

dermatitis herpetiformis, acne vulgaris, lichen planus, adenoma sebaceum, porokeratosis, psoriasis, metastatic carcinoma of the omentum, portal cirrhosis, and mesenteric spreads from guinea pigs. In these conditions most of the mast cells, the distribution of which was demonstrated by toluidine blue stain, showed acid-fast granules in the corresponding sections stained by Fite-Faraco-haematoxylin stain.

Morphology, evolution, cytochemistry, functions in health and disease, etc., of the mast cells have been reviewed by Asboe-Hansen¹ and Riley². It is generally agreed that the mast cell granules contain an acidic mucopolysaccharide and substances closely related to hyaluronic acid and heparin. These cells are also believed to contain histamine. The present investigation shows that the granules contain a substance that shows an acid-fast character with the Fite-Faraco stain.

In the course of his work on the early lesions of leprosy, Khanolkar³ was the first to record the presence of certain cells that showed acid-fast granules (fuchsinophil cells) in sections stained by the Fite-Faraco method. The morphology and distribution of these cells as described by him and illustrated with drawings in colour in his paper appear to be identical with the mast cells seen in our tissue sections. Khanolkar regarded these granules as partly digested *Mycob. leprae* and the cells as adventitial cells or macrophages.

Our observations indicate that these cells are tissue mast cells, the granules of which have now been shown to contain among other substances an acid-fast material and that such mast cells are seen in a wide variety of conditions unrelated to mycobacterial infection.

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¹ Asboe-Hansen, G., in *Intern. Rev. Cytol.*, edit. by Bourne, G. H., and Danielli, J. F. (Academic Press, New York, 1954).

² Riley, J. F., *The Mast Cells* (E. and S. Livingstone, Edinburgh and London, 1959).

³ Khanolkar, V. R., *Indian Council. Med. Res. Spec. Rep.*, No. 19 (New Delhi, 1951).

Utilization of Cell Chromosome Number for Diagnosing Cancer Cells in Effusion

THE karyological study of cancer cells has recently been a field of increased interest. While noting that most human cancer cells have abnormal idiograms, the various contributions have been concerned largely with the theoretical nature of cancer and have provided only a minimal amount of clinically useful information¹⁻⁵. Jacob⁶ has suggested that malignancy may be diagnosed by chromosome counts. It therefore appears worthwhile to emphasize that determining the approximate number of chromosomes of cells frequently will provide accurate diagnoses of cancer.

In the work reported here 49 effusions from pleural or peritoneal spaces were analysed for chromosomes. The absolute diagnosis in each instance was determined by the patient's hospital course. The cancerous effusions were primarily those with ovarian or uterine tumours, but also included are effusions from patients with cancer of the stomach, lung and breast.

The laboratory technique requires minimal experience. The effusion fluid is slowly centrifuged, and the cell button is resuspended in 0.5 per cent sodium citrate for 20 min. the cells then fixed in 50 per cent acetic acid. The cell suspension is stained with acetic orcein and a drop of fluid then squashed between cover-slip and slide. Using a phase microscope, the preparation is searched for cells and a minimum of 10 cells must be counted.

For the purposes of 'marker' chromosome examination and stem line determination, it is necessary to photo-

graph the better chromosome figures and prepare idiograms. This laborious step is not required if the purpose is only to determine the presence of cancer. Most cancer cells are readily identified because of their abnormal chromosome patterns.

Cancer cell chromosome counts in this work have ranged from 24 to more than 250. The majority have been between 54 and 84. In these human cancer preparations, many different chromosome numbers were found and there was frequent absence of a clear-cut model number (stem line) of chromosomes. Idiogram analysis will often show unusually long acrocentric chromosomes which are apparently associated with cancer.

The fluids were evaluated at the same time with standard cytological techniques for cancer cells. The results are shown in Table 1. In 5 out of 16 examples of benign effusion, inadequate chromosome preparations were found and no diagnosis could be made. In only 2 of the 31 cancerous effusions was this true.

Table 1. DIAGNOSIS OF CANCER CELLS IN EFFUSIONS, USING CHROMOSOMAL AND CYTOLOGICAL TECHNIQUES

Source	Total cases studied	Chromosome study		Cytological study		Preparations inadequate for chromosome study
		Correct diagnosis	Incorrect diagnosis	Correct diagnosis	Incorrect diagnosis	
Non-cancer effusions	16	11	0	16	0	5
Cancer effusions	33	26	5	31	2	2

Five out of 33 chromosomal preparations of malignant cells were incorrectly diagnosed, either because the tumour had nearly normal chromosome patterns or because poor karyological technique obscured the total number of chromosomes. Fluids from two patients were incorrectly diagnosed as showing 'no malignant cells' by the cytological technique but were correctly diagnosed as cancer by chromosome analysis.

Improved results could be obtained by short-term culture techniques and by more detailed analysis of the cell preparations. But such modifications change a simple technique into an expensive and laborious procedure which can offer no competition to the standard cytological techniques. While a significant number of malignant effusions were called benign, no false positive diagnoses were made.

In addition to adding a simple collaborative technique to the usual cytological procedures, chromosome study has the advantage of contributing knowledge to our understanding of cancer.

This work was supported in part by a grant from the American Cancer Society Institution.

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⁵ Sandberg, A., Ishihara, T., Miwa, T., and Hauschka, T., *Cancer Res.*, **21**, 678 (1961).

⁶ Jacob, G., *Lancet*, ii, 724 (1961).

IMMUNOLOGY

A Freezing-thawing Technique for Concentrating Antibodies in Serum

WHEN frozen serum is centrifuged, thawing due to air friction takes place and the heavier molecules are precipitated. By this means it is possible to concentrate the proteins and electrolytes present in serum.

Bovine serum containing *Brucella abortus* agglutinins was placed in 10-ml. amounts in 10-ml. conical centrifuge tubes and the tubes were frozen overnight at -10°C . The tubes were then centrifuged at 3,800 r.p.m. for 30 min at