

Mosaic Hemoglobin Types in a Pair of Cattle Twins

Abstract. Erythrocyte mosaicism for hemoglobin types is demonstrated in a pair of dizygotic cattle twins. Both twins were of hemoglobin type AB in the direct tests. However, when hemoglobin types were ascertained on the two populations of red cells comprising the mosaic, one population proved to be hemoglobin type A and the other, type B. Since the ratio of the two populations of red cells was approximately 60:40, there was no evidence from the direct tests suggesting mosaicism for hemoglobin types. The results, as might be expected on theoretical grounds, provide evidence that synthesis of hemoglobin of one type in a mixed clone is not influenced by synthesis of hemoglobin of another type.

Three hemoglobin phenotypes A, B, and AB controlled by a pair of co-dominant autosomal alleles *Hb<sup>A</sup>* and *Hb<sup>B</sup>* are recognized in cattle (1). The question has arisen whether dizygotic mosaic cattle twins (that is, dizygotic twins which share each other's blood-forming tissues as a consequence of chorionic vascular anastomosis) may exhibit mosaicism for hemoglobin types just as they do for blood types. The problem is readily amenable to solution, since it is possible by means of differential hemolysis (2) to separate from the same animal populations of red cells which differ in blood types. As an example, consider a mating in which both parents are of genotype *Hb<sup>A</sup>Hb<sup>B</sup>* and one of the resulting twins is of genotype *Hb<sup>A</sup>Hb<sup>A</sup>* and the other *Hb<sup>B</sup>Hb<sup>B</sup>*.

Some of the hematopoietic tissue in each twin would then be of genotype *Hb<sup>A</sup>Hb<sup>A</sup>* and some of genotype *Hb<sup>B</sup>Hb<sup>B</sup>*. If the red cells deriving from

each of the genotypically distinct tissues are produced in approximately equal numbers, we should expect that the hemoglobin type of each twin would be AB and would appear to be indistinguishable in electrophoretic pattern from AB derived solely from hematopoietic tissue of genotype *Hb<sup>A</sup>Hb<sup>B</sup>*. Our problem would then be to show that hemoglobin type AB in such twins is in reality a result of the mixing, in vivo, of red cells of hemoglobin types A and B. Such an example has now been found in a pair of purebred, female Jersey twins 2 years of age. We report here the demonstration of mosaic hemoglobin types in these twins.

The red cells of each twin were typed with our collection of cattle blood-typing reagents in accordance with routine procedures described elsewhere (3). Hemolysates were analyzed for hemoglobin types after the starch-gel method of Gahne *et al.* (4). The plasma samples were analyzed for transferrin

types by means of a starch-gel method described by Kristjansson (5). The results of these tests, which are summarized in Table 1, indicated that the twins were of like blood and hemoglobin types, with erythrocyte mosaicism indicated in tests with reagents for blood factors F<sub>1</sub> in the F-V system and R' in the R'-S' system. One of the twins (coded 750A) was of transferrin type AD and the other (750B) was type DD, thereby clearly excluding monozygosity (4).

When the red cells of each twin were subjected to differential hemolytic tests with F and R' reagents, it was shown that F antibodies lysed approximately 60 percent of the red cells in each twin whereas R' antibodies lysed approximately 40 percent, thereby indicating that one of the two populations of red cells possessed blood factor F<sub>1</sub> but not R' whereas the other possessed R' but not F<sub>1</sub>.

After differential hemolysis with F and R' reagents, the residual non-hemolyzed red cells were washed in saline solution. Samples were then subjected to blood typing tests and analysis of hemoglobin types. Table 2 shows that the red cells remaining after differential hemolysis with F antibodies were of hemoglobin type B whereas those remaining after hemolysis with R' reagent were of hemoglobin type A. Conversely, when the hemoglobin types were determined on the supernatants obtained after differential hemolysis with F and R' reagents, the types were A and B, respectively.

As already indicated, there was nothing from the direct analysis of hemoglobin types in these dizygotic mosaic twins which would suggest that one was actually of genotype *Hb<sup>A</sup>Hb<sup>A</sup>* and the other of genotype *Hb<sup>B</sup>Hb<sup>B</sup>*. This was because the ratio of the two kinds of red cells comprising the mosaic closely approximated 1 : 1. These results were essentially indistinguishable from results obtained on similar runs with a 1 : 1 pool of hemoglobin A and hemoglobin B. With ratios greater than, say, 2 : 1, we should expect that one of the two hemoglobin bands would appear somewhat thicker and more densely stained, thereby suggesting mosaicism in the direct tests.

The tests performed in this study give no indication as to which of the twins is of genotype *Hb<sup>A</sup>Hb<sup>A</sup>* and which is of genotype *Hb<sup>B</sup>Hb<sup>B</sup>*. As in the case of blood group genotypes (6), the geno-

Table 1. Blood, hemoglobin (Hb), and transferrin (Tf) types in a pair of dizygotic mosaic cattle twins with mosaicism in red cell types indicated (by F<sub>1</sub> and R') in tests for blood factors F<sub>1</sub> and R'.

Blood types in the genetic systems*										Hb and Tf types	
A	B	C	F-V	J	L	M	S	Z	R'-S'	Hb	Tf
Twin 750A											
A <sub>1</sub> H	BO <sub>x</sub> E' <sub>1</sub> I'	WX <sub>2</sub>	F <sub>1</sub> V <sub>1</sub>	-/-	-/-	-/-	H'	-/-	R'S'	AB	AD
Twin 750B											
A <sub>1</sub> H	BO <sub>x</sub> E' <sub>1</sub> I'	WX <sub>2</sub>	F <sub>1</sub> V <sub>1</sub>	-/-	-/-	-/-	H'	-/-	R'S'	AB	AA

\*The -/- symbol indicates no reactions in the indicated systems.

Table 2. Blood and hemoglobin (Hb) types of the two populations of red cells separated after differential hemolysis with antibodies for blood factors F and R', respectively. The two populations differed in blood factors F<sub>1</sub> and R' and in hemoglobin types.

Blood types in the genetic systems										Hb types
A	B	C	F-V	J	L	M	S	Z	R'-S'	
Cells recovered after lysis with F reagent										
A <sub>1</sub> H	BO <sub>x</sub> E' <sub>1</sub> I'	WX <sub>2</sub>	V <sub>1</sub> /V <sub>1</sub>	-/-	-/-	-/-	H'	-/-	R'/S'	B
Cells recovered after lysis with R' reagent										
A <sub>1</sub> H	BO <sub>x</sub> E' <sub>1</sub> I'	WX <sub>2</sub>	F <sub>1</sub> /V <sub>1</sub>	-/-	-/-	-/-	H'	-/-	S'/S'	A

types of cattle twins which exhibit mosaicism for hemoglobin types can be established only on the basis of breeding tests.

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4 May 1964

### Protein-Bound Iodine in Serum of Rats Breathing 99 Percent Oxygen

**Abstract.** Exposure of rats to 99 percent oxygen for 24 to 72 hours resulted in a significant fall in protein-bound iodine in serum. The fall was most prominent in the group treated for 72 hours and was not, however, associated with any detectable microscopic changes in thyroid structure.

The influence of the thyroid gland on oxygen toxicity was first noted in 1937 by Campbell (1), who described increased survival in thyroidectomized rats exposed to 6 atmospheres of oxygen, while administration of exogenous thyroxine to normal rats enhanced toxicity. Similar findings (2) have been reported with regard to the elevated mortality and pulmonary damage induced by increased oxygen concentrations at atmospheric pressure. In addition, the measurable degree of protection afforded rodents by hypophysectomy can be counteracted by the administration of thyroid extract (3).

Despite these implications of thyroid participation, determinations of endogenous glandular activity and morphology have to our knowledge not been reported in previous investigations on animals exposed to oxygen-enriched environments. Our study, representing part of an overall investigation on the effects of pure oxygen systems programmed for use in spacecraft, was therefore designed to evaluate the

protein-bound iodine (PBI) in serum and to study the thyroid histology in rodents inhaling 99 percent oxygen at 1 atmosphere pressure for periods of 24 to 72 hours.

Adult male albino rats (Wistar strain) weighing  $240 \pm 9$  grams were used throughout the study and maintained on a diet of Purina rat chow (containing 1 part of iodine per million) and water as desired. Initially groups of 5, 6, and 20 rats were placed for 24, 48, and 72 hours, respectively, in a closed-system (4) environmental chamber in which they were exposed to  $99 \pm 0.5$  percent oxygen at  $22^\circ$  to  $26^\circ\text{C}$  with a relative humidity of 41 to 57 percent. The experiment was then repeated with two groups of ten rats each, exposed for 24 and 48 hours, respectively, to conditions identical with those in the first series. The carbon dioxide was maintained at less than 0.2 percent by circulation of the chamber atmosphere through lithium hydroxide. Total pressure was kept at 25 mm-Hg above ambient to insure that all leaks should be "outboard." Immediately after removal from the chamber the rats were lightly anesthetized with diethyl ether, and blood was obtained from the abdominal aorta. A group of 24 rats maintained in individual cages in room air served as controls, and this group was treated identically. Serum PBI was determined by the method of Moran (5). Upon completion of aortic puncture, the thyroid was removed from each animal, fixed in formalin, sectioned, and stained with hematoxylin and eosin. The lungs were also examined for gross pathologic changes, and pleural fluid, if present, was withdrawn and its volume was measured.

The animals kept in oxygen for 48 hours or more appeared hyperpneic and lethargic (6), and at autopsy had characteristic diffuse areas of pulmonary consolidation. Ten of the rats died during the 72-hour-exposure period and were not used for blood or thyroid studies. Detectable pleural effusions amounting to 4 to 8 ml of serous fluid were noted in 80 percent of the rats surviving 72 hours in oxygen. Control animals in room air had no evidence of gross pulmonary changes or pleural effusion.

The results of the protein-bound iodine studies are summarized in Fig. 1. The data at 24 and 48 hours represent the combined results of two series of experiments. After only 24 hours, ex-

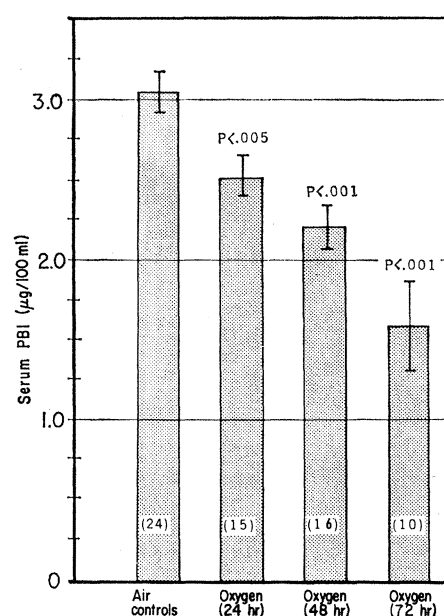


Fig. 1. Mean values for protein-bound iodine (PBI) in serum of control rats and rats exposed to 99 percent oxygen. The figures within each bar represent the number of rats in that group. Significance levels for differences from the control group are given above each bar. The vertical lines at the top of the bars represent the standard error (S.E.  $\pm 1$ ).

posure to 99 percent oxygen, a highly significant ( $P < .005$ ) fall in protein-bound iodine was noted. With longer exposure periods (48 and 72 hours), this decline became more striking and its statistical significance ( $P < .001$ ) increased. Microscopic examination of the excised thyroids revealed cuboidal epithelium and normal amounts of colloid with no detectable differences between air controls and the rats inhaling oxygen. However, the exposure periods (no more than 72 hours) may have been insufficient to allow development of detectable structural changes.

Diminution in protein-bound iodine may result from direct antithyroid action, suppression of thyrotropin secretion, or alteration in peripheral utilization and clearance of thyroid hormones. Reduction in thyroxine-binding capacity of plasma proteins may also be contributory. The mechanism responsible for the fall in hormone concentration accompanying oxygen inhalation remains to be determined.

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