UNSTABLE RIBONUCLEIC ACID IN MAMMALIAN BLOOD CELLS[1]

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There is considerable evidence to suggest that an RNA fraction exists in bacterial cells which may serve as an intermediate in the transfer of the genetic information for coding amino acid sequence in protein molecules (Volkin and Astrachan, 1956; Brenner et al, 1961; Gros et al, 1961; Nomura et al, 1960; Hurwitz et al, 1961; Wood and Berg, 1962). This "messenger" RNA, as postulated by Jacob and Monod (1961), has a high rate of renewal and, under certain conditions, associates with ribosomes. The present study has sought evidence for this type of RNA in mammalian blood cells. These cells are of particular interest because of the striking differentiation involved in the formation of the red cells, platelets and white cells. Evidence has been obtained that an RNA fraction with metabolic characteristics similar to that of "messenger" RNA exists in white blood cells of rabbits.

METHODS

Blood obtained from normal rabbits, anticoagulated with heparin, was centrifuged, the plasma removed and the cells washed twice with a solution of (Solution 1) NaCl 1.5×10^{-1}M, KCl 6.2×10^{-3}M, KH_2PO_4 1×10^{-6}M, glucose 5.5×10^{-3}M, and ferrous ammonium citrate 7.6×10^{-6}M; the pH was adjusted to 7.4 by addition of $NaHCO_3$. For the experiments with cell populations containing a high percentage of reticulocytes, blood was obtained from rabbits injected with 10 mgm of phenylhydrazine per kg body weight each day for 5 days prior to bleeding.

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In the experiments with the blood of normal rabbits, the top 5% of the column of centrifuged cells enriched with leucocytes (the "buffy coat" layer), was decanted and used for the incubation with the radioactive substrate. In the experiments with blood from the phenylhydrazine treated rabbits, the "buffy coat" layer was removed and discarded, and the remaining cell mixture employed. The washed cells were suspended in solution 1 and the concentration of red cells, white cells, nucleated red blood cells and reticulocytes determined (Marks et al, 1960). The cell suspensions were incubated at 37°C for 3 min., allowing for temperature equilibration, and the isotopically labelled substance, 8-^{14}C-guanine or ^{32}P, was then added and incubation continued an additional period of time as indicated below. The reaction was terminated by addition of 4 volumes of an ice-cold solution of Tris hydroxyl methylaminomethane, 5 x 10^{-3}M, pH 7.5 containing $MgSO_4$, 1 x 10^{-3}M, sodium lauryl sulfate 0.15 M, bentonite, 120mg/liter; 8-oxyquinoline, 1gm/liter; and naphtholene 1,5-disulfonate, 5gm/liter. The RNA was purified by phenol treatment, addition of desoxyribonuclease, 5γ/ml, and ethanol reprecipitation (Gros et al, 1961). The RNA was examined by the sucrose gradient centrifugation technique (McQuillen et al, 1959).

RESULTS

The RNA purified from "buffy coat" cells labelled with 8-^{14}C-guanine, after sedimentation in a sucrose gradient, distributed into three major peaks, one sedimenting at a relatively slow rate (4S) (a, Figure 1) presumably representing "transfer" RNA and two (c and d) sedimenting more rapidly, corresponding to ribosomal RNA. The radioactivity in these samples was present in two components; one (a) coincided with the peak of "transfer" RNA, and the other (b), which sedimented more rapidly, did not coincide with the major peaks of UV absorption (Figure 1A).

When cell populations containing a large percentage of reticulocytes were incubated with 8-^{14}C-guanine, sedimentation analysis of the isolated RNA (Figure 1B) also revealed a distribution of radioactivity in two peaks (a, b), component b not coinciding with the major peaks of ribosomal RNA (c and d).

Incubation of preparations of cells with 8-^{14}C-guanine for 5 min., did not result in detectable incorporation of radioactivity into the RNA.

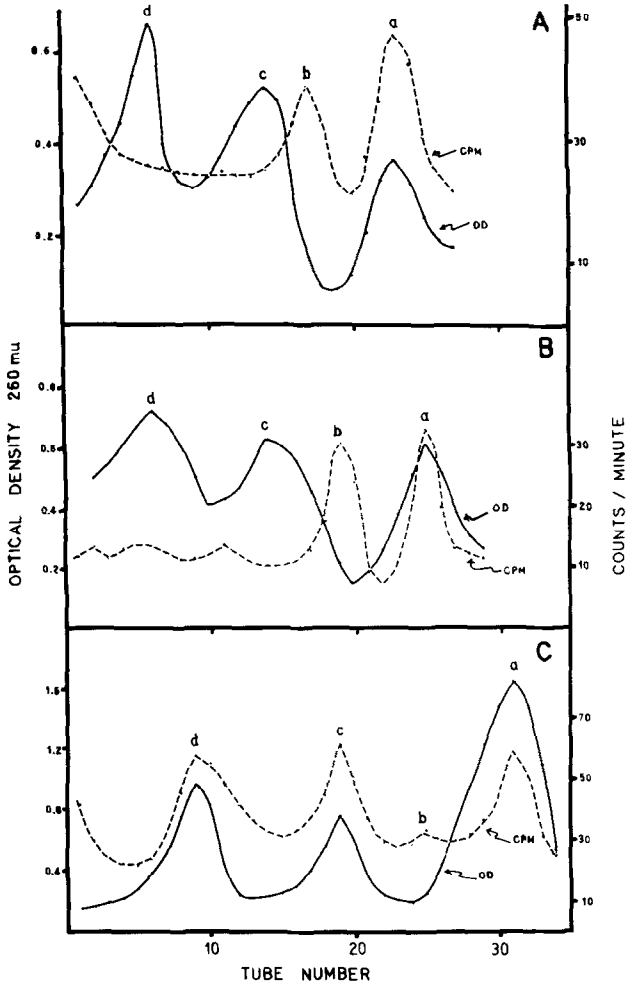

Figure 1. SEDIMENTATION OF PURIFIED RNA LABELLED WITH ^{14}C-GUANINE.

(A) - RNA extracted from "buffy coat" cell preparations incubated in a concentration of 2 x 10^8 white cells and 3 x 10^{10} red cells of which 1.2 x 10^9 were reticulocytes per 10 ml with 50 μc of 8-^{14}C-guanine (7.7 μc/mg) at 37°C for 30 min.

(B) - RNA extracted from a cell population containing 2 x 10^8 white cells and 2 x 10^{10} red cells of which 1.8 x 10^{10} were reticulocytes incubated with 50 μc 8-^{14}C-guanine at 37°C for 30 min.

(C) - RNA extracted from "buffy coat" cell population of the same cell concentration as in A which was incubated for 30 min. with 8-^{14}C-guanine, then resuspended with unlabelled guanine (1 x 10^{-4}M) and incubated for an additional 120 min. at 37°C.

In all experiments, the purified RNA was layered on top of a 24 ml sucrose gradient (5 to 20 per cent) in a Spinco SW 25 swinging-bucket tube. It was centrifuged for 13 hours at 23,000 rpm at 4°C. Fractions of gradient were then collected and analyzed for absorbancy at 2,600 A and the radioactivity in 5 per cent trichloracetic acid precipitates.

The two types of cell preparations were incubated under the conditions indicated in Figure 1 with 3 mc of ^{32}P (added as H_3PO_4) for 3 min. With both types of cell preparations, the patterns of distribution of radioactivity in material made soluble in cold trichloracetic acid by ribonuclease after incubation with ^{32}P were similar to those observed in the experiments with ^{14}C-guanine. The finding of comparable patterns for the distribution of radioactivity after labelling for 30 min. with ^{14}C-guanine and only 3 min. with ^{32}P could be due to differences in the metabolism of guanine and inorganic phosphate or the greater sensitivity of the ^{32}P experiments in detecting the labelled RNA because of the much higher level of radioactivity employed. In 4 experiments with ^{14}C-guanine labelling for 30 min. and 11 experiments with ^{32}P labelling for 3 min., the average S value of the faster sedimenting peak of radioactivity varied between 9S and 16S. This could reflect an instability of the labelled RNA fraction.

When cells that had been incubated with 8-^{14}C-guanine for 30 min. were then removed from the radioactive medium by centrifugation, resuspended in a solution containing unlabelled guanine ($1 \times 10^{-4}M$) and incubated for an additional 120 min., the radioactivity coincided with both peaks of ribosomal RNA (c and d, Figure 1C) and "transfer" RNA (a, Figure 1C), but little or none remained in the region of peak b. This suggests that the newly synthesized RNA undergoes active metabolic renewal and is converted or decomposed and utilized to form ribosomal RNA.

The "buffy coat" preparations contained, on the average, the same number of white cells, but less than 1/10 the number of reticulocytes as the blood from the phenylhydrazine treated rabbits. The content of platelets in both types of cell preparations were similar. The total radioactivity recovered in the RNA isolated from these two types of cell preparations was approximately the same. Assuming that the white cells of normal and phenylhydrazine treated rabbits have similar rates of RNA synthesis, these data suggest that the white cells make the major contribution to the observed pattern of labelled RNA.

Further evidence in support of this conclusion was obtained in studies with preparations in which a better separation of white cells and red cells from

blood of normal rabbits was achieved employing a dextran flotation technique (Marks et al, 1960). The RNA purified from preparations of cells enriched with white cells (containing 6 x 10^7 white cells and 3 x 10^9 red cells of which 3.5 x 10^8 were reticulocytes, per ml) had a pattern of distribution of incorporated label from ^{14}C-guanine similar to that illustrated in Figure 1A. On the other hand, the preparations of cells relatively poor in white cells and 6-fold enriched with reticulocytes (containing 6 x 10^6 white cells and 4.9 x 10^{10} red cells of which 2.1 x 10^9 were reticulocytes, per ml) failed to incorporate detectable radioactivity into the RNA. Similar results were obtained when ^{32}P was employed as the labelled substrate, except that with the latter cell preparation a peak of radioactivity corresponding to the "transfer" RNA was observed.

Analysis of the distribution of radioactivity in the nucleotides of the purified RNA, labelled with ^{32}P, by methods of column chromatography, electrophoresis and paper chromatography, (Hurlbert et al, 1954) showed radioactivity in each of the four nucleotides, adenylic, uridylic, cytidylic and guanylic acids[4]. This provides further evidence that the radioactivity incorporated into the RNA reflects newly synthesized molecules.

DISCUSSION

This investigation shows that mammalian blood cells contain an RNA component which turns over rapidly relative to the major RNA fraction. The present study does not definitely resolve whether the newly formed RNA is present in both reticulocytes and leucocytes. The data strongly suggest that it is primarily formed by the white cells and that reticulocyte RNA is renewed, if at all, much less rapidly. It is possible that the RNA which provides specificity information for hemoglobin synthesis is formed prior to the maturation of erythroid cells to the reticulocyte stage. A similar suggestion has been made by Nathans et al (1962). Experiments with reticulocyte cell-free systems have indicated that the non-particulate fraction may contain specificity information (Lamfrom, 1961; Kruh et al, 1961) but that the information is not essential for hemoglobin synthesis (Bishop et al, 1961;

von Ehrenstein and Lipmann, 1961). An RNA polymerase is present in preparations of blood cells similar to those employed in this study (Marks, 1961). The presence in mammalian cells of an RNA fraction with characteristics similar to that of "messenger" RNA has also been observed in studies with human amniotic cells (Cheng, 1961), thymus nuclei (Sibatani et al, 1962) and liver nuclei (Hiatt, 1962).

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