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The Presence of Histone H1° in Human Tissues[1]

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SUMMARY

Histone H1° was found to be present in a number of human tissues. It constituted 50% of the total 5% perchloric acid-soluble histone in human breast, 35% in thyroid, 12 to 18% in adrenal, and 7% in parathyroid tissues. The quantities of histone H1° in these human tissues were large compared with the amounts found in rat liver (8%), calf thymus (0%), and HeLa cells (0%).

Although the quantity of histone H1° was found to vary from one type of tissue to another, it was essentially constant in normal, hyperplastic, and neoplastic human thyroid tissues.

INTRODUCTION

Because of the problems involved in obtaining human tissues, the histones of human cells have not been as extensively studied as those of other organisms. Previous work (5, 7, 10) indicates that human tissues contain the 5 major histone fractions[3] found in other mammalian species. The presence of a 6th-histone fraction, designated H1°, has not previously been reported in humans. Histone H1° has a high content of lysine and, along with the major lysine-rich histone H1, is soluble in 5% perchloric acid (14). This protein was originally reported to be present in nonreplicating tissues of the calf, mouse, and rat and to be absent in actively dividing cells (14). This inverse relationship to cellular proliferation is supported by studies of rat liver and pancreas; this protein is present therein under normal conditions but decreases in quantity during regeneration when DNA is being synthesized (2, 11). Recently, it was reported (8) that histone H1° was present in a series of rat hepatomas, with its concentration inversely related to the growth rate of the tumor (8).

We have been studying the histones isolated from human adrenal, thyroid, parathyroid, and breast tissues obtained by surgical removal from patients suffering from a variety of endocrine and other medical disorders and we have found that they contain large quantities of H1°, compared with the amounts found in calf thymus, rat liver, and HeLa cells. The histones of a series of normal, hyperplastic, and neoplastic human thyroids have also been studied in order to determine whether the quantity of histone H1° is lower than normal in disease states involving abnormal proliferation of thyroid cells.

MATERIALS AND METHODS

Tissues. Tissues were obtained from 13 different patients (Table 1). Thyroid tissues included 1 nontoxic adenoma, 3 diffuse hyperplastic glands (Graves' disease), and 2 carcinomas. Normal thyroid tissue was also obtained from 2 patients.

An adrenal adenoma was obtained from a patient with Cushing's syndrome, and the contralateral suppressed adrenal tissue from this patient was also studied. Normal adrenal tissue was obtained from another patient, and benign adenomas of both breast and parathyroid glands were also analyzed.

HeLa cells were kindly supplied by Dr. T. W. Borun of Fels Research Institute, Temple University School of Medicine.

Isolation of Nuclei. The tissues were placed in ice immediately after removal from the patients. A pathologist examined the tissue and removed a sample for microscopic examination. Within 15 min, the process of isolating nuclei was under way. All steps were carried out at 4°. The tissues were minced with scissors and homogenized in 0.25 M sucrose containing 0.003 M $CaCl_2$ in a Sorvall Omnimixer. In order to avoid damaging the nuclei, the lowest possible speed of the Omnimixer was used, and the homogenate was examined frequently with a phase contrast microscope. Nuclei were isolated from the homogenate by filtration and centrifugation, according to the procedure of Allfrey *et al.* (1). Further purification was achieved by centrifuging the nuclei through 2.4 M sucrose, as described by Chaveau *et al* (4). The nuclear pellets were quickly frozen with a Dry Ice-acetone mixture and stored at −40°.

Isolation of Histones. All steps were carried out at 4°. The nuclei were washed 3 times with a solution containing 80 mM NaCl, 20 mM EDTA, 1% Triton X-100, and 0.05 M sodium bisulfite (pH 7.2). They were pelleted by centrifugation at $600 \times g$ for 10 min and then were washed 3 times with 0.15 M NaCl plus 0.05 M sodium bisulfite. Histones were extracted from the pelleted chromatin with 0.25 N

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[3] The terminology for histones used in this paper was employed at the 1974 Gordon Conference. Histone H1 is the same as Fl, according to the nomeclature of Johns (6) and of Phillips and Johns (15); H3 is F3; H2B is F2b; H2A is F2a2; and H4 is F2al.

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H_2SO_4 plus 0.05 M sodium bisulfite and dialyzed against 0.9 N acetic acid.

Determination of Protein. Protein was determined by the method of Lowry *et al.* (9), using calf thymus histone as a standard.

Gel Electrophoresis. Gel electrophoresis was performed according to the method of Panyim and Chalkley (13), utilizing 15% polyacrylamide gels, 2 M urea, and 0.9 N acetic acid. Approximately 60 μg of histone were applied to gels 25 cm long, and electrophoresis was performed for 26 hr at 190 V. The gels were stained for 18 hr in 0.2% Amido black dissolved in 70% ethanol in 0.9 N acetic acid. Destaining was performed electrically. The absorbance of the stained histone bands was determined with a Gilford spectrophotometer equipped with a gel scanner. The quantity of protein in each band was determined by tracing the bands on uniform-weight paper and cutting out and weighing the area under each curve.

Perchloric Acid Extraction. An aliquot of the histone solution was made 5% with perchloric acid, chilled in ice for 30 min, and centrifuged at 600 × *g* for 15 min. The supernatant solution was dialyzed against 0.9 N acetic acid and subjected to electrophoresis, as described above.

RESULTS

Gel Electrophoresis of Histones Isolated from Human Tissues. When histones isolated from human tissues were subjected to electrophoresis on polyacrylamide gels, 6 major fractions were observed (Charts 1 and 2). Five of these fractions correspond to those usually found in mammalian tissues: H1, H3, H2B, H2A, and H4 (in order of increasing mobility). A minor subband can be seen in the H4 region which corresponds to acetylated H4 (12). In the H3 region, subbands were also found; these correspond to previously reported histone H3 species (12).

In addition to these 5 major histone fractions, a 6th major fraction appeared in the human tissues (Charts 1 and 2). This 6th protein, which migrated to a position between the H1 and H3 histones, was electrophoretically distinct from the other 5 fractions and corresponds in electrophoretic mobility to H1°. All the human tissues examined contained a substantial amount of this histone (Table 1). However, it was not present in cultured HeLa cells or in calf thymus, and it appeared in comparatively small quantities in rat liver.

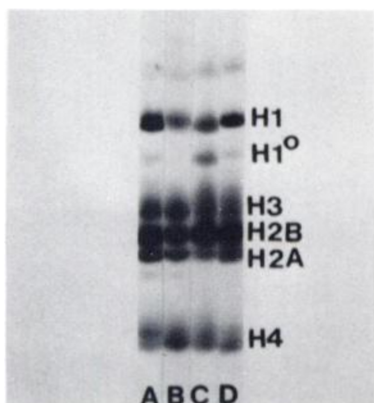

Chart 1. Separation of histones by polyacrylamide gel electrophoresis. The gels that contained 15% acrylamide, 2 M urea, and 0.9 N acetic acid were run for 26 hr at 190 V and stained with Amido black. Only the 10-cm portion of the gel containing the histone bands is shown. *A*, rat liver; *B*, HeLa cells; *C*, human thyroid; and *D*, human parathyroid.

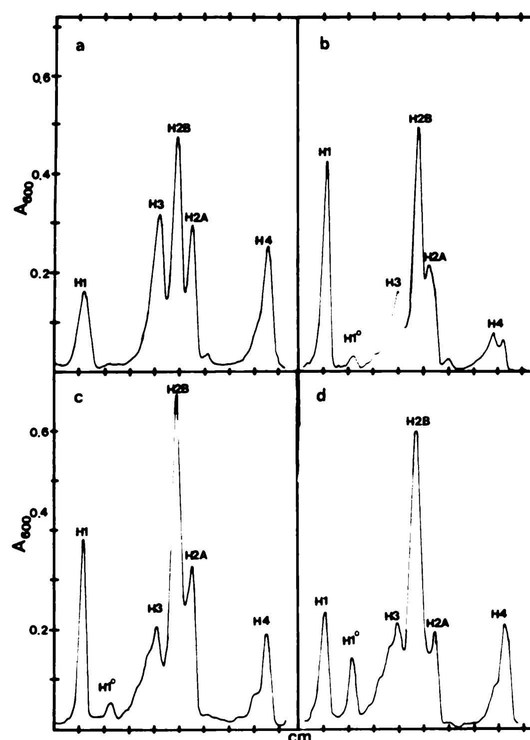

Chart 2. Densitometric tracings of gels shown in Chart 1. *a*, HeLa cells; *b*, rat liver; *c*, human parathyroid; and *d*, human thyroid.

Since 5% perchloric acid selectively extracts histones H1 and H1°, the human histones were treated with this acid. The results of gel electrophoresis of the perchloric acid extract are shown in Charts 3 and 4. Both this 6th human fraction and the major lysine-rich histone (H1) were soluble in 5% perchloric acid. Therefore, because of its electrophoretic mobility and its perchloric acid solubility, we tentatively concluded that this 6th human histone fraction is H1°.

Comparison with Thyroglobulin. Since we first observed this 6th histone fraction in thyroid tissue, we were concerned that it might be a nonhistone contaminant, possibly thyroglobulin. However, when thyroglobulin was subjected to electrophoresis, the only band observed (even when 200 μg were applied to the gel) ran a short distance from the origin, 10 cm behind the histone region.

Comparison with Blood Proteins. We subsequently found that other human tissues (adrenal, parathyroid, and breast) also contained a protein electrophoretically similar to H1°. Since we were not able to perfuse these tissues with 0.9% NaCl solution, we examined 0.25 N H_2SO_4 extracts of human blood in order to determine whether this protein might be a contaminant from blood. We found that human

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Table 1

The amount of H1° calculated as percentage of the total 5% perchloric acid-soluble histone (H1 + H1°)

Histones were isolated from each tissue, subjected to electrophoresis, and the amount of protein in each fraction was determined by weighing the area under the appropriate curve of a densitometric tracing of the stained (Amido black) gel.

Source of histones	Tissue diagnosis	Amount of H1° as % of total 5% perchloric acid-soluble histone
E. D.	Breast adenoma	50
J. S.	Normal thyroid	25
J. R.	Normal thyroid	38
M. O.	Thyroid adenoma	33
S. G.	Hyperplastic thyroid	25
J. B.	Hyperplastic thyroid	33
S. M.	Hyperplastic thyroid	41
J. D.	Papillary thyroid carcinoma	36
F. M.	Papillary thyroid carcinoma	37
R. E.	Normal adrenal	18
G. S.	Suppressed adrenal	16
G. S.	Adrenal adenoma	12
R. L.	Parathyroid adenoma	7
G. T.	Parathyroid adenoma	7
HeLa	Cervical carcinoma (culture)	0
Calf	Normal thymus	0
Rat	Normal liver	8

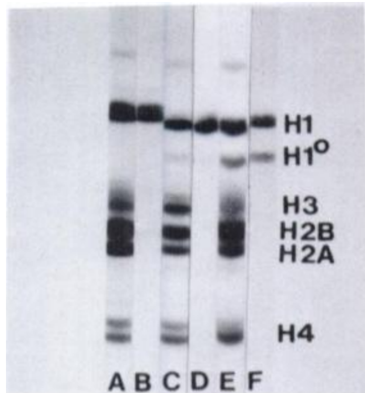

Chart 3. Electrophoretic patterns of whole histones and 5% perchloric acid-soluble histones. *A*, calf thymus; *B*, perchloric acid-soluble calf thymus; *C*, rat liver; *D*, perchloric-acid soluble rat liver; *E*, human thyroid; and *F*, perchloric acid-soluble human thyroid.

plasma contained no proteins that band in the histone region. However, 2 bands were obtained from human red blood cells, which appeared just behind and in front of the H1° fraction. The major band moved more slowly than H1, but the minor band appeared in the same position as H1°. These red blood cell fractions were identical in position to bands obtained from purified bovine globin. However, they were not soluble in 5% perchloric acid as were the H1 and H1° fractions. Furthermore, our method of isolating and purifying nuclei eliminated all traces of red blood cells as determined by phase contrast microscopy.

Quantitation of Histone H1°. The amount of H1° in various human tissues was calculated as the percentage of the total 5% perchloric acid-soluble fraction (H1 + H1°). The largest amount of H1° (50%) was found in a human breast adenoma. In human thyroid tissues, H1° constituted one-third of the total perchloric acid-soluble fraction. In the adrenal, it was 12 to 18% and in parathyroid tissue, only 7% (Table 1).

DISCUSSION

A quantitative study of the amount of histone H1° in various tissues has not previously been reported. Panyim and Chalkley (14), who examined a series of calf, mouse, and rat tissues, gave a quantitative value only for calf lung. Other laboratories have focused on 1 or 2 tissues (11, 16) or have not presented quantitative data (3, 8). Because the techniques used vary, particularly the staining procedures, it is difficult to compare the reports from these laboratories on the quantity of histone H1° in different tissues.

We have examined a number of tissues utilizing a standard staining technique and have found that the amount of histone H1° varies from one type of tissue to another (Table 1), ranging from cells in which it is completely absent (calf thymus and HeLa cells) to those in which it constitutes 50% of the total perchloric acid-soluble histone (human breast adenoma).

The function of histone H1° has not been definitely established. Panyim and Chalkley (14) originally suggested

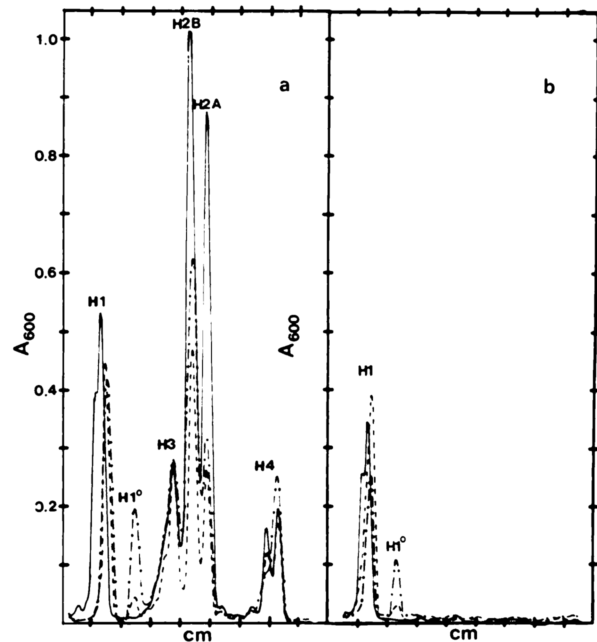

Chart 4. Densitometric tracings of gels shown in Chart 3. *a*, whole histones; *b*, 5% perchloric acid-soluble histones; ——, calf thymus; - - - -, rat liver; and – - – -, human thyroid.

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that it is not present in replicating cells. Our observation that actively proliferating HeLa cells do not contain H1° supports this conclusion. However, in recent studies, H1° has been found in tissues which *are* replicating. Lea *et al.* (8) have observed H1° in 3 different rat hepatomas, Marsh and Fitzgerald (11) have found it in regenerating rat pancreas, and we have observed it in hyperplastic and neoplastic human thyroid tissues.

Marsh and Fitzgerald (11) have suggested that H1° is an inhibitor of DNA synthesis. If this is the case, then the quantity of H1° relative to total histone should be inversely related to the number of cells in the tissue that are in the process of replicating. Such a relationship to replication has been observed in rat hepatomas, in which the quantity of H1° is inversely related to their growth rate (8), and in regenerating pancreas wherein the quantity of H1° is lower than in normal pancreas (11). The thyroid series we have studied fits this pattern. These tissues are growing abnormally, but at a very slow rate. At any given time, the number of cells that are replicating is very small and the quantity of histone H1° in the tissue is essentially the same as in normal thyroid tissue.

Although the accumulated evidence is consistent with an inverse relationship of H1° to cell division, the variation in the amount of this protein in different tissues remains to be explained. Why, in tissues wherein replication is occurring either not at all or to only a limited extent, does the quantity of H1° vary from 7 to 50% of the perchloric acid-soluble histone? Perhaps the function of this protein is more complex than the initial studies indicate. Further studies of its cellular role are warranted.

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