Chromium^{51} Elution from Hemoglobin and Intact Erythrocytes of Adults, Infants and Patients with Cooley's Anemia.* (24284)

MARION E. ERLANDSON, IRVING SCHULMAN, BEATRICE WALDEN AND CARL H. SMITH
Department of Pediatrics, The New York Hospital-Cornell Medical Center

Stimulated by the discrepancy in values for life span of infant erythrocytes when measured by the Ashby and chromium^{51} technics (1), Suderman *et al.* recently demonstrated an accelerated rate of chromium^{51} elution in cord-hemoglobin solutions when compared with adult hemoglobin solutions(2). If the rapid elution rates found in cord-hemoglobin solutions are attributable to the presence of large amounts of fetal hemoglobin, similarly rapid elution rates would be expected in hemoglobin specimens from patients with homozygous Cooley's anemia where high percentages of fetal hemoglobin are found. If excessive elution also occurs from intact red blood cells in such patients, previous estimates of erythrocyte longevity in patients with Cooley's anemia might have to be invalidated when based on the chromium^{51} technic. Therefore, experiments were designed to compare chromium^{51} elution rates from intact erythrocytes and hemoglobin solutions from normal adults, infants, and patients with Cooley's anemia.

Methods. Venous blood was collected in acid-citrate-dextrose solution using 2 ml ACD per 10 ml blood. In the preparation of hemoglobin solutions, chromium^{51} as sodium chromate was introduced immediately into the blood-anticoagulant mixtures which were then incubated for 90 minutes at room temperature. Three microcuries of chromium^{51} were added for each ml of blood. (The amount of isotope employed was the amount routinely used in this laboratory for injection specimens in *in vivo* erythrocyte survival studies.) The tagged red blood cells were then separated by centrifugation, washed 3 times with isotonic sodium chloride, and hemolyzed by addition of water. The stroma was removed by agitation with toluene and subsequent centrifugation and filtration. The clear filtrates of hemoglobin solution were adjusted to approximately 12 g per 100 ml water. Counting specimens and dialysis specimens were prepared as described for the labeled erythrocytes.

In the preparation of intact erythrocyte suspensions, the cells were separated from the plasma-ACD mixture by centrifugation and resuspended in that amount of isotonic saline necessary to adjust the hemoglobin concentration to approximately 12 g per 100 ml of suspension. The plasma was saved. The saline suspensions of red blood cells were tagged with 3 microcuries of chromium^{51} per ml of suspension. At the end of a 90-min. incubation period ascorbic acid (2 mg per ml) was added. The cells were then separated from the saline by centrifugation, washed 3 times with fresh isotonic saline and resuspended in the donor's plasma-ACD mixture adjusting the hemoglobin concentration to 12 g/100 ml.

For both the hemoglobin solutions and erythrocyte suspensions the subsequent procedures were as follows: A 100% specimen was obtained by diluting 1 ml of the original material to 100 ml in water, from which a 4 ml counting specimen was removed and placed in a screw-top vial. Individual 1 ml aliquots of the original material were placed into Visking dialysis tubing. The specimens were dialyzed against isotonic saline for periods of 16 to 118 hours. The dialysis fluid was changed 3 times per 24 hours. One dialysis bag was removed at 16 hours, and one at each 24 hours thereafter. The contents of the dialysis bags were transferred quantitatively by multiple water rinses to final 1:100 dilutions. Four ml specimens of the final solutions were used in counting. Six duplication experiments showed the possible error of the method to be 3%. The 4 ml specimens were counted in a well-type scintillation

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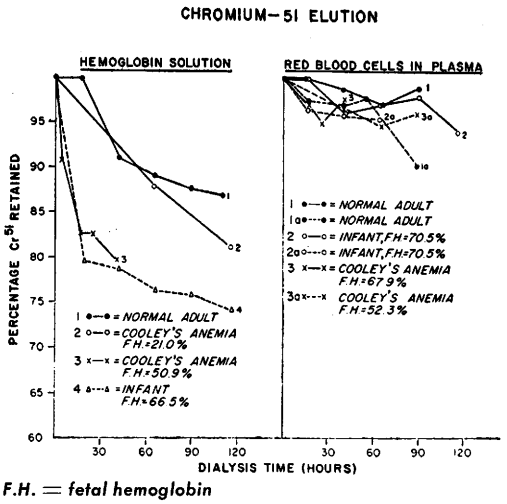

FIG. 1. Rates of chromium^{51} elution from hemoglobin solutions and red blood cells from normal adults, infants, and patients with Cooley's anemia when dialyzed against isotonic NaCl.

counter and correction for radioactive decay was accomplished by recounting the 100% specimen concomitantly with each subsequent specimen.

Results. Hemoglobin solutions were obtained from one adult (fetal hemoglobin = <2%), one infant (fetal hemoglobin = 66.5%), and 2 patients with Cooley's anemia (fetal hemoglobin = 21% and 50.9%). As may be seen in Fig. 1 (left), a markedly greater rate of elution of chromium^{51} occurred from the infant's than from the adult's specimen. Elution from the specimen containing 21% fetal hemoglobin obtained from a patient with Cooley's anemia was only slightly greater than that from the adult's specimen; that from the patient with Cooley's anemia containing 50.9% fetal hemoglobin was almost as rapid as that occurring from the infant's specimen. Therefore rapid rates of elution occurred in specimens containing fetal hemoglobin whether obtained from an infant or from patients with Cooley's anemia. The rate of elution appeared to be directly related to the percent fetal hemoglobin present.

Intact tagged erythrocytes were prepared from blood specimens from 2 normal adults (fetal hemoglobin = <2%), from 2 infants (fetal hemoglobin = 70.5 and 70.5%), and from 2 patients with Cooley's anemia (fetal hemoglobin = 52.3% and 67.9%). The rapid rates of elution found with hemoglobin solutions were not demonstrated; the content of fetal hemoglobin within the erythrocyte produced no significant difference in rate of chromium elution. (Fig. 1, right).

Discussion. Chromium^{51} tagged red blood cells have been used extensively for estimation of erythrocyte longevity in many disorders including the abnormal hemoglobin syndromes and Cooley's anemia(3-4). Elution of chromium^{51} from tagged normal erythrocytes has been investigated *in vivo* by simultaneous erythrocyte survival studies comparing the chromium^{51} technic and the Ashby method of differential agglutination, and *in vitro*, by dialysis(5-9). However, elution has not been studied in suspensions of cells containing abnormal hemoglobins or fetal hemoglobin.

Hollingsworth reported that estimates of mean life span of fetal erythrocytes were shorter using the chromium^{51} technic than those previously reported utilizing the method of differential agglutination(1). Since the life span of fetal erythrocytes could not be correlated with the percent fetal hemoglobin present in the cells, it is unlikely that the differences were secondary to aberrations in chromium elution from the cells resulting from the presence of fetal hemoglobin. Data available for comparison of erythrocyte longevity by the chromium^{51} and differential agglutination methods in patients with Cooley's anemia have not demonstrated differences in results obtained by the 2 technics. The 50% survival times of erythrocytes from 3 untransfused patients with homozygous intermediate Cooley's anemia were reported by Kaplan and Zuelzer as 21, 22, and 27 days by the Ashby method(10). The 50% survival times in 3 comparable patients by the chromium^{51} technic were reported from this laboratory as 19, 22, and 27 days(4). Corrections for elution were based on rate of elution previously demonstrated for normal erythrocytes.

Suderman *et al.* observed by *in vitro* dialysis experiments that chromium did elute more rapidly from solutions of hemoglobin ob-

tained from cord specimens than from specimens from normal adults(2). This has been confirmed in the present studies. Elution rate has been shown to be directly related to percent fetal hemoglobin present. Rapid rates of elution were also present in hemoglobin solutions containing fetal hemoglobin obtained from patients with Cooley's anemia. Elution from intact erythrocytes has previously not been investigated. In the present studies no clear cut difference in elution could be demonstrated between labeled intact red blood cells from adults, infants, and patients with Cooley's anemia.

Elution of chromium from normal erythrocytes has been shown to occur at a level of approximately 1% per day(5-9). It would be difficult to demonstrate a significant difference in rate of elution from erythrocytes of normal adults and from cells containing large percentages of fetal hemoglobin by dialysis where the error of the method may be of the same order of magnitude as the possible difference in rate of elution. Evidence thus far does not support the existence of any influence by fetal hemoglobin on rate of chromium elution from intact erythrocytes. A final solution to this problem might be obtained by studies of survival of erythrocytes from patients with Cooley's anemia in normal recipients simultaneously by the differential agglutination and chromium^{51} technics.

Summary. Accelerated rates of chromium^{51} elution from hemoglobin solutions containing fetal hemoglobin have been shown to be present in specimens obtained from an infant and from patients with Cooley's anemia. This acceleration has been shown to be directly related to the percent fetal hemoglobin present. Rapid rates of chromium^{51} elution could not be demonstrated to occur from intact labeled erythrocytes containing large amounts of fetal hemoglobin studied by the technic of dialysis.

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Inhibition of Bilirubin Conjugation *in vitro.** (24285)

WILLIAM J. WATERS, ROBERT DUNHAM, AND WILLET R. BOWEN
(Introduced by L. K. Diamond)
Department of Pediatrics, State University of New York College of Medicine at Syracuse

In recent years we have been interested in the problem of bilirubin metabolism in the newborn and the mechanism of production of bilirubin encephalopathy as well as its prevention(1). Recent studies have suggested an impairment of substrate production and conjugation of bilirubin as a glucuronide in the newborn period(2). In the light of this knowledge, the reports of jaundice associated primarily with 30 mg or more of Synkavite in 2-3 kg newborn infants(3,4) stimulated us to assess the possible role of the latter in inhibition of the conjugation mechanism leading to hyperbilirubinemia. Recent reports would also suggest another possible mechanism of action, namely, increased hemolysis of newborn red cells(5). The report of Silverman *et al.*(6), further stimulated us to test a num-

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