	75.2
C	0	—	77.6
Without lymph hearts, humidity constant			
B	48	68.5	75.9
C	48	70.3	75.3
C	86	71.7	74.9
Without lymph hearts + water present			
A	24	70.2	81.5
C	30	72.6	83.4
B	48	72.4	80.6
B*	48	68.7	78.0
C	48	73.7	80.8

A. Data from Foglia and Gerschman.

B. Data from Dr. Mazzocco on mixed samples from many animals.

B*. Special water recovery method.

C. Data averaged from many single determinations in present experiments.

Conclusions. Toads without lymph hearts kept in constant humidity showed no change in body weight, whereas other animals kept in the presence of water showed a gain in weight of as much as 20% daily. With a constant body weight the fluid redistribution can be assumed to be internal. In both series of experiments there was a marked increase in red cell volume to a point where flow even in large vessels was impeded.

The fall in plasma and lymph protein, and the rise in body fluid, potassium content and tissue water are much greater when water is absorbed through the skin by contact.

When the normal flow of fluid from plasma to tissue space to lymph channels to vascular system is blocked by lymph heart destruction, there is a marked increase in the interstitial fluid at the expense of the plasma. The failure of this edema fluid to return to the blood vessels in these experiments is a primary and uncomplicated cause of death.

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The Effect of Phosphatides on Utilization of Vitamin A and Carotene.

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The problem of the utilization of carotene and vitamin A as influenced by the oil or fat in the diet has been investigated repeatedly during recent years.

Sherman[1] studied the effect of various oils on the utilization of carotene and found that the addition of 0.1 ml of cottonseed oil daily and especially soybean oil to the diet greatly improved the growth response of vitamin A deficient rats receiving 1 and 2 μg carotene daily.

Quackenbusch, Cox and Steenbock[2] reported that tocopherol promoted the response to carotene and concluded that tocopherol functioned in the intestinal tract as an antioxidant.

Sherman[3] reported that carotene was destroyed in the intestinal tract in the absence of tocopherol.

Quackenbusch, Cox and Steenbock[4] provided further evidence that tocopherol is essential for the utilization of carotene in the intestinal tract but reported even better results with a soybean oil distillate.

1 Sherman, W. C., *J. Nutrition*, 1941, **22**, 153.

2 Quackenbusch, F. W., Cox, R. P., and Steenbock, H., *J. Biol. Chem.*, 1941, **140**, civ.

3 Sherman, W. C., *Fed. Proc. Soc. Biol. Chem.*, 1942, **1**, 134.

4 Quackenbusch, F. W., Cox, R. P., and Steenbock, H., *J. Biol. Chem.*, 1942, **145**, 169.

As it is known that the antioxidant activity of tocopherol *in vitro* is greatly influenced by cephalin[5] it seemed advisable to investigate this problem further. The evidence presented by our experiments indicates that this synergism between cephalin and tocopherol exists also *in vivo* but that other factors may be involved in the utilization of carotene and vitamin A by the rat.

Experimental. Female albino rats, Sherman strain, from our laboratory colony, 28 to 30 days old, weighing approximately 60 to 66 g, were kept in individual metal cages provided with raised screen floors. The basal diet used had the following percentage composition: casein Labco, 20; cerelose, 72; salts, Osborne and Mendel, 4; cottonseed oil, 4. This diet was supplemented with thiamine 2 mg, riboflavin 4 mg, pyridoxine 4 mg, nicotinic acid 1 mg, choline 500 mg and 200 mg of Viosterol per kilogram of diet. Synthetic calcium pantothenate was given orally, 100 μg daily. The rats were weighed daily during the depletion period and 3 times a week during the 4-week period when the test substances (phosphatides and soybean oil) were included in the diet. The purified diets and mixtures were freshly prepared each week and stored at 4°C. Food consumption was determined.

Discussion. Although our basal diet was similar to the diet used by Sherman, Steenbock, *et al.*, we failed to get the expected growth response with the rats receiving 2 U.S.P. units carotene or vitamin A and a diet containing 4% cottonseed oil which should have been sufficient to provide the necessary tocopherol. However, our diet differed from those mentioned before in that both authors used yeast or rice bran extract as a source of the B complex in their diet while we used the synthetic vitamins mentioned above. The striking response of our rats to 1% soybean phosphatides,* and to a certain degree to 0.3% soybean oil, indicates the presence of an unknown factor, possibly also present in yeast or the B complex and essential for the utilization of vitamin A and that this factor is not present in cottonseed oil.

That factors of the B complex may be essential for the utilization of vitamin A was proved by Popper[6] who showed that choline deficiency interferes with vitamin A utilization. However, in our experiments, enough choline was provided to exclude this possibility. It may be noted that the presence of a hypothetical factor for the utilization of vitamin A and carotene has been suspected before[7] and it was claimed that palm kernel meal, coconut cake and acetone-extracted herring roe are rich in this factor.

It can be seen from Chart 1 that the vitamin A-depleted rats gave essentially the same type of growth response with 2 units of carotene as with 2 units of vitamin A (cod liver oil) when 1% of soybean phosphatides was added to the diet. As these soybean phosphatides contain considerable amounts of soybean oil (up to 30%) two groups of rats were given the equivalent amount of soybean oil (0.3%) instead of the phosphatides in the diet. Although the rats receiving soybean oil plus 2 units of vitamin A showed a gain in weight, they failed to equal that obtained with the phosphatides. After the third week the rats receiving soybean oil instead of soybean phosphatides started to lose weight while those receiving phosphatides continued to gain. Even more striking was the fact that the animals receiving phosphatides were in much better condition at the end of the 4-week test period than the rats receiving only soybean oil. The fur of the soybean oil rats appeared shaggy and dry while that of the phosphatides rats was sleek and glossy.

The above experiments were repeated substituting 4% soybean phosphatides in the place of 1% phosphatides in the diet. The increased intake of soybean phosphatides did not appreciably change the gain in weight.

In addition, two groups of vitamin A de-

[5] Swift, C. E., Rose, W. G., and Jamieson, G. S., *Oil and Soap*, 1942, **19**, 176.

* We are indebted to the American Lecithin Co., Elmhurst, L.I., N.Y., for furnishing us with commercial soybean phosphatides. These soybean phosphatides are composed of about 20 to 25% lecithin, 25 to 30% cephalin fraction, including some carbohydrate, about 15% inositol phosphatides and a carrier of 30% soybean oil.

[6] Popper, H., and Chinn, H., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 202.

[7] Morton, R. A., *Ann. Rev. Biochem.*, 1942, **11**, 371.

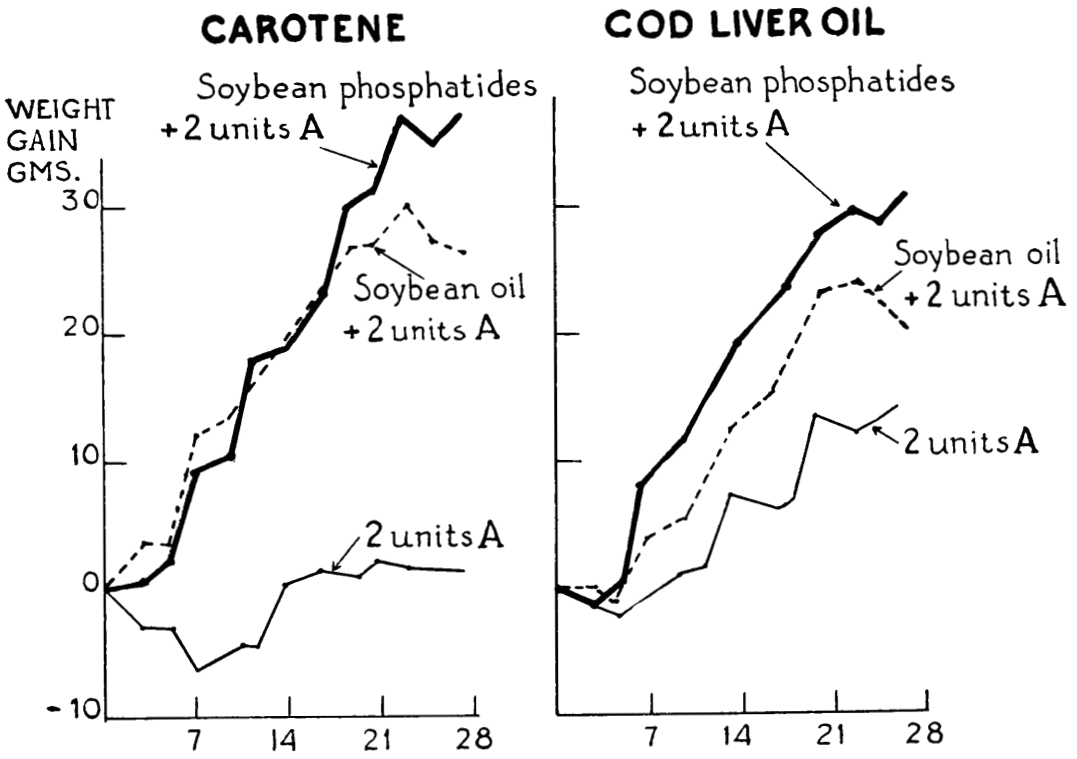

CHART 1.

pleted rats were given soybean phosphatides without vitamin A. These rats lost weight even more rapidly than those receiving the basal diet without vitamin A. This response may be due to the strong lipotropic effect of soybean lecithin.

Summary. Vitamin A-depleted rats fed a vitamin-free diet, supplemented with synthetic B vitamins and 2 U.S.P. units of vitamin A in the form of carotene, failed to gain weight in spite of the presence of 4% cottonseed oil in the diet. Satisfactory growth was obtained when soybean phosphatides (1%) were added to the diet. An amount of soybean oil (0.3%) equivalent to that contained in the soybean phosphatides used also promoted growth but to a lesser degree. Rats fed the vitamin A-deficient diet supplemented with soybean oil and 2 units of vitamin A appeared in poor condition and began to lose weight after the third week.

Substitution of cod liver oil in place of carotene as the source of vitamin A in the diet, with addition of soybean oil or soybean phosphatides resulted in a growth response similar to that obtained with carotene. However, cod liver oil alone also caused some gain in weight.

In the presence of soybean phosphatides, carotene was as well utilized as vitamin A under the conditions of our experiment.