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.

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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.

For the composition of the trichrome stain the reader is referred to Foot's description.[2] The only modifications found desirable in adapting this stain to the vaginal smear were an increase in staining time with light green to 8 minutes and a reduction in the strength of acetic acid from the original 1 per cent. to 0.25 per cent. The acetic acid solution is made up fresh weekly. The other solutions will, with an occasional filtration to keep them clear, stain 1,200 to 1,500 slides satisfactorily. The exact procedure of staining is as follows:

(1) Fix slide while wet in 95 per cent. alcohol: ether (1:1) and carry through alcohols to water.
(2) Harris hematoxylin—2 minutes.
(3) Rinse 3–4 times in water and let stand 5 minutes in running water.
(4) Ponceau-acid fuchsin-orange G—5 minutes. Rinse 3–4 times in water.
(5) Phosphotungstic acid (3 per cent.)—10 minutes. Rinse 3–4 times in water.
(6) Light green—8 minutes. Do not wash.
(7) Acetic acid (0.25 per cent.)—3 minutes. Do not wash.
(8) Dehydrate, clear in xylol, and mount in damar.

Analysis of the action of individual components indicates the possibility of some simplification of the stain. Ponceau de xylidene, acid fuchsin and orange G were all taken up by the cornified cells and contributed to their final color. The most satisfactory staining of the cornified cells, using these dyes separately, was obtained with Ponceau de xylidene. Orange G is, however, desirable because of its staining of red cells. Phosphotungstic acid is essential because of its action as a mordant in fixing and intensifying the color produced by the preceding solution. Light green acts as a counterstain for the non-cornified cells. When used alone it will stain cornified cells a more intense green, but is unable to displace the other dyes once they have entered the cell. The staining with hematoxylin can be omitted, the nuclei then taking a red stain. Dioxane can be used instead of the alcohols as with tissue.

A more detailed description of the nuances of morphology and color revealed by this stain in smears from various normal and pathological states will be given elsewhere.

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A RUBBER CAST OF THE DOGFISH SPIRAL VALVE

THE spiral valve of the dogfish is a feature of most courses in comparative anatomy. It is difficult to visualize the spiral course taken by the food, and the large absorbing surface of the intestine is seldom appreciated. The structure and function of the spiral valve is well demonstrated by a rubber cast, which takes but a few minutes to make. The intestine from pylorus to rectal gland is removed from a fresh or formalin-preserved dogfish. The pyloric end is attached to a faucet and the contents completely flushed out by a slow but positive stream of water. This process is aided by gentle manipulation, and should be continued until the water is clear. After removal from the faucet the intestine is carefully squeezed to remove as much water as possible, and latex[1] is injected through the pyloric end under about 200 mm pressure. The large intestine should be tied off when latex flows out, and gentle manipulation assists the even distribution of the latex, mixing it completely with the water remaining inside. When the intestine is thoroughly turgid, the pylorus is tied off under pressure and the whole preparation hardened in 2 per cent. acetic acid for ten days. It is a simple matter to dissect away the tissue from around and between the flexible spirals, leaving a rubber cast of the interior of the intestine. The quality of the rubber improves if the cast is washed for a few hours in tap water and allowed to dry at room temperature for a day or two. The spiral may then be stretched, twisted or even unrolled without becoming permanently deformed, and is a striking demonstration specimen.

K. D. ROEDER

TUFTS COLLEGE

[1] Turtox Latex supplied by The General Biological Supply House.

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[2] *Amer. Jour. Path.,* 14: 245, 1938.