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.

ammonium sulfate indicate that the leukocytosis-promoting factor seems primarily linked with the pseudoglobulin fraction of exudates. Precipitation of an alkaline exudate at one third saturation with $(NH_4)_2SO_4$ yields an inactive fraction; the average increase of the white cell counts in several experiments being 21.3 per cent. This would again support the fact that the LPF is probably not a euglobulin. Treatment of the exudate with 14 per cent. Na_2SO_4 produces an inactive material, and thus seems likewise further to substantiate this inference. On the other hand, the dialyzed precipitate resulting from preliminary salting out of the exudate with $(NH_4)_2SO_4$ at one-half saturation, is highly active. The results of five experiments indicate that the average increase in the number of circulating leukocytes with this globulin fraction is 88.9 per cent. Similar fractionation of normal blood serum fails completely to produce any material which manifests any augmentative effect on the number of circulating leukocytes.

These observations, therefore, support the view that the leukocytosis-promoting factor is either a globulin or at least that it is closely linked with the pseudoglobulin fraction of exudates.[4] It is conceivable that the increase in the alpha-globulin and therefore in the high value of the α-globulin/albumin ratio recently found by Longsworth, Shedlovsky and MacInnes[5] in the blood sera of patients afflicted with various inflammatory processes, may be referable to a discharge from the site of inflammation into the circulation of the leukocytosis-promoting factor. It is to be recalled in this connection that the LPF seems to favor the outpouring of immature granulocytes from the bone marrow.[2,3] Studies are now in progress in an endeavor to purify further this globulin-like substance in exudates which *per se* offers an adequate explanation for the mechanism of leukocytosis accompanying inflammation.

VALY MENKIN[6]

HARVARD MEDICAL SCHOOL

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A NEW TECHNIC FOR STAINING VAGINAL SMEARS[1]

IN the studies from this laboratory which have demonstrated the value of the vaginal smear method for detecting the action of estrogenic, androgenic and gonadotropic hormones in man, the staining technic employed was the conventional hematoxylin-eosin method which, with the addition of waterblue as a counterstain, was originally applied to the vaginal smear by Papanicolaou. While, as with fixed tissue, this stain is satisfactory for the morphological details of the vaginal secretion, it leaves something to be desired as regards the detection of cornification. As the extent of cornification is a measure of the degree of ovarian function and one of the most important indices in the vaginal smear, it was thought worth while to investigate other staining methods which might be more specific as regards this chemical cytoplasmic change.

Of a number of methods tested, a modification of the Masson trichrome stain was found to offer distinct advantages in this respect over the hematoxylin-eosin-waterblue method. Four months' experience with several thousand vaginal smears taken from patients with normal and abnormal menstrual cycles, in pregnancy, and in menopause and amenorrhea during treatment with various sex hormones, permits me to recommend this staining procedure highly to other workers in the field.

The advantages of the trichrome stain lie in its specific and reliable detection of cornification and in its production of a sequence of contrasting color changes in the cells under the influences of estrogens, whether endogenously produced or administered as therapeutic agents. These changes resemble a chemical titration in their sharpness. The addition of the color changes to the morphological alterations in the vaginal secretion contributes greatly to the ease and certainty of the interpretation of the smear.

The changes are seen most strikingly in menopause or amenorrhea, when, as a result of estrogenic therapy, an atrophic smear is transformed to the estrous or follicular type. The cells of the typical atrophic smear usually stain a lavender or pale blue with the trichrome stain. In less atrophic smears, the prevailing tint is a pale greenish-blue. Following the administration of estrogens, the cells, in addition to undergoing morphological changes, become progressively greener. This definite greenish coloration persists up to cornification, at which stage the cells abruptly change to a brilliant orange red. During the normal menstrual cycle, and in amenorrhea following the use of gonadotropic hormones, similar sharp transitions occur and permit the ready detection of ovulatory reactions.

[3] V. Menkin, *Am. Jour. Path.*, 16: 13, 1940; "Dynamics of Inflammation," Macmillan Company, New York, 1940 (in press).

[1] Aided by a grant from the Josiah Macy, Jr., Foundation.

[4] The persistence, however, of a positive Molisch, along with the usual tests for proteins, in the present state of purification of the material does not preclude the possibility of a carbohydrate as a prosthetic group.

[5] L. C. Longsworth, T. Shedlovsky and D. A. MacInnes, *Jour. Exper. Med.*, 70: 399, 1939.

[6] With the technical assistance of Mr. M. A. Kadish and Miss Irene Lapouse.