PROTEIN SYNTHESIS IN NUCLEI OF AVIAN ERYTHROCYTES*

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The ability of avian red blood cells to incorporate amino acids into heme and globin in vitro is a well-established phenomenon (1, 4, 5). It has also been shown by Wiggans et al. (1960) that the incorporation of labeled amino acids into globin continues to increase for several hours after the cells have been transferred to a medium containing no label. They postulated a precursor non-protein material to account for this "formation" of protein from a nondiffusible substance. Using the same system, we have obtained evidence that the source of isotopic material within the cell to account for this increase lies within the nucleus.

METHODS

Fresh red cells from the heparinized whole blood of pigeons were separated from the plasma and buffy coat by centrifugation and washed three times with saline at 0 - 4° C. Incubations were carried out at 37° with the red cells suspended in Krebs-Ringer bicarbonate buffer containing C^{14} labeled amino acid in a concentration of 0.5 μc./ml. of incubation mixture.

Following incubation an excess of carrier amino acid was added to the mixture which was immediately chilled to 0.°C. The cells were centrifuged off and washed twice with cold 0.9% NaCl. In some experiments the cells were lysed with distilled water, and globin from the cytoplasm was isolated according to the procedure of Anson and Mirsky (1930). The protein of the nuclear pellet was precipitated with 5% trichloroacetic acid (TCA). In other

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experiments the cells were lysed with saponin and the nuclei washed with .25 M sucrose - .0030 M $CaCl_2$ solution until the supernatant was water-clear. The proteins from both nuclear and supernatant fractions were then precipitated with 5% TCA.

All proteins were washed three times with TCA, the third wash at 91°C. for fifteen minutes. They were then purified by solution in 0.4 N NaOH, re-precipitation in 5% TCA, extraction with 1:1 alcohol:ether, and finally suspended in 1 ml. 95% ethanol for plating on 1 inch aluminum planchets. The radioactivity was counted in a gas-flow end-window counter. The specific activities thus obtained were corrected to uniform self-absorption at 10 mg. of protein. Total protein activities were calculated for some experiments after determining gravimetrically the total protein weights of both nuclear and cytoplasmic fractions.

RESULTS AND DISCUSSION

The method we used for isolating globin is the same one used by Wiggans et al. in showing the continued incorporation of radioactivity even after transferring the cells to a "cold" medium. This method involves hemolyzing the cells with water, emulsifying the hemolysate with toluene to remove lipids, then centrifugation to separate the layers. This gives a crystal clear red solution of hemoglobin overlaid by a layer of toluene and overlying a pellet of nuclei and cell ghosts. The supernatant solution is used for the isolation of globin. Upon precipitating the protein from the washed nuclear pellet and comparing its radioactivity with that of the globin isolated from the overlying hemoglobin solution, we found a significantly greater specific activity in the nuclear fraction (Table I). The same relationship of activities was also found when the nuclei were separated following hemolysis with saponin and the TCA precipitates of nuclear and supernatant fractions counted.

Further studies were done to ascertain the changes in total activity of nuclear and cytoplasmic fractions with time. As shown in Figure I, the nuclear protein shows a rapid rise in activity during the first two minutes of incubation. During this time their total activity exceeds that of the

TABLE I

	Specific Activities (c.p.m./mg.)	
	L-Phenylalanine-1-C^{14}	L-Histidine-1-C^{14}
Nuclear Protein	70	148
Globin	52	97
$\frac{\text{Nuclear Activity}}{\text{Globin Activity}}$	1.4	1.5

cytoplasmic protein even though the ratio of nuclear to cytoplasmic protein is only 1:9. After two minutes there is a relatively sharp break in the nuclear curve to a new slower rate of incorporation. At the same time, the total activity of the cytoplasmic protein overtakes that of the nucleus and continues to rise at the same almost constant rate which has characterized its curve from time zero. Thus labeled protein is apparently transferred from nucleus to cytoplasm during the course of time.

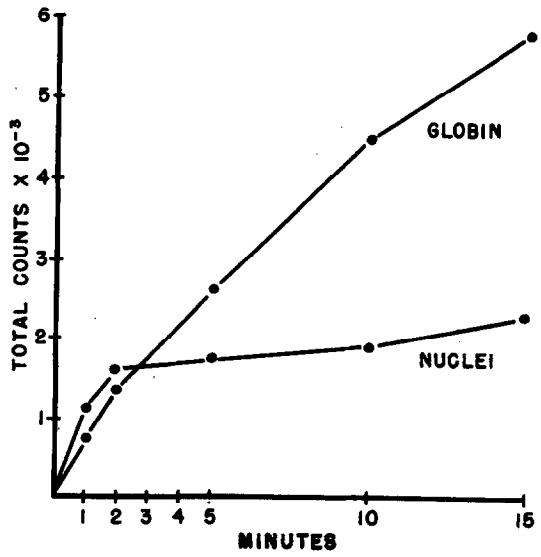

Figure I

Since it has been shown that globin activity continues to increase with time even after a second suspension in "cold" medium after almost all intracellular free label has diffused out, and since, after all, hemoglobin accounts for the major portion of the cytoplasmic protein, it seems likely from our additional evidence that globin is synthesized entirely in the nucleus and transferred to the cytoplasm. The initial sharp rise in nuclear activity probably represents the complete labeling of the globin pool, followed by a slower rate of labeling of other pools within the nucleus.

The possible synthesis of hemoglobin within the avian nucleus has been suggested by Davies (1961) on the basis of electron microscope data. Experiments are in progress to identify the protein which becomes labeled initially in the nucleus.

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