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Chemical reactivity of a macromolecule as a function of its age

That hemoglobin molecules are altered on aging has been suggested by KUNKEL AND BEARN[1] and by ROSA, DREYFUS AND SCHAPIRA[2] who found that the speed of migration on electrophoresis of hemoglobin increased as it aged. EDWARDS *et al.*[3] reported that the dialyzed macromolecular fractions derived from young or old erythrocytes have a different oxyhemoglobin saturation.

The development of centrifugal and hemolytic techniques for the separation of young and old erythrocytes or of their lysates[4–6] has led to a probing of the enzymological and morphological differences of these cells[7,8]. Using a serial osmotic-hemolysis procedure[6], we have recently shown[9] that young erythrocytes are preferentially labeled with ^{51}Cr. Is this preferential labeling a function of a greater permeability to ^{51}Cr by the younger cells, of the different size of red cells of different ages, of young red cells' greater metabolic activity, or is it dependent on changes caused by aging in the hemoglobin molecule?

Rats were chosen for these experiments because of the ease with which their hemoglobin can be recrystallized. Animals of the Sprague-Dawley strain weighing between 300 and 400 g were injected intravenously *via* the femoral vein with about 10 μC of ^{59}Fe in the form of ferrous citrate and the blood was collected in heparin or acid–citrate–dextrose solution 2 days later. The red cells were washed 5 times at room temperature with isotonic saline and the serial osmotic-hemolysis procedure of SIMON AND TOPPER[6] was used to obtain separately lysates enriched with respect to young or old cells. Since lysates corresponding to younger cells have higher specific activities, the age of cells could be verified by determining the specific activity of the hemolysate (*i.e.*, the number of ^{59}Fe counts/min/absorbancy unit at 540 mμ). The hemoglobin in the tubes containing the most concentrated lysate of the younger cells or of the older cells was recrystallized from water–acetone using the method of MUIR, NEUBERGER AND PERRONE[11]. It was found that the best recrystallizations were obtained when the hemoglobin concentration was not less than 5 absorbancy units (at 540 mμ on the Beckman DU Spectrophotometer). The recrystallized hemoglobin was redissolved in water saturated with CO_2. Solutions containing identical amounts of younger or older hemoglobin were diluted to equal volumes and the hemoglobin labeled by standard methods with similar amounts of ^{51}Cr (about 0.01 μC/absorbancy unit) and then treated with sodium ascorbate. Hemoglobin was precipitated by adding solid ammonium sulfate to the saturation[12] point and allowed to stand in the cold for 2 h; it was centrifuged, dissolved in water and dialyzed overnight. The specific activity of hemoglobin with respect to ^{51}Cr was determined using a scintillation well-counter with a Baird-Atomic pulse-height analyzer and the above-mentioned spectrophotometer.

As may be seen in Table I the younger hemoglobin is found to be preferentially labeled. MEIJERING AND HUISMAN[10] have shown that different hemoglobins bind ^{51}Cr to differing extents. It appears, therefore, that certain structural requirements for chromium binding are not met by all hemoglobins. It may be that aging so modifies the rat hemoglobin that it can no longer bind the chromium to the same extent as in its youth. Although considerable work has been done on the nature of the hemoglobin–chromium interaction[13], no definite conclusions have as yet been reached.

The larger ratio of ^{59}Fe specific activity of younger/older hemoglobin as compared

TABLE I

SPECIFIC ACTIVITY OF YOUNGER AND OLDER RAT HEMOGLOBIN LABELED WITH ^{59}Fe *in vivo* AND WITH ^{51}Cr *in vitro*

Specific activities given are counts/min of the isotope in question per absorbancy unit (at 540 mμ). See text for details.

Rat	*Age of Hb*	^{59}Fe *Specific activity*	*Ratio of ^{59}Fe specific activity of younger/older Hb*	^{51}Cr *Specific activity*	*Ratio of ^{51}Cr specific activity of younger/older Hb**
No. 3	Younger	527		218	
	Older	111	4.74	78	2.80
No. 4	Younger	569		232	
	Older	178	3.20	112	2.07
No. 7	Younger	726		302	
	Older	115	6.31	206	1.47
No. 8	Younger	167		274	
	Older	14	11.9	251	1.09

* Average of this ratio for the 17 rats investigated in separate experiments was 1.42.

to the ^{51}Cr ratio in each rat (see Table I) is due to ^{59}Fe labeling only the younger hemoglobin while ^{51}Cr labels all hemoglobin with the younger molecules being labeled to a greater extent.

It might be mentioned that the specific activities of all hemoglobin fractions (young or old) with respect to ^{59}Fe remain constant during the recrystallization procedure although a portion of the hemoglobin is generally lost in this step. This indicates that hemoglobin of all ages behaves similarly on recrystallization.

It appears that our previous results[9] are due to differences in the chemical reactivity of young and old hemoglobin. This finding supports the suggestion that structural alterations on macromolecular aging take place.

Medical Research Programs, Veterans Administration Hospital, Long Beach, Calif. and Department of Physiological Chemistry, University of California, School of Medicine, Los Angeles, Calif. (U.S.A.)

HARRY WALTER

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