to 13.4 kg, were operated upon and all of the pancreas removed save for a small portion in the region of the lesser duct. Patterns of pancreas tissue cut to the size of the remnants left weighed from 1 to 2 g so that the pancreatectomy was 90 to 94% complete. The diet of all the animals was similar and consisted of 600 g meat, 100 g bread, 400 cc milk, 25 g suet, 25 g bone meal, and 10 g brewer's yeast. This was fed in 2 meals and insulin was given after each feeding. The urine was examined daily for sugar and the dose of insulin adjusted to limit the glycosuria to less than 1 to 2 g per day. The insulin requirement of these animals is given in Table I together with a representative series of animals with total pancreatectomy for comparison. It is evident that dogs with partial pancreatectomy require definitely more insulin than after complete pancreatectomy and that occasionally animals may be found such as D 373 and D 430 where exceptionally large doses are tolerated. These animals were given 11 and 10 units of regular insulin per kg of body weight per day for long periods. This would correspond to 700 units for a 70-kg man and comes within the range of so-called insulin resistance.

Young[3] found that dogs made permanently diabetic by injections of anterior pituitary extracts also required more insulin than depancreatized animals on the same diet. At the present time we have two dogs in the laboratory made diabetic by the intravenous injection of alloxan and these require 32 and 36 units of regular insulin daily which is perhaps somewhat greater than for depancreatized animals at the same period.

It is likely that the somewhat better digestion and absorption in the animals with pancreas remnants is a factor in their greater insulin tolerance but this is probably not the complete explanation. The phenomenon recalls the greater tolerance of normal animals to the administration of thyroxin as compared with the same animals after thyroidectomy.

[3] Young, F. G., *New England J. Med.*, 1939, **221**, 635.

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Effect of Tocopherols and Soybean Phosphatides on Utilization of Carotene.

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Slanetz and Scharf[1] have reported recently that soybean phosphatides have a sparing action on carotene and vitamin A. Rats fed a limited quantity of A-vitamins grew faster when soybean phosphatides were fed simultaneously. If this enhanced growth were due to the phosphatides in the preparation fed, it would reveal a new and important factor for the better utilization of vitamin A. At present, the only natural factors known to possess the power of vitamin A synergy are the tocopherols, either singly or as members of a covitamin E complex.

It occurred to us that the synergy observed in Slanetz and Scharf's experiment may have been due to the tocopherols contained in the crude soybean phosphatides employed. Calculations followed by analysis of a similar phosphatide product suggested that the soybean oil or the crude phosphatides in their experiment each contributed about the optimum daily supplement of tocopherols and would thus provide sufficient explanation for the synergism observed.

Experiments now to be reported in which the phosphatides have been treated for removal of tocopherols, indicate that the precipitated phosphatides are substantially inactive. At the same time, the materials removed from the phosphatides and established

* Communication No. 55. Covitamin Studies, Part IV.

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

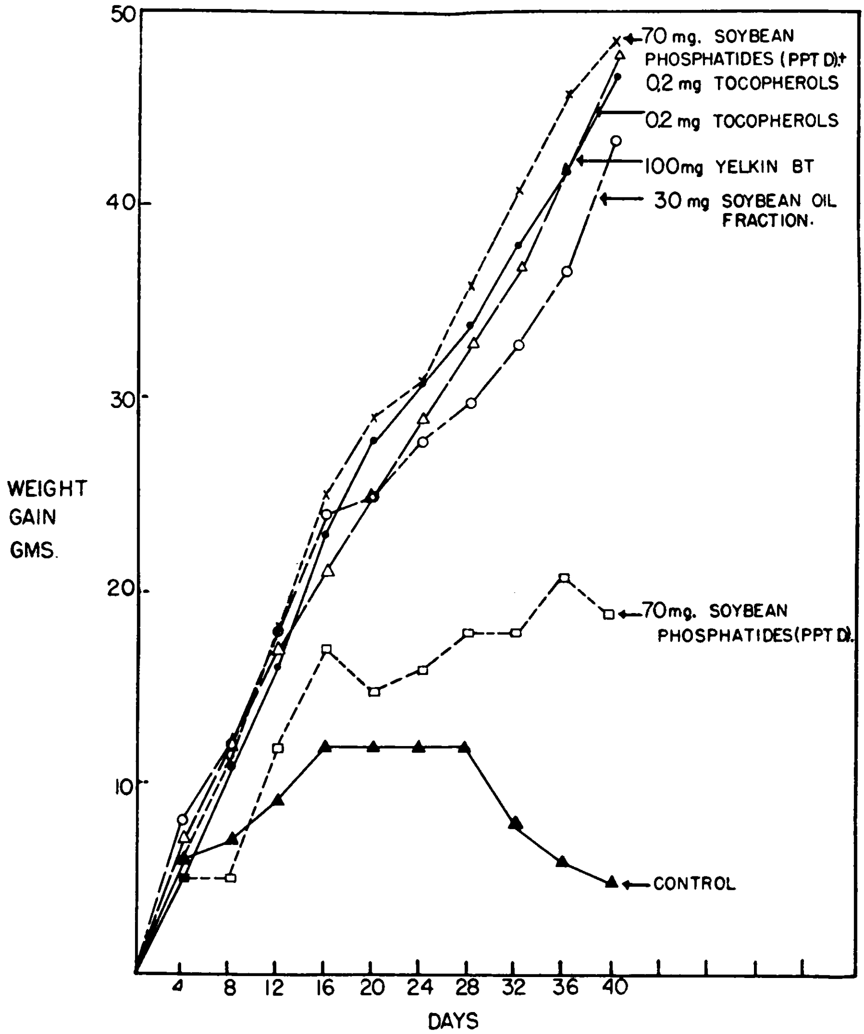

FIG. 1.
Growth of vitamin A depleted rats fed 1.2 μg carotene per day with and without protective agents.

to contain tocopherols are found to give the same growth enhancement as the crude phosphatides or natural mixed tocopherols.

Experimental. The diet used throughout this experiment contained casein (vitamin A-free), 18%; salt mixture (U.S.P. No. 2), 4%; yeast (dried Anheuser-Busch Strain G), 8%; starch, 30%; Cerelose, 35%; lard, 5%. The vitamin D content was 3 units per gram of diet supplied by activated ergosterol, A.R.P.I. Process. This diet was known to be very low in vitamin A and vitamin E potency.

Young male rats were depleted of vitamin A on this diet in the usual manner. At depletion, groups of 10 rats each were given 1.2 μg carotene (S.M.A. Corp.: 90% β + 10% α) daily together with supplements of soybean phosphatides and tocopherols, as listed in Fig. 1. The crude soybean phosphatide, Yelkin BT, was obtained from the same source† used by Slanetz and Scharf.

Preparation of Pure Phosphatides. Yelkin BT was dissolved in ether and the phosphatides precipitated by acetone. The treat-

† American Lecithin Company, Elmhurst, Long Island. This product contains about 30% soybean oil.

ment was repeated and the precipitate transferred to olive oil. The oil, containing a known weight of the precipitated phosphatides, was fed to the rats from a calibrated dropper.

The ether-acetone extract of the crude phosphatides, hereafter referred to as soybean oil fraction was concentrated by evaporation to remove the ether and acetone. The concentrate was fed as such to one group of depleted rats.

The amounts of tocopherols present in the crude phosphatide and fractions were determined by the Emmerie and Engel[2] method and results are given in Table I.

TABLE I.
Tocopherol Content of Soybean Phosphatides by Chemical Analysis (Emmerie and Engel).

Material analyzed	Tocopherol content
	%
Yelkin BT (whole)	0.2
Soybean Oil Fraction	0.6
Precipitated phosphatides	0.01

Thus, all groups of rats except the control and one of the groups fed 70 mg precipitated phosphatides received 0.2 mg of tocopherols daily.

Discussion. It is evident from Fig. 1 that of the agents studied the tocopherols were chiefly responsible for the increase in weight gains over that of the control group. The rats fed 0.2 mg tocopherols per day showed weight gains as great as the rats fed 30 mg of the soybean oil fraction or 100 mg Yelkin BT. Both fractions contained 0.2 mg tocopherols by analysis. The addition of 70 mg precipitated phosphatides to the 0.2 mg tocopherol supplement did not improve the protective power of the tocopherols. However, the supplement of 70 mg of precipitated phosphatides was responsible for maintaining early growth gains after 4 weeks at a time when the rats receiving 1.2 μg carotene alone began to lose weight. This might be explained by the small quantity of tocopherols (0.01%) which survived the two precipitations of the phosphatides.

Slanetz and Scharf reported cessation in growth after three weeks of the rats fed soybean oil, but continued growth of the rats fed commercial soybean phosphatides. This observation may be explained in two ways. Either the soybean oil did not contain as much tocopherol as was present in the soybean phosphatides or their diet was deficient in factors which were present in soybean phosphatides but not in the soybean oil. The inability of rats fed 2 I.U. carotene to grow puzzled these workers. They suggested that their supplement of the B-vitamins, namely, thiamine, riboflavin, pyridoxine, niacin, choline, and pantothenic acid, was inadequate and that probably an unknown factor is present in soybean phosphatides, soybean oil, and yeast which is essential for the utilization of vitamin A and carotene and, furthermore, that this factor is not present in cottonseed oil. Cottonseed oil, presumably refined, constituted 4% of their diet. If the rats ate 7 g of the ration per day and the cottonseed oil contained 0.04% vitamin E, the rats would have received only about 0.1 mg of vitamin E per day. Previous work[3] has shown this amount to be borderline or inadequate for maximum growth when low levels of carotene were fed. Thus, it is probable that additional quantities of tocopherols would have resulted in growth of the animals fed the low level of carotene.

Slanetz and Scharf stated that "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." Comparison of our rats fed tocopherols with those fed Yelkin BT, soybean oil fraction, or soybean phosphatides plus tocopherols failed to show any difference in body condition or appearance of the fur between these groups.

The able assistance of Miss Joann Beers in managing the experimental animals is acknowledged.

Summary. The vitamin A-enhancing property of crude soybean phosphatides is due principally to the tocopherols present in these phosphatides.

[2] Emmerie, A., and Engel, C., *Rec. trav. chim.*, 1938, **57**, 1351.

[3] Hickman, K. C. D., Harris, P. L., and Woodside, Marian, *Nature*, 1942, **150**, 91.