SYNTHESIS OF PROTEIN AND RNA IN PIGEON ERYTHROCYTES

(UDC 612.111.19.015.348-019 + 598.65-111.105)

B. K. Khuskivadze and V. S. Tongur

Laboratory of Nucleic Acid Biochemistry (Head, Dr. Chem. Sci. V. S. Tongur),
Institute of Biological and Medical Chemistry (Dir. Member of the Acad. Med. Sci. USSR,
Prof. V. N. Orekhovich), Academy of Medical Sciences USSR, Moscow
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 4,
pp. 42-43, April, 1966
Original article submitted November 30, 1964

In our previous work [1], we demonstrated that in the red formed elements of the bone marrow of mammals, protein (hemoglobin) synthesis is not retarded by the addition of actinomycin—a known inhibitor of RNA synthesis —to the medium. This permitted the conclusion that in spite of the presence of a nucleus (or its remains at the late stages of development) in these cells, evidently there is no synthesis of messenger RNA, and protein synthesis is accomplished on account of "ready-made" messenger RNA, probably synthesized at the early stages of their development.

It remained unclear whether the same occurs in the erythrocytes of birds, which contain nuclei at all stages of development, or whether they carry no functional load with respect to RNA synthesis, like the nuclei of mammal reticulocytes.

The purpose of this work was to investigate protein and RNA synthesis in pigeon erythrocytes.

Bird erythrocytes have been little studied in this aspect. It is known that the nuclei of pigeon erythrocytes produce proteins, from which they are discharged into the cytoplasm [3]. With respect to RNA synthesis in the nuclear erythrocytes, it has been found by autoradiographic investigations [2] that in the development of chick erythrocytes, the incorporation of labeled uridine ends at a time when the incorporation of labeled amino acids into proteins is still continuing. From this, it seems that it should follow that, as in mammals, at the end of the development of the red blood cells, their nuclei no longer synthesize RNA.

EXPERIMENTAL PROCEDURE

The blood of normal pigeons, collected with citrate, was washed twice with salt solution, removing the upper layer of cells containing leucocytes, after each centrifuging.

In experiments with the incorporation of leucine, the cells were suspended in a medium containing a set of *l*-amino acids, glucose, tris-buffer, and Mohr's salt in the recommended concentrations [4].

The suspension was brought to a concentration of 10^8 cells per ml and incubated with shaking at 37°. In a count on smears stained according to Romanovskii per 10,000 erythrocytes in the suspension to be incubated, there were no more white cells. Aurantine (10 μg/ml) was added to the experimental samples, while the same volume of physiological saline was added to the controls. After 2 h of incubation 0.2 microcurie of l-leucine-C^{14} with activity 5.5 microcuries/micromole was introduced into all the samples, each containing 4 ml of suspension, and incubated for another 2 h.

In the experiments with incorporation of uridine, the erythrocytes were suspended in medium No. 199, 0.12 microcurie of labeled uridine with specific activity 4.4 microcuries and aurantine in a concentration of 10 μg/ml were added to the sample, and the mixture incubated for 3 h.

The incorporation of leucine was established according to the radioactivity of washed TCA precipitates with a parallel determination of the protein according to Lowry. The incorporation of uridine was determined according to the radioactivity of the alkaline hydrolyzate after fractionation of the TCA precipitate according to Thannhauser.

Effect of Aurantine (Actinomycin C) on the Incorporation of Leucine and Uridine into Proteins and RNA of Pigeon Erythrocytes

Expt. No.	Leucine-C^{14} (counts/min/mg)		Leucine-C^{14} (counts/min/sample)	
	control	exptl.	control	exptl.
1*	747	739	–	–
2	451	483	–	–
3†	416	430	–	–
4	–	–	189	10
5	–	–	152	14

* Number of counts is given for an aliquot sample, and not per mg protein.

†Addition of puromycin at a dose of 30 μg/ml in the control reduced the incorporation to 23 counts/mg protein.

The results of our experiments are cited in the table.

EXPERIMENTAL RESULTS

As can be seen from the table, the reticulocytes of birds incubated in vitro synthesize protein. The addition of aurantine, which acts like actinomycin D, does not reduce protein synthesis, and this shows that it does not depend upon the penetration of fresh messenger RNA, the synthesis of which is inhibited by actinomycin. At the same time, there is a small but distinctly pronounced incorporation of labeled uridine in the nuclear erythrocytes, which is evidence of RNA synthesis; moreover, this incorporation is almost entirely stopped by actinomycin.

Thus, here we are dealing with RNA synthesis on the DNA template, i.e., in the nucleus. The incorporation of labeled uridine into mature chick erythrocytes could not be detected [2] by an autoradiographic method. The conclusion of the authors, that the nuclei of bird erythrocytes are incapable of synthesizing RNA, is evidently incorrect in the light of the data that we obtained.

The results of our experiments may be explained in two ways: 1) an amount of messenger RNA was accumulated in bird erythrocytes such as to suffice for prolonged protein synthesis, in spite of the stoppage of RNA synthesis by actinomycin; 2) the observed incorporation of labeled uridine reflects the synthesis not of messenger RNA but of some other RNA. Actinomycin blocks this synthesis. In this case also, the amount of messenger RNA is sufficient for protein synthesis.

Direct experiments on the characteristics of the RNA synthesized in nuclear erythrocytes may give an answer to the question of the correctness of one hypothesis or another.

LITERATURE CITED

1. B. K. Khuskivadze and V. S. Tongur, Vopr. Med. Khimii, No. 1 (1966), p. 37.
2. C. L. Hammel and S. P. Bessman, Proc. nat. Acad. Sci., 21, Washington (1962), p. 441.
3. I. L. Cameron and D. M. Prescott, Exp. Cell. Res., 30 (1963), p. 609.
4. J. B. Lingrel and H. Borsook, Biochemistry, 2, Washington (1963), p. 309.