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Chromatographic Identification of Lysins in Normal Tissues and Tumours of Mice

THE two hæmolytic materials found in the normal tissues of the mouse and man, and also in mouse and human tumours, have been partially identified as being higher fatty acids (or their soaps) and lysolecithin; the former give a characteristic ultra-violet fluorescence, and the latter are soluble in ethanol but not in cold ether[1,2]. This communication concerns their more complete identification by silica-gel thin-layer chromatography.

The separations were carried out on glass plates, 20 × 5 cm, coated with silica-gel according to standard procedure. Small quantities (0·01 ml.) of known solutions of fatty acids or lipids, or of unknown solutions (1 gm tissue in 5 ml. ethanol) of the ethanol soluble material of mouse tissues or tumours, are spread across the lower end of the plate; after drying, the plates are placed vertically in methanol–chloroform, which is allowed to ascend for about 18 cm. The plates are made in duplicate, so that one of each can be developed with iodine vapour to show the position of the unsaturated fatty acids, etc., and these positions are marked on the duplicate plates. Transverse strips, each 5 mm wide, are scraped off the plates at various levels, for example, at regions to which the fatty acids have migrated, and also at regions in which there is no reaction with iodine (blanks). The scraped-off material is received in test tubes, one for each scrape; 1 ml. of redistilled ethanol is added to each, and after 15 min the tubes are centrifuged and the supernatant fluid of each is poured off into porcelain cupels, one for each test-tube. The cupels are dried for 60 min at 60° C; 1 ml. of a suspension of washed red cells (the cells of 50 mm³ of blood to 10 ml. of saline) is added to each, and they are transferred to hæmolysis tubes. These are kept in a water-bath at 37° C, with hourly shaking, and the time for complete hæmolysis is determined for each.

The difficulty lies in avoiding the appearance of irregular lytic regions on the plates. Control plates (blanks) must show no hæmolytic regions, regions which do not stain with iodine should be negative, and many regions which stain with iodine are also not lytic. Avoidance of the appearance of irregular hæmolytic regions requires a standardization technique in which the samples do not come into contact with hands, a cell suspension buffered at *p*H 7, and redistilled ethanol for extraction.

The chromatographic plates usually show 6–8 components which stain with iodine vapour, but the only regions which produce hæmolysis in 10 h or less are: (*a*) a region which corresponds to the position of oleic acid; and (*b*) a region which corresponds to the position of lysolecithin.

Comparing plates of ethanol extracts of normal mouse tissue (liver, lung) with those of ethanol extracts of C_3H mouse tumour in the same concentration (g tissue/g extraction medium), the number and position of the regions which stain with iodine vapour are the same, and the same regions are hæmolytic, but the intensity and size of the regions corresponding to oleic acid and lysolecithin are greater for the extracts of liver and lung than for the extracts of tumours; measurement of fluorescence also shows the concentration of fluorescent material to be two to six times greater in the mouse liver and lung (male or female) than it is in the female breast tumour. The hæmolytic activity of the mouse tumour, however, is usually several times greater than that of normal mouse tissue[3,4]. That the lysin-inhibitor complex[5,6] in normal mouse tissues is more strongly bound than that in tumours is a possibility to be investigated.

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Electrophoretic Patterns of Lysed Fibrin formed by Homologous and Heterologous Thrombin

THROMBIN[1] and fibrinogen[2] have been shown to exhibit species specificity. Recently attention has been directed to the possible removal of intravascular clots by fibrinolytic agents. Since high concentration of thrombin produces lysis of fibrin[3], it was of interest to investigate the effect of homologous and heterologous reagents.

In each of 2 tubes, equal volumes of fibrinogen (1 g/100 ml.) and thrombin (500 units/ml.) were quickly mixed and incubated at 37° C. Lysis was prevented in the control by the immediate addition of equal volume of 10 M urea (dissolves fibrin) in 0·1 M sodium acetate/acetic acid buffer *p*H 4·85, while in the test this reagent was only added after lysis had occurred, usually in 2½–4 h. In order to minimize the precipitation of fibrin and its lysed products, paper electrophoresis was carried out using 0·1 M sodium acetate/acetic acid buffer *p*H 4·85 (no final polymerization of fibrin at *p*H 4). For reasonable separation of components a potential of 260 V was applied for 28 h. After staining with azocarmine *B*, the strips were scanned with a chromoscan.

There was no demonstrable difference between the electrophoretic patterns of lysed and unlysed fibrin in urea on using homologous thrombin and fibrinogen (Table 1). On the other hand, the use of heterologous reagents resulted in the appearance of a new band moving towards the anode. This band was well illustrated on using bovine thrombin and human fibrin (Fig. 1). Increasing the concentration of thrombin did not alter the