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Isolation of histone proteins from rat normal and tumour blood plasma

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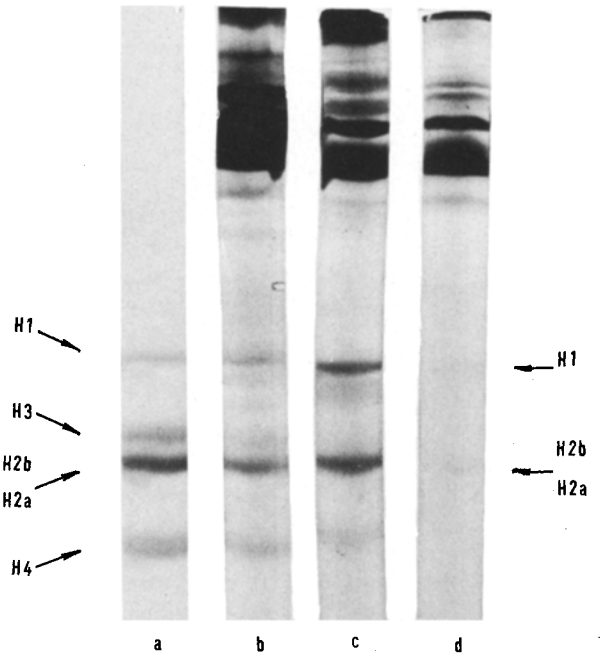
Summary. Histone proteins were isolated from both normal and tumour rat blood plasma: H1, (H2b + H2a) for normal plasma and H1, H3, (H2b + H2a) and H4 for tumour one.

Recently it has been reported that exogenous histones influence cell functions[1,2] and their intracellular structures (lysosimes, mitochondria)[3,4]. Observing the immunological aspects of the inhibitory tumour growth effect of DMS[5], we have established plasma histone-like proteins. The purpose of the present study is isolation and electrophoretic analysis of these histone-like proteins.

Material and methods. Blood plasma from normal Wistar rats and from rats with transplantable 'Joshida' fibrosarcome (diameters of tumour 4–5 cm) was obtained in the usual way by centrifugation at 4 °C. Trichloroacetic acid was then added to a final concentration of 18%. The precipitate was homogenized with 10 vol. of 0.25 M sucrose, 0.1 M tris-HCl pH 7.4 and 0.003 M $CaCl_2$. The homogenate was centrifuged and the sediment was washed twice in the same solution, combining homogenization with centrifugation. The sediment obtained from the last washing was homogenized with 0.25 M H_2SO_4 and was extracted twice more in a similar manner. The combined supernatants were clarified by filtering and histone-like proteins were precipitated by adding 6 vol. ethanol for 18 h at − 10 °C. The precipitate was dissolved in 0.9 N CH_3COOH and 10 M urea. Whole histone was extracted with 0.25 M H_2SO_4 from rat liver chromatin according to Spelsberg and Hnilica[6] and was used as a standard. Acrylamide gel electrophoresis of histones was carried out according to Panyim and Chalkley[7] in 6.25 M urea, 0.9 N acetic acid, pH 3.2. The gels were stained with amido black.



Electrophoretic patterns of histones and cationic plasma proteins. From left to right: a) Histones; b) normal blood plasma + histones; c) tumour blood plasma; d) normal blood plasma.

Results and discussion. Gel electrophoresis of a tumour plasma sample shows 4 cationic protein bands of the same electrophoretic mobility as rat liver chromatin histones: H1, H3, (H2b + H2a) and H4 (figure, c, a). Normal plasma separated into 2 cationic bands corresponding to H1 and (H2b + H2a), which are very faintly visible and difficult to detect compared to the standard histone fractions (figure, d, a). Whole chromatin histone was added to normal plasma immediately before electrophoresis in order to establish if histone mobility is influenced by plasma proteins. In the sample thus obtained, the added whole chromatin histone has the same electrophoretic pattern as that of standard histone and tumour plasma histone (figure, b, a, c). We presume that these blood plasma histone proteins are probably extracted from nucleoproteins present in blood. On the other hand, the former could possibly be histones which have directly entered circulation. In both cases the origin of these histone proteins is most likely related to cell destruction under physiological or pathological conditions. The complicated interaction existing between blood cells and nucleoproteins or histones[1,2] accounts for the difficult isolation of these histone proteins, as well as for the variations in the data we have obtained. What their biological significance is, or if they have only a pathological role in increased cell destruction, is a problem which requires further study.

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