Differences in the Quantitative Distribution of Lysine-Rich Histones in Neoplastic and Normal Tissues[1] (35741)

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The chromatin of eukaryotic cells contains DNA and histone in approximately equal amounts on a weight basis. Evidence has been obtained which suggests that histones function both as structural elements of the chromatin (1–6) and as regulators of genetic expression in higher organisms (7–10). Recent work (11–13) has established a direct correlation between the distribution of lysine-rich histones and phenotypic expression in normal tissues. It was shown that there were qualitative differences among the lysine-rich histones of different species, and quantitative differences between certain of the corresponding components of different tissues of the same species. In the present paper, data are presented which show that neoplastic tissues of the rat and calf contain the same lysine-rich histones as normal tissues, and that there are distinct quantitative differences between certain of the corresponding components of neoplastic and normal tissues.

Materials and Methods. Male, Albino rats (200 to 250 g) bearing the Novikoff ascites hepatoma and bovine lymphosarcoma were the sources of neoplastic tissue for the isolation of lysine-rich histones. Lysine-rich histones were extracted from normal and neoplastic tissues, and the crude lysine-rich histone preparation was fractionated by gradient elution chromatography on Amberlite IRC-50 ion-exchange resin with a shallow gradient of guanidinium chloride as described previously (13). For convenience, the first 600 ml of effluent volume were excluded from the chromatographic profiles presented in the figures and chromatographic components were numbered in an arbitrary fashion for descriptive purposes. Normalization of the chromatographic profiles and quantitative determinations of the chromatographic components and their statistical evaluation were carried out as previously reported (13). Absorbance was determined with a PMQ II Zeiss spectrophotometer.

Results. Representative chromatographic profiles of the whole lysine-rich histone fractions of Novikoff hepatoma and normal rat liver are shown in Fig. 1. A comparison of these profiles showed that each tissue contained the same complement of lysine-rich histones, but some of the components, especially component 3, were present in different amounts in the two tissues. The lysine-rich histone components of Novikoff hepatoma were subjected to polyacrylamide gel electrophoresis and co-chromatography with appropriate subfractions from nontumor tissues of the rat, as reported for normal rat liver, thymus, spleen, and kidney tissues (13). No differences could be detected between the corresponding components of normal and neoplastic tissues by these criteria. Amino acid analysis of the lysine-rich histone components of Novikoff hepatoma further substantiated the idea that there were no differences between the tumor components and those of normal rat tissues (13). In addition, component 3 of Novikoff hepatoma was found to contain a single methionine residue having the same susceptibility to reaction with cyanogen bromide as observed for component 3 of four different, normal rat tissues (13).

The crude lysine-rich histone preparation from bovine lymphosarcoma and bovine spleen were fractionated by ion-exchange chromatography and representative elution profiles are given in Fig. 2. Both tissues con-

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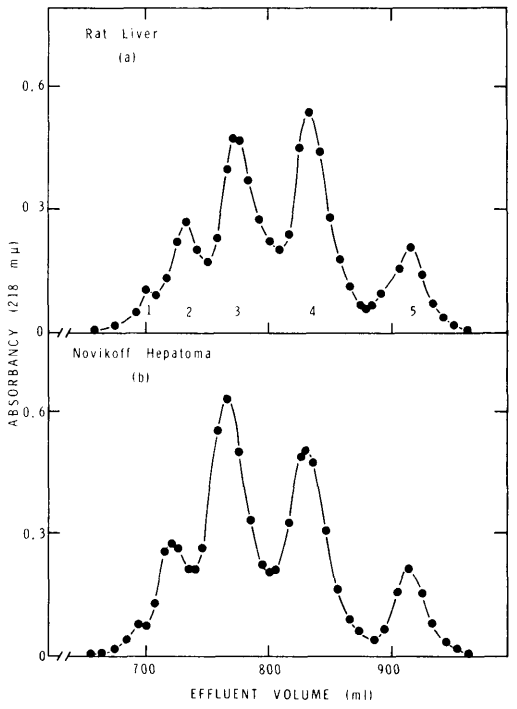

FIG. 1. Chromatographic profiles for the lysine-rich histone fraction of rat liver and Novikoff hepatoma. Chromatography was performed on Amberlite IRC-50 ion-exchange resin with a linear gradient of guanidinium chloride (7 to 14%) containing 0.1 *M* sodium phosphate buffer, pH 6.8 (total volume of 1700 ml). The column (2.3 × 26 cm) was eluted at a flow rate of 12 ml/hr and 4 ml fractions were collected. (a) rat liver, 28 mg; (b) Novikoff hepatoma, 26 mg. The elution profiles were normalized at the peak absorbance value of component 5 of rat liver.

tained the same complement of lysine-rich histones but quantitative differences between certain of the corresponding components, particularly components 1 and 2, were evident. Furthermore, polyacrylamide gel electrophoresis, co-chromatography and amino acid analysis studies similar to those described for bovine spleen, thymus, and liver (13) showed that by these criteria the chromatographic components of the tumor tissue were identical to the corresponding components from normal bovine tissues.

The quantitative differences observed in the tissue distribution of the lysine-rich histone components of Novikoff hepatoma and bovine lymphosarcoma, and a nontumor tissue of each species are summarized in Table I. Component 1 of bovine spleen was significantly increased in amount (25%) compared to component 1 of bovine lymphosarcoma, and component 2 of bovine lymphosarcoma was increased approximately twofold compared to component 2 of the spleen. In addition, the amount of component 3 of Novikoff hepatoma was significantly increased (28%) compared to component 3 of normal rat liver.

Discussion. In the neoplastic state, certain aspects of the functional control of gene expression must be abnormal. Given the postulated role of histones in the control of gene transcription (7–10), the finding in this work that there is a different quantitative distribution of lysine-rich histones in neoplastic tissues compared to normal tissues is correlated with the different phenotype of these tissues. In fact, this correlation would seem stronger

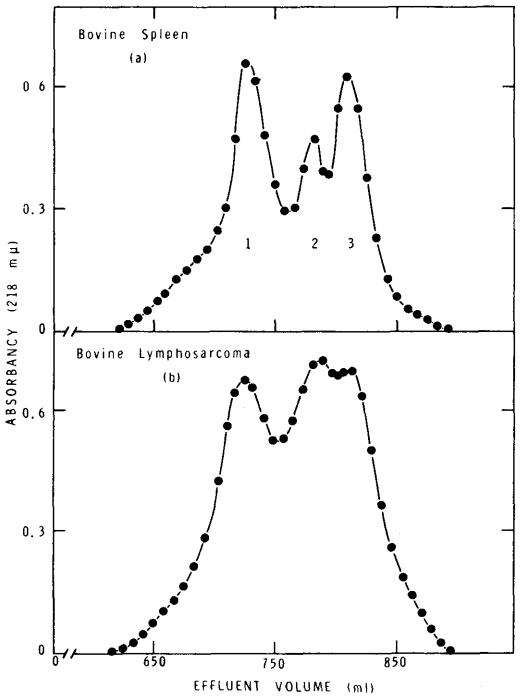

FIG. 2. Chromatographic profiles (Amberlite IRC-50) for the lysine-rich histone fraction of bovine spleen and bovine lymphosarcoma. The conditions were the same as described in the legend to Fig. 1: (a) bovine spleen, 20 mg; (b) bovine lymphosarcoma, 24 mg. The elution profiles were normalized at the peak absorbance value of component 1 of bovine spleen.

TABLE I. Distribution of Chromatographic Components of Lysine-Rich Histone Fractions of Normal and Neoplastic Tissues.

Tissue	Chromatographic components[a]				
	1	2	3	4	5
Bovine spleen[b]	49 ± 2	18 ± 2	33 ± 3		
lymphosarcoma	39 ± 2	32 ± 4	29 ± 3		
Rat liver[b]	4 ± 0.5	14 ± 2	32 ± 2	36 ± 2	16 ± 1
Novikoff hepatoma	2 ± 0.9	11 ± 2	41 ± 1	35 ± 2	11 ± 2

[a] Values reported as percentage of total lysine-rich histone fraction. Each value is the mean ± standard error of three different preparations ($N = 3$) each of which was chromatographed two times (see Figs. 1 and 2).

[b] Similarly numbered components of different species do not necessarily correspond to the same histone.

in view of the observation (11–13) that there are quantitative differences in the distribution of lysine-rich histones even among different normal tissues of the same species.

Other observations lend support to the idea that different concentrations of lysine-rich histones are a reflection of the involvement of these proteins in the control of different functional cell states. Fambrough *et al.* (14) have shown an increase in the relative proportion of the lysine-rich histone class of pea cotyledons during germination, and recently Stellwagen and Cole (15) showed that there were small but significant changes in the relative amounts of lysine-rich histone components of rabbit mammary gland as the time progressed from pregnancy to lactation. In addition, using an organ culture system of rabbit mammary gland, Hohmann and Cole (16) demonstrated specific hormonal effects on the incorporation of radioactive lysine into specific lysine-rich histone components.

Lysine-rich histones can induce marked conformational changes in the DNA of nucleohistone preparations (5). The presence of different relative amounts of lysine-rich histones might reflect different transcriptional states of the chromatin from different tissues. On the other hand, there is support for the idea that particularly the lysine-rich histones are important in the maintenance of the structural integrity of interphase chromatin (2) as well as metaphase chromosomes (3).

It should be mentioned that changes in the relative amounts of various histone components may not necessarily reflect fundamental changes in the population of a given histone fraction. Thus, specific side-chain modifications such as acetylation (17), methylation (18), oxidation–reduction (19) and phosphorylation (20), might be expected to alter the distribution of histone molecules characteristic of a given cell state. The elucidation of the significance of different relative amounts of lysine-rich histone components in normal and neoplastic tissues must await the findings of further investigations.

Summary. The data obtained in the present study show that neoplastic tissues of the rat and calf contain the normal complement of lysine-rich histones, and that there are distinct quantitative differences between certain of the corresponding components of neoplastic and normal tissues. These results are discussed in relation to the postulated roles of histones as regulatory and/or structural elements of the genetic apparatus in higher organisms.

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