Acid Soluble Components in RNA Prepared from Human Leukemic White Cells. (27979)

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(Introduced by Nathaniel I. Berlin)
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This work was undertaken following reports(1,2) that the RNA from frog ova prepared by a phenol method contained unusual acid soluble components releasing 2′ 3′ nucleotides following alkaline digestion. It was natural to suspect, therefore, that the RNA from other immature cells, such as leukemic white cells, might have similar components. Indeed such has proven to be the case. However, even though the phenol method has become a standard means of preparing biologically active RNA(3,4), the acid soluble components in this instance seem to represent materials released during the process of preparation rather than any unusual RNA's intrinsic to the leukemic cells.

Materials and methods. White cells were obtained in large amounts, mainly by the technic of plasmapheresis(5) and were separated from the red cells by either centrifugation for 20 minutes at 50 × *g* or dextran sedimentation at 4°C. All subsequent operations were performed at 4°C unless otherwise specified. The supernate was centrifuged at 1500 × *g* for 10 minutes. The cellular pellet was resuspended in 2-3 volumes of 0.05 M sodium phosphate buffer pH 6 containing 0.5% 1, 5-naphthalene-5-disulphonate(6) and homogenized in a Servall omni-mixer or Waring blendor for 30 seconds. The homogenate was then immediately mixed with an equal volume of redistilled 90% phenol and mechanically stirred for 20 minutes. Following centrifugation at 3500 × *g* for 20 minutes, the aqueous phase was removed and the residue washed once with distilled water. The combined aqueous phases were made 2% in potassium acetate and 2 volumes of 95% ethanol at −20°C added. The preparation was kept at −20°C for 18 hours.

The precipitate was collected by centrifugation, dissolved in a minimal amount of distilled water, and dialyzed for 6-8 hours against 3 changes of 50 volumes each of distilled water. The dialyzed solution was promptly freeze-dried.

Prior to analysis the lyophilized material was resuspended in 12.5 ml of distilled water and clarified by centrifugation at 100,000 × *g* for one hour. One-tenth volume of 5N perchloric acid was added and the acid soluble fraction removed by centrifugation. Following reneutralization and the removal of salt, this fraction was digested in 0.5 M KOH for 18 hours at 37°C. The precipitate (acid precipitable fraction) was also alkaline digested.

Chromatography of these digests was performed on 0.6 × 7 cm Dowex-1 × 8 formate, 200-400 mesh, columns using the elution scheme of Cohn (Fig. 1)(7). The eluates from each mononucleotide region were evaporated to dryness and desalted when necessary by sublimation. The material was then chromatographed on Whatman #1 paper in 2 dimensions: absolute ethanol: 2% boric acid:NH_4OH, 6:3:1(8) followed by methanol:concentrated HCl:water, 7:2:1(9). The

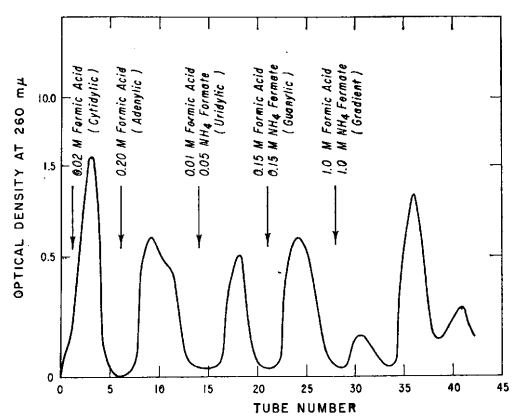

FIG. 1. Chromatography of alkaline digest of acid soluble fraction of RNA from white cells of chronic myelocytic leukemia on Dowex-1 × 8 formate.

TABLE I. Specific Activity (cpm/OD unit at 260 mμ) of 2′ 3′ Nucleotides Following P^{32} Administration to a Patient with Chronic Myelocytic Leukemia.

Fraction	Without added RNA	With added RNA	% dilution
Acid soluble			
adenylic	183 ± 7*	108 ± 10	42
cytidylic	425 ± 38	185 ± 5	56
Acid precipitable			
adenylic	180 ± 3	82 ± 3	55
cytidylic	430 ± 7	182 ± 3	58

* 2 standard deviations.

nature of each ultraviolet absorbing spot was verified by analysis of its spectra following elution from paper.

Measurements of radioactivity were performed on a liquid scintillation counter (Tri-Carb) after dissolving the material to be assayed in a dioxane-containing solution(10).

Results. The amount of acid soluble material as measured by absorption at 260 mμ in the whole RNA preparation from the 4 major types of leukemia (acute and chronic lymphocytic and myelocytic) ranged from 12-36% with a median of 23% in 15 separate determinations. Fifty to 60% of this alcohol-precipitable, non-dialyzable, acid-soluble material was converted to 2′ 3′ nucleotides by alkaline digestion in chronic myelocytic leukemia, only 15-20% in chronic lymphocytic leukemia, and a variable amount in the acute leukemias. No 2′ 3′ nucleotides could be detected in the acid-soluble fraction prior to alkaline digestion. The remainder of the material was eluted from the Dow-1 column by stronger concentrations of salt solution (Fig. 1) and has not been further identified.

To determine whether the acid soluble and acid precipitable RNA fractions were distinct the specific activities of the 2′ 3′ nucleotides were measured 2 days following a therapeutic dose of 3 mc P^{32} (Na_2PO_4) given to a patient with chronic myelocytic leukemia. Prior to homogenization his white blood cells were divided in 2 equal portions and 30 mg of rabbit liver RNA known to contain no alkali-releasable, acid-soluble 2′ 3′ nucleotides was added to one portion. Both portions were then processed in the usual manner. The results (Table I) demonstrated that the nucleotide specific activities of both the acid soluble and acid precipitable fractions were diluted to about the same degree when non-radioactive RNA was added. Furthermore, the specific activities of these 2 fractions were the same prior to dilution.

Discussion. These results indicate that the acid-soluble components found in the RNA of leukemic white cells prepared by the phenol method are formed during the isolation procedure. They most likely represent the products of phenol-resistant ribonuclease activity since ribonucleases capable of degrading RNA to oligonucleotides have been found in a variety of animal leukemic and tumor cells(11).

Summary. Phenol-prepared RNA from human leukemic white cells contains degradation products. Interpretations regarding the significance of "unusual" RNA components from such sources, therefore, have to be made with great caution.

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