Endogenous Production of Carbon Monoxide in Normal and Erythroblastotic Newborn Infants

M. JEFFREY MAISELS, AMBADAS PATHAK, NICHOLAS M. NELSON, DAVID G. NATHAN, and CLEMENT A. SMITH

From the Laboratory for Neonatal Research, Boston Hospital for Women, Division of Hematology of the Children's Hospital Medical Center, and Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115

ABSTRACT The endogenous production of carbon monoxide (Vco) in newborn infants was measured by serial determinations of blood carboxyhemoglobin during rebreathing in a closed system. Mean Vco in nine full-term infants was 13.7 ± 3.6 μl CO/kg per hr (sd), and in four erythroblastotic infants Vco ranged from 37 to 154 μl CO/kg per hr preceding exchange transfusion. Mean red cell life-span (MLS) and total bilirubin production were calculated from Vco. MLS in normal newborns was 88 ± 15 days (sd), and bilirubin production was 8.5 ± 2.3 mg/kg per 24 hr. This is more than two times the amount of bilirubin normally produced in the adult per kilogram of body weight. Normal infants achieved a net excretion of bilirubin of at least 5.6 ± 2.3 mg/kg per 24 hr (sd) as calculated from the bilirubin production and the measured rise in serum bilirubin concentration.

The measurement of Vco should prove valuable in the study of red blood cell survival and bilirubin metabolism in the newborn infant.

INTRODUCTION

The recent studies of Coburn and coworkers (1–3) have confirmed the original observations by Sjöstrand (4–6) that carbon monoxide (CO) is endogenously produced in normal man, and that approximately 1 mole of CO is produced per mole of heme catabolized (2).

Interest in neonatal hemoglobin turnover has led previous investigators (7–10) to measure blood carboxy-

Dr. Maisels' present address is the Department of Hematology, Walter Reed Army Institute of Research, Washington, D. C. 20012.

This work was presented in part at the Annual Meeting of the Society for Pediatric Research, Atlantic City, New Jersey, May 1969.

Received for publication 2 January 1970 and in revised form 22 June 1970.

hemoglobin (COHb)1 levels in normal newborn infants and in those with jaundice of hemolytic and non-
hemolytic origin. In hemolytic disease of the newborn they demonstrated an elevated level of COHb which was assumed to be due to an increase in CO production (Vco) resulting from increased hemoglobin destruction. Although a correlation has, indeed, been shown to exist between blood COHb and Vco (11, 12) the relationship is too uncertain to derive an accurate indication of hemoglobin turnover from COHb (11).

Wranne (13–15) and Fällström (12, 16) have measured the pulmonary excretion of CO in newborn infants. This measurement may not accurately reflect CO production because of the difficulty in achieving a steady state between Vco, the critical measurement, and the rate of CO excretion via the lung (11).

We have adapted Coburn's rebreathing system for the measurement of Vco (1, 17) to the study of newborn infants. With this technique an alteration in CO production can be measured within minutes after it has occurred (18), and we have been able to calculate hemoglobin catabolism, red blood cell survival, and bilirubin production in the newborn infant.

METHODS

Rebreathing circuit

The closed rebreathing circuit is shown in Fig. 1. The method of sealing the infant's face into the rebreathing mask is similar to that described by Cross (19). The infant's face emerges through a pneumatic cuff (a) which seals against the face and the surrounding plastic ring (b) onto which a lid is sealed by spring clips. A stop cock (e) allows gas sampling and CO addition at the end of the procedure (see below). The blower (g) circulates air at approximately 15 liters/min. At

1 The terms COHb, COHb%, and COHb per cent saturation are used interchangeably throughout this paper. All imply the per cent of hemoglobin saturated with carbon monoxide.

this rate of flow through the CO₂ absorbent (f) (650 ml of Baralyme),* less than 1% CO₂ is detectable in the circuit. Humidified oxygen is added via a 50 ml syringe at e in amounts sufficient to maintain a constant circuit volume.

A thin walled rubber tube, 36 cm long and 6 cm in diameter, is sealed inside a solid plastic tube (c) as illustrated. Rubber stoppers at either end have an outlet for the circulating gas and an additional inlet at one end for oxygen (e). This device allows the addition of oxygen when required without the use of a rubber bag (1) which could act as "dead space" and prevent complete circulation of CO₂ throughout the system. A Krogh spirometer (d) is connected to an outlet from the plastic tube and reflects volume changes occurring in the circuit. The use of the spirometer outside of the circuit allows the volume of the system to be kept as small as possible (about 2 liters).

Conduct of study

The infant was placed in the system, and the oxygen tension was adjusted to approximately 150 mm Hg. Thereafter, as oxygen was consumed by the infant, it was replaced so as to maintain circuit volume constant as measured by the spirometer. Oxygen tension was monitored frequently throughout the study* and adjusted, if necessary, by the addition of extra oxygen. After rebreathing had continued for at least 15 min (1, 17, 20) the first blood sample was taken. Subsequent samples were taken at 30-min intervals for 1 hr. Blood was taken ananaerobically either from an indwelling, size 21, scalp vein needle inserted into an antecubital vein, or from free flowing arterialized heel prick samples, the same method of sampling being used throughout a given study. 1 ml of blood was sufficient for duplicate analyses.

Total time involved in the study including the initial equilibration, determination of the dilution factor (see below), insertion of an indwelling needle, and settling the baby into the system, was usually close to 3 hr, by which time most full-term infants were showing signs of restlessness, crying, and/or hunger. When these occurred, leaks* were apt to develop around the baby's face. At the end of the period of observation of the rate of increase of COHb, 0.92 ml (STPD) of 99.5% CO gas* was added to the system at e (Fig. 1), and a final blood sample was taken 45 min later. The maximum blood CO level measured after the addition of CO was 0.736 ml/100 ml or 3.48% of hemoglobin saturated with CO(1).

Blood analysis

The blood samples were analyzed for CO by liberating bound CO with acidified potassium ferricyanide and a hemo-lyzing agent (Triton X-100; Rohm and Haas Co., Philadelphia, Pa.). The liberated blood gases were then extracted under vacuum using the modified microgasometer described by Natelson and Stellate (21) followed by injection into a gas chromatograph (Perkin-Elmer 154L). The method of gas injection was modified from Farhi, Edwards, and Homma (22) where the connecting tube leading from the upper two-way

* Baralyme® is obtainable from Warren E. Collins, Inc., Braintree, Mass.
* IL 113 pH/gas analyzer PO₃ electrode; Instrumentation Laboratory, Inc., Watertown, Mass.
* Leaks could be detected by a number of means, including rapid fall of the spirometer bell, and could be corrected by increasing the inflation of the pneumatic cuff seal or by wedging the infant's face into the cuff more firmly by means of an inflatable pillow.
* CO 99.5%; Matheson Co., Inc., East Rutherford, N. J.

![FIGURE 1 Rebreathing circuit showing pneumatic cuff (a), plastic ring (b), plastic tube with thin-walled rubber tube (c), spirometer (d), stopcocks (e), CO₂ absorbent (f), variable speed blower (g), and oxygen source.](image-url)
Calculations

CO production. The $\dot V_{CO}$ was calculated according to the equation

$$\dot V_{CO} = \Delta COHb\% \times \frac{CO_d}{\Delta COHb\%}$$

(1)

where $\dot V_{CO}$ is CO production in milliliters per hour (STPD), and $\Delta COHb\%$ is the average hourly increase in the per cent saturation of hemoglobin with CO (1).

The term $CO_d/\Delta COHb\%$ is the dilution of added CO in the body and is determined by adding 0.92 ml (STPD) of 99.5% CO to the circuit (CO$_D$), and therefore to the body stores, and measuring the resultant increase in the blood COHb per cent ($\Delta COHb\%$).

Mean red cell life span (MLS). In the steady state, the mean red cell life span (MLS) is expressed by the equation

$$MLS\text{ (days)} = \frac{T_{bmea} \text{ (µmoles)}}{\dot V_{bmea} \text{ (µmoles/24 hr)}}$$

(2)

where $T_{bmea}$ is the total circulating heme and $\dot V_{bmea}$ is the rate of breakdown of circulating heme (17).

Total circulating hemoglobin ($T_{bH}$) was determined by dividing the dilution factor $CO_d/\Delta COHb\%$ (see equation 1) by 1.34 (20). $T_{bH}$ is derived from the total hemoglobin in grams ($g_{TH}$) as follows:

$$T_{bH} \text{ (µmoles)} = \frac{g_{TH}}{0.017}$$

(3)

where the factor 0.017 is grams of hemoglobin per µmole.

The denominator in equation 2 is derived directly from $\dot V_{CO}$ and a correction made for the "early labeled" CO peak; that is, CO not produced by senescent circulating red cells (see Discussion). In this study it was assumed that only 75% of the measured $\dot V_{CO}$ was derived from breakdown of circulating red cells (26). Equation 2 then becomes:

$$\text{MLS$_{corr}$} = \frac{0.75 \times \dot V_{CO} \times 44.6 \times 24}{g_{TH}/0.017}$$

(4)

where $\dot V_{CO}$ is in milliliters per hour STPD, $T_{bH}$ is in grams, and MLS$_{corr}$ is the mean life span corrected for early labeled CO. The calculation of both total hemoglobin and of $\dot V_{CO}$ (see equation 1) requires a measurement of CO dilution. This term, therefore, cancels, and the measurement of total circulating hemoglobin by CO dilution and any errors involved therein should not affect the calculation of mean life span.¹⁰

$$\text{MLS$_{corr}$} = \frac{5.46 \times 10^{-3}}{\Delta COHb\%}$$

(5)

Bilirubin production, retention, and excretion. $\dot V_{CO}$ has been shown to reflect bilirubin production in man (27). Bilirubin production was therefore calculated directly from the measured $\dot V_{CO}$ (µmoles/hr) and the molecular weight of bilirubin:

$$\text{Bilirubin production} (\text{mg/kg per 24 hr}) = \frac{\dot V_{CO} \text{ (µmoles/hr)} \times 0.585 \times 24}{\text{body weight (kg)}}$$

(6)

where the factor 0.585 is the mg of bilirubin per µmole.

¹⁰ The rate of rise of COHb will itself be affected by the true size of the total CO pool. When this pool differs significantly from the CO binding capacity of the total circulating hemoglobin (in circumstances where significant amounts of CO are bound outside the circulation), the calculation of circulating hemoglobin cannot be based on CO dilution.

The "retention" of intravascular bilirubin was calculated from the measured rise in serum bilirubin concentration per 24 hr and the plasma volume.

The total bilirubin space has not been measured in the normal newborn infant, but studies in adults suggest that it is equal to about twice the plasma volume (28). Total body bilirubin was therefore calculated by multiplying the total intravascular bilirubin by two. Excretion of bilirubin was assumed to equal production minus retention.

Plasma volume was calculated from the red cell volume (RCV) and the whole body hematocrit (0.87 X venous hematocrit) (29). RCV was determined from CO dilution and the hematocrit (30).

$$\text{RCV ml} = \frac{CO_d}{\text{measured increase in CO ml/ml}} \times \text{hematocrit}$$

(7)

Subjects

Nine normal newborn infants of 40-41 wk gestation were studied in the first 3 days of life. Permission for the studies was obtained from their informed mothers. None of the mothers received barbiturates during labor (18) or volatile anesthetics during delivery (9). Ages at the time of study ranged from 27 to 57 hr, and the maximum bilirubin concentration in any of these infants before their discharge on the 5th day was 8 mg/100 ml. Two of the mothers smoked. Their infants were 40 and 47 hr old, respectively, at the time of study, and their levels of blood COHb were well within the normal range (7-9).

Four infants with erythroblastosis were studied immediately before the first exchange transfusion. Three of these infants were delivered vaginally; one was an infant of a diabetic mother who was delivered by elective cesarean section under spinal anesthesia. One mother smoked up to the time of delivery, and the possible effect of this on $\dot V_{CO}$ is discussed below. Rebreathing periods in these infants varied from 30 min to 1 hr.

RESULTS

CO production and mean red cell life span (MLS). The results of studies of CO production in normal and erythroblastotic infants are shown in Fig. 2. The individual data are presented in Tables I and II. The mean rate of CO production in the normal infants was 13.7 ± 3.6 µl CO/kg per hr (SD). In erythroblastotic infants $\dot V_{CO}$ ranged from 37 to 154 µl CO/kg per hr. The MLS of the nine normal infants was 88 ±15 days (SD).

Bilirubin metabolism. Fig. 3 shows the results of bilirubin production plotted against bilirubin retention in the normal infants.

Reference to Fig. 3 shows that if none of the infants were capable of excreting bilirubin, production would equal retention and all points would fall on the line of identity. The fact that all points fall below the line implies that all of these infants were capable of excreting some bilirubin. The mean rate of bilirubin production in the normal infants was 8.5 ± 2.32 mg/kg per 24 hr (SD), and mean excretion was 5.6 ± 2.29 mg/kg per 24 hr (SD). Bilirubin production in the erythroblastotic infants ranged from 23 to 96 mg/kg per 24 hr.
DISCUSSION

These studies provide direct measurements of CO production in newborn infants and demonstrate the feasibility of applying this technique to the study of a variety of clinical conditions in the neonatal period. \( \dot{V}_{CO} \) in normal newborns is about twice that in adults when expressed per kilogram of body weight. This can be explained by the more rapid turnover of a larger relative mass of circulating hemoglobin as well as a greater contribution from heme catabolism outside of the circulation (26, 33). The MLS of 88 days corresponds well with nearly all of the published data on MLS in the newborn determined by various methods (33–36). The results also suggest that the newborn infant's ability to excrete bilirubin may be much greater than has been previously appreciated. These conclusions are based on calculations that are dependent upon certain assumptions and upon accurate measurement of CO accumulation \( \dot{V}_{CO} \) in the blood of newborns.

To assess the accuracy and reproducibility of the \( \dot{V}_{CO} \) measurement would require repeated studies on individual infants within a short space of time which was not possible. Analysis of CO in duplicate blood samples was satisfactory, but one important source of measurement error may have resulted from the clinical circumstances which necessitated the construction of regression lines from only three samples. This uncertainty may have contributed to the rather high standard deviation of the regression lines in the normal infants which varied from 0.006 to 0.046 COHb per cent saturation per hour (equivalent to a measured \( \dot{V}_{CO} \) of 1.3–9.9 \( \mu l/kg \) per hr). The high initial COHb in infant Con (Table II) may have been influenced by maternal smoking, but the elevated \( \dot{V}_{CO} \) reflected hemolysis as indicated by the reticulocyte count of 22%.

The calculation of MLS (equation 2) assumes that a steady state existed. Normal newborn infants may show significant changes in blood volume and hematocrit during the first 24 hr of life mainly due to changes in plasma volume (31, 37, 38), but red cell volume remains stable during the first 3 days of life (31) and no real decrease in hemoglobin concentration occurs until some time between the 1st and 3rd wk of life (39). In view of

**TABLE 1**

| Infant | Gestation | Age | Weight | Reticulocytes | Hemo- | Dilution | Initial | Hourly | Mean red |
|--------|-----------|-----|--------|--------------|--------|----------|---------|--------| cell life |
|        | wk        | hr  | kg     | %            | globin | factor   | COHb    | increase | span     |
|        |           |     |        | %          | ml     | %        | %       | \( \mu l/kg/hr \) |        |
| Faz    | 41        | 27  | 1.16   | 6.0        | 19.3   | 66.1     | 0.545   | 0.059   | 12.3     | 93       |
| Blo    | 40        | 29  | 3.28   | 5.4        | 19.3   | 79.0     | 0.567   | 0.089   | 21.5     | 61       |
| Fit    | 41        | 38  | 3.57   | 7.4        | 21.0   | 98.3     | 0.494   | 0.052   | 14.5     | 105      |
| Wal    | 40        | 40  | 3.36   | 5.6        | 19.6   | 72.1     | 0.447*  | 0.066   | 14.1     | 83       |
| Bra    | 41        | 48  | 3.23   | 5.4        | 19.5   | 53.5     | 0.610   | 0.052   | 8.6      | 106      |
| Wo     | 40        | 54  | 3.32   | 4.8        | 18.9   | 75.2†    | 0.405   | 0.071   | 16.0     | 77       |
| Wil    | 41        | 55  | 3.52   | 4.8        | 19.3   | 82.0†    | 0.791   | 0.058   | 13.5     | 95       |
| Sia    | 40        | 55  | 3.54   | 7.0        | 16.5   | 71.5     | 0.636   | 0.056   | 11.3     | 98       |
| Gai    | 40        | 57  | 3.29   | 8.0        | 16.5   | 51.2     | 0.580*  | 0.073   | 11.3     | 75       |
| Mean ±SD | 40.4  | 44.8| 3.36   | 6.0±       | 18.9±  | 72.1±    | 0.564±  | 0.064±  | 13.7±   | 88.1±    |

COHb = per cent saturation of hemoglobin with CO; \( \dot{V}_{CO} \) = CO production.
* Mother a smoker.
† Calculated from estimated blood volume based on venous hematocrit (31).
TABLE II
CO Production in Erythroblastotic Infants

<table>
<thead>
<tr>
<th>Infant</th>
<th>Blood group incompatibility</th>
<th>Gestation wk</th>
<th>Age hr</th>
<th>Weight kg</th>
<th>Reticulocytes %</th>
<th>Hemoglobin g/100 ml</th>
<th>COHb mL</th>
<th>Dilution factor</th>
<th>Initial COHb %</th>
<th>Hourly increase in COHb µl/kg/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sul</td>
<td>Rh</td>
<td>38</td>
<td>2</td>
<td>2.98</td>
<td>14.6</td>
<td>12.8</td>
<td>38.4</td>
<td>1.131</td>
<td>0.286</td>
<td>36.9</td>
</tr>
<tr>
<td>Lam</td>
<td>Rh</td>
<td>36</td>
<td>3</td>
<td>2.75</td>
<td>10.8</td>
<td>11.5</td>
<td>31.7</td>
<td>0.610</td>
<td>0.355</td>
<td>40.9</td>
</tr>
<tr>
<td>Cam</td>
<td>A-O</td>
<td>37</td>
<td>3</td>
<td>3.60</td>
<td>17.3</td>
<td>15.8</td>
<td>60.9</td>
<td>1.502</td>
<td>0.434</td>
<td>73.5</td>
</tr>
<tr>
<td>Con</td>
<td>Rh</td>
<td>37</td>
<td>5</td>
<td>2.68</td>
<td>22.2</td>
<td>15.5</td>
<td>41.7</td>
<td>5.739†</td>
<td>0.992</td>
<td>154.3</td>
</tr>
<tr>
<td>Mean values</td>
<td></td>
<td>37</td>
<td>3.2</td>
<td>3.00</td>
<td>16.2</td>
<td>13.9</td>
<td>43.2</td>
<td>2.246</td>
<td>0.517</td>
<td>76.4</td>
</tr>
</tbody>
</table>

COHb = per cent saturation of hemoglobin with CO; \( \bar{V}_{CO} \) = CO production.

* Calculated from estimated blood volume of 75 ml/kg (31, 32).
† Mother a smoker.

This and the brief duration of the study, the steady-state assumption is probably valid.

The determination of total circulating hemoglobin from CO dilution assumes that CO dilution measures circulating red cell volume. Red cell volumes measured by the CO method are 6–16% higher than that measured by the use of \( ^{51} \text{Cr} \) (30, 40). This has been attributed to the binding of some of the administered CO by extravascular substances, chiefly myoglobin. No simultaneous measurements using CO and \( ^{51} \text{Cr} \) have been performed in newborn infants. The newborn has a relatively small muscle mass and probably less myoglobin relative to his hemoglobin mass; therefore, we did not apply a correction for nonhemoglobin binding of CO. The calculated mean red cell volume in the seven infants in whom CO dilution was measured was 45 ml/kg which is only 7% greater than the figure of 41.9 ml/kg calculated from the data of Mollison, Veall, and Cutbush in 33 infants with hematocrits between 40.2 and 66.2% (29).

The turnover of bilirubin has not previously been measured in newborn infants because of the difficulty in achieving steady-state conditions and the undesirability of administering labeled bilirubin to infants. Bilirubin...
is mainly produced by catabolism of circulating hemo-
globin, but also by heme turnover in several other areas
including the bone marrow and any tissue containing
molecules with heme as a prosthetic group (41), of
which the liver appears to be the most important source
(42-44). The bilirubin from sources other than circulat-
ing hemoglobin is commonly referred to as the “early
labeled peak” (44, 45). Our calculations of bilirubin
turnover depend on the assumption that the production
of CO and bilirubin are proportional. Engel, Berk,
Rodkey, Howe, and Berlin (27) measured $V_{CO}$ and
endogenous bilirubin production in normal subjects and
patients with hemolytic disease. They found an excellent
correlation ($r = 0.96$) between $V_{CO}$ and bilirubin
production.

An early labeled CO peak as well as early labeled
stercobilin has been demonstrated in patients with in-
effective erythropoiesis (45). Two studies have been
reported concerning the early labeled peak in newborn
infants. Vest, Strebel, and Hauenstein (26), using
glycine-$^{15}$N in two full-term infants calculated that at
least 21-25% of bile pigment excreted in the feces was
not derived from senescent erythrocytes. Vest (33)
further reported that in two premature infants this
fraction was more than 30%.

Jaundice in the normal newborn infant has been
attributed to the inability of the liver to conjugate
bilirubin due to decreased activity of the glucuronyl-
transferase enzyme (46-48) in the face of a relatively
“normal” rate of bilirubin production. However, our
results indicate that normal newborns produce bilirubin
at more than twice the adult rate (per kilogram per
24 hr). Recent studies have suggested that inability to
conjugate bilirubin may not be the most important
rate limiting step in the excretion of bilirubin in the
newborn (49-51). Failure of bilirubin uptake and
excretion (51) and increased bilirubin production
possibly play important roles in this complex problem.
Adults produce about 250 mg of bilirubin per day (52)
(3.6 mg/kg per day), and the adult’s liver may be
capable of excreting 10 times the normal rate of
bilirubin (53). Billing, Cole, and Lathe (54) calculated
that small newborn infants have only 1-2% of the
normal adult capacity for bilirubin excretion. Pearson
(34) has calculated bilirubin production from red cell
survival studies in newborns and points out that, based
on serum bilirubin values normally found on the 3rd day
of life, the liver in the newborn infant must have “con-
siderable ability to conjugate and excrete bilirubin.”
Our findings support this conclusion and suggest that
normal full-term infants have at least 15% of the adult
capacity for bilirubin excretion.

We did not calculate bilirubin turnover in the erythro-
blastotic infants because of rapidly changing bilirubin
values and uncertainty regarding albumin binding
capacity in these infants. In the normal infants (maxi-
num serum bilirubin, 8 mg/100 ml), the primary bind-
ing sites for bilirubin on albumin would not be saturated
(55). Our calculations of bilirubin turnover do not con-
sider the possibility of an enterohepatic circulation of
bilirubin in the newborn (56). If such a circulation con-
tributes significantly to the bilirubin load, the ability
of the newborn to excrete bilirubin must be even greater
than our calculations imply.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge the technical assistance
of Miss Ruth Cherry, Miss Marilyn Boyer, and Miss Josephine
Neveska. We are grateful to Doctors W. D. Cochran, M. Sears,
and D. Muirhead of the Boston Hospital for Women for
permission to study their patients, and to Dr. S. Vivona,
Walter Reed Army Institute of Research, for his statistical
advice. We also wish to thank SSG Roland Lewis and
the Medical Audio-Visual Department, Walter Reed Army
Institute of Research, for their help.

This work was supported by U. S. Public Health Service
grants HD 00050, HD 02777, and Tl-Am05581 and by a grant
from the John A. Hartford Foundation, Inc. Dr. Nelson is the
recipient of a Research Career Development Award from the
National Institute of Child Health and Human Development.
Dr. Nathan is the recipient of U. S. Public Health Service
Research Career Development Award K03 AM35361.

REFERENCES

Invest. 42: 1172.

Effect of erythrocyte destruction on carbon monoxide pro-

1967. The production of carbon monoxide from hemoglobin

4. Sjöström, T. 1949. Endogenous formation of carbon mon-
oxide in man under normal and pathological conditions.

5. Sjöström, T. 1951. Endogenous formation of carbon mon-
oxide. The CO concentration in the inspired and expired air

6. Sjöström, T. 1951. The in vivo formation of carbon mon-

levels in hemolytic disease of the newborn. J. Pediat.
61: 709.

of carbon monoxide in newborn infants. I. Nonicteric and
icteric infants without blood group incompatibility. Acta

of carbon monoxide in newborn infants. II. Rh hemolytic

of carbon monoxide in newborn infants. III. ABO incompat-

11. Coburn, R. F., R. E. Forster, and P. B. Kane. 1965. Con-
siderations of the physiological variables that determine the

6  M. J. Maisels, A. Pathak, N. M. Nelson, D. G. Nathan, and C. A. Smith


