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PATHOLOGY

Cytolytic Effect of Homogenates of Mouse Adenocarcinomas on White Cells

Homogenates of the adenocarcinomas of mice (females of the *C3H* strain) hæmolyse red cells of mouse and of man. This is due, in part at least, to the higher fatty acids which can be extracted from the tumours and also from normal mouse tissue[1]. The question which now arises is whether these higher fatty acids cytolyse white cells.

Cytolysis of white cells is more difficult to demonstrate than hæmolysis of red cells. After washing white cells derived from man (preferably with a white cell count of 10,000–20,000) or leukæmia in man, placing them on 'Millipore' filters impregnated with the higher fatty acids, and then trying to stain them, it became apparent that this method, although advocated for the detection of tumour cells, is unsatisfactory for investigating the changes in white cell cytology produced by the higher fatty acids or by tumour homogenates. The following technique was finally adopted.

A solution of $M/10$ ricinoleic acid in benzene (28 mgm./ml.) is diluted so as to give $M/20$ . . . . $M/100$ . . . . $M/800$ solutions of the fatty acid in benzene. To a series of small test tubes, 0·2 ml. of each solution is added; the solutions in the tubes are then dried at 60° C., and the tubes are cooled to room temperature. A volume of 0·2 ml. of a freshly prepared white cell suspension with a known volume concentration (ρ = about 0·03 is convenient) is added to each tube. The tubes are incubated in a water bath at 37° C., and the tube containing the highest concentration of fatty acid ($M/20$) is removed from the water bath after 15 min. and centrifuged for 1 min.; a film of the sedimented white cells is made, and stained with MacNeil's tetrachrome. The cells are examined with oil-immersion. If cytolysis has occurred, little or no cellular detail can be made out; if cytolysis has not occurred, the nucleus and cytoplasm appear relatively normal. After 30 min. the tube with the next highest fatty acid content is removed, and the process is repeated; this is continued at intervals of 1 hr. up to 5 or 6 hr., when it will be found that a sufficiently low concentration of fatty acid (for example, $M/600$) does not produce cytolysis even after these long times.

At the same time as this 'standardization' part of the experiment is carried out, 5 or 6 tubes, to which have been added 0·2 ml. of a Soxhlet extraction of tumour or normal tissue in benzene, are dried at 60° C., cooled and incubated in the water bath at 37° C. After 0·2 ml. of white cell suspension has been added, one is removed every hour, films are made from the centrifuged cells, and the presence or absence of cytolysis is determined by examining the stained film. After some hours, for example 5 hr., about the same amount of cytolysis will be found as occurs in a certain concentration of ricinoleic acid in 5 hr., for example $M/500$ ricinoleic acid. Suitable control tubes must be included.

Suppose that after 5 hr. the cytolysis in the tube containing tumour extract is about the same as that in $M/500$ ricinoleic acid, and suppose that 1 gm. of tissue has been extracted in 25 ml. of benzene. One ml. of extract is then equivalent to 1 ml. of ricinoleic acid in a concentration of 0·56 mgm./gm. wet weight of tissue or 2·8 mgm./gm. dry weight of tissue. In fact, 3 gm. of tissue had been extracted, and so the fatty acid content, in terms of dry tissue weight, is 0·93 mgm./gm. of (dry) tissue extracted. The method is not exact, principally because it depends on the presence or absence of cytolysis as determined by microscopic examination, but it can nevertheless be relied on to a power of 2.

The mean of the fatty acid contents, in terms of dry tissue weight, was found to be 0·52 mgm./gm. of (dry) tissue extracted, the standard error of the mean being $\pm$ 0·13. The mean is therefore 520 γ/gm. of (dry) tissue or only about one-tenth of the quantity of fatty acids found by fluorescence. It is also about ten times greater than the quantity of fatty acids which produces slow lysis of red cells. All the C_{18} fatty acids found by fluorescence measurements are certainly not involved in the cytolysis of white cells, but the quantities of fatty acids involved in the cytolysis of white cells is much greater than the quantity involved in the production of hæmolysis. The reason for this is probably that not all the fatty acid can react with cell surfaces because its solubility in the cell components is limited; if this is so, the amount of fatty acid which reacts with the white cell is about ten times that which reacts with the red cell, but only about one-tenth of the fatty acid present in the systems.

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Extraction of the Higher Fatty Acids from Human Malignant Tumours

C_{18} or higher fatty acids can be identified in and extracted from mouse mammary carcinomas and from mouse lymphomatous tissue[1–3]. Similar substances can be extracted from normal mouse tissue, but they are usually less hæmolytic than are the substances extracted from tumours and lymphomas[3]. The method used for estimating the quantity of the higher fatty acids is based on their fluorescence in benzene when treated with rhodamine *B* and uranyl nitrate in