liver following the technique of Wright and Welch.[7] The monkey livers were obtained from two vitamin M-deficient animals (*Macaca mulatta*) with leucopenia and anemia. One of these (M1) had died some hours previously from intestinal obstruction, while the other (M2) died after having been given 40 mg of xanthopterin in four days in a belated attempt to prevent its death. Control experiments with liver alone and with xanthopterin added to liver were made. The xanthopterin was synthesized by the method of Purrmann.[3] Folic acid was assayed by the method of Mitchell and Snell,[16] using *S. lactis R* as the test organism. Values obtained in representative experiments are given in the table. Each series of experiments was made on one uniform batch of dispersed liver.

It can be seen from the table that yeast, which has a relatively low preformed folic acid content, is rich in the substances which give rise to folic acid when incubated with fresh liver; 15-fold or greater increases over the preformed folic acid were obtained. The high value of 89 μgm of extra folic acid produced per gram of yeast may be lower than the total amount of precursors present since it could hardly be expected that the synthesis would go to completion under the conditions of the experiment. Such a value for the precursors of folic acid is in much better agreement with the known high vitamin M content of this yeast than is the value for preformed folic acid (4.2 μgm per gram of yeast[11]).

Also supporting the belief that there is a relationship between vitamin M and the precursors of folic acid was the finding that livers from the two vitamin M-deficient monkeys were strikingly low in preformed folic acid. As predicted from our feeding experiments,[11] extra folic acid was produced from xanthopterin by the liver of the animal not receiving the pterin (M1). The small total increase may have been due to the fact that the liver was not fresh. Liver from the other animal (M2) produced extra folic acid from yeast and from xanthopterin when yeast was also added.

As expected from the finding of O'Dell and Hogan,[9] chicken liver failed to produce any extra folic acid (as measured by *S. lactis R* stimulation) from xanthopterin alone. Surprisingly, however, it produced folic acid from xanthopterin in the presence of yeast, and also from yeast alone. If folic acid is identical or isotelic[17] with vitamin B_c this would seem to indicate that the *S. lactis R* method of assay is not suitable for measuring vitamin B_c activity in some crude materials. The difference in behavior of livers from chickens and rats toward xanthopterin may be due to the absence of a required substance from chicken liver which is contained in variable amounts in rat livers. Such a substance is evidently present in yeast.

It would appear from our findings and those of Wright and Welch[7] that from a nutritional standpoint the determination of "potential" (preformed plus extra) folic acid may be of much greater significance than the determination of preformed folic acid.

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[16] H. K. Mitchell and E. E. Snell, University of Texas Publication, 4137: 36, 1941.

[17] For an explanation of the term "isotelic" see R. J. Williams, SCIENCE, 98: 386, 1943.

VITAMIN A TOXICITY AND HYPOPROTHROMBINEMIA

RODAHL and Moore[1] have recently shown that the toxicity of bear and seal liver to humans may be due to a hypervitaminosis A. The effects of an overdosage of vitamin A have been studied by a number of investigators[2,3,4,5] and they are in general agreement on certain of the symptoms, which are roughening of the skin, rarefaction of bones, alopecia and profuse internal hemorrhage, often subcutaneous.

In an investigation of the mechanism responsible for the spontaneous hemorrhage caused by hypervitaminosis A, it was found that overdosage of vitamin A causes a pronounced hypoprothrombinemia. Doses of approximately 15,000 units of vitamin A per day produced a demonstrable hypoprothrombinemia in white rats within a period of ten days. Both the alcohol and the ester forms of vitamin A were fed in doses of 35,000 to 40,000 units per day, and appear to have had equal effect on the prothrombin level. Carotene in doses of 40,000 units per day was without effect on the prothrombin level in the blood.

This effect of vitamin A overdosage can be controlled by a simultaneous daily administration of vitamin K. A daily dose of 25 micrograms of vitamin K will maintain the normal prothrombin level in the blood of animals fed overdosages of vitamin A. Levels of 10 micrograms or less did not give complete protection.

One experiment will serve to illustrate the results obtained. Sixty-four white rats, having an average body weight of 135.3 grams, were divided into eight groups. Seven groups were fed a synthetic diet con-

[1] K. Rodahl and T. Moore, *Biochem. Jour.*, 37: 166–168, 1943.

[2] L. J. Harris and T. Moore, *Biochem. Jour.*, 22: 1461, 1928.

[3] K. Schuebd, *Med. Welt*, 11: 705, 1937.

[4] E. B. Vedder and C. Rosenberg, *Jour. Nutrition*, 16: 57–68, 1938.

[5] J. A. Collazo and J. S. Rodriguez, *Klinische Woch.*, 12: 1732, 1768, 1933.

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.

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[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.