

BAL, M.	1957.	<i>J. Clin. Invest.</i> , 30, 871.
BOTHWELL, T. H., FIRZIO-BIROLI, G., AND FINCH, C. A.	1958.	<i>J. Lab. Clin. Med.</i> , 51, 24.
BRATLEY, F. G., BURROUGHS, H. H., HAMILTON, D. M., AND KERN, C.	1931.	<i>Amer. J. Med. Sci.</i> , 182, 507.
CRUZ, W. O., HAHN, P. F., AND BALE, W. F.	1941-42.	<i>Amer. J. Physiol.</i> , 135, 595.
GIBLETT, E. R., MOTULSKY, A. G., CASSERD, F., HOUGHTON, B., AND FINCH, C. A.	1956.	<i>Blood</i> , 11, 1118.
JANDL, J. H., AND KAPLAN, M. E.	1960.	<i>J. Clin. Invest.</i> , 39, 1145.
KALDOR, I.	1954a.	<i>Austral. J. Exp. Biol. Med. Sci.</i> , 32, 795.
" "	1954b.	<i>Ibid.</i> , 32, 801.
" "	1958.	<i>Ibid.</i> , 36, 173.
KALDOR, I., AND POWELL, MARJORIE	1957.	<i>Ibid.</i> , 35, 123.
LAURELL, C.-B.	1952.	<i>Pharmacol. Rev.</i> , 4, 371.
MORGAN, E. H., AND CARTER, G.	1960.	<i>Austral. Ann. Med.</i> , 9, 209.
MUIR, R., AND DUNN, J. S. . .	1914-15.	<i>This Journal</i> , 19, 417.
" " " " " " " " " " " "	1915-16.	<i>This Journal</i> , 20, 41.
PALMER, J. G., EICHWALD, E. J., CARTWRIGHT, G. E., AND WINTROBE, M. M.	1953.	<i>Blood</i> , 8, 72.
RAMSAY, W. N. M.	1958.	<i>In Advances in clinical chemistry</i> , ed. by H. Sobotka and C. P. Stewart, New York and London, vol. 1, p. 2.
SCHADE, A. L., AND STENGLE, J. M.	1959.	<i>J. Lab. Clin. Med.</i> , 54, 593.
SHODEN, A., GABRIO, B. W., AND FINCH, C. A.	1953.	<i>J. Biol. Chem.</i> , 204, 823.
SHODEN, A., AND STURGEON, P. .	1958.	<i>Amer. J. Path.</i> , 34, 1139.
WALSH, R. J., ARNOLD, BARBARA J., LANCASTER, H. O., COOTE, MARGARET A., AND COTTER, HELEN	1953.	<i>Austral. Med. Res. Council. Spec. Rep. Ser. no. 5, Sydney.</i>

NOTE: THIS DOCUMENT DID
NOT COME FROM PPG FILES

BB 0005526

07:41 99

* Present address: Pneumoconiosis Research Unit, Llandough, South Wales.

3. Extract and Winbury's (1955) metachromatic staining reaction. 0.1 per cent. solution of azure "A" MacNeal (Rovector) in 30 per cent. of alcian blue was used.

4. Hale's colloidal iron method (Hale, 1946). At the suggestion of Dr D. J. Winslow, Mowry's modification (1958) of this technique was adopted.

5. McManus' (1916) periodic acid-Schiff method (P.A.S.). In a few cases the results obtained by this method were confirmed by the use of the Hotchkiss (1948) technique.

6. Steedman's (1950) alcian blue method. Later Mowry's modification (1956) was used.

7. The method of Dempsey *et al.* (1947) for demonstrating metachromasia at varying pH levels, with solutions of 0.1 per cent. azure "A" buffered to a reaction of pH 2.0, 3.0, 3.5 and 4.0 with Walpole's HCl/acetate buffer.

Hyaluronidase treatment. Two enzyme preparations were used:

(i) Testicular enzyme (Benger's Hyalase), at a strength of 75 turbidity-reducing units (TRU) per ml. in M/5 acetate buffer at pH 5.6. Sections were incubated for 18 hr at 37° C.

(ii) Streptococcal enzyme (generously donated by Lederle Ltd, New York). This was used at a strength of 50 TRU per ml. in M/5 acetate buffer at pH 5.6. Sections were incubated at 37° C. for 4½ hr.

After treatment with the above solutions, control and digested sections were stained by both Hale's colloidal iron and the metachromatic techniques.

Chemical determination of hyaluronic acid. This depends upon the production of a mucin clot on the addition of acetic acid to diluted pleural fluid, a method very kindly communicated to us by Dr Karl Meyer. Samples of fluid were diluted with 2.5 volumes of water and 50 per cent. acetic acid added to a concentration of 0.2 per cent. The presence of hyaluronic acid is shown by the formation of a typical synergetic clot; this is inhibited by previous incubation of the fluid with hyaluronidase for 30 min. at 38° C. We have also used an unpublished method, also provided by Dr Meyer, for the quantitative isolation of hyaluronic acid. This involves the precipitation of the hyaluronic acid (with its protein), digestion with pepsin and trypsin, and subsequent isolation of the polysaccharide.

RESULTS

From the table it may be seen that the secretion in all 16 of the mesotheliomas was metachromatic, and stained by Hale's method and alcian blue. The metachromasia and Hale-staining were abolished after incubation with testicular hyaluronidase. Only 2 of the 16 were P.A.S.-positive. In contrast the sections of the adenocarcinomas gave strongly positive results with P.A.S., Hale's method, and alcian blue. Metachromatic secretion was observed in most of the adenocarcinomas studied. In none of the adenocarcinomas with metachromatic or Hale-positive secretion was the staining reversed by treatment with either hyaluronidase. Streptococcal hyaluronidase applied to the sections removed the metachromasia in 13 out of the 16 mesotheliomas and markedly reduced it in the remaining 3. In the mesotheliomas the secretion stained a magenta colour (a "gamma" metachromasia), whereas in the adenocarcinomas the metachromatic substance stained purple ("beta" metachromasia). The metachromasia exhibited by the mesotheliomas is presumably due to the presence of

HYALURONIC ACID IN MESOTHELIOMAS

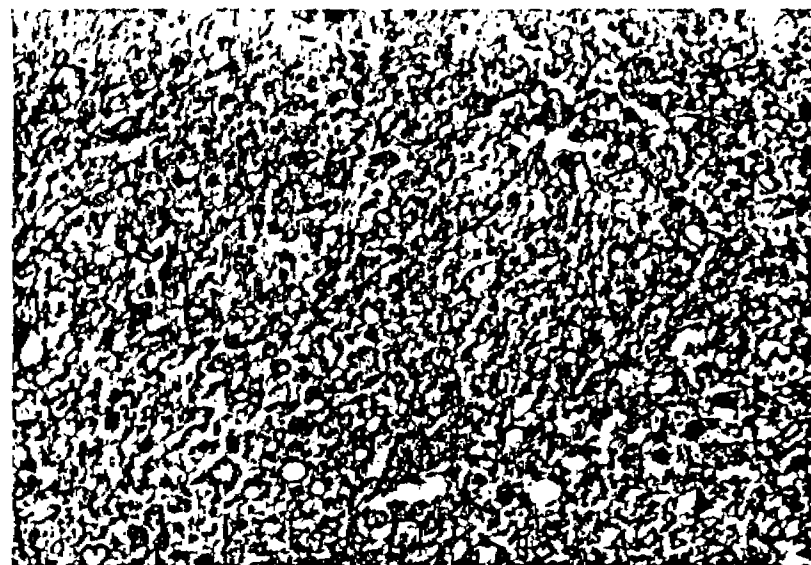


FIG. 1.—Mesothelioma stained by Hale's method. $\times 120$.

NOTE: THIS DOCUMENT DID
NOT COME FROM PPG FILES



FIG. 2.—Another section from the same block, stained by Hale's method after treatment with testicular hyaluronidase. $\times 120$.

carboxyl groups in the hyaluronic acid molecule, which results in a weaker metachromatic effect than that observed, for example, in cartilage and other tissues containing acid polysaccharide.

The amount of metachromatic substance in the mesotheliomas was extremely variable and followed no definite pattern. For example, in some tumours large cystic spaces were filled with stained secretion, in others this secretion was seen in the smaller spaces but not in the larger. In the more cellular tumours only a few clefts containing this substance were seen.

TABLE
Histochemical staining of secretions

	Type of tumour			
	Diffuse mesothelioma	Pulmonary adeno-carcinoma	Gastro-intestinal adeno-carcinoma	Metastatic adeno-carcinoma
No. of tumours examined	16	4	3	2
P.A.S. positive, not removed by diastase	2	4	3	2
Metachromatic with azure "A"	10	4	2	2
Metachromasia removed by testicular hyaluronidase	16	0	0	0
Metachromasia removed by streptococcal hyaluronidase	13 (markedly diminished in 3 others)	0	0	0
Hale positive	10	4	3	2
Hale-positive secretion removed by testicular hyaluronidase	15 (markedly diminished in 1 other)	0	0	0
Alcian blue positive	16	4	3	2

The effect of pH on metachromasia was tested in 7 mesotheliomas and 3 of the peripheral pulmonary adenocarcinomas. The differentiation was not as clear as was expected, for although 6 of the mesotheliomas showed no metachromasia below pH 4, one was metachromatic at pH 3.5. Two of the 3 adenocarcinomas were metachromatic at pH 1, but the third became so only at pH 4.

At the time of our earlier work on this subject we were impressed by the stromal metachromasia exhibited by some of the mesotheliomas. We now consider this as a non-specific feature of the rapidly growing connective tissue and its ground-substance (Taylor and Saunders, 1957). Chemical studies (Watson and Pearce, 1950) indicated that this stromal substance consisted of a hyaluronic-acid chondroitin-sulphate complex. We were able to confirm this in part, by showing that the stromal substance in the mesotheliomas and in the proliferating fibrous tissue provoked by certain carcinomas had its metachromasia and Hale's colloidal iron staining completely removed by testicular hyaluronidase but only diminished by the streptococcal enzyme.

NOTE: THIS DOCUMENT DID
NOT COME FROM PPG FILES

CG 14762

1 BB 0005528 1

Clinical estimations. Hyaluronic acid was isolated from the pleural fluids of 5 cases of confirmed mesothelioma of the pleura in concentrations varying from 0.02 to 1.25 per cent. In 3 of these cases the yield of the acid increased as the disease progressed; in a fourth, a steady rise was observed until about two months before death, when none could be recovered. The disappearance of hyaluronic acid in such cases might be due to the formation of a gel of polysaccharide which could no longer be aspirated along with the pleural fluid. No false positives for hyaluronic acid have yet been obtained from a large number of pleural fluids including specimens from idiopathic effusions, tuberculous infections and from malignancies other than mesotheliomas. Dr Karl Meyer (personal communication) has found the polysaccharide in pleural fluids from ten cases of mesothelioma.

DISCUSSION

In reviewing the sections of pleural tumours that had been submitted to various pathological laboratories throughout South Africa prior to 1956, a number that had originally been diagnosed as adenocarcinomas were found to have histological features consistent with those of mesotheliomas. It is possible that a similar situation has existed elsewhere, as in a number of cases of carcinoma complicating asbestosis the tumours were described as being pleural adenocarcinomas in which no primary site of origin had been found in the lung (Owen, 1951; Bonser, Faulds and Stewart, 1955). Similarly, no primary site of origin was found in several of the peritoneal tumours described by Keal in 1960. It is hoped that the methods described will help to give a clearer indication of the number of mesotheliomas that have occurred in association with exposure to asbestos dust.

The main difficulty in applying the histochemical tests described here is the circumstance that the majority of specimens are received in formol-saline unless the presence of a mesothelioma has been previously suspected on clinical and radiological grounds. In formalin-fixed tissue we have found the P.A.S. method most useful in differentiating between peripheral pulmonary adenocarcinomas and mesotheliomas. In our experience pulmonary adenocarcinomas have always contained P.A.S.-positive secretion, whereas the mesotheliomas are usually negative. In addition to those mesotheliomas mentioned in the table, we have used the P.A.S. method on a further 20 of these tumours that had a compound tubular structure which could be confused with that of adenocarcinoma, and in which the tissue had been fixed in formol-saline. Of these only 5 contained P.A.S.-positive secretion and in every case this was removed by incubation with diastase. It is only in the 2 cases previously described that the P.A.S.-positive secretion was not removed by this method. One of these was similar to the mesotheliomas of "mesenchymal type" described by McCaughey (1958). The other case although classified as a mesothelioma has several other features that would suggest that it was a

peripheral adenocarcinoma: the Hale-positive secret was not entirely removed by incubation with either enzyme, and metachromasia appeared below pH 4. The specimen was from a man who had worked on an asbestos mine for many years, and at thoracotomy the appearance of the tumour was typical of a mesothelioma. A lung biopsy taken at the time showed the histological features of asbestosis.

It is not yet possible to say whether the methods described in this paper will differentiate between peritoneal mesotheliomas and metastatic carcinoma.

The isolation of hyaluronic acid from pleural fluid has been a useful diagnostic aid in some cases, but has been negative in a number of cases in which the diagnosis has subsequently been proved histologically and histochemically. It is clear that only positive results are of any use. No false positives have been obtained in human cases in our series, although several were obtained in pleural fluids from mice that had died of a streptococcal pneumonia. Hyaluronic acid was demonstrated histochemically in the acute pleural exudate of these animals.

SUMMARY

Diffuse pleural mesotheliomas secrete material that stains as an acid mucopolysaccharide and can be removed by prior digestion with either testicular or streptococcal hyaluronidase. Chemically, this material can be quantitatively precipitated by acetic acid, and its presence confirmed by prior treatment of pleural fluid with hyaluronidase. These methods have been used to differentiate between diffuse pleural mesothelioma and peripheral pulmonary adenocarcinoma, especially in asbestosis.

We wish to thank Dr Ian Webster, the Sub-director of this unit, for allowing us facilities for carrying out this investigation, and Miss Tony Le Mare for technical assistance.

REFERENCES

- | | | |
|--|-------|--|
| BLIX, G. | 1951. | <i>Acta Soc. Med. upsalien.</i> , 56, 47. |
| BONSER, GEORGINA M., FAULDS, J. S., AND STEWART, M. J. | 1955. | <i>Amer. J. Clin. Path.</i> , 25, 126. |
| DEMPSEY, E. W., BUNTING, H., SINGER, M., AND WISLOOKI, G. B. | 1947. | <i>Anat. Rec.</i> , 98, 417. |
| HALE, C. W. | 1940. | <i>Nature (Lond.)</i> , 157, 802. |
| HOTCHKISS, R. D. | 1948. | <i>Arch. Biochem.</i> , 16, 131. |
| KEAL, E. E. | 1960. | <i>Lancet</i> , 2, 1211. |
| KRAMER, H., AND WINDRUM, G. M. | 1955. | <i>J. Histochem. Cytochem.</i> , 3, 227. |
| MCCAUGHEY, W. T. E. | 1958. | <i>This Journal</i> , 76, 517. |
| MC MANUS, J. F. A. | 1946. | <i>Nature (Lond.)</i> , 158, 202. |
| MEYER, K., AND CHAFFEE, ELEANOR | 1939. | <i>Proc. Soc. Exp. Biol. Med.</i> , 42, 7. |
| " " " " " " " " | 1940. | <i>J. Biol. Chem.</i> , 133, 83. |
| MOWRY, R. W. | 1950. | <i>J. Histochem. Cytochem.</i> , 4, 407. |
| " " " " " " " " | 1958. | <i>Lab. Invest.</i> , 7, 560. |
| OWEN, T. K. | 1951. | <i>Brit. J. Cancer</i> , 5, 382. |

NOTE: THIS DOCUMENT DID NOT COME FROM PROPER FILE