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Human Leukemic Polyribosomes.* (31942)

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The important discovery that polyribosomes (PRS) are connected by strands of messenger RNA led to the finding that synthesis of proteins occurs on the polyribosome complex(1,2,3). Our knowledge of mammalian cells is largely based on studies with reticulocytes, rat liver and HeLa cells, in which PRS appear to be held together by a relatively stable messenger RNA(1). Aging of reticulocytes results in a decreased capacity to synthesize protein and a progressive decrease in PRS(4).

There are no reports on PRS profiles of lymphocytes or granulocytes and the number of tissues examined is still insufficient for an adequate comparison of normal with malignant cells. The purpose of this paper is to report the analysis of PRS in normal granulocytes and in a variety of human leukemic cell types. The PRS patterns of these human leukocytes differ from most other mammalian cells which have been studied.

Materials and methods. PRS preparations were made from human leukemic leukocytes (1 normal, 3 acute granulocyte leukemia [AGL], 4 chronic granulocytic leukemia [CGL], and 3 chronic lymphocytic leukemia [CLL]). Heparinized whole blood was allowed to sediment for 30-45 minutes at room temperature and the leukocyte-rich plasma layer was then removed by aspiration. Labeled PRS were prepared after incubating leukocytes for 30 minutes at 37°C with 2 μc/ml uridine-H^3 (Sp. Act. 3.62/c/mM New England Nuclear Corp.). A minimum of 9.0×10^8 cells were used per preparation. Incubation was omitted whenever non-labeled preparations were made.

Polyribosomes were prepared from leukocytes by two different methods. Method I was reported by Penman *et al*(5) for the preparation of HeLa cell PRS. This procedure utilized sodium deoxycholate (DOC) treated postmitochondrial supernatants layered over linear sucrose gradients of 5-20% (w/w). Method II, a technique originally used by Wettstein *et al*(3), was modified and adapted for this work as follows: after incubation, the cells were chilled to 4°C then washed twice with normal saline. The cells were suspended in RSB buffer (RSB buffer = 10^{-2} *M* Tris pH 7.4; 10^{-2} *M* KCL: and 1.5×10^{-3} *M* Mg Cl_2) 1:10 (v/v) for approximately 10 minutes and subsequently homogenized in a T-Dounce homogenizer. During homogenization, the cells were examined for breakage by light microscopy and homogenization was stopped when most of the cells were broken. The homogenate was then centrifuged at 27,000 $\times$ *g* for 30 minutes, and the microsomal supernatant thus obtained diluted with medium A without sucrose (1:1 v/v). DOC was added to a final concentration of 0.005 mg/ml. The mixture was allowed to stand for 10 minutes, and then layered above a discontinuous sucrose gradient(3). Centrifugation was carried out at 105,000 $\times$ *g* for 2½ hours in a Spinco model L ultracentrifuge. After centrifugation, the material found in the bottom of the 2 *M* sucrose layer, plus any other pelleted material found at the bottom of the tubes, was suspended in medium A without sucrose and concentrated by recentrifugation. Ribosomes thus obtained were dissolved in a minimum volume of medium A and 10-20 OD units (260 mμ) layered over 7-34% (w/w) exponential sucrose gradients. The gradients were centrifuged at 25,000 RPM for 2½ hours on an SW 25.1 rotor. Gradient analysis was performed by punc-

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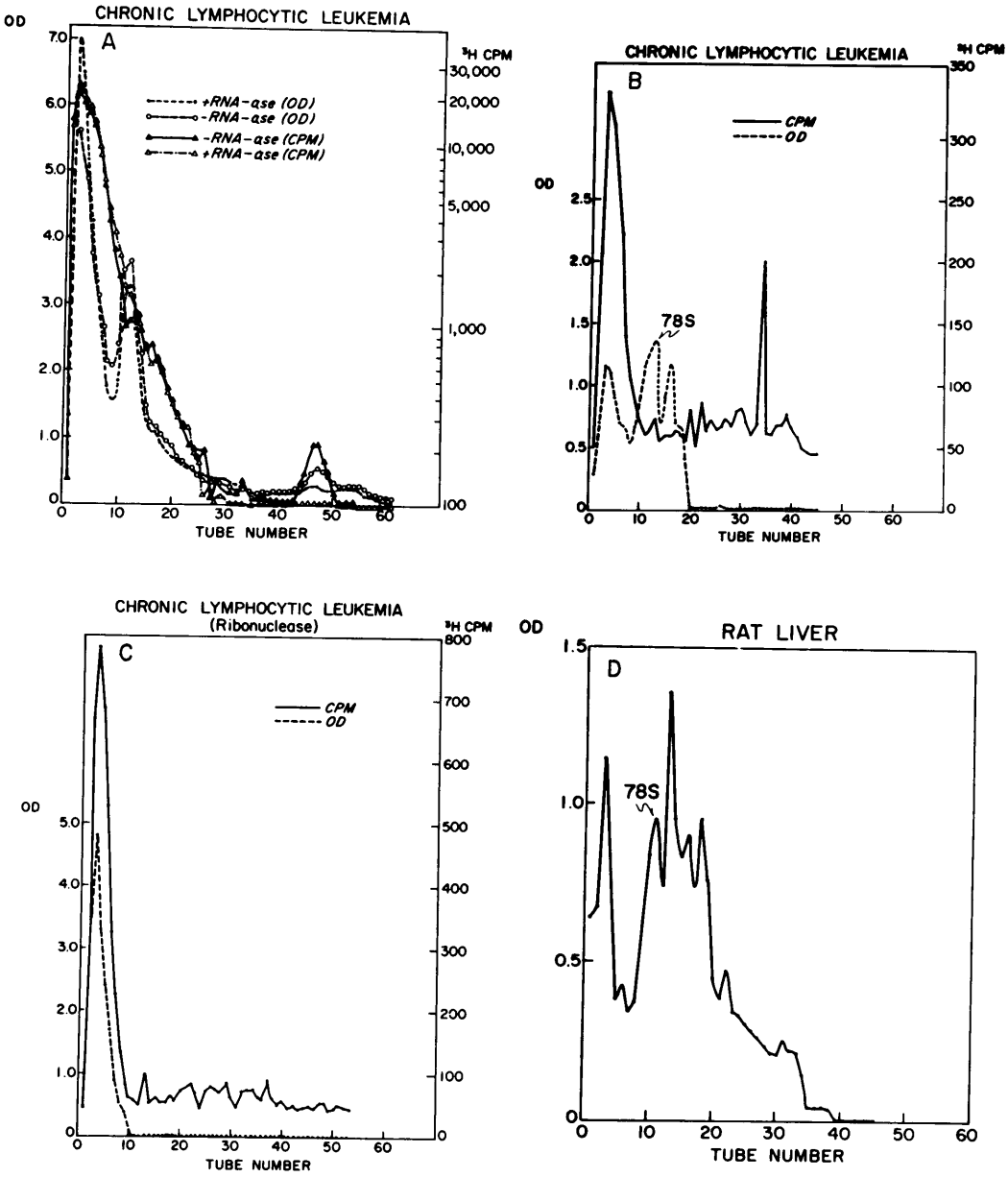

FIG. 1A. CLL Ribosomes. Fifteen ml of plasma containing 1.2 × 10^9 cells were incubated in presence of uridine-^{3}H (2 μc/ml). The differential cell count was 98% lymphocytes and 2% granulocytes. Ribosomes were prepared according to Method 1 (13). Half of the ribosomal material was incubated with ribonuclease (1 μg/ml of mitochondrial supernatant) for 10 min at 0°C. The ribosomes were separated using a 5-20% linear sucrose gradient which was centrifuged at 25,000 rpm for one hour using a 25.1 swinging bucket rotor.

FIG. 1B. 30 ml of plasma containing 5.4 × 10^8 cells were incubated in the presence of uridine-^{3}H (2 μc/ml). The differential cell count was 97% lymphocytes and 3% granulocytes. Ribosomes were prepared according to Method 11 and layered onto a 7-34% logarithmic sucrose gradient.

FIG. 1C. An aliquot of the preparation shown in 1B was incubated in presence of ribonuclease 0.5 μg/OD for 20 min at 37°C. Six OD units were layered onto the logarithmic gradient.

FIG. 1D. Rat Liver Ribosomes. Ribosomes were prepared as described in text according to Method 11. The ribosomal preparation was layered onto a 7-34% logarithmic sucrose gradient.

turing the tubes and pumping the material upward at a constant rate through an LKB flow monitor with a 260 mμ filter. Fractions of 25 drops (approximately 0.55 ml) were obtained on a fraction collector, and the radioactivity determined on a Beckman liquid scintillation spectrometer by adding 10 ml of scintillator to each fraction. The counting solution contained 150 g napthalene, 10 g PPO (2,5 Diphenyloxazole) and p-Dioxane (Fisher) to a total volume of 1.0 liter. A quench correction factor was calculated for each sucrose gradient tube after the addition of a known sample of uridine^{-3}H to each tube.

Rat liver PRS were prepared using Method II except that homogenization was carried in 2 volumes of Littlefield's medium(6) and the post-mitochondrial supernatant was not further diluted. Gradient analysis was performed as described. Ribosome preparations were examined by electron microscopy.†

Results. Fig. 1a shows patterns of PRS obtained from CLL cells using Method I. The overall absorbancy pattern resembles that of HeLa cell polyribosomes(5), except that with the latter a much larger fraction of PRS was obtained. Polyribosomes made from CLL with this technique were found to have large amounts of ribosomal subunits as well as 78S monomers. Subunits were highly labeled in comparison with the heavier particles. The effect of ribonuclease (RNA-ase) on this CLL ribosomal preparation was to degrade the small amount of PRS, resulting in a shift of the optical density and the radioactivity to the lighter regions of the gradient.

Because of the difficulty in observing polyribosomes from leukemic cells with Method I, all subsequent experiments were performed using Method II. Fig. 1b illustrates the type of gradient obtained from CLL leukocytes in which the bulk of the optical density is found in ribosomal subunits, monomer (78S) and dimer particles with very small amounts of heavier particles present. Although the quantity of PRS was small there were significant uridine^{-3}H counts incorporated into the heavier regions. A large amount of radioactivity was found in the ribosomal subunits in contrast to monomers and dimers. Ribo-

†Electron microscopy of ribosomal pellets was kindly performed by Dr. S. Vogel, Dept. of Pathology Duke Univ.

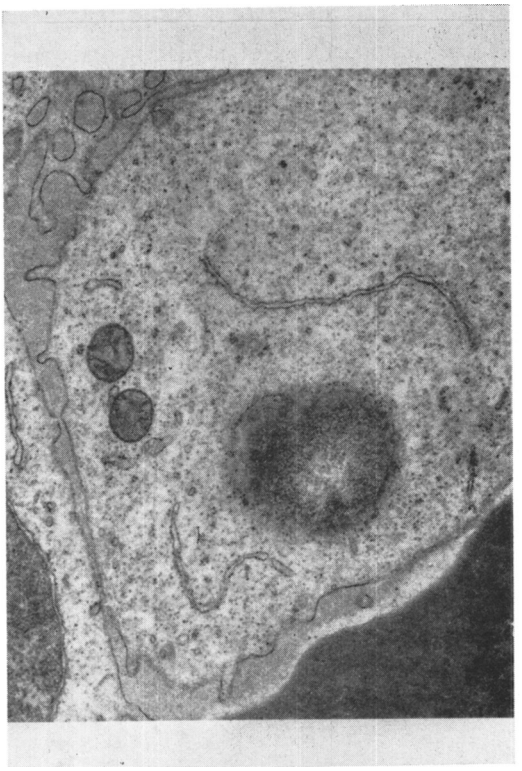

FIG. 2A. Low power electron micrograph (3500 × magification) of a CLL lymphocyte obtained prior to the isolation procedure shown in Fig. 1B.

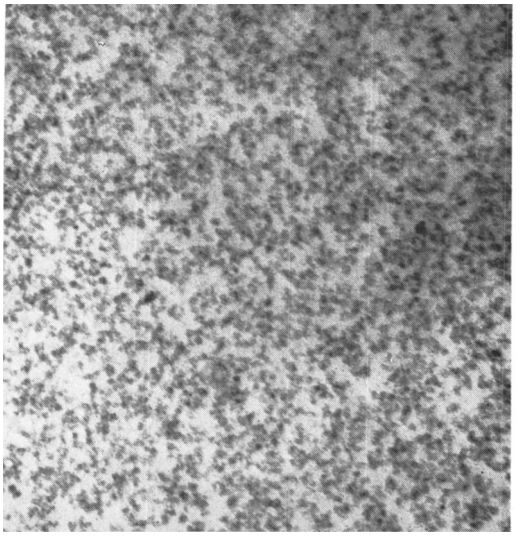

FIG. 2B. Electron microscopic preparation (84,000 × magnification) taken from the CLL preparation analyzed in Fig. 1B.

nulcease treatment of CLL ribosomes resulted in the disappearance of monomer and dimer peaks and an increase in the optical density and label of the ribosomal subunits (Fig. 1c). For a comparison with the gradient patterns, Fig. 2a shows an electron micrograph of a lymphocyte from which the above preparation was made and Fig. 2b shows the appearance of the isolated ribosomes. These visual techniques confirm the marked preponderance of single particles compared with PRS. In order to compare the ribosomal profile of leukemic lymphocytes with a more standard rat liver preparation, a typical rat liver ribosome profile is shown in Fig. 1d.

Fig. 3a represents the ribosome profile of normal granulocytes which were found to have fewer PRS peaks than rat liver but similar subunit, monomer and dimer peaks. The optical density pattern of ribosomes obtained from AGL leukocytes was intermediate between the pattern of normal granulocytes

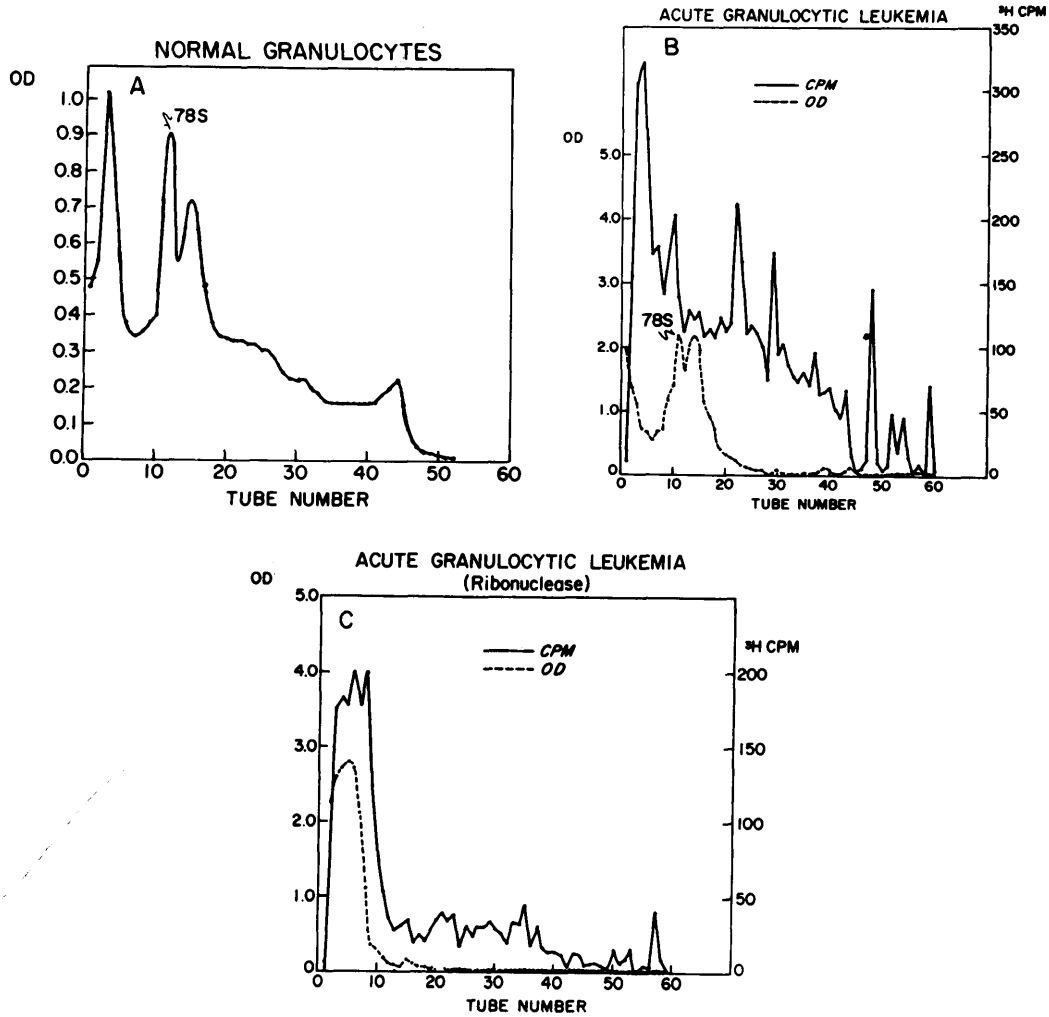

FIG. 3A. Normal Granulocyte Ribosomes. Ribosomes were prepared (Method 11) from normal granulocytes taken from a patient with leukocytosis of infection. A 7-34% logarithmic sucrose gradient was used in the experiment.

FIG. 3B. AGL Ribosomes. 5.1 × 10^9 cells were incubated in 34 ml of plasma containing uridine-^{3}H (2 μc/ml). Ribosomes were isolated as described in Method 11.

FIG. 3C. AGL with Ribonuclease. A1 aliquot of the ribosomal preparation shown in Fig. 3B was incubated with ribonuclease (1 μg/OD) for 15 minutes at 37°C and then layered onto the logarithmic sucrose gradient.

and CLL cells; having fewer PRS than the former and more than the latter (Chart 3b). The distribution of the RNA label in AGL was characterized by numerous peaks distributed over the entire range of the gradient except in the monomer-dimer regions where there was low specific activity. By contrast the specific activity of polysomes and ribosomal subunits was high. Ribonuclease treatment caused the expected shift of optical density and radioactivity to the lighter portions of the gradient (Fig. 3c).

Fig. 4a illustrates the optical density pattern and uridine-^{3}H incorporation of CGL ribosomes. The uniquely high concentration of polysomes was confirmed in 3 separate untreated patients, with as many as 10 separate preparations from one subject providing highly reproducible gradients. It may be seen that the amount of uridine-^{3}H incorporation into CGL polyribosomes was low compared with AGL preparations. Ribonuclease treatment caused the expected shift in optical density and radioactivity (Fig. 4b).

Discussion. There is considerable evidence to indicate that the functional unit of protein synthesis is the polyribosome. The early studies of Tissieres *et al*(7,8) with *E. coli* ribosomes suggested that both the 70S and 100S particles are active in protein synthesis only when combined with RNA containing the genetic information. Similar conclusions were reached from experiments with mammalian reticulocytes(9) and it was further reported

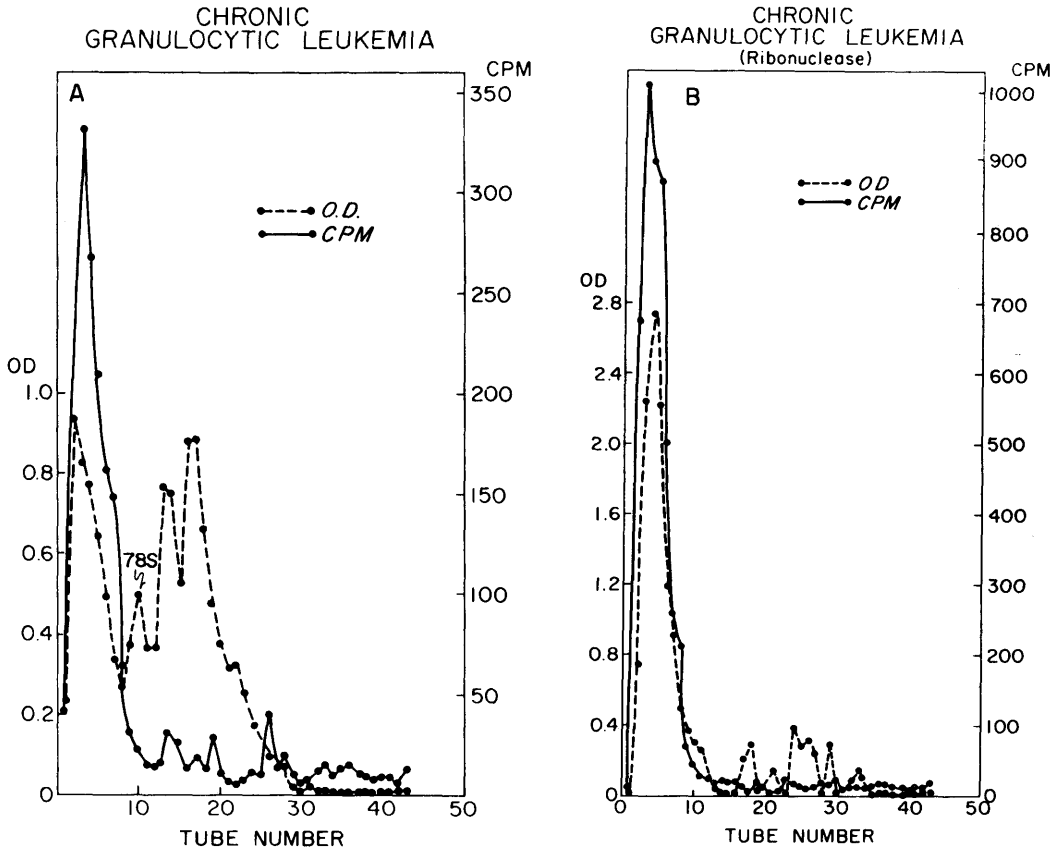

FIG. 4A. CGL Ribosomes. 6.2 × 10^9 cells were incubated in 64 ml of plasma containing uridine-^{3}H (5 μc/ml). The cell differential was polymorphonuclear leukocytes 20% stabs 15%, metamyelocytes 30%, myelocytes 25%, promyelocytes 3%, myeloblasts 2%,basophils 4%, eosinophils 2%, Ribosomes were isolated according to Method II prior to logarithmic gradient centrifugation.

FIG. 4B. An aliquot of the ribosomal preparation shown in Fig. 4A was incubated with ribonuclease (2 μg/OD) for 10 minutes at 37%C prior to gradient analysis.

that monosomes which are inactive in protein synthesis can reaggregate to form PRS which are active in protein synthesis(10). These and other studies indicate that PRS are indeed the site of protein synthesis as recognized by Rich and other investigators(1-3, 11,12).

In contrast to the above reports, other studies suggest that monomers and dimers, rather than PRS, are the active units in gamma globulin synthesis by rabbit spleen cells(13,14). Similar studies with lymph node homogenates indicate that either ribosomes or polyribosomes may be active in protein synthesis(15-17). Furthermore, it has been shown that ^{14}C labeled N-antibody is primarily associated with the 81 S phage band (18), and that monosomes are very active in the synthesis of complete hemoglobin chains (19).

There are no data available concerning the PRS pattern of normal human nucleated cells or of human tumor cells with which to compare the data presented in this report. Webb *et al*(20) studied the PRS pattern of 3 hepatomas and compared them with normal rat liver. They found the hepatomas to have a higher proportion of monomers and dimers than did the normal rat liver which contained more PRS.

The present analyses of sucrose gradients of PRS from human AGL and CLL cells show a large fraction of ribosomal subunits, distinct monomer and dimer peaks, and relatively little optical density in the heavier PRS regions. Further, the data clearly show the absence of a significant uridine incorporation in the monomer-dimer areas. In contrast, the ribosomal subunits, and the heavier polysomes (although very small in quantity, and often undetectable) do possess a high specific incorporation of uridine-^{3}H (CPM/OD). The finding of RNA labeling in PRS regions suggests that the optical density results cannot be explained on the basis of endogenous ribonuclease activity, as the counts would have likewise been displaced toward the monosome or subunit region (ribosome preparations were also assayed spectrophotometrically for ribonuclease and no activity was detected). The addition of RNA-ase did in all instances result in the expected shift in radioactivity. These observations suggest that the ribosomal subunits are either derivatives of PRS resulting from breakdown during isolation, or that they are ribosomal precursors. Their incorporation into complete ribosomes would appear to be direct, without involving intermediate synthesis of single ribosomes. Bishop (21) reported that the 60 S and the 40 S particles are native components of reticulocytes, and that they are much more active than monomers in polyU-directed incorporation. The possibility that these ribosomal subunits are the active components for initiation of polypeptide synthesis should be considered.

High monomer-dimer peaks were found in acute granulocytic and chronic lymphocytic leukemias. It appears that high concentrations of these particles are not unique to malignant cells because they were also found in normal granulocytes. In addition, a decrease in PRS cannot be considered to be a characteristic of the leukemic process because PRS were demonstrated in CGL. In this form of leukemia, the cells are morphologically well differentiated, and the PRS content appears to be even greater than normal granulocytes and approaches that of rat liver. There was substantially less uridine incorporation into CGL PRS than in acute leukemia, suggesting the possibility of a more stable messenger RNA in CGL, providing that ribosomes are indeed held together by messenger RNA.

It has been pointed out that there are numerous technical problems involved in the isolation of PRS. Despite differences in isolation procedure and in degree of ribonuclease inactivation, there is a surprisingly good correlation between the sucrose gradient patterns of isolated ribosomes and their appearance in the intact cell as estimated by electron microscopy(4,22). The present studies also indicate a reasonable correlation between these two methods of PRS analysis. Both methods illustrate major differences between human leukocytes and tissues such as rat liver and rabbit reticulocytes.

Work is in progress to define the kinetics of uridine and amino acid labeling of subunits and completed ribosomes. Comparison of chronic and acute leukemias should serve to discriminate these metabolic alterations which

are fundamental to the leukemic process from those associated with a more uncontrolled malignancy.

Summary. Ribosomes were prepared from normal human granulocytes, acute and chronic leukemias and rat liver. These preparations were analyzed for polyribosome (PRS) profile by means of sucrose density gradient centrifugation. None of the leukemic cell preparations had as high a proportion of PRS as did rat liver. Normal granulocytes and chronic granulocytic leukemic (CGL) cells had a greater fraction of PRS than did acute granulocytic (AGL) or chronic lymphocytic leukemic (CLL) cells. Most of the AGL and CLL particles were ribosomal subunits, monomers, and dimers. With 30′ of uridine-^{3}H labeling of all types of leukemic cells, the highest specific activity was in the subunits and PRS regions with low specific activity in the monomer-dimer regions. The data suggests that the monomers and dimers are not intermediates for the formation of PRS.

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Vitamin D Deficiency in Adult Quail and Chickens and Effects of Estrogen And Testosterone Treatments.*† (31943)

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The effects of vitamin D deficiency on various aspects of the reproductive processes in mature hens have been described by Turk and McGinnis(1,2). These workers, using Single Comb White Leghorn hens, observed that vitamin D deficient hens remained in good physical condition, even though they laid very few eggs. The limited number of eggs laid by these hens had thinner shells and were smaller in size. Taylor and Hertelendy (3) suggested that vit. D is required for estrogen to increase plasma calcium, phospholipid and blood total lipids in mature roosters.

In a continuation of studies on vitamin D

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