tration of the alcohol. To rule out the possible effect of increased absorption of vitamin A which might possibly have been present in the gastro-intestinal tract, the experiment was repeated with six normal dogs that had been fasted for forty-eight hours. These dogs received amounts of ethyl alcohol varying between 15 ml and 60 ml in a 20 per cent. aqueous solution by stomach tube. An increase in the vitamin A content of the serum was observed in all cases as is shown in Table I. In two of the dogs with fistulas of the thoracic duct, the vitamin A content of the lymph was determined and no increase proportionate to the increase in the blood was noted.

To rule out further the possible effect of absorption of vitamin A from the gastro-intestinal tract, one dog was given ethyl alcohol in Ringer's solution intravenously. A rise in the vitamin A content of the serum followed, similar to that observed in the dogs already mentioned. In another dog, ethyl alcohol injected directly into the portal vein produced a marked rise in the vitamin A content of the hepatic vein. This observation suggests a direct effect of the alcohol on the liver. However, another possibility which should be considered is this: Ethyl alcohol may injure the epithelium of the gastro-intestinal tract or the other tissues. The injured tissue may produce a substance X, which enters the blood, passes to the liver or other vitamin A stores and liberates the vitamin.

In three of the dogs a biopsy of the liver was performed and there seemed to be a positive correlation between the increase of vitamin A in the serum after the administration of the alcohol and the concentration of vitamin A originally present in the liver. It is hoped that this correlation may lead to the development of methods for the study of the vitamin A reserves in the body. If further observations confirm a direct action of ethyl alcohol on the liver, this fact may possibly aid in the study of liver function. Further work in this direction is now in progress in this laboratory.

It is also planned to study the effect of alcohols of the homologous series, methyl, ethyl, propyl, butyl and higher alcohols such as cetyl and aldehydes, acids, acetates, lactates, phosphates, etc., to determine whether they have the capacity for mobilizing vitamin A.

SAMUEL W. CLAUSEN
WILLIAM S. BAUM
AUGUSTA B. MCCOORD
JOHN O. RYDEEN
BURTIS B. BREESE

DEPARTMENT OF PEDIATRICS,
SCHOOL OF MEDICINE AND DENTISTRY
OF THE UNIVERSITY OF ROCHESTER

THE ISOLATION OF PHYTIN FROM SOIL[1]

THE general behavior of soil organic phosphorus in extraction and fractionation is explainable on the basis that phytin is present in soil. However, the presence of phytin in soil has not heretofore been demonstrated. It has been shown[2] that phytin, added to silica sand cultures, is readily attacked by soil microorganisms. This led to the conclusion that phytin could not accumulate in soil.

We have found that, when phytin was added to soil cultures, it did not readily decompose, but behaved in a manner similar to our so-called soil "nucleic acid" preparations.[3] Moreover, analyses of soil "nucleic acid" showed the ratio of nitrogen to phosphorus to be very much lower than would be expected from such material. A large proportion of the soil organic phosphorus is stable to alkaline hydrolysis and also comparatively resistant to acid hydrolysis.[3] We have found also that organic phosphorus may be precipitated from an acid hydrolysate of soil by ferric chloride, and that part of the phosphorus of the soil "nucleic acid" is precipitable by ferric chloride in acid solution. These facts strongly indicate the presence of phytin in soil.

The material precipitated by ferric chloride from soil "nucleic acid" preparations was obviously not a pure substance. Oxidation with alkaline hypobromite destroys most of the organic matter of soil extracts, but does not liberate phosphate from a major part of the soil organic phosphorus, as was shown by Dean.[4] Investigation showed that phytin is quite stable to alkaline hypobromite. By means of this treatment of an alkaline soil extract, coupled with ferric chloride precipitation, we have been able to prepare a small quantity (about 1.25 g) of a faintly yellow-colored product which contained about 25 per cent. of the organic phosphorus of the original soil. Similar material was obtained from a soil "nucleic acid" preparation.

The phosphorus : iron ratios of the preparations are given in Table I along with corresponding data for ferric phytate prepared from wheat-bran phytin. All the ferric salts were precipitated from acid solution by an excess of ferric chloride. Solutions of the sodium salts were obtained by decomposing the ferric salts with sodium hydroxide. Phosphorus : iron ratios were also determined by acidifying these solutions to 0.6 per cent. hydrochloric acid and titrating with ferric chloride to the thiocyanate end-point. The ratios determined by titration are in agreement with those reported by Rather.[5] It is noteworthy that phytin combines with much more iron than is indicated by titration to the thiocyanate end-point.

[1] This work was financed in part through a grant from the National Research Council, Ottawa, Canada.

[2] J. T. Auten, *Soil Science*, 16: 281–294, 1923.

[3] C. L. Wrenshall, W. J. Dyer and G. R. Smith, *Sci. Agr.*, 20: 266–271, 1940.

[4] L. A. Dean, *Jour. Agr. Sci.*, 28: 234–246, 1938.

TABLE I

THE P : Fe RATIOS OF SOIL AND BRAN PREPARATIONS

Material examined	Phosphorus : iron ratio	
	By analysis of the Fe salt	By $FeCl_3$ titration of the Na salt
Soil preparation (1)	0.76	1.27 1.19
Soil preparation (2) (two bromine treatments) ..	0.69	
Bran preparation (1)	0.71	1.12
Bran preparation (2)	0.67	1.20
Bran preparation (3) (bromine treated)	0.68	

A solution of the sodium salt of the soil preparations gave the Fischler and Kurten[6] test for phytin. The same solution was subjected to the action of phosphatase (intestinal mucosa extract) and of phytase (bran extract) with the results shown in Table II.

TABLE II

DEPHOSPHORYLATION BY INTESTINAL AND BRAN EXTRACTS (RESULTS EXPRESSED AS PERCENTAGE OF THE TOTAL ORGANIC P)

Substrate	Intestinal extract (pH 8.5)			Bran extract (pH 4.8)	
	Days incubated at 37° C.			Days incubated at 37° C.	
	3	6	10	3	7
Nucleic acid	52.4	70.5	75.0	72.2	72.2
Na salt of soil preparation	9.0	19.6	27.0	70.5	89.4
Na phytate (bran) ..	0.0	1.4	5.6	81.5	85.3
Na phytate, bromine treated (bran) ..				83.0	95.1
Fe phytate, bromine treated (bran) ..				0.0	2.7

Intestinal extract has very little action on phytin, while bran extract vigorously attacks both phytin and nucleic acid but has no appreciable action upon ferric phytate.

The data in Tables I and II would appear to confirm the identity of the soil preparation as ferric phytate.

We have now obtained indications that phytin is promptly fixed in acid soils, presumably by combining with iron. This would help to explain the accumulation and the low availability to plants of the organic phosphorus in Quebec podsol soils, since ferric phytate apparently is resistant to attack by enzymes, probably because of its low solubility.

A detailed account of these experiments will be published elsewhere at an early date.

W. J. DYER
C. L. WRENSHALL
G. R. SMITH

CHEMISTRY DEPARTMENT,
MACDONALD COLLEGE,
QUEBEC

[5] J. B. Rather, *Ark. Agr. Exp. Sta. Bul.* 135, 1917.

[6] F. Fischler and F. H. Kurten, *Biochem. Zeit.*, 254: 138–147, 1932.

ON THE NATURE OF THE LEUKOCYTOSIS-PROMOTING FACTOR OF INFLAMMATORY EXUDATES[1]

IN earlier studies the writer has shown that there exists in inflammatory exudates of animals a leukocytosis-promoting factor (abbreviated as the LPF), capable *per se*, when injected into the circulating blood of normal dogs, of inducing a marked rise in the number of white cells.[2,3] The presence of this factor offers an explanation for the mechanism of leukocytosis frequently accompanying inflammatory processes. The active principle is essentially indiffusible and thermolabile.[2,3] Heating the exudate to 60° C. inactivates its leukocytosis-promoting property. These facts are compatible with the possibility that the LPF is a protein. This original view has now been further verified. The present observations indicate that the factor is either a globulin or that it is at least closely associated with that class of proteins. The details of all the observations will be reported *in extenso* elsewhere. The essential facts, however, can be briefly summarized as follows:

The normal range of variation in white blood cell counts of several dogs over a period of about six hours yielded an average maximum increase of 26.2 per cent. The effect of injecting intravascularly 20 to 30 cc of an exudate into these same animals induced an increase in counts averaging 77.2 per cent. This indicates, as found previously,[3] that the LPF of exudates causes roughly a threefold increase in the absolute number of circulating leukocytes.

Dialysis of the exudative material favors the separation of the euglobulins. This fraction introduced into the circulating blood stream of dogs leaves the level of leukocytes essentially unaltered; the average increase in a series of experiments being 29.1 per cent. The residual cloudy supernatant material, after removal of the euglobulins, contains the active factor. This fraction yields in animals an average increase in leukocyte counts of 64.4 per cent. The LPF seems primarily to be associated with the pseudoglobulin fraction, for separating the albumins after treatment with $(NH_4)_2SO_4$ at half saturation fails to alter the activity of the material. The albumins *per se* induce only an average increase of 2.7 per cent. in the leukocyte count. This figure is even considerably lower than that encountered in the range of normal fluctuation. The nucleoproteins, obtained presumably by precipitation of the above supernatant cloudy material by adjusting the pH between 4.2 to 4.5 with dilute acetic acid, are likewise practically inactive.

Further studies by fractional salting out with

[1] Aided by grants from the Milton Fund of Harvard University, the International Cancer Foundation and the Dazian Foundation for Medical Research.

[2] V. Menkin, SCIENCE, 90: 237, 1939.