MUCOPOLYSACCHARIDE IN THE SECRETION OF VERNAL CONJUNCTIVITIS* ITS USE AS A DIAGNOSTIC TEST

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THE stringy character of the secretion in vernal conjunctivitis has long been known. It was first described by Herbert (1903), who noted the formation of a pseudo-membrane ("fibrinous") on the everted eyelid, when the lid was kept maintained everted for several minutes. Lyons (1937) was of the opinion that the pseudo-membrane was a better diagnostic sign than the eosinophilia and used provocative procedures to obtain it. Strebel (1933) gave a description of the strings in the secretion according to which the centre of the string is structureless, hard, and with a peripheral mantle in which cells are imbedded. Lehrfeld and Miller (1939) referred to the stickiness of the strings as "chewing gum mucus".

These peculiarities of the secretion of vernal conjunctivitis cannot be explained by the assumption of an abundance of fibrin exudation, since other fibrinous exudates are not as stringy or sticky. Sary (1957) described the presence of a mucopolysaccharide on the surface of the mucous membranes of the respiratory tract, and proved that the amount of mucopolysaccharides was increased in allergic conditions.

It was, therefore, considered possible that some mucopolysaccharide may account for the character of the secretion in vernal conjunctivitis. Since the quantities of conjunctival secretion are usually minute it was necessary to resort to a new microtest for mucopolysaccharides developed in this laboratory (Blumenkrantz, 1957).

Material and Methods

(A) *Smears*.—These were taken at various times from 150 patients known to suffer from vernal conjunctivitis. All these cases were under treatment in our special clinic for vernal conjunctivitis and the diagnosis was based on objective findings in the palpebral or bulbar conjunctiva. Similar smears were taken from fifty normal eyes and from fifty other patients suffering from various conjunctival conditions as a control series.

In each case the smear was taken from the conjunctiva of the everted upper eyelid. A wire loop was used lightly scratching the surface of the conjunctiva five or six times. No drops nor provocative methods were used. The smears were

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examined fresh or at most within several hours. The secretion on the slide was suspended in a drop of distilled water and a drop of toluidine blue 0.1 per cent. was added. The precipitate obtained was examined under the low-power microscope.

Results were considered "positive" if there was a relative abundance of a granular precipitate in the form of particles or of fibres coloured violet instead of blue (metachromasia). This precipitate is similar both in colour and morphology to that obtained when toluidine blue is added to pure hyaluronic acid.

Results were considered "negative" if there was very little precipitate which was practically all blue and therefore showed no metachromasia.

Results were considered "atypical" when there was abundance of a precipitate of a similar granular structure but not metachromatic.

(B) *Paper Chromatography*.—Secretion from cases of vernal conjunctivitis, from normal conjunctivae, and hyaluronic acid were submitted simultaneously to paper chromatography. Six cases of vernal conjunctivitis were taken thus, and four patients with normal conjunctivae as controls. The conjunctival secretions and the hyaluronic acid were submitted to hyaluronidase digestion for 36 hours at 37°C. before the paper chromatography tests. Silver nitrate was used as the spraying agent.

Results

Smears.—All 150 smears taken from the vernal conjunctivitis cases and submitted to the toluidine blue microtest were "positive". This was so even in the bulbar cases in which secretion was often scarce.

All the smears from normal conjunctivae were either "negative" or "atypical".

Of the fifty various conjunctival conditions twenty-five were "atypical", twenty "negative", and five "positive". Of the five "positive", one was a fresh lime burn, and the other four were of undetermined aetiology (Table).

TABLE
RESULTS WITH TOLUIDINE BLUE MICROTEST

Condition	Smears			
	Number	Negative	Atypical	Positive
Vernal Conjunctivitis	150	0	0	150
Normal	50	46	4	0
Other	50	20	25	5

Paper Chromatograph.—Similar spots were obtained with hyaluronic acid and with the secretions from the six cases of vernal conjunctivitis, but the secretions from the four cases without vernal conjunctivitis gave dissimilar spots.

Discussion

The fact that all the vernal conjunctivitis smears were consistently "positive" and that all the smears from normal conjunctivae and from patients with other conjunctival conditions were either "negative" or "atypical" suggests that the microtest described above is a simple and valuable aid in the diagnosis of vernal conjunctivitis. It is easy to perform and gives more consistent results than any other known diagnostic test, including the provocative tests for the appearance of strings and the presence of eosinophilia.

This test is based on the relative abundance of a mucopolysaccharide in the secretion of vernal conjunctivitis. In describing the microtest, Blumenkrantz (1957) suggested that hyaluronic acid could be identified according to the colour and morphology of the precipitate. Since the precipitate obtained with the secretion from cases of vernal conjunctivitis is similar to that obtained when toluidine blue is added to pure hyaluronic acid, it is suggested that the mucopolysaccharide in the secretion of vernal conjunctivitis is hyaluronic acid. The results of the paper chromatography test tend to confirm this view.

The reason for the abundance of a mucopolysaccharide in the secretion in vernal conjunctivitis is not clear. Altshuler and Angevine (1949) showed that the formation of metachromatic material was closely related to the processes of hyalinization and amyloid degeneration. Godtfredsen (1949) showed local production of hyaluronidase in various allergic conditions. Vernal conjunctivitis causes hyalinoid degeneration of the connective tissue of the conjunctiva, and is at least a "secondary allergic condition" as described by Gutmann (1945). A local production of hyaluronidase in vernal conjunctivitis may explain the partial digestion of the mucopolysaccharide within the conjunctiva and its appearance in the secretion.

Summary

- (1) Secretions from 150 cases of vernal conjunctivitis were submitted to the toluidine blue microtest for mucopolysaccharides together with control smears from 100 other cases.
- (2) Secretions from cases of vernal conjunctivitis and from a number of controls were tested by paper chromatography together with hyaluronic acid after digestion with hyaluronidase.
- (3) The results indicate that a specific test for vernal conjunctivitis may be based on the presence in the secretion of a mucopolysaccharide.
- (4) The role of hyaluronic acid and hyaluronidase in the pathogenesis of vernal conjunctivitis is discussed.

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REFERENCES

- ALTSHULER, C. H., and ANGEVINE, D. M. (1949). *Amer. J. Path.*, **25**, 1061.
 BLUMENKRANTZ, N. (1957). *Clin. Chem.*, **3**, 696.
 GODTFREDSEN, E. (1949). *Brit. J. Ophthalm.*, **33**, 721.
 GUTMANN, M. J. (1945). *Acta med. orient. (Tel-Aviv)*, **4**, 150.
 HERBERT, H. (1903). *Brit. med. J.*, **2**, 735.
 LEHRFELD, L., and MILLER, J. (1939). *Arch. Ophthalm. (Chicago)*, **21**, 639, 1070.
 LYONS, F. M. (1936). "Observations on the Pathogenesis of Spring Catarrh", 11th Annual Report, Giza Memorial Ophthalmological Laboratory, Cairo, **11**, 121.
 STARY, Z. (1957). *Clin. Chem.*, **3**, 557.
 STREBEL, J. (1933). *Klin. Mbl. Augenheilk.*, **91**, 512.