Erythrocyte survival in the newborn infant, as measured by chromium\textsuperscript{51} and its relation to the postnatal serum bilirubin level

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Though the last years have brought a considerable increase in our knowledge of bilirubin metabolism, the exact mechanisms which cause the icterus of the newborn infant are still controversial. Investigations undertaken after the discovery of Billing, Cole, and Lathe,\textsuperscript{1} Schmid,\textsuperscript{2} and Talafant\textsuperscript{3} that bilirubin is excreted as a glucuronide have shown that the excretory capacity of the liver for bilirubin is diminished in the newborn period because of an insufficiency of liver glucuronyl transferase\textsuperscript{4-7} and possibly also of the glucuronic acid donor, UDPGA (uridyl-diphosphate-glucuronic acid).\textsuperscript{8}

No such unanimity exists with regard to the rate of bile pigment production after birth, which depends chiefly on destruction of erythrocytes and the role it plays in the genesis of neonatal jaundice. Until a few years ago it was widely accepted that the improvement in oxygen saturation seen after birth as compared with the low oxygen content of cord blood at delivery would bring about a rapid destruction of superfluous red cells.\textsuperscript{9-10} This increased breakdown would lead to the accumulation of bile pigment in the serum and hence to neonatal jaundice.\textsuperscript{11-12}

Newer studies have raised some doubts about this concept. However, because of the difficulty of getting accurate information on the rate of red cell destruction either by recording hemoglobin concentration and number of reticulocytes simultaneously or by measuring the excretion of bile pigments in the stool and urine, the question remained open.

It seemed to us that by direct measurement of red cell survival it would be possible to eliminate some of the difficulties encountered in the above-mentioned methods of estimation of red cell destruction. The present study was undertaken to find out if there is a relationship between the rate of erythrocyte destruction and the bilirubin level in the serum after birth. We are aware that half-life measured by tagging cells with chromium\textsuperscript{51} might not give a true survival time in the newborn period. However, for comparative measurements of red cell survival, as in this study, it is very suitable. An advantage of the method is that autologous transfusion is possible, so that incompatibility reactions can be excluded.
METHODS

Four cubic centimeters of blood was obtained from 33 premature infants (weights from 1,280 grams to 2,480 grams) and 6 full-term newborn ones (weights between 2,570 grams and 4,000 grams) either by umbilical vein catheter or by vein puncture. Labeling with chromium$^{51}$ was done by standard methods.$^{14,15}$ The average activity used for tagging was 3 μc per kilogram of body weight. After incubation the labeled erythrocytes were injected intravenously to the same infant from whom they were originally taken (autologous transfusion). The radioactivity of a sample taken 24 hours after marking was regarded as 100 per cent. Further measurements were made twice a week and continued until activity had fallen below 30 to 40 per cent. The necessary amount of blood (0.1 c.c.) was obtained by heel puncture. Counting was done in a scintillation counter with the use of a well-type crystal.

Bilirubin concentration in the serum was estimated by a micromodification of the method reported by Jendrassik and Gröf.$^{16}$ As a rule, measurement of bilirubin levels were done daily during the first days of life, later on at greater intervals.

The red cells of 5 infants were labeled on the day of birth, of 8 on the second, of 9 on the third, of 10 on the fourth, and of 7 between the fifth and the thirteenth day of life.

RESULTS

Table I shows the erythrocyte half-life for the individual infants grouped according to the day of life on which the experiment was started. As can be seen, the spread of values is biggest on the third and fourth day after birth, ranging from 14.5 to 36 days. Six values are clearly within the range found in adults (26 to 35 days). The red cell survival of infants tagged on the first and second day and again between the fifth and the thirteenth day of life does not show such a large variability (range 15 to 27 days). With two exceptions the half-lives in this group of infants are shorter than in adults. We cannot explain this difference at present. With the use of the t-test of Fisher$^{17}$ the mean of values for the third and fourth days (24.1 days) was compared with that of values for the first, second, and fifth to thirteenth days (20.3).

There was no significant difference between these 2 results: P was between 0.1 and 0.05.

Fig. 1. Relationship between red cell half-life and maximum bilirubin concentration in the serum. For each individual the half-life of the red cell in days is plotted against serum bilirubin concentration in milligrams per cent.
In the few full-term infants we also encountered rather wide variations in the apparent half-times of the individual chromium$^{51}$-survival experiments (19 to 31 days). Two of the 6 measurements are in the adult range.

In Fig. 1 the red cell half-life in days is plotted against the maximum bilirubin concentration in the serum determined in the individual infants. If hemolysis of red cells after birth is a major factor in the production of neonatal jaundice, one would expect to find higher bilirubin levels in the individuals with shorter survival times. No such relationship is evident. In fact, we observe a big variation of chromium$^{51}$ half-times in individuals with identical or similar bilirubin levels. Statistical analysis confirms this impression. The correlation coefficient $r$ in this case is $-0.196$ and $P > 0.1$. From this then, no significant relation between bilirubin levels and the rate of erythrocyte destruction could be established.

In Fig. 2, the duration of hyperbilirubinemia, i.e., the time during which the bilirubin level in the serum was above 2 mg. per cent, is compared with the half-life of the chromium$^{51}$-tagged red cells. The value of 2 mg. per cent serum bilirubin was chosen because in most infants the bile pigment concentration was not followed any longer when it had fallen below that mark. As in Fig. 1, the survival times vary greatly for individuals with a similar duration of elevated bilirubin levels. There is no specific trend evident in the sense that hyperbilirubinemia of long duration was paired with short erythrocyte survival. The correlation coefficient $r$ in this case is $-0.255$. So there is no indication of an interrelation of the duration of hyperbilirubinemia and half-life of erythrocytes in the newborn period. $P$ is $> 0.1$.

We also tested whether both variables, duration and level of hyperbilirubinemia, were dependent on erythrocyte destruction. However, if one multiplies the peak bilirubin level and duration of jaundice (divided by 2) and compares this "area" of

### Table I. Half-life of chromium$^{51}$-labeled erythrocytes grouped according to the day of life on which the red cells were marked

<table>
<thead>
<tr>
<th>Day of life on which red cells were tagged</th>
<th>Premature infants</th>
<th>Full-term newborn infants</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
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<tr>
<td>Half-life of Cr$^{51}$-tagged red cells in days</td>
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<tr>
<td>24</td>
<td>27</td>
<td>36</td>
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<td>24</td>
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<td>15.5</td>
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<tr>
<td>18</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>14.5</td>
<td>20</td>
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</tr>
<tr>
<td>Average</td>
<td>19.4</td>
<td>21.6</td>
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</table>

In the few full-term infants we also encountered rather wide variations in the apparent half-times of the individual chromium$^{51}$-survival experiments (19 to 31 days). Two of the 6 measurements are in the adult range.
hyperbilirubinemia with the red cell half-life in each case, no significant relation results. The correlation coefficient r is -0.314 and P > 0.05, a value which is statistically not significant.

**DISCUSSION**

The present study confirms the finding of Hollingsworth, Gilardi and Miescher, and Foconi and Sjölin that the apparent half-life of red cells tagged with chromium is shorter in the newborn period than in adults. In addition it is shorter in premature infants than in full-term newborn infants. With an average half-life of 22.1 days in premature and 24.1 days in full-term infants our values are a little above those reported by Hollingsworth and Foconi and Sjölin, who, using heterotransfusion in adults, found a mean of 16 days in the former and 22.8 days in the latter group. There is also a difference from the values given by Kaplan. He found a normal half-life (25 to 35 days) for 5 infants whose red cells were labeled during the first week of life but shortened survival in most infants aged 3 to 9 weeks, and in some aged 10 to 16 weeks.

The finding that the half-life of chromium-labeled red cells in most newborn infants is shortened as compared with that of adults does not necessarily prove that there is increased red cell breakdown in the newborn period. It is possible that the elution of chromium from red cells, which in adults amounts to about 1 per cent per day, proceeds at a more rapid rate in infants. Suderman, White, and Israels found a more rapid elution from cord hemoglobin than from adult hemoglobin solution. This was confirmed by Erlandson and colleagues. But these authors, as well as Kaplan reported that, in vitro, the rate of chromium elution from intact erythrocytes in newborn infants was the same as in adults. This is in contrast to results by Foconi and Sjölin, who found an elution of 2.2 per cent per day for placental red cells of premature infants and 2.6 per cent for full-term ones as compared to 1 per cent with adult cells. The question of the rate of chromium elution from fetal red cells, therefore, remains open.

There are some results, however, which make it likely that the survival of fetal erythrocytes is somewhat shorter than of adults or older children. Studies making use of the method of differential agglutination have shown that, at least in premature infants, fetal red cells have a shorter life span. The existence of a difference between the survival time of erythrocytes from premature and full-term infants, which is borne out by both chromium labeling and differential agglutination, also supports the notion of a shortened survival of fetal red cells. From the data published we may assume that the life span of erythrocytes is about 70 to 90 days in premature infants and somewhat longer in full-term newborn ones.

How then would this increased erythrocyte turnover influence the genesis of neonatal jaundice? Billing, Cole, and Lathe have estimated that the excretory capacity of the liver in the newborn period is only about 1 per cent of that in adult life. Our own studies, with the use of the percentage excretion of N-acetyl-p-aminophenol as an index of the conjugating capacity of the liver, have given an average of about 10 per cent in premature infants and 20 per cent in full-term newborn infants of the percentage found in adults. Often, in individual cases in the first days of life, the excretion was much less. Compared with such a degree of glucuronyl transferase insufficiency of the liver, it seems improbable that a slight increase in erythrocyte destruction by one fourth to one fifth should make a noticeable contribution to the accumulation of bile pigment in the serum after birth.

In confirmation of this conclusion, most newer studies trying to elucidate a relationship between the decrease in the number of erythrocytes or the hemoglobin concentration and the level of serum bilirubin after birth have given entirely negative results. In addition they have shown...
that the biggest fall in hemoglobin concentration takes place in the second or third week of life, at a time when the bilirubin concentration in the serum has already decreased. In the first days after birth hemoglobin number and red cell number by a fall in red cell production, reflected by a decrease in the number of reticulocytes in the periphery and of normoblasts in the bone marrow. The decline in erythrocyte number and hemoglobin concentration after the first week of life can readily be explained by a fall in red cell production, reflected by a decrease in the number of reticulocytes in the periphery and of normoblasts in the bone marrow. The data presented corroborate and extend these findings, inasmuch as no relation between erythrocyte survival on the one hand and peak bilirubin level in the serum, duration of hyperbilirubinemia, or the product of these variables on the other hand could be established. From these data we may assume that physiologic differences in the normal rate of destruction of red cells do not play an important role in the genesis of neonatal jaundice. It is understood that this statement does not apply to cases of blood group incompatibility where the precipitous hemolysis of course greatly contributes to the accumulation of bile pigment in the serum.

SUMMARY

The present study was undertaken to determine whether increased hemolysis after birth is a factor in the genesis of physiologic icterus neonatorum. Erythrocyte destruction was followed in 33 premature and 6 full-term infants after autologous transfusion of red cells labeled with chromium. Simultaneously, the course and duration of elevated serum bilirubin levels were recorded. The half-life of tagged erythrocytes varied between 14.5 and 36 days in the premature infants (average 22 days) and between 19 and 31 (average 24 days) in the full-term newborn infants. If increased hemolysis were responsible for neonatal jaundice, one would expect that infants with a short red cell survival would show a higher bilirubin concentration or a hyperbilirubinemia of longer duration than infants with a lower rate of erythrocyte destruction. No such relation was evident in this study.

REFERENCES