

Endogenous Production of Carbon Monoxide in Normal and Erythroblastotic Newborn Infants

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ABSTRACT The endogenous production of carbon monoxide (\dot{V}_{CO}) in newborn infants was measured by serial determinations of blood carboxyhemoglobin during rebreathing in a closed system. Mean \dot{V}_{CO} in nine full-term infants was $13.7 \pm 3.6 \mu\text{l CO/kg per hr (SD)}$, and in four erythroblastotic infants \dot{V}_{CO} ranged from 37 to $154 \mu\text{l CO/kg per hr}$ preceding exchange transfusion. Mean red cell life-span (MLS) and total bilirubin production were calculated from \dot{V}_{CO} . MLS in normal newborns was 88 ± 15 days (SD), and bilirubin production was $8.5 \pm 2.3 \text{ mg/kg per 24 hr}$. This is more than twice 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 $5.6 \pm 2.3 \text{ mg/kg per 24 hr (SD)}$ as calculated from the bilirubin production and the measured rise in serum bilirubin concentration.

The measurement of \dot{V}_{CO} 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-

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 (\dot{V}_{CO}) resulting from increased hemoglobin destruction. Although a correlation has, indeed, been shown to exist between blood COHb and \dot{V}_{CO} (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 \dot{V}_{CO} , the critical measurement, and the rate of CO excretion via the lung (11).

We have adapted Coburn's rebreathing system for the measurement of \dot{V}_{CO} (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 cuff² (a) which seals against the face and the surrounding plastic ring (b) onto which a lid is sealed by spring clips. A stop cock (c) 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

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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.

² Dunlop Company, Ltd., Manchester, England.

this rate of flow through the CO₂ absorbent (f) (650 ml of Baralyme)³ 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 CO 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 study⁴ 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 anaerobically 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, leaks⁵ 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 gas⁶ 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 hemolyzing 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

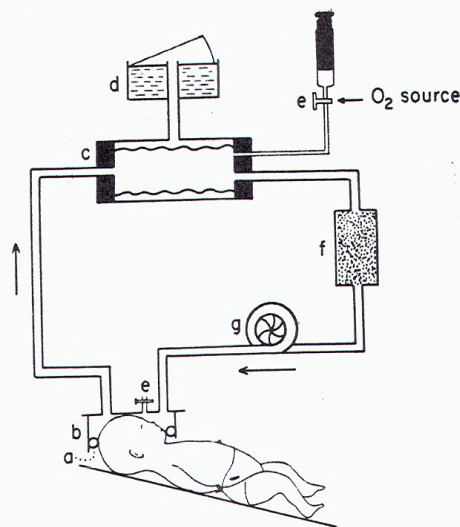


FIGURE 1 Rebreathing circuit showing pneumatic cuff (a), plastic ring (b), plastic tube with thin-walled rubber tube (c), spirometer (d), stopcocks (e), CO₂ absorbent (f), variable speed blower (g), and oxygen source.

stopcock of the microgasometer is connected to two limbs of a four-arm, four-way stopcock. Blood samples of 0.24 ml were drawn up followed by reagents in the following order: 0.025 ml of 1 N lactic acid, 0.024 ml of 10% potassium ferricyanide, 0.1 ml of a solution containing two parts 30% Triton X-100 in saline and one part caprylic alcohol. After the sample and reagents were brought down into the lower reaction chamber under vacuum, the mixtures were agitated for 4 min using the automatic shaker. The extracted gas was then transferred into the carrier stream of helium by the method described by Farhi et al. (22). Helium at a flow of 50 ml/min carried the gas through the water⁷ and CO₂⁸ absorbers into the gas chromatograph. Oxygen, nitrogen, and CO were separated on a $\frac{1}{8}$ inch \times 6 ft column of 13x molecular sieves. Column (and detector) temperature and pressure were 30°C and 10 PSIG, respectively. Voltage change across the thermistor detector bridge was recorded by a 1 mv potentiometric recorder (E. 11. Sargent & Co., Springfield, N. J.; model S-72180-25).

The apparatus was calibrated by introducing a 3 ml sample of CO⁹ of known concentration into the microgasometer and thence to the chromatograph. The blood COHb saturation was calculated by dividing CO content in milliliter per 100 ml (STPD) by the CO capacity. The CO capacity was determined by multiplying the hemoglobin concentration in grams per 100 ml by 1.34 (23). All analyses were performed in duplicate or triplicate. SD of the analysis was $\pm 0.04\%$ COHb (coefficient of variation 1.7%). Hemoglobin was measured as cyanmethemoglobin (24). Serum bilirubin concentration was measured by the method of White, Haidar, and Rheinhold (25).

⁷ Drierite; W. A. Hammond Drierite Company, Xenia, Ohio.

⁸ Ascarite; Arthur H. Thomas Co., Philadelphia, Pa.

⁹ CO 0.0950%; analyzed by and obtained from Matheson Co., Inc., East Rutherford, N. J.

³ Baralyme^R is obtainable from Warren E. Collins, Inc., Braintree, Mass.

⁴ IL 113 pH/gas analyzer PO₂ electrode; Instrumentation Laboratory, Inc., Watertown, Mass.

⁵ 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.

⁶ CO 99.5%; Matheson Co., Inc., East Rutherford, N. J.

Calculations

CO production. The \dot{V}_{CO} was calculated according to the equation

$$\dot{V}_{CO} = \Delta COHb\% \times \frac{CO_D}{\Delta COHb\%_D} \quad (1)$$

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 $CO_D/\Delta COHb\%_D$ 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 ($\Delta COHb\%_D$).

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

$$MLS \text{ (days)} = \frac{T_{heme} \text{ (}\mu\text{moles)}}{\dot{V}_{heme} \text{ (}\mu\text{moles/24 hr)}} \quad (2)$$

where T_{heme} is the total circulating heme and \dot{V}_{heme} is the rate of breakdown of circulating heme (17).

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

$$T_{heme} \text{ (}\mu\text{moles)} = \frac{gT_{Hb}}{0.017} \quad (3)$$

where the factor 0.017 is grams of hemoglobin per μ mole.

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:

$$MLS_{corr} = \frac{gT_{Hb}/0.017}{0.75 \times \dot{V}_{CO} \times 44.6 \times 24} \quad (4)$$

where \dot{V}_{CO} is in milliliters per hour STPD, T_{Hb} 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

$$MLS_{corr} = \frac{5.46 \times 10^{-2}}{\Delta COHb\%} \quad (5)$$

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} (μ moles/hr) and the molecular weight of bilirubin:

$$\text{Bilirubin production (mg/kg per 24 hr)} = \frac{\dot{V}_{CO} \text{ (}\mu\text{moles/hr)} \times 0.585 \times 24}{\text{body weight (kg)}} \quad (6)$$

where the factor 0.585 is the mg of bilirubin per μ mole.

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 ($0.87 \times$ venous hematocrit) (29). RCV was determined from CO dilution and the hematocrit (30).

$$RCV \text{ ml} = \frac{CO_D}{\text{measured increase in CO ml/ml}} \times \text{hematocrit.} \quad (7)$$

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 $13.7 \pm 3.6 \mu\text{l CO/kg per hr}$ (SD). In erythroblastotic infants \dot{V}_{CO} ranged from 37 to $154 \mu\text{l CO/kg per hr}$. 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 $8.5 \pm 2.32 \text{ mg/kg per 24 hr}$ (SD), and mean excretion was $5.6 \pm 2.29 \text{ mg/kg per 24 hr}$ (SD). Bilirubin production in the erythroblastotic infants ranged from 23 to 96 mg/kg per 24 hr.

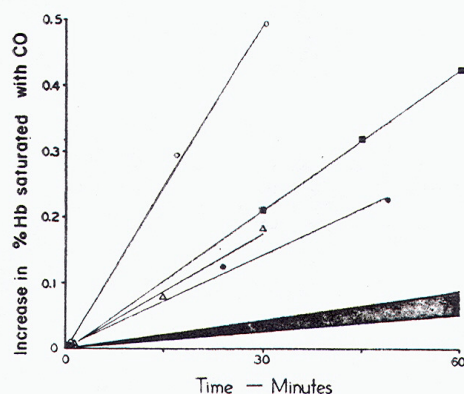


FIGURE 2 Increase in per cent hemoglobin saturated with CO in four infants with erythroblastosis fetalis in the rebreathing system. Regression lines were drawn by the least square method. Shaded area represents range of values found in nine normal full-term infants. Infants (see Table II): Con, open circles; Cam, squares; Lam, triangles; Sul, closed circles.

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 circula-

tion (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 μ 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 I
CO Production in Normal Infants

Infant	Gestation	Age	Weight	Reticu- lo- cytes	Hemo- globin	Dilution factor	Initial COHb	Hourly increase in COHb	\dot{V}_{CO}	Mean red cell life span
	wk	hr	kg	%	g./100 ml	ml	%	%	μ l./kg./hr	days
Faz	41	27	3.16	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
Sla	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 \pm SD	40.4	44.8	3.36	6.0 \pm	18.9 \pm	72.1 \pm	0.564 \pm	0.064 \pm	13.7 \pm	88.1 \pm
				1.16	1.47	14.41	0.114	0.0118	3.64	15.1

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

Infant	Blood group incompatibility	Gestation	Age	Weight	Reticulocytes	Hemoglobin	Dilution factor	Initial COHb	Hourly increase in COHb	\dot{V}_{CO}
		<i>wk</i>	<i>hr</i>	<i>kg</i>	<i>%</i>	<i>g/100 ml</i>	<i>ml</i>	<i>%</i>	<i>%</i>	<i>$\mu\text{l/kg/hr}$</i>
Sul	Rh	38	2	2.98	14.6	12.8	38.4*	1.131	0.286	36.9
Lam	Rh	36	3	2.75	10.8	11.5	31.7*	0.610	0.355	40.9
Cam	A-O	37	3	3.60	17.3	15.8	60.9	1.502	0.434	73.5
Con	Rh	37	5	2.68	22.2	15.5	41.7	5.739†	0.992	154.3
Mean values		37	3.2	3.00	16.2	13.9	43.2	2.246	0.517	76.4

COHb = per cent saturation of hemoglobin with CO; \dot{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}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}Cr have been performed in newborn infants. The newborn has a relatively small muscle mass and probably less myo-

globin 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

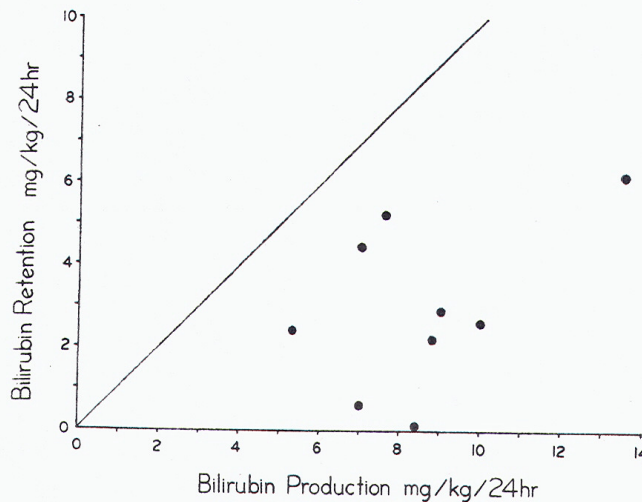


FIGURE 3 Bilirubin production plotted against retention in nine normal full-term infants (closed circles). The line drawn is that on which points would fall if production equaled retention (i.e., no excretion of bilirubin occurring).

is mainly produced by catabolism of circulating hemoglobin, 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 circulating 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 \dot{V}_{CO} and endogenous bilirubin production in normal subjects and patients with hemolytic disease. They found an excellent correlation ($r = 0.96$) between \dot{V}_{CO} and bilirubin production.

An early labeled CO peak as well as early labeled stercobilin has been demonstrated in patients with ineffective erythropoiesis (45). Two studies have been reported concerning the early labeled peak in newborn infants. Vest, Strebelt, 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 "considerable 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 erythroblastic infants because of rapidly changing bilirubin

values and uncertainty regarding albumin binding capacity in these infants. In the normal infants (maximum serum bilirubin, 8 mg/100 ml), the primary binding sites for bilirubin on albumin would not be saturated (55). Our calculations of bilirubin turnover do not consider the possibility of an enterohepatic circulation of bilirubin in the newborn (56). If such a circulation contributes significantly to the bilirubin load, the ability of the newborn to excrete bilirubin must be even greater than our calculations imply.

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