Human Leukaemic Cells

DETERMINATION OF TRACE ELEMENTS IN NUCLEIC ACIDS AND HISTONES BY NEUTRON-ACTIVATION ANALYSES

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Trace metals were measured by neutron-activation analyses in purified nucleic acids and histone(s) of lymphocytes from patients with acute lymphocytic leukaemia or infectious mononucleosis, and from normal donors. DNA isolated from lymphocytes of a patient with infectious mononucleosis and a normal donor showed a high content of Cr^{2+} , Sb^{2+} , Fe^{2+} , and Zn^{2+} , whereas DNA of lymphoblasts from a patient with acute lymphocytic leukaemia had a lower content of these trace metals, but the Co^{2+} content was 20-fold higher than in DNA of normal donor lymphocytic cells. Total histones from leukaemic cells had higher contents of most of the trace metals except for Zn^{2+} , which was present in lesser concentration than in histones from normal donor lymphocytic cells. Lysine-rich (F1) histones showed lower contents of Cr^{2+} , Sb^{2+} and Co^{2+} , whereas arginine-rich (F3) histones had significantly higher contents of these trace metals. These observations may be of interest in that F3 histones more effectively inhibit RNA synthesis in human lymphocytic cells than do other species of histones.

The presence of trace metals in eukaryotic cells has been known for some time (Vallee, 1955), and there is some evidence that they may influence regulatory mechanisms (Chesters, 1972; Williams & Loeb, 1973). Their presence in DNA (Andronikashvili *et al.*, 1970; Fiske & SubbaRow, 1925; Huff *et al.*, 1964; Wacker *et al.*, 1959; Wacker & Vallee, 1959; Weser *et al.*, 1969) and RNA of several species of cells (Andronikashvili *et al.*, 1970, 1972; Rubin, 1972; Wacker *et al.*, 1959; Wacker & Vallee, 1959; Williams & Loeb, 1973) has been documented. Zn^{2+} appears to be required for the induction and continuation of DNA synthesis (Lieberman *et al.*, 1963; Rubin, 1972; Springgate *et al.*, 1973; Weser *et al.*, 1969; Williams & Loeb, 1973).

The trace-metals content of nucleic acids from tumour tissue (Sarcoma M-1 and Walker 256 carcinoma) has been reported (Andronikashvili *et al.*, 1970, 1972, 1974) as have those thought to be required for the growth of tumours (Addink & Frank, 1959; De Wys *et al.*, 1970; McQuitty *et al.*, 1970; Petering *et al.*, 1967; Rubin, 1972). Thus the trace-metal con-

tent of subcellular components isolated from human lymphocytic cells of non-malignant and malignant origins may be of some interest. The present paper describes the quantitative determination, by neutron-activation analyses, of trace metals in nucleic acids and histone(s) of human lymphocytes isolated in continuous culture from patients with acute lymphocytic leukaemic or infectious mononucleosis, and normal donors.

Materials and Methods

Cells

The origins and isolation *in vitro* of these human lymphocytic cells (Table 1) have been described (Foley *et al.*, 1965, 1968). All cells were cultivated in large-volume suspension cultures in Eagle's minimal essential medium modified for spinner culture (Eagle, 1959) and supplemented with 10% (w/v) whole foetal calf serum (Microbiological Associates, Bethesda, MD, U.S.A.) Cultures were maintained in continuous exponential growth at population densities of 2×10^6 and 4×10^6 cells/ml, with a generation time of approx. 24 h, by successive withdrawal and replacement of substrate (Foley *et al.*, 1965, 1968).

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Isolation and purification of DNA and RNA

The cells were homogenized with a loose-fitting Teflon pestle for 1 min in 0.01 M-sodium EDTA/0.01 M-sodium acetate buffer, (pH 5.1), containing 0.5% sodium dodecyl sulphate. An equal volume of 88% (v/v) liquid phenol (Schwartz/Mann, Orangeburg, NY, U.S.A.) saturated with the same buffer was then added. The suspension was incubated and shaken in a water bath at 60°C for 10 min. After cooling in an ice bath, and centrifugation in a Sorvall centrifuge at 2000g for 10 min, the aqueous layer was separated and extracted twice with the phenol solution for 5 min. The aqueous phase was collected and dialysed against water overnight, and the RNA precipitated with 2–2.5 vol. of ethanol containing 2% (w/v) KCl and 0.1 vol. of 2 M-NaCl (Wulff *et al.*, 1975). DNA was isolated at room temperature as described previously (Desai *et al.*, 1971). The cell pellet was washed thoroughly with 0.14 M-NaCl solution and was treated with 45% (v/v) ethanol containing 5% sodium dodecyl sulphate. NaCl crystals were added to bring the solution to 1 M. After gently stirring for 30 minutes the solution was centrifuged to eliminate the sodium dodecyl sulphate and proteins. The solution containing DNA strands was further treated with RNAase (ribonuclease) and Pronase (Worthington Biochemicals Corp., Freehold, NJ, U.S.A.) to eliminate any RNA and protein contaminants. This procedure was repeated several times until the final DNA purification was achieved (Desai *et al.*, 1971).

Isolation and purification of histones

The nuclei isolated from cell pellet were further washed several times with cold 88% (v/v) ethanol containing 0.01 M-HCl (Fisher Chemical Co., Silver Springs, MD, U.S.A.). The nuclei were re-sedimented by centrifugation, and extracted with 0.2 M-HCl for 30 min. The histones were extracted and purified as described previously (Desai & Foley, 1970a,b; Desai *et al.*, 1973).

Precautions were taken to ensure that reagents and glassware used in these procedures were free of metals (Auld *et al.*, 1974).

Neutron-activation analyses

As described elsewhere (Andronikashvili *et al.*, 1970, 1972, 1974), individual freeze-dried samples of nucleic acids or histones were sealed in quartz ampoules and irradiated in the central channel of the IRT-2000 reactor, in the neutron integral flux in the order of 10^{19} neutrons/cm². Control samples of each trace metal were included with each preparation. After irradiation for 21–28 days, gamma-ray spectra were measured in a high-resolution germanium/lithium detector, as described previously (Andronikashvili *et al.*, 1974). A typical gamma-ray spectrum

of a sample of DNA so obtained has been published (Andronikashvili *et al.*, 1974). The gamma-ray spectrum of a histone species is illustrated in Fig. 1.

Results

Trace metals in nucleic acids

DNA of leukaemic (CCRF-CEM) cells contained less Cr²⁺, Fe²⁺ and Zn²⁺ than did that of lymphocytes from a patient with infectious mononucleosis (CCRF-SB) or a normal donor (AMC-30). DNA of normal lymphocytic cells (AMC-30) contained four times as much Zn²⁺ as did DNA of leukaemic (CCRF-CEM) cells (Table 1), whereas the Co²⁺ content of DNA from leukaemic cells was more than 20 times that of DNA from a patient with infectious mononucleosis or normal donor cells. Similarly, RNA of leukaemic (CCRF-CEM) cells showed a higher content of Co²⁺, Sb²⁺, Fe²⁺ and Zn²⁺, than did that of CCRF-SB cells (Table 1).

Trace metals in total histone and histone species

Total histones of CCRF-CEM and CCRF-HSB-9 cells contained higher concentrations of Cr²⁺, Sb²⁺, Fe²⁺ and Co²⁺ than did AMC-30 cells, whereas the total histones of CCRF-CEM cells had the lowest Zn²⁺ content (Table 2). Analyses of individual species of histones revealed striking differences in lysine-rich (F1) and arginine-rich (F3) histones with respect to Zn²⁺, Fe²⁺, Cr²⁺ and Co²⁺ content. The highest concentrations of Zn²⁺ and Fe²⁺ were in the F3 histone CCRF-CEM, CCRF-HSB-9 cells as compared with F1 histones (Table 2). F2a and F2b histone species exhibited marked variations in Zn²⁺, Fe²⁺, Cr²⁺ and Co²⁺ content (Table 2).

Discussion

With the exception of Zn²⁺ (Vallee, 1955), the role(s) of trace metals remain(s) largely undefined, but some may relate to transcription mechanisms, as suggested by the relationship of Zn²⁺ to the synthesis of nucleic acids (Chesters, 1972; Fujioka & Lieberman, 1964; Lieberman *et al.*, 1963; Rubin, 1972; Vallee, 1955; Wacker *et al.*, 1959; Wacker & Vallee, 1959; Williams & Loeb, 1973), its role in tumour DNA and RNA re-duplication (Andronikashvili, 1972), and its presence in DNA polymerase (Auld *et al.*, 1974; Livingston *et al.*, 1973; Slater *et al.*, 1971; Springgate *et al.*, 1973) and RNA polymerase (Scrutton *et al.*, 1971). Thus although these preliminary studies with only a few lines of human lymphocytic cells (all of which were maintained in the same culture substrate) are in themselves inconclusive, certain of the observed differences in trace-metal con-

Fig. 1. Gamma-ray spectrum of F2a histone (isolated from CCRF-CEM cells, cf. Tables 1 and 2) obtained on high-resolution Ge(Li) detector (vol. 55 cm³)

The clearly resolved lines of many radioisotopes can be used for the quantification of the different trace metals (Andronikashvili *et al.*, 1974).

Table 1. *Trace-metals content of nucleic acids isolated from some human lymphocytic cells*

CCRF-CEM cells are from a patient with acute lymphoblastic leukaemia (Foley *et al.*, 1965). CCRF-SB cells are from a patient with classical infectious mononucleosis superimposed on pre-existing acute lymphoblastic leukaemia. SB cells were isolated directly in culture (Foley *et al.*, 1968); HSB-9 cells were isolated from the same peripheral blood specimen by direct implantation in neonatal Syrian hamsters (Adams *et al.*, 1968; Foley *et al.*, 1968). SB and HSB cell lines exhibit distinctly different biological properties (Adams *et al.*, 1968, 1970); SB is non-tumourigenic in untreated neonatal Syrian hamsters and resembles cell lines derived from other patients with infectious mononucleosis (Adams *et al.*, 1970), whereas HSB is tumourigenic in untreated neonatal Syrian hamsters (Adams *et al.*, 1970) and resembles CEM cells (Adams *et al.*, 1968). We thank Dr. M. Benyesh-Melnick, Baylor University College of Medicine, Houston, TX, U.S.A. for providing the culture of AMC-30 cells which was derived from the bone marrow of a normal pediatric patient.

Source of nucleic acid	Content (mg/g of nucleic acid)				
	$10^6 \times [\text{Cr}^{2+}]$	$10^7 \times [\text{Sb}^{2+}]$	$10^6 \times [\text{Fe}^{2+}]$	$10^6 \times [\text{Zn}^{2+}]$	$10^8 \times [\text{Co}^{2+}]$
DNA from:					
CCRF-CEM cells	2.7 ± 0.1	0.46 ± 0.6	18.5 ± 2.1	10.2 ± 0.1	2.40 ± 0.04
CCRF-SB cells	6.1 ± 0.6	20.1 ± 1.0	81.9 ± 12.7	14.6 ± 0.7	7.2 ± 1.3
AMC-30 cells	4.0 ± 0.5	22.0 ± 1.2	36.6 ± 5.1	46.9 ± 2.9	9.9 ± 1.4
RNA from:					
CCRF-CEM cells	6.1 ± 0.8	20.0 ± 1.7	46.2 ± 7.5	27.5 ± 3.1	17.0 ± 3.0
CCRF-SB cells	2.0 ± 0.3	9.9 ± 0.8	28.0 ± 4.9	7.3 ± 0.8	8.1 ± 1.2

Table 2. *Trace-metal content of histones isolated from some human lymphocytic cells*

For details of cells, see Table 1.

Histones	Content (mg/g of histone)				
	$10^6 \times [\text{Cr}^{2+}]$	$10^7 \times [\text{Sb}^{2+}]$	$10^6 \times [\text{Fe}^{2+}]$	$10^6 \times [\text{Zn}^{2+}]$	$10^8 \times [\text{Co}^{2+}]$
CCRF-CEM cells					
Total	19.0 ± 1.5	53.4 ± 4.0	203.0 ± 1.4	12.5 ± 1.4	179.0 ± 14.0
F1	8.4 ± 1.0	6.9 ± 0.8	56.0 ± 9.0	5.3 ± 0.8	30.6 ± 4.3
F3	28.1 ± 2.0	26.6 ± 1.9	274.0 ± 25.0	62.6 ± 4.3	52.3 ± 4.7
F2a	113.0 ± 15.0	35.2 ± 4.6	412.0 ± 62.0	61.3 ± 8.6	450.0 ± 63.0
F2b	30.4 ± 7.9	41.1 ± 10.7	302.0 ± 91.0	189.0 ± 49.0	456.0 ± 123.0
CCRF-HSB-9 cells					
Total	22.8 ± 3.7	24.4 ± 3.7	276.0 ± 50.0	63.8 ± 9.4	285.0 ± 45.0
F1	11.9 ± 1.2	559.0 ± 56.0	302.0 ± 36.0	58.8 ± 6.4	55.0 ± 7.0
F3	10.7 ± 1.5	527.0 ± 68.0	1164.0 ± 163.0	128.0 ± 17.0	67.0 ± 10.0
F2a	4.3 ± 0.4	10.8 ± 0.9	68.9 ± 8.3	50.5 ± 3.2	66.0 ± 7.0
F2b	25.0 ± 2.0	11.7 ± 0.9	176.0 ± 18.0	36.1 ± 3.2	131.0 ± 12.0
AMC-30					
Total	6.2 ± 0.4	7.0 ± 0.4	195.0 ± 16.0	44.4 ± 2.7	38.8 ± 3.1

tent warrant more extensive investigation, for example, the relatively low Zn^{2+} and the high Co^{2+} content of the DNA, RNA and histones of leukaemic (CCRF-CEM) lymphoblasts.

It has been suggested (Fujioka & Lieberman, 1964; Lieberman *et al.*, 1963; Vallee, 1955; Wacker *et al.*, 1959) that the phosphate groups of nucleic acids would be the most likely sites for interactions with trace metals. Although there were striking similarities in content and composition with respect to the major nucleotides of DNA and RNA isolated from the cells considered herein (Desai *et al.*, 1973; Desai & Foley, 1970a), there were significant differences in the con-

tent of minor and methylated bases in RNA isolated from these cells (Wulff *et al.*, 1972, 1975), which may relate to these differences in trace-metals content.

The trace-metal content of the total histone and histone and histone species derived from these cells affords further interesting speculation that trace metals may be involved in the interactions between histones and DNA (especially at the binding sites of histones to DNA), which affect transcription characteristics. Numerous reports indicate that metals bind to nucleic acids (Fujioka & Lieberman, 1964; Lieberman *et al.*, 1963; Rubin, 1972; Scrutton *et al.*, 1971; Springgate *et al.*, 1973; Vallee, 1955; Wacker

et al., 1959; Wacker & Vallee, 1959; Williams & Loeb, 1973), and the observations presented here clearly demonstrate for the first time the presence of trace metals in nuclear proteins. Other studies (Desai *et al.*, 1973; Desai & Foley, 1971) indicate that histones more effectively inhibit or repress RNA synthesis in isologous* as compared with homologous† recipient cells, a differential inhibitory activity which may reflect differences in the affinities between histones and the DNA of recipient cells. The presence of different amounts of trace metals in total histones of different cells may relate to differences in the inhibitory effects of isologous and homologous total histones on RNA synthesis (Desai & Foley, 1971).

The high concentrations of Cr^{2+} , Fe^{2+} and Co^{2+} in histones F3 may further support observations (Desai *et al.*, 1973; Desai & Foley, 1971) that arginine-rich F3 histones inhibit or repress RNA synthesis in human lymphocytic cells, perhaps by binding to DNA sites which serve as primers for RNA synthesis. The lysine-rich F1 histones have a high content of Zn^{2+} and lower contents of Fe^{2+} and Cr^{2+} , and are of particular interest, since these histone species are less inhibitory for RNA synthesis. Distinct differences in Zn^{2+} , Fe^{2+} , Cr^{2+} and Co^{2+} contents which were found among intermediate species of histones F2a, F2b similarly may relate to the binding of these histones to DNA.

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