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*POSSIBLE SOMATIC CELL MATING IN TWIN CATTLE WITH ERYTHROCYTE MOSAICISM**

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Erythrocyte mosaicism or chimerism in cattle twins is a condition in which there is a mixture of genetically different tissues forming antigenically distinct blood cells within an individual. Owen[1] proposed that vascular anastomoses between twin embryos permitted a reciprocal exchange of primordial hematopoietic tissues so that each twin possesses erythrocytes formed by its own tissues as well as those formed by tissue derived (transplanted) from its co-twin.[2–4] Presumably, tissues that give rise to histocompatibility antigens must be exchanged also, since dizygotic twins with erythrocyte mosaicism usually accept each other's skin grafts.[5, 6] Karyotypic chimerism[7] and transferrin chimerism[8, 9] may also exist. Erythrocyte mosaicism has been described in sheep,[10] humans,[11] chickens,[12] marmosets,[13] and mink.[14] It is of interest because of its significance to the phenomenon of immunologic tolerance[15] and "radiation chimerism."[16]

The proportions of the blood types in twins with erythrocyte mosaicism are essentially the same in each twin.[17–20] They may be equal in both twins or unequal so that one twin may possess more of its co-twin's blood type than of its own. The genotype of a twin can be recognized only by the blood types of its progeny.[21]

This paper presents some new observations on chimerism in cattle twins. Two phenomena have been discovered: (1) the proportion of the two cell types may change markedly with time, and (2) some kind of genetic exchange, possibly resulting from somatic cell mating, may occur between the hematopoietic tissues of the mixture yielding a cell type containing a new combination of antigens.

Materials and Methods.—The techniques of recognizing mosaicism of blood types in twins has been termed differential hemolysis.[17, 18, 22, 23] The proportions of the two cell types can be determined, following differential hemolysis, by a spectrophotometric method[18] which measures the fraction of cells not lysed by a given reagent.

Results.—*Changes in the proportions of mixed blood types:* It had been assumed that the proportions of the two cell types in chimeric twins remained constant. However, upon testing blood from chimeric twins tested previously, it was obvious that the proportions had changed. The results of differential hemolysis tests on ten sets of twins with erythrocyte mosaicism are shown in Table 1. The time

TABLE 1

RESULTS OF DIFFERENTIAL HEMOLYSIS TESTS SHOWING CHANGES IN PROPORTIONS OF ERYTHROCYTE TYPES IN CATTLE CHIMERIC TWINS*

Case no.	Twin no.	First Tests				Subsequent Tests			
		Ages	No. tests	Type I	Type II	Ages	No. tests	Type I	Type II
100	272A	1 mo–3 yr	2	90	10	8–11 yr	15	Nt	Nt†
	272B			90	10			2‡	2
137	645	2–3 mo	2	70	30	8–11 yr	2	5	95
	646			70	30			5	95
247	86	12–16 mo	3	80	20	4–9 yr	8	95	5
	88			80	20			95	5
379	186J	1–6 mo	3	80	20	4–6 yr	3	55	45
	187J			85	15			Nt	Nt
419	298	2 yr	1	65	35	3–5 yr	2	74	26
	299			65	35			70(75)§	30(25)§
453	22	15 mo	1	75	25	4 yr	1	75	25
	23			75	25			75	25
454	24	14 mo	1	55	45	4 yr	1	40	60
	25			55	45			25	75
455	26	6 mo	1	80	20	4 yr	1	93	7
	27			70	30			80	20
464	306	7 mo	1	93	7	15 mo–4 yr	2	88 (98)**	12 (2)**
	307			70	30			55	45
470	318	6 mo	1	50	50	1–4 yr	2	60	40
	319			75	25			85	15

* All twins are females except those of Case 100.
† Not tested—dead.
‡ A new type appeared, accounting for 96% of cells (see text).
§ At 3 yr = 70:30; at 5 yr = 75:25.
** At 15 mo = 88:12; at 4 yr = 98:2.

interval between tests varied from one to eight years. Only the twins of Case 453 showed no shift in the proportions of cells between the first and subsequent tests. The twins of Case 137 were tested at two and three months of age and contained a 70:30 mixture at each test. They were retested at eight and at 11 years of age, and at both times their bloods showed a substantial shift to a 5:95 proportion. One or both twins of six additional cases (247, 379, 454, 455, 464, and 470) showed a 10 per cent or greater change in the proportions of the two cell types between the first test and later tests. Twin no. 298 of Case 419 showed a 9 per cent shift, whereas its co-twin, no. 299, showed a 5 per cent shift when retested at three years and a 10 per cent shift in the same direction when tested again at five years. Finally, twin no. 306 of Case 464 showed a peculiar fluctuation, having shifted 5 per cent in one direction from the original when tested at 15 months and another 10 per cent in the opposite direction when tested again at four years of age, whereas its co-twin (307) changed 15 per cent at 15 months and was at the new level when retested at four years.

Experimental error cannot account for the changes noted, since duplicate tests at earlier and later ages with several reagents gave concordant results, and since most of the changes exceeded 10 per cent (at least twice the technical error[18]).

There is considerable variation in the magnitude of the changes observed, ranging from 5 to 65 per cent (excluding Case 100). Monthly or quarterly tests would be required to determine the age at which a change might be expected, and whether the change reaches a stable equilibrium.

It is important to note that the direction of the change was usually the same in *both* twins of a pair. Furthermore, the direction of the shift with respect to the initially predominant type (arbitrarily called Type I) seems random. There were twins in which the majority type at the time of the first tests remained the majority type at the time of subsequent tests despite an increase or decrease, and cases in which the majority type became the minority type at subsequent tests.

The occurrence of a new ("recombinant") cell type presumably from somatic cell mating: Twin 272B of Case 100 is especially interesting because at eight years of age the proportions of his blood types had changed from 90 per cent of Type I and 10 per cent of Type II to approximately 2 per cent of each type (Table 1); the remaining 96 per cent of the cells in this chimera were of a new type containing antigens on the same cell which were previously on different cells in the mixture. Unfortunately, the co-twin 272A was not available after the tests made at three years of age. These twins were routinely blood-typed at one month and again at three years of age. The abbreviated results of these tests and of tests on their parents are shown in Table 2. The twins had identical blood types with clear

TABLE 2

ORIGINAL BLOOD TYPES* OF BULL TWINS AND THEIR PARENTS—CASE 100

	Pertinent Genetic Systems			
	B	C	L	S
Sire 40A	$GY_2E_1'/(I_2)$†	C_1X_3/C_1X_1L'	$L/-$§	SH'/H'
Dam 42‡	$BO_1/(I_2)$	C_1X_3/WX_2	$L/-$	$H'/-$
Twin 272A	$\overset{\pm}{B}\overset{\pm}{O}_1/\overset{\pm}{G}\overset{\pm}{Y}_2\overset{\pm}{E}_1'/(I_2)$	$C_1\overset{\pm}{X}_1\overset{\pm}{L}'/\overset{\pm}{W}X_2/C_1X_3$	$\overset{\pm}{L}/$	$\overset{\pm}{S}H'/$
Twin 272B	$\overset{\pm}{B}\overset{\pm}{O}_1/\overset{\pm}{G}\overset{\pm}{Y}_2\overset{\pm}{E}_1'/(I_2)$	$C_1\overset{\pm}{X}_1\overset{\pm}{L}'/\overset{\pm}{W}X_2/C_1X_3$	$\overset{\pm}{L}/$	$\overset{\pm}{S}H'/$

* The ± indicates weak reaction suggesting erythrocyte mosaicism.
† The I_2 factor was assumed from progeny tests since reagent was not originally available.
‡ Dam 42 is also daughter of 40A.
§ Dash represents absence of known antigenic factor.

evidence of erythrocyte mosaicism at four independent blood group loci (B, C, L, and S systems). The phenogroups GY_2E_1', C_1X_1L', and SH' were transmitted only by the sire, whereas the phenogroups BO_1 and WX_2 were transmitted only by the dam. At the time of the first tests, the I_2 reagent was not available, but later tests showed that the cells of all four individuals of this family had I_2. Also, the dam (42) was the daughter of the sire (40A), and his genotype as shown was well established by progeny tests. Since 40A did not transmit the phenogroup GY_2E_1' to his daughter (42), he must have transmitted the alternative group I_2.

Erythrocyte mosaicism was confirmed by differential hemolysis tests made on bloods obtained from both twins at one month and at three years of age. As shown in Table 3, it was possible at that time, using each of 12 different reagents, to distinguish the two distinct cell populations in each twin. Following treatment of different samples of cells from each twin with the reagents for G, Y_2, or E_1' (B

TABLE 3

PERCENTAGE CELLS HEMOLYZED* AFTER TREATMENT WITH BLOOD-TYPING REAGENTS CASE 100—TWIN 272B†

System	B						C				L	S	
Reagent	G	Y_2	E_1'	B	O_1	I_2	C_1	X_1	L'	W	L	S	H'
Age: 1 mo–3 yr	10	10	10	90	90	NT‡	100	10	10	90	10	90	100
8–11 yr	98	98	98	98	98	4	100	98	98	98	98	98	100

* Results of two differential hemolysis tests within first age period and of 15 tests within second age period; two or more of each reagent were used in each test.
† Co-twin 272A was not available at second age period, but gave results as shown at first age period.
‡ Not tested.

system), 10 per cent of the cells were hemolyzed. Similar results were obtained following treatment with X_1, L', and L reagents. Therefore, one population of cells, representing 10 per cent, contained the antigenic factors G, Y_2, and E_1' of the B system, X_1 and L' of the C system, and L of the L system. Similarly, it was found that the other population of cells, representing 90 per cent, contained the factors B and O_1 of the B system, W of the C system, and S of the S system. Since 100 per cent of the cells were hemolyzed after treatment with C_1 and H' reagents, the cells of *both* populations contained these factors.

The results of blood-typing tests on samples of cells obtained after differential hemolysis tests are shown in Table 4. When tested at three years of age, twin 272B had sired several offspring, so it was possible to establish his genotype as Type II, representing 10 per cent of his cells. Tests on progeny of 272B born after he was older than three years also established that he was transmitting the gene for the factor I_2.

Five years later, when 272B was eight years old, a sample of his cells was retested. It was noted that the hemolytic reactions for factors of Type II which were previously weak (partial) and typical of mosaicism were now strong (almost complete). In fact, a sample of his cells obtained when he was nine years old was tested as an unknown by 12 different laboratories of the United States and Europe participating in the cattle blood-typing standardization tests sponsored by the U.S. Department of Agriculture.[24] Not a single laboratory reported mosaicism of this blood sample and each laboratory, without exception, recorded the blood as Type III of Table 4. Extensive differential hemolysis tests were performed using many of the same reagents under the same conditions used five years previously. A summary of these results and others obtained over a three-year period is presented in Table 3. These results show that reagents which previously hemolyzed only 10 per cent of the cells were now hemolyzing 98 per cent of them. Likewise, reagents which previously hemolyzed 90 per cent were now hemolyzing 98 per cent. No changes were observed with reagents which previously had given either complete or no

TABLE 4

BLOOD TYPES IN CHIMERIC TWIN 272B AT FIRST AND SECOND AGE PERIODS*

Age period	Cell types†	Proportion (%)	Genetic Systems: B	C	L	S
1 mo–3 yr	I	90	$BO_1/(I_2)$‡	WX_2/C_1X_3	–/–	$SH'/$
	II	10	$GY_2E_1'/(I_2)$	C_1X_1L'/C_1X_3	L/L	$H'/$
8–11 yr	I	2	BO_1/I_2	WX_2/C_1X_3	–/–	$SH'/$
	II	2	GY_2E_1'/I_2	C_1X_1L'/C_1X_3	L/L	$H'/$
	III§	96	GY_2E_1'/BO_1	C_1X_1L'/WX_2	$L/$	$SH'/$

* See footnotes to Table 3.
† Based on pedigree and progeny tests, Type I was the genetic type of twin 272A, and Type II the genetic type of twin 272B.
‡ The I_2 factor was assumed from pedigree and progeny tests.
§ "Hybrid" cell type (see text).

hemolysis. The results of differential hemolysis tests on known artificial mixtures (95:5) of cells containing one or more of the same factors as the blood of 272B were within two per cent of the expected values, indicating that the test procedure was accurate. It is obvious from these results that a new cell type (Type III, Table 4) containing a different combination of antigenic factors was present in the blood of 272B.

It was not possible to obtain a definitive blood type on a pool of unhemolyzed cells remaining after treatment of the blood of 272B with the reagents for factors unique to Type II (G, Y_2, E_1', X_1, L', and L) because for unexplained reasons the complement controls (cells plus complement only) usually hemolyzed completely. However, since an average of two per cent of the cells remained unhemolyzed after treatment with these reagents, a cell type lacking these factors must have existed and was presumably Type I.[25] Unhemolyzed cells recovered after treatment with reagents detecting factors unique to Type I (B, O_1, W, and S) had the complete phenotype of Type II cells. Also, since only four per cent of the cells were hemolyzed after treatment with I_2 reagent (2% Type I plus 2% Type II), there were sufficient residual cells to obtain a clear blood type of them. These residual cells reacted completely with *all* of the reagents used, except I_2, indicating strongly that the remaining 96 per cent of the cells were Type III.

In summary, as shown in Table 4, the shift in the proportion of cells in the original mixture at the first age period to the second age period included the appearance of a new cell type. Note that the phenogroups BO_1, WX_2, and the factor S which were previously unique to Type I cells, and the phenogroups or factors GY_2E_1', X_1L' (of the phenogroup C_1X_1L') and L which were previously unique to Type II cells were later found on the same cell, called Type III. The phenogroup I_2 (B system), which was previously detectable on both Type I and Type II cells was not detectable on Type III cells, suggesting that it had been "lost" in the formation of the new type. It is not possible to say if the phenogroup C_1X_3 (C system), which was detectable on both Type I and Type II cells, was "lost" in the formation of Type III cells, since Type III cells have phenogroups C_1X_1L' and WX_2 which contained the same (C_1) or overlapping (X_1 and X_2) specificities. Possible "losses" of factors at the L and S loci could not be detected because the genotypes of the three different cell types were not sufficiently different.

Discussion.—From the data presented in this paper, it appears that the mixture of the two kinds of red cells in chimeric twins is not always stable. Whether a change in the proportions of blood types is a universal phenomenon is an open question. This phenomenon cannot be explained simply as a partial loss of immunologic tolerance, since the direction of the change is the same in both twins of a pair.

Dunsford and Stacey[26] reported about a 10 per cent decrease in the proportion of the "grafted" population of cells in a human chimeric twin four years after the original tests were made and after the twin had given birth to two children. This twin's brother died shortly after birth, so we do not know if a similar shift might have occurred in his blood. Recently, Slee[27] reported that erythrocyte mosaicism "regressed" in a pair of chimeric sheep twins, but tolerance for reciprocal skin grafts seemed to be sustained.

It is possible that the changes are due to aging or any process upsetting the equilibrium between the two hematopoietic tissues resulting in a selective advantage of one type of tissue over the other. Irradiation[28] might be expected to hasten these changes in chimeric twins. Preliminary results[29] suggest that sublethal irradiation of twin cattle causes a substantial shift in the proportions of cell types. It may be pertinent also that tolerance in rats is lost following sublethal irradiation.[30, 31]

The results of the differential hemolysis tests (Table 3) on the blood of twin 272B leave little doubt that there are three cell types in the mixture and that one type is a "hybrid" or "recombinant" containing antigenic factors which were originally on different cells. The most likely explanation of these results is that the "hybrid" cell type resulted from some kind of somatic cell mating of the two different hematopoietic stem cells.

The possibility of somatic cell mating as a tool for the genetic analysis of somatic cells was proposed by Lederberg.[32] The parasexual cycle of filamentous fungi[33] is an example of the fusion of nongerminal cells (haploid nuclei in multinucleate mycelia). Fusion of mammalian tumor cells grown *in vitro* was reported first by Barski and co-workers[34] and later by Sorieul and Ephrussi.[35] Ephrussi and Sorieul[36, 37] suggested that somatic cell mating may not be a rare event. They consider three possible prerequisites for the "mating" process: (1) the tendency for fusion may be a genetic property of particular cells; (2) neoplastic cells may mate readily because of a unique cell surface structure; and (3) mating may be related to polykaryocytosis observed in tissue cultures, resulting from cell fusions of virus-infected (altered cell surface) and noninfected cells. Finally, these authors propose that segregation takes place following hybridization, probably by accidental loss of chromosomes. Recently, Gershon and Sachs[38] confirmed and extended Ephrussi and Sorieul's findings of somatic cell hybridization of mouse tumor tissues growing in mixed cultures.

If the third cell type in the blood of the chimeric twin described in this report arose from somatic cell mating, it would represent the first example of hybridization of mammalian diploid somatic cells *in vivo*. Paralleling the phenomenon occurring in tumor tissue cultures, the hybrid cells in the blood of the chimera seemingly exhibit a selective advantage over the parental types in that they now represent at least 96 per cent of the cell population. There is a suggestion that the overgrowth of hybrid cells took place gradually because the hybrid cells represented only about 90 per cent of the population when first detected three years ago. In contrast to the data of Ephrussi and Sorieul,[37] our data, obtained with a Coulter counter, indicate that the hybrid cells are not detectably larger than the parental cells. Short-term cultures of peripheral blood (lymphocytes) yielded normal karyotypes ($2n = 60$) with XY sex chromosome constitution. Thus, there were no quantitative changes in the chromosome number of the lymphocytes. Whether or not the progenitors of the nonnucleated terminal erythrocytes are diploid is unknown. The health records of this chimera showed that during the first month after birth, he suffered severe scours which may have been of viral etiology[39] and may have altered the surface of his hematopoietic cells thereby enhancing mating.

Since the hybrid cells behave serologically and are the same size as normal diploid cells, segregation very probably occurred following the fusion of the parental

cells. Two mechanisms may have operated separately or in combination: (1) accidental chromosome loss as proposed by Ephrussi and Sorieul,[37] or (2) tetrapolar mitosis. (The latter mechanism was suggested to us by Dr. S. Ohno.) The finding of XX diploid cells in the chimera's bone marrow would strongly support tetrapolar mitosis as the mechanism.

One may consider alternative hypotheses to explain the occurrence of the hybrid cell type. Phenotypic mixing of the sort manifested by the Lewis blood group substances in man[11] might be invoked. This seems unlikely since the antigenic substances involved in the present study do not ordinarily occur in soluble form. Inhibition tests with the serum of the chimera did not reveal serologically active blood group substances. It would be difficult to understand the disappearance of the I_2 antigenic factor in Type III cells (Table 4) if they had originated by simple phenotypic mixing. In fact, the loss of I_2 is compatible with the evolution of a basically tetraploid hybrid toward a basically diploid cell type in which only two alleles of a given blood group system occur. Somatic mutation is not likely to account for these results, since it would require simultaneous mutations at two independent loci, at least. Errors in blood typing of dam and/or sire of the chimera are excluded because the blood types of a large number of their progeny were consistent with the phenotypes and genotypes of the dam and sire. The chimera did not receive a blood transfusion, thus excluding the unlikely possibility of a postnatal hematopoietic transplant. Unfortunately, the hemoglobin and transferrin loci were not useful markers in these studies.

Perhaps the most reasonable alternative hypothesis is that the dam carried fraternal triplets and that the blood of one triplet showed the same type as the "hybrid" cell. If so, this third embryo must have been resorbed sometime after the formation of its hematopoietic system and before parturition. Stormont[40] reported a single-born heifer with erythrocyte mosaicism, and suggested that she was a twin *in utero*. If the hybrid type present in the chimera's blood was derived from a resorbed embryo, these cells must have existed, during the first three years, in a very minor proportion to have gone undetected; then, after eight years, they became the predominant (96%) type. The only data shedding light on this hypothesis come from the records of the reproductive history of the dam of the chimera. These records indicate the presence of only two corpora lutea at the time the reproductive organs were examined approximately two weeks after breeding. This observation fails to indicate that more than two eggs had been shed from the ovary.

During the eleven years that the chimera has been studied, he has had 39 offspring. Studies of their blood types indicated that his blood group genes (for Type II of Table 4) have segregated normally. None of his offspring contained blood groups of the co-twin's type.

Summary.—The proportions of the two antigenic types of cells in cattle chimeric twins may change with time. The direction and magnitude of change is usually the same in both members of a pair. Consequently, they are not likely the result of an abrogation of immunologic tolerance. One chimeric twin was found at three years of age whose blood contained two antigenic types: 10 per cent representing his own genotype and 90 per cent the genotype of his co-twin. When the blood of this chimera was retested at eight years of age, three blood types were found:

the two "parental" types, each representing two per cent, and a "hybrid" type representing 96 per cent of the cell population. It is postulated that the "hybrid" cell type resulted from "mating" between the two hematopoietic tissues in the chimeric mixture and that the hybrid type had a distinct selective advantage.

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*BIOSYNTHESIS OF A LYSINE-RICH HISTONE IN ISOLATED CALF THYMUS NUCLEI**

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Interest has been revived recently[1, 2] in the concept[3] that histones function as regulators of cell metabolism and differentiation at the level of DNA-primed synthesis of nucleic acids. If histones do, in fact, act physiologically in this control function, then a new problem arises concerning the control of histone synthesis itself, "Quis custodiet ipsos custodes."[3a] An initial phase in the answering of this question is the determination of the site and mechanism of histone biosynthesis. Since the nucleus is known to contain histones, it is reasonable to look to this organelle as the site of their biosynthesis.

General protein biosynthesis in the nucleus has been studied by Allfrey *et al.*,[4-6] and they have demonstrated the existence of a nuclear system in calf thymus which incorporates amino acids into the general (trichloroacetic acid-precipitable) protein of the nucleus during *in vitro* incubations. While the nuclear system resembles the cytoplasmic system of protein biosynthesis in the steps of amino acid activation and polymerization, the nuclear and cytoplasmic systems can be distinguished. Among the documented differences[5] are the insensitivity to RNase and dependence upon Na^+ in the case of thymocyte nuclei. The calf thymus nuclear system of Allfrey *et al.*, with these distinguishing characteristics, was used in the present work to test the nucleus as the site of biosynthesis of histone.

Of the total proteins of the cell nucleus, the histones represent a relatively simple group of highly basic proteins distinct from other nuclear proteins. This distinction is based classically on the extractability of histones from nuclei by acid. Although most studies of protein biosynthesis have measured the incorporation of radioactive amino acids into even more broadly defined protein groups (e.g., trichloroacetic acid precipitates), the classically defined histones seemed too general a fraction for the purposes of the present experiments. Random polymerization of amino acids could lead to the production of basic polypeptides which were not authentic histones, and so it was felt necessary to study amino acid incorporation into a naturally occurring histone. The histone whose biosynthesis was studied in the present work was, therefore, identified with authentic lysine-rich histone by chromatography, electrophoresis, and amino acid analysis. Brunish and Luck[7] reported an incorporation of amino acids into histone which was apparently nonenzymic, and they stressed the importance of demonstrating incorporation into normal peptide linkage as a criterion of true protein synthesis. This criterion has been applied in the present work.