

THE EFFECT OF EXCHANGE  
TRANSFUSION ON ENDOGENOUS  
CARBON MONOXIDE PRODUCTION  
IN ERYTHROBLASTOTIC INFANTS

M. JEFFREY MAISELS, M.B., B.Ch.  
Hershey, Pa.

AMBADAS PATHAK, M.D.  
and

NICHOLAS M. NELSON, M.D.  
Boston, Mass.

From the Laboratory for Neonatal Research,  
Boston Hospital for Women, and Department  
of Pediatrics, Harvard Medical School

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## *The effect of exchange transfusion on endogenous carbon monoxide production in erythroblastotic infants*

*In order to elucidate the mechanism of the "late" postexchange transfusion bilirubin rebound, we measured endogenous carbon monoxide production in infants with erythroblastosis fetalis before and after exchange transfusions. The rate of carbon monoxide production was markedly increased before the first exchange transfusion and remained elevated until two, or in one case until three, exchange transfusions had been completed. Normal rates of carbon monoxide production were found when falling levels of serum bilirubin concentration indicated that further exchange transfusions were not necessary. These findings suggest that a continued increase in heme turnover is largely responsible for the "late" bilirubin rebound which occurs after exchange transfusions.*

M. Jeffrey Maisels, M.B., B.Ch.,\* Hershey, Pa., Ambadas Pathak, M.D.,  
and Nicholas M. Nelson, M.D., Boston, Mass.

DURING exchange transfusion, extravascular bilirubin equilibrates with plasma bilirubin.<sup>1,2</sup> Immediately after the exchange, further equilibration takes place which is complete within 30 minutes<sup>3</sup>; there is always some degree of bilirubin rebound which varies in rapidity and extent.

*From the Laboratory for Neonatal Research, Boston Hospital for Women, and Department of Pediatrics, Harvard Medical School.*

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*\*Reprint address: Department of Pediatrics, Milton S. Hershey Medical Center, Hershey, Pa. 17033.*

In a significant percentage of cases of hemolytic disease of the newborn infant,<sup>4,5</sup> a further increase in serum bilirubin concentration occurs after the initial rebound, necessitating one or more further exchange transfusions. This increase in serum bilirubin concentration after a new equilibrium is reached must, therefore, represent the addition of newly formed bilirubin to the body stores. Either bilirubin is being produced at a relatively normal rate but is not being cleared, or there continues to be an excessive rate of bilirubin production. It has been widely assumed that increased bilirubin production is the primary mechanism responsible, although documentation of this is limited.<sup>6</sup> Until recently, no measurements

Table I. Clinical and laboratory data for CO and bilirubin production before and after exchange

| <i>Case No.</i>                  | <i>Gestation<br/>(wk.)</i> | <i>Weight<br/>(Kg.)</i> | <i>Age at study<br/>(hr.)</i> | <i>Exchange<br/>No.</i> |
|----------------------------------|----------------------------|-------------------------|-------------------------------|-------------------------|
| 1                                | 36                         | 2.75                    | 3                             | Before 1                |
|                                  |                            |                         | 14                            | After 1                 |
|                                  |                            |                         | 42                            | After 2                 |
|                                  |                            |                         | 74                            | After 3                 |
|                                  |                            |                         | 88                            | After 4                 |
| 2                                | 38                         | 2.98                    | 2                             | Before 1                |
|                                  |                            |                         | 48                            | After 1                 |
|                                  |                            |                         | 120                           | After 2                 |
| 3                                | 35                         | 2.27                    | 29                            | Before 2                |
|                                  |                            |                         | 156                           | After 3                 |
| 4                                | 38                         | 2.84                    | 43                            | Before 2                |
|                                  |                            |                         | 92                            | After 2                 |
| Mean normal values <sup>11</sup> | 40                         | 3.36                    | 44.8                          |                         |

\*Calculated from CO production.<sup>11</sup>

of heme turnover have been made to help define the factors involved.

Carbon monoxide (CO) is endogenously produced in normal man by the catabolism of heme.<sup>7</sup> In vivo studies in man<sup>8</sup> have shown that approximately 1 mole of CO is produced per mole of heme catabolized. Furthermore, 1 mole of bilirubin is produced for every mole of CO<sup>9, 10</sup> so that the measurement of CO production can be used to determine heme turnover and bilirubin production.<sup>11</sup> The development of a method for measuring endogenous CO production in newborn infants<sup>11</sup> has allowed us to study a group of erythroblastotic infants before and after exchange transfusion in order to further understand the mechanism of the "late" bilirubin rebound following exchange transfusions.

#### METHODS

The technique for measuring CO production has been fully described in a previous publication.<sup>11</sup> In principle, the method involves the serial determination of blood carboxyhemoglobin\* (COHb) while the infant rebreathes in a closed system. The use of a rebreathing system prevents the normal excretion of CO via the lungs; the resultant

increase of CO in the blood is measured over a fixed time span. CO production is then calculated from the rate of increase of blood COHb and the dilution of CO in the body.

The blood samples were analyzed for CO by gas chromatography as previously described.<sup>11</sup> Hemoglobin was measured as cyanmethemoglobin<sup>12</sup> and serum bilirubin concentration by the method of White and associates.<sup>13</sup> Bilirubin production was calculated directly from CO production.<sup>11</sup>

**Patients.** Fourteen studies were performed on six infants with erythroblastosis fetalis due to Rh or AO incompatibility (Table I). In four infants CO production was measured before the first exchange transfusion (mean age 3.2 hours), and the results of these studies have been reported.<sup>11</sup> In two of these infants and two others not studied before the first exchange, CO production was measured before the succeeding exchanges and again when it was apparent that no further exchange would be necessary. Exchange transfusions were performed according to standard techniques<sup>14</sup> using fresh (less than 24 hours) heparinized blood. The indication for exchange was generally based on the level of indirect serum bilirubin, using the predictive curves of Allen and Diamond.<sup>14</sup> An average of 200 ml. was exchanged per kilo-

\*The per cent of hemoglobin saturated with carbon monoxide.



transfusion

| Reticulocytes<br>(%) | Hemoglobin<br>(Gm./100 ml.) | CO production<br>( $\mu$ l/Kg./hr.) | Bilirubin<br>production*<br>(mg./Kg./24 hr.) | Serum bilirubin<br>(mg./100 ml.) |
|----------------------|-----------------------------|-------------------------------------|--|----------------------------------|
| 10.8                 | 11.5                        | 40.9                                | 25.6   | 5.4                              |
| 2.2                  | 15.0                        | 28.6                                | 17.9   | 8.6                              |
| 0.8                  | 15.0                        | 33.0                                | 20.7   | 17.8                             |
| 0.6                  | 14.2                        | 16.8                                | 10.5   | 18.2                             |
| 0.6                  | 14.3                        | 18.0                                | 11.3   | 13.4                             |
| 14.6                 | 12.8                        | 36.9                                | 23.1   | 6.1                              |
| 11.0                 | 11.6                        | 34.3                                | 21.5   | 15.3                             |
| 1.4                  | 13.8                        | 19.4                                | 12.1   | 13.0                             |
| 8.2                  | 13.9                        | 43.9                                | 27.5   | 14.6                             |
| 1.0                  | 14.6                        | 9.1                                 | 5.7  | 8.5                              |
| 5.3                  | 13.4                        | 26.8                                | 16.8   | 14.2                             |
| 3.6                  | 14.6                        | 14.1                                | 8.8  | 13.0                             |
| 6.0                  | 18.9                        | 13.7 $\pm$ 3.64                     | 8.6 $\pm$ 2.32                               |                                  |

gram of body weight ( $2.4 \times$  calculated blood volume).

# RESULTS

The results of studies before and after repeat exchanges are shown in Fig. 1, and the individual data are presented in Table I. In all infants CO production was markedly increased (normal values  $13.7 \pm 3.64 \mu$ l CO per kilogram per hour)<sup>11</sup> both before and after the first exchange transfusion, and in one (Case 1) CO production remained elevated after two exchanges. In another, (Case 2) at the time of the second study at 48 hours of life, CO was being produced at essentially the same rate as before the first exchange at age three hours. In three of the infants CO production returned to normal after two exchanges, and in one, (Case 1) after three exchanges. Ages at which normal CO production was first recorded ranged from 74 to 156 hours.

# DISCUSSION

These results suggest that in erythroblastic infants there is a marked increase in heme turnover which continues in spite of one, or in some cases two, exchange transfusions. A 2.4 volume exchange will remove about 90 per cent of the circulating red blood cells<sup>2</sup>; we have calculated that catabolism

of the sensitized cells remaining, if it continued at the same rate as before the exchange, would not account for the level of increased CO production found after the first exchange (in Cases 1 and 2).

Various mechanisms may explain the persistently increased level of heme catabolism (as reflected by the elevated CO production) found after exchange transfusion:

Pools of sensitized red cells may exist in the bone marrow or spleen which are not removed by the exchange transfusion and are subsequently released into the circulation and rapidly catabolized.

The bone marrow may continue to produce cells which are then sensitized by antibodies persisting in the circulation. That red cell production continues is suggested by the findings of marrow hyperplasia in such infants<sup>10</sup> and the continued presence of an increased number of reticulocytes after some exchange transfusions<sup>9</sup> (see Table I).

It is also possible that a significant contribution to CO and bilirubin production is that arising from the "early-labeled peak."<sup>16, 17</sup> The "early-labeled peak" refers to that portion of bilirubin (and CO) produced which does not arise as a result of the catabolism of circulating red blood cells but arises from heme turnover in other areas—mainly the bone marrow (ineffective eryth-

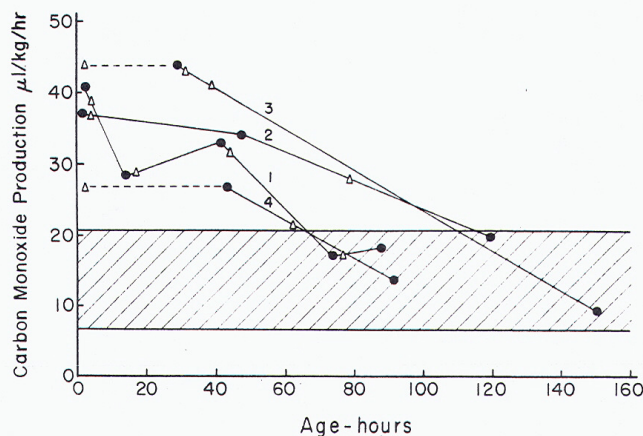


Fig. 1. Effect of exchange transfusion on CO production. The triangles represent exchange transfusions and the dots, measurement of CO production. The dotted line in Infants 3 and 4 indicates that CO production was not measured before the first exchange. The hatched area is the mean  $\pm$  2 standard deviations for CO production in normal full-term infants.<sup>10</sup>

ropoiesis) and the liver.<sup>16</sup> In normal infants the early-labeled peak appears to account for about 25 per cent of the bilirubin<sup>18</sup> produced. Berk and associates<sup>19</sup> have shown that in the absence of a defect in heme biosynthesis, the proportion of total bilirubin production contributed by the early-labeled peak remains constant, even in hemolytic diseases. Considering that these infants produce enormous amounts of bilirubin (see Table I) and that 25 per cent of this production may be from a source outside of the circulation and therefore not affected by exchange transfusion, it is not difficult to imagine that this may significantly contribute to the increased production of CO found after an exchange transfusion.

Hemolysis of the transfused red cells is generally considered to be an important mechanism contributing to bilirubin rebound after exchange transfusions. The commonly used "push-pull" technique of exchange transfusion may cause damage to the donor red cells leading to an increase in hemolysis.<sup>6, 20</sup> It has been suggested that using a "drip" method for exchange transfusion may significantly decrease the necessity for re-

peat exchanges by decreasing mechanical damage to the red cells.<sup>5</sup>

During 1968, in this nursery, of 84 infants requiring exchange transfusions, 4 of 15 (27 per cent) with nonhemolytic jaundice were exchanged twice (but none required more than two exchanges), whereas 45 of 69 infants (65 per cent) with hemolytic disease required a second exchange ( $p < 0.01$ ), and 23 of these needed three or more exchanges. This suggests that while the procedure itself is important and may contribute to the increased bilirubin production, other factors, as discussed above, must play a role in the higher incidence of significant bilirubin rebound in infants with hemolytic disease.

Finally, while increased bilirubin production appears to be the major factor in the "late" bilirubin rebound, decreased hepatic clearance may also occur as was seen in the fourth study on the first infant; at 74 hours of age, the rate of bilirubin production was normal but the rising serum bilirubin concentration necessitated a further exchange transfusion. In the other infants studied, once the bilirubin production rate had returned to normal the serum bilirubin concentration decreased, indicating that the



liver in these infants could adequately clear the normal bilirubin load. None of the infants had elevated levels of direct reacting bilirubin.

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