Bilirubin: Acute Effects in Newborn Rhesus Monkeys

Abstract. *Sustained concentrations of serum bilirubin of over 30 mg/100 ml were associated with decreased concentrations of molecular oxygen, measured polarographically in the peritoneum of infant monkeys. This effect may precede the intracellular action of bilirubin and contribute to the greater severity of experimental manifestations of hyperbilirubinemia with antecedent asphyxia than of asphyxia alone.*

The observation that bilirubin decreases oxygen uptake and uncouples oxidative phosphorylation was documented in experiments with tissue homogenates, tissue cultures, and unicellular animals (*1*); its toxicity in vivo is assumed to be a consequence of these effects. The similarities between human infants and newborn monkeys with respect to bilirubin metabolism have been noted (*2, 3*). Experimentally, production of hyperbilirubinemia did not result in kernicterus in newborn monkeys, but hyperbilirubinemia following a period of asphyxiation did so (*4*). This prompted us to conduct the following study in vivo.

Four healthy 2- to 5-hour-old monkeys (*Macaca mulatta*), delivered by cesarean section, and one 2-week-old spontaneously born monkey were selected from a caged breeding colony; pregnancies were uncomplicated, and the infants required no resuscitation.

The animals were restrained on a soft towel over a heating pad, a 2-cm right paramedial incision was made under procaine anesthesia (without epinephrine), and the peritoneum was opened. A fine platinum oxygen electrode (*5*) was placed beneath loops of bowel, and the wound was closed in layers, fixing the electrode in place. Catheters were inserted into the umbilical vein of the newborn monkeys and into a femoral vein of the 2-week-old animal.

We used the polarographic technique in which continual measurement is made of the diffusion current produced by electroreduction of molecular oxygen at a platinum cathode (*6*). This diffusion current is a function of the number of oxygen molecules reaching the active surface of the cathode; the electrode and its calibration have been described in detail (*7*). A 1-hour stabilization period was allowed before starting each experiment and the calibration before and after each experiment did not vary significantly. The variability in the electrode measurements in the peritoneal fluid both before and after infusion was no greater than ± 5 percent in any experiment.

Records were obtained from an electrocardiograph (ECG) by arm-leg leads, and the heart rate was obtained from a cardiotachometer fed by the R waves of the ECG. The respiratory rate and temperature were obtained with physiological pressure transducers and thermister probes (*8*). All recordings were made simultaneously and continuously on a polygraph (*8*), and steady base lines were recorded for all measurements for the hour prior to infusing the bilirubin solution.

Crystalline bilirubin (*9*) was dissolved in an aqueous solution of 0.52 g of NaCl and 0.5 g of Na_2CO_3 to provide a solution containing 200 mg/100 ml. Infusions of 2 to 4 mg/100 g were administered through the catheter in umbilical or femoral veins. Previous studies of bilirubin tolerance in monkey infants had indicated that this dose was sufficient to maintain high concentrations of the substance in the serum (*3*).

Before and after each infusion, peripheral venous samples were obtained so that the indirect-reacting bilirubin could be determined by the Malloy-Hsia method (*10*) adapted for ultramicrotechniques (*11*). Experiments lasted 3.5 to 10 hours, after which the animals, still in good condition, were killed rapidly by perfusion-fixation with a solution containing formaldehyde (*12*). In two additional newborn animals and in a 1-week-old animal, identical experiments were conducted over 8 to 10 hour periods, with the NaCl-Na_2CO_3 aqueous solvent being infused without bilirubin in volumes corresponding to the bilirubin infusions on a weight basis.

Within 15 minutes after the bilirubin infusions which maintained bilirubin concentrations in the serum between 30 and 45 mg/100 ml, the concentration of oxygen molecules, as measured by the electrode, decreased; by 1 hour this concentration had stabilized at the lower level (Table 1). This decrease was associated with no significant changes in heart rate, rectal temperature, or respiratory rate. Infusion of the NaCl-Na_2CO_3 solvent without bilirubin did not affect the concentration of oxygen molecules.

Postmortem examination of the animals infused with bilirubin revealed that the tissues, including the peritoneum, were stained yellow. The brains had a diffuse yellow color without gross staining of nuclei. Histological sections showed intravascular deposition of bilirubin crystals, but no bilirubin was observed within cells. No cellular alterations such as chromatolysis were observed in the brains of these animals.

The elevation of bilirubin concentrations in the serum to over 30 mg/100 ml alone resulted in a decrease in the oxygen concentration in the peritoneal fluid as estimated by the polarographic technique. This concentration may have induced capillary vasoconstriction, thus accounting for the trapping of bilirubin crystals in peripheral vessels as well as the decrease in the concentration of oxygen molecules measured by the electrode. We cannot exclude the possibility that this decrease in oxygen resulted from a decrease in cardiac output or ventilation, though it seems unlikely in these preparations

Table 1. Decrease in oxygen concentration 1 hour after infusion of bilirubin solution. All body temperatures were recorded from a rectal probe. The basal temperature was the rectal temperature recorded during the 1-hour stabilization period. The environmental temperature was maintained at 30 to 31°C. The percentage decrease in O_2 is the change from the base line polarographic O_2 recording (100 percent).

	Before infusion			After infusion			
Monkey No.	Body temp. (°C)	Heart rate (per min)	Resp. rate (per min)	Body temp. (°C)	Heart rate (per min)	Resp. rate (per min)	Decrease in O_2 (%)
282	31.5	215–225	95–110	31.5	180–220	105–180	42
279	34.0	170–180	70–80	34.8	160–188	72–80	54
280	36.0	255–270	75–88	37.0	240–290	120–165	20
271	35.0	215–230	80–95	35.1	210–225	80–90	32
276	33.0	180–215	82–90	33.2	180–215	80–90	30

(Table 1). Furthermore, bilirubin solutions do not decrease the oxygen-carrying capacity of intact red cells (*13*).

The magnitude of the Bohr effects in human fetal hemoglobin and that of rhesus monkeys are similar (*14*), but the fact that the infusions of alkaline solvent alone did not change the oxygen concentration measured by the electrode indicates that the lowered oxygen concentration in the tissues was not secondary to a shift in the dissociation curve for hemoglobin caused by a change in *p*H.

Kernicterus was reproduced in newborn monkeys by combining the effects of hyperbilirubinemia with antecedent injury—that is, asphyxia, and the clinical manifestations of it were more severe than those produced by asphyxia alone (*4*, p. 56). In conjunction with those observations, our findings support a hypothesis that high serum bilirubin concentration in vivo may result in a decrease in the concentration of oxygen in the tissues, and thereby make cells more susceptible to the inward diffusion of bilirubin. This effect may precede the intracellular action of bilirubin on oxygen uptake and phosphorylation.

RICHARD E. BEHRMAN
Department of Pediatrics,
Johns Hopkins University,
Baltimore, Maryland

EMERSON HIBBARD
Department of Biology,
California Institute of Technology,
Pasadena

References and Notes

1. R. Day, *Pediatrics* **17**, 925 (1956).
2. R. Lester, R. E. Behrman, J. F. Lucey, *ibid.* **32**, 416 (1963).
3. J. F. Lucey, R. E. Behrman, A. L. Warshaw, *Am. J. Diseases Children* **106**, 350 (1963).
4. J. F. Lucey, R. E. Behrman, E. Hibbard, E. O. Esquivel de Gallardo, W. F. Windle, *Exptl. Neurol.* **9**, 43 (1964).
5. Siliclad.
6. L. E. Clark, G. A. Misrahy, R. P. Fox, *J. Appl. Physiol.* **3**, 85 (1958); G. A. Misrahy, A. V. Berans, J. F. Spradley, V. P. Garwood, *Am. J. Physiol.* **199**, 959 (1960).
7. S. V. Pose, R. Caldeyro-Barcia, R. E. Behrman, *Apartado del Libro: V Congreso Medico del Uruguay* **3**, 1183 (1962).
8. Sanborn Co.
9. Eastman Kodak Co.
10. H. T. Malloy and K. A. Evelyn, *J. Biol. Chem.* **19**, 481 (1937).
11. Spinco and Coleman.
12. C. J. Bailey and W. F. Windle, *Exptl. Neurol.* **1**, 467 (1959).
13. A. Abrahamov, *Kernicterus* (Univ. of Toronto Press, Toronto, 1951), p. 79.
14. R. E. Behrman, C. J. Heller, F. C. Battaglia, A. E. Hellegers, *Quart. J. Exptl. Physiol.* **48**, 258 (1963).
15. The experiments were performed in the Laboratory of Perinatal Physiology, National Institute of Neurological Diseases and Blindness, San Juan, Puerto Rico, and Bethesda, Maryland.

17 March 1963

Transpiration: Its Effects on Plant Leaf Temperature

Abstract. *A definite and significant temperature gradient exists over the area of a tomato leaflet and is apparently due to the variation in transpiration across the leaflet. Transpiration is most effective at low velocities of air movement, but when the velocity increases, convection becomes the dominant factor. Leaves in which the stomata are allowed to open naturally in response to light attain temperatures about 5°C lower than leaves in which the stomata are forced to remain closed.*

Despite the fact that the phenomenon of transpiration has been the object of a great deal of study (*1*) there remains some uncertainty about the magnitude of its role in determining the temperature of the plant leaf. Schull (*2*) concluded that transpiration played a significant part in temperature regulation since he found it to be the foremost means of energy dissipation. Curtis and Clark (*3*), however, stated that the influence of transpiration on leaf temperature is slight. Similarly, Ansari and Loomis (*4*) concluded that the effects of transpiration are small, and that convection and radiation are the major factors in heat exchange. Most recently, Wolpert (*5*), through a theoretical analysis, showed that under normal conditions transpiration could account for approximately one-fourth of the heat removed from a leaf, indicating that transpiration is at least a significant factor in the heat exchange phenomena of leaves.

In view of this lack of agreement, additional work on the role played by transpiration in the temperature regulation of plant leaves was necessary. We carried out such a study using the tomato plant, *Lycopersicon esculentum*, L. Leaf temperature was determined by inserting a thermocouple made from two 36-gauge wires under the lower epidermal surface of the tomato leaflet, the insertion depth being about 1 cm in a direction parallel to the leaf surface. This insertion technique caused some local cell damage but, in comparison with other techniques, proved to be most accurate and reliable. The environment for these experiments was a growth chamber in which the relative humidity was maintained at a constant 50 percent. Air velocity and light were varied so that their individual effects on the leaf temperatures could be determined.

The existence of a temperature gradient across a tomato leaflet was established by measuring temperatures at five or six selected locations over the surface of several leaflets under otherwise constant conditions. Equivalent temperatures at corresponding locations were verified, so that the data from the several leaflets could be correlated. The results of a typical isothermal plot are illustrated in Fig. 1. Environmental conditions for these tests were 24.2°C, still air, and illumination at 660 lumen/m^2 (60 ft-ca). The temperature variation over the leaflet in this instance was approximately 1.0°C, the leaflet being coolest near the midrib and warmest near its extremities. With one notable exception, the results for all other leaflets tested under the same environmental conditions were virtually the same, within ±0.1°C. Those leaflets which had started to dry out and become yellow had a much smaller temperature gradient, often less than 0.5°C in magnitude. Also, the average temperature of these leaflets was nearer room temperature. No significant variation in leaf temperature (that is, no greater than 0.1°C) was noted from one leaflet to another, from one leaf to another, or from one plant to another when the temperatures were measured at corresponding locations on leaflets of tomato plants in the same condition.

Next, two series of tests were made in which a fan and ducting arrangement were used to produce a controlled velocity of air over the leaflets. In the first series of tests a high light intensity [24,750 lumen/m^2 (2250 ft-ca)] was obtained by using a 1000-watt incandescent bulb in a reflector placed about 30 cm from the leaflet. In the second series of tests the light intensity was much lower, 605 lumen/m^2 (55 ft-ca), which is equivalent to normal indoor lighting. Since the air temperature was held constant for each test, it was a suitable reference with which to compare the changes in leaflet temperature. Thus, the results presented in Fig. 2 are the temperature differences between the leaflet and air, plotted against wind velocity.

In curve 1, Fig. 2, the equilibrium temperature is much lower than in curves 2 and 3, because the leaflet was heated to 21°C above the room temperature of 25°C by the incandescent bulb which caused some wilting. In curve 6 the response was more accentuated than in curves 4 and 5 because the thermocouple in this test