taining 71 per cent. sucrose, 20 per cent. vitamin-free casein, 4 per cent. Osborne and Mendel mineral salt supplement, 3 per cent. Celluration and 2 per cent. of a suspension of vitamins A, D and E in Mazola oil. The eighth group was fed ground Purina dog chow. All the animals received daily supplements of the available pure components of the B complex vitamins.[6] Vitamin A was administered by means of fish liver oil concentrate (potency, 825,000 units per gram) or a preparation containing molecular distilled vitamin A (potency, 202,400 units per gram) mixed in the basal diets twice weekly to minimize vitamin A loss through oxidation. The dosage of vitamin K (2 methyl-3-phytyl-1,4-naphthoquinone) was given orally each day. Blood samples for prothrombin determinations were obtained by heart puncture after the animals were on the test diet for ten days, except in the case of those animals showing severe toxicity symptoms. From such animals blood samples were taken two to three days before the conclusion of the test. Prothrombin determinations were made according to the method of Campbell, Smith, Roberts and Link,[7] using 50 per cent. plasma dilution levels.

TABLE 1

Group	Vitamin A per 100 gms of basal ration	Vitamin K	Average clotting time
1	400,000 units (ester)*	none	5.69 min.
2	400,000 units (ester)*	100 γ per day	0.37 "
3	88,000 units (alcohol)*	none	0.39 "
4	176,000 units (alcohol)*	"	2.51 "
5	352,000 units (alcohol)*	"	5.12 "
6	352,000 units (alcohol)†	"	5.54 "
7	352,000 units (alcohol)*	25 γ per day	0.35 "
8	704,000 units (alcohol)*	none	7.17 "

* Basal ration, synthetic.
† Basal ration, ground dog chow.

The animals on this test consumed approximately 10 grams of food daily. Hypothrombinemia was produced within a 10-day period in white rats when fed 17,600 units of vitamin A per day. However, 8,000 to 9,000 units per day did not have any significant effect. At higher levels of vitamin A intake, 35,000 to 40,000 units per day, prothrombin levels of the blood were reduced within the comparatively short period of ten days to such an extent that many of the animals died of cerebral hemorrhage. This effect was obtained with a diet of natural ingredients (dog chow) as well as with the purified casein-sucrose ration. No difference between the effects of the alcohol or ester form of the vitamin was observed.

As shown hypoprothrombinemia is prevented by the administration of 25 micrograms of vitamin K daily. The absolute requirement of the white rat for vitamin K is unknown. This species normally requires no dietary source of this factor, since vitamin K is presumably supplied by the intestinal flora. Fecal counts on the animals showed no significant differences in either the total counts or *E. coli* counts.

Although the experiments indicate that an overdosage of vitamin A results in a hypoprothrombinemia, which can be corrected by the daily administration of vitamin K, there is no evidence that vitamin K is effective in preventing any of the other toxicity symptoms occasioned by hypervitaminosis A. The mechanism by which the overdosage of vitamin A causes hypoprothrombinemia requires further investigation.

ROBERT F. LIGHT
RUTH P. ALSCHER
CHARLES N. FREY

THE FLEISCHMANN LABORATORIES,
STANDARD BRANDS, INC.,
NEW YORK, N. Y.

[6] The composition of this supplement is the same as that used by Light, Cracas, Olcott and Frey, *Jour. Nutrition*, 24: 427, 1942

[7] H. A. Campbell, W. L. Smith, W. L. Roberts and K. P. Link, *Jour. Bio. Chem.*, 138: 1–20, 1941.

COTTONSEED PHOSPHOLIPIDS[1]

WOOLLEY[2] has recently described the properties of an inositol-containing phospholipid from soybeans to which he gives the name "soybean lipositol." Observations made during an investigation now discontinued indicate that the phospholipids from the cottonseed resemble soybean lipositol more closely than they do other phospholipids previously described as plant constituents.

A commercial cottonseed phospholipid preparation[3] was stirred with changes of acetone until the phospholipids were in the form of a fine reddish yellow powder. The powder was centrifuged, dried and dissolved in purified ethyl ether. A small amount of insoluble material was removed by centrifugation, and the ether solution was then poured into an excess of acetone. The insoluble fraction was a stable, bright yellow, powdery solid (54 per cent. yield). The results of analyses of this preparation are compared in Table 1 with the data of previous investigators.

The cottonseed phospholipid had an ash content of 10 to 11 per cent. Attempts to remove inorganic salts from the preparation by washing, using the technique suggested by Channon and Foster,[7] resulted in some fractionation as determined by P and N determinations, but did not reduce the ash content. Spectrophotographic analyses of the ash showed strong lines for B and Mg, detectable amounts of Ca, Na, K and Si, and a trace of Zn. The original concentrate had been prepared with the use of boric acid as described

[1] Contribution XLV from the Cotton Research Foundation Fellowship, Mellon Institute.

[2] D. W. Woolley, *Jour. Biol. Chem.*, 147: 581, 1943.

[3] Dr. M. Mattikow, Refining Inc., Charlotte, N. C., kindly furnished a generous sample of the cottonseed phospholipid concentrate.

TABLE 1

Phospholipid	Phosphorus Per cent.	Nitrogen Per cent.	Ash Per cent.	Fatty acids Per cent.
Cottonseed (present investigation) ...	2.9	1.2	10–11	48
Cottonseed (Thurman[4]) ...	2.6–2.9	0.96	–	–
Cottonseed (Hilditch and Zaky[5]).	2.8	1.6	–	48
Soybean lipositol (Woolley[2])	3.1	0.98	12.1	45
Soybean (Hilditch and Pedelty[6])	3.7	1.4	–	70
Wheat germ (Channon and Foster[7])	3.0	1.5	4.3	56
Lecithin (theory[7]) .	4.0	1.8	none	70

by Thurman.[8] The phospholipid contained 0.6 per cent. Mg, 0.3 per cent. Ca, and 0.03 per cent. K.[9]

Fifteen to 20 per cent. of the phospholipid could be dissolved by extracting with many changes of hot alcohol. This "lecithin" fraction contained 3.1 per cent. P, 1.5 per cent. N, and 2.5 per cent. ash.

After either acid or alkaline hydrolysis, 41 per cent. of the nitrogen was present in the form of amino nitrogen.

The phospholipid was readily soluble in water, and in ether and petroleum ether after the addition of small amounts of water. In 5 per cent. concentration, the solution in water was dark-colored with oil-like viscosity and wetting properties. It possessed antioxidant activity of a high order. When assayed in a preparation of ethyl esters of cottonseed oil fatty acids, 0.1 per cent. increased the induction period from 17 to 195 hours at 70°. The role of lipositols and metallic phosphatidates as antioxidants deserves further study.

The relatively low unsaturation (Iodine Number, 100) of the cottonseed phospholipid fatty acids is responsible, at least in part, for its stability.[10] This property and its availability make it a promising material for further investigation of the composition of plant phospholipids.

T. D. Fontaine supplied many analytical data in this study.

HAROLD S. OLCOTT[11]

MELLON INSTITUTE,
PITTSBURGH, PA.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

PROPOSED USE OF STARCH SPONGES AS INTERNAL SURGICAL DRESSINGS ABSORBABLE BY THE BODY

AN absorbent dressing which, when saturated with a medicament and placed in an incision or a deep wound, would be slowly dissolved and absorbed by the body with gradual release of the medicament would be of considerable value. Sponges obtained by freezing starch pastes[1] have characteristics which suggest their use for such a purpose. As the authors have no facilities for experimentation with animals or for clinical studies, the preparation and characteristics of the starch sponges are described here, with the hope that others may be able to test the medical value of these materials.

Starch sponges are quickly and easily prepared. The following procedure is recommended: A 5 per cent. suspension of purified cornstarch is pasted by heating and then sterilized by heating in an autoclave for 15 to 20 minutes at 15 pounds per square inch gauge pressure. The autoclaved paste is placed in shallow pans or other containers as desired, and frozen slowly, preferably at a temperature just below 0° C. The higher the freezing temperature, the coarser and stronger the resulting sponge. When freezing is complete, the paste is removed from the freezer and allowed to thaw. The resulting spongy mass may be cut into pieces of the required size and shape. Sponges having different textures may be prepared by varying the pasting and freezing conditions, and the kind and concentration of starch used.

Sterile sponges can be prepared by autoclaving the paste and carrying out the rest of the preparation under aseptic conditions, or the final product can be simultaneously toughened and sterilized by immersion in 70 per cent. alcohol. Dried sponges can be sterilized by autoclaving, similarly to absorbent cotton.

Starch sponges are highly absorbent. They will take up 15 to 18 times their own weight of aqueous or alcoholic solutions. Unlike cotton, they are firm enough to retain the absorbed liquid during gentle handling.

These sponges can be air-dried at temperatures up to 105° C., to light, rather brittle masses which return

[4] B. H. Thurman, U. S. Patent No. 2,201,061, 1940.
[5] T. P. Hilditch and Y. A. H. Zaky, *Biochem. Jour.*, 36: 815, 1942.
[6] T. P. Hilditch and W. H. Pedelty, *Biochem. Jour.*, 31: 1964, 1937.
[7] H. J. Channon and C. A. M. Foster, *Biochem. Jour.*, 28: 853, 1934.
[8] B. H. Thurman, U. S. Patent No. 2,150,732, 1939.
[9] Microchemical analyses by C. Tiedcke, New York, N. Y.
[1] The sponge-like character of frozen starch paste was reported by E. A. Scharling, *Ann.*, 49: 315, 1844.
[10] B. H. Thurman, U. S. Patents No. 2,182,767, 1939; 2,201,064, 1940.
[11] Present address: Western Regional Research Laboratory, Albany, Calif.