Red Cell 2,3-Diphosphoglycerate and Oxygen Affinity in Newborn Goats and Sheep (35992)

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The decrease in oxygen affinity of blood of lambs and kids during the first week after birth (1, 13, 14) has been ascribed to a decrease of the intracellular pH (9) and also to the increase in concentration of 2, 3-diphosphoglycerate (2,3-DPG), which is presumed to act by its direct interaction with hemoglobin and perhaps by lowering the intracellular pH (2). The change in oxygen affinity during this early postnatal period cannot be accounted for by any significant increase in adult hemoglobin (2, 3, 10, 11, 14). The present study was undertaken to determine in more detail the changes which occurred in the concentrations of 2,3-DPG in the red cells of young goats and sheep before and after birth, and to examine the interaction between 2,3-DPG and the hemoglobins of these animals.

Materials and Methods. Blood was obtained from kids and lambs at various intervals after birth. Fetal blood samples were collected during cesarean section before severing the umbilical vessels. 2,3-DPG was determined following chromatography of red cell extracts on Dowex I-X2 (200-400 mesh) by the method of Loos (12) and by the automated enzymatic procedure described by Grisolia *et al.* (8); agreement between the two methods was within 5%. The percentages of fetal hemoglobin (Hb F) were determined by DEAE Sephadex chromatography (11). The methods used for the determination of oxygen affinity of whole blood and hemolysates have been described previously (10). Hemolysates were freed from organic phosphates by dialysis against 0.2 *M* NaCl for 24 hr followed by dialysis against distilled water for a further 24 hr. The oxygen affinities of these stripped hemolysates were examined in 0.1 *M* phosphate at varying pH and pCO_2, and at 37°. 2,3-DPG (as the pentacyclohexylammonium salt) was added to hemolysates in a ratio of 2.0 moles/mole of Hb; use of the salt rather than free 2,3-DPG does not alter the results (5).

Results. The postnatal decrease in oxygen affinity, shown in Fig. 1, corresponds with results which have been described by other workers (1, 2, 10, 13, 14); in both species the P_{50} increased from 20 mm Hg in the fetus to 30 mm Hg in the adult. Blood samples from two fetal goats and one newborn goat 1 hr old, had almost identical oxygen affinities (P_{50} values: 21.8, 19.8, and 19.6 mm Hg, respectively); whereas the affinity of blood from the newborn goat, 6 days old, was identical with that of an adult goat (P_{50} values: 30.1 and 29.5 mm Hg, respectively); the age of the newborn sheep (Fig. 1, Sheep 2) was estimated at 12 hr and the P_{50} value of blood from this animal (28.8 mm Hg) was between the fetal and adult values (19.0 and 31.6 mm Hg, respectively).

In the 6 day old goat at least 70% of the total hemoglobin would be the fetal type and only 10 to 20% would be the β^C containing hemoglobins (Fig. 2a), which, in the goat, have an affinity for oxygen lower than the adult type (Fig. 1, Goat 6). This observation makes it improbable that the early decrease in oxygen affinity is directly related to the presence of these β^C hemoglobins.

In two goat fetuses, which were almost ready to be born, the concentrations of 2, 3-DPG were 7.3 and 7.4 μmoles/g of Hb; in younger fetus, which still lacked complete hair covering, the concentration was 6.4 μmoles/g Hb. Blood samples were taken from one newborn goat within 1 hr of birth and subsequently at 6 to 12 hr intervals. Immedi-

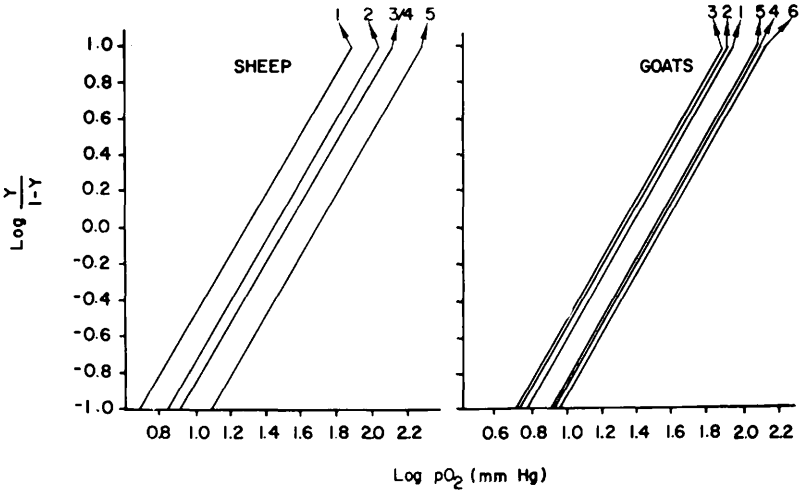

FIG. 1. Oxygen equilibrium curves of blood samples from fetal, newborn, and adult sheep, and goats with different hemoglobins: temp, 38°; pH 7.3. The logarithm of the ratio, saturated/unsaturated hemoglobin $\{\log [y/(1 - y)]\}$ is plotted against the logarithm of the oxygen tension [log pO_2 (mm Hg)]. Sheep: (1) fetal; (2) newborn; (3, 4) adult with Hb A or Hb C; (5) adult with Hb B. Goat: (1) young fetus; (2) fetus near term; (3) newborn, 1 hr; (4) newborn, 6 days; (5) adult with Hb AB; (6) anemic adult with Hb C.

ately after birth and for the first 6 hr the amount of 2,3-DPG remained low (3.0–3.5 μmoles/g Hb), but increased abruptly to a maximum value of nearly 25.0 μmoles/g Hb by the third day (Fig. 2b). Adult values of less than 1.0 μmoles/g Hb were observed after 50 days.

The concentrations of 2,3-DPG were also determined in the red cells of 17 young goats and 22 young lambs varying in age from 3 to 55 days. Values greater than 20 μmoles/g Hb were observed in both species at 3 days, and they decreased concurrently with the decrease in the percentage of Hb F, so that adult values were recorded from about 40 days.

It is tempting to relate the decreased oxygen affinity to the increased concentrations

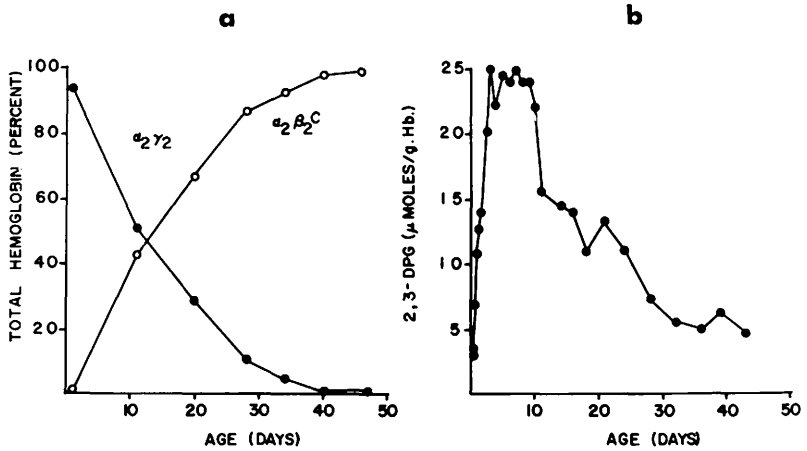

FIG. 2a. The changes in the percentages of γ and β^C containing hemoglobins in a newborn goat from birth to 50 days. (b) The changes in the concentrations of 2,3-diphosphoglycerate (μmoles/g Hb) in red cells of a newborn goat from within 1 hr after birth to 50 days.

TABLE I. The Effect of 2,3-DPG on the Oxygen Affinities of Goat Hemoglobins. The affinity is expressed as the log P_{50} at pH 6.8.

Conditions of expt.	log P_{50} at pH 6.8		
	Hb AB[a]	Hb C[a]	Hb F[a]
pCO_2: 36 mm Hg, no DPG added	1.64	1.69	1.34
pCO_2: 36 mm Hg, DPG added	1.69	1.72	1.39

[a] Hemoglobin concentration between 5 and 8 g/100 ml.

of 2,3-DPG. However, when 2,3-DPG was added to hemolysates of goats, which had been stripped of red cell phosphates, only a small effect was observed on the oxygen affinity (Table 1). In no case was the increase in the P_{50} value greater than 10%, and it occurred in the presence and absence of CO_2. No change in the Bohr effect was observed.

Discussion. The small increase in P_{50} after addition of 2,3-DPG to adult goat hemoglobins has also been observed by Bunn (4); our data indicate that goat Hb F reacts similarly. This observation is not unexpected because the comparable amino terminal residue of the human β chains which participates in the binding of 2,3-DPG [(4) and references quoted] is lacking in both the γ chain of goat Hb F and the β chain of goat Hb A, (15). It is possible that one role of 2,3-DPG in these young animals is to lower the intracellular pH as suggested by Battaglia *et al.* (2). However, even though 2,3-DPG does not apparently bind to goat Hb F *in vitro*, an additional mechanism whereby 2,3-DPG binds to the hemoglobin inside the red cell may also be considered.

The mechanism by which the oxygen affinity is reduced during the first 6 to 10 days after birth appears to be similar in both sheep and goats. Subsequently, the β^C containing hemoglobins become predominant in the young goat (Fig. 2a); whereas in young lambs Hb C is seldom over 5% of the total hemoglobins (11). Goat Hb C has a P_{50} value even higher than the normal adult hemoglobins whereas the P_{50} of sheep Hb A and Hb C are almost identical (Fig. 1). During the period of rapid growth and postnatal adjustment the young goat is served by a switch to the production of an emergency type of hemoglobin with a low oxygen affinity and high unloading capabilities; in the sheep with Hb A other forms of physiological compensation, such as increased cardiac output, have evolved (4, 5), while the very low affinity of sheep Hb B (Fig. 1, Sheep 5) may be adequate by itself.

Summary. Red cell 2,3-diphosphoglycerate is present in the red cells of fetal sheep and goats. Its concentration increases from 6 hr after birth to maximum values between 3 to 10 days. This increase occurs at the same time as a decrease in oxygen affinity. *In vitro* experiments failed to show a direct effect of 2,3-DPG on the oxygen affinity of fetal hemoglobin of goats.

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