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Marrow Chimerism in Marmosets

Abstract. In the femoral marrow of three adult marmosets, two male *Callithrix jacchus* and one female *Leontocebus rosalia,* a number of opposite-sexed metaphases were found. It is inferred that this chimeric state resulted from intrauterine placental anastomoses between heterosexual twins. The lack of the freemartin effect in connection with this chimerism is discussed, and the structural nature of the Y chromosome in the Callithricidae is described.

The mosaic constitution of circulating blood in twin cattle was first demonstrated by Owen (*1*). It is the result of placental anastomoses between fraternal twins through which hematopoietic precursor cells are exchanged in embryonic life. These immature elements settle in the fraternal twin partner and, by virtue of aquired tolerance, continue to propagate throughout life in the new host and are instrumental in producing reciprocal tissue tolerance (*2*). If the metaphases are opposite-sexed, the resulting population of red cells may be detected by blood typing.

Similar chimerism has been demonstrated on rare occasions in human fraternal twins (*3–5*), but placental anastomoses are extremely uncommon in human fraternal twins (*3*) if they occur at all—they have never been demonstrated unequivocally (*6*). Wislocki (*7*) examined the placentas in 19 marmoset pregnancies and reviewed previously recorded observations. Of 40 pregnancies, 87.5 percent were twin pregnancies. Wislocki's data indicate that fraternal twinning is the rule in marmosets and that early fusion of the blastocysts results in the establishment of placental anastomoses between the twins. Despite these vascular channels, he points out, in marmosets the female partner of heterosexual twins is never a freemartin, whereas in cattle it usually is (*8*).

Ryan *et al.* (*9*) have recently presented support for their hypothesis that the enzymic constitution of the primate placenta may explain the nonoccurrence of freemartins in marmosets and in the rare instances of blood chimerism in man. According to their view in the primate the placenta may be able to convert the male gonadal hormones passing through it to estrogens, a faculty that the cattle placenta has not been shown to possess.

Despite the gross appearance of anastomotic channels in the marmoset placenta (*7, 9*), actual mixing of fetal blood has not yet been demonstrated. This report describes the occurrence of chimerism in the bone marrow of three adult marmosets, which is held to be the result of prenatal exchange of circulating hematopoietic tissue (*10*).

Six marmosets were available for study, but only five of these could be utilized. The mature animals [four male *Callithrix* (or *Hapale*) *jacchus,* one male and one female *Leontocebus rosalia*] were injected intramuscularly with colchicine (0.07 mg per gram of body weight) 5 to 6 hours prior to sacrifice through intraperitoneal injection of Nembutal. The male *L. rosalia* died, before marrow studies could be made, from infection with *Prosthenorchis elegans* (*11*); however, chromosome preparations were made from tissue cultures of the kidney. In the other five animals both femora were removed and the marrow was aspirated and placed for 30 minutes in 7 ml of hypotonic solution (Earle's solution and water, 1:4). After centrifugation the cells were fixed in a mixture of acetic acid and methanol (1:30) and subsequently treated as leukocyte cultures according to the method of Moorhead *et al.* (*12*). The air-dried cells were stained with aceto-orcein, and random mitoses were photographed by an individual who had no concern with the outcome of the experiment, selection being made only to obtain well-spread, intact cells. The sex chromosomes were identified by karyotyping the males, and subsequently karyotypes were prepared for doubtful cells, as indicated in Table 1.

The results of chromosomal analysis in five animals are shown in Table 1. In two of the five marmosets (M_3, M_4) all the cells analyzed contained the XY sex chromosomes expected in male monkeys, while in the three other marmosets cells were chimeric with respect to the sex chromosomes. Numerous polyploid cells were seen in M_6, few in the other animals. Figures 1 and 2 are representative male karyotypes for the two genera. As may be seen (Fig. 1), the Y chromosome of *Callithrix jacchus* is exceedingly small and thus is readily recognizable in well-spread metaphases. Consequently, chimerism in this genus is easily established through analysis of metaphase plates. The Y chromosome of *Leontocebus rosalia* (Fig. 2) is a small metacentric element, and karyotype analysis was more important in this genus for recognizing the chimeric nature of the animal's marrow. It is of course possible that M_3 and M_4 as well as the other three marmosets of Table 1 were members of pairs of fraternal twins but that their chimeric constitution could not be recognized because of the possibly isosexual nature of the twins. Alternatively, these animals could have been singletons, although Schultz (*13*) finds that twin pregnancy is the rule in marmosets in the wild state, singletons having been born only in captivity.

These results indicate prenatal exchange of marrow elements among heterosexual twins in marmosets; however, it is impossible to assess adequately the quantitative aspects of admixture, whereas this has been possible in the human chimeras. While it is not likely that selection of the metaphases in this study was biased, the number of metaphases analyzed is probably too small to give representative results. Thus, the findings of an 18-percent admixture of cells from the fraternal twin in M_2 and of a 34-percent admixture in M_6 may not indicate the true state of chimerism in these animals. Likewise, the finding of occasional "drumsticks" in polymorphonuclear leukocytes of the male chimeric monkeys is inadequate as a basis for quantitative assessment of the degree of chimerism, whereas a finding of "drumsticks" did provide such a basis in the studies of human blood chimerism reported by Woodruff (*14*). In Woodruff's studies, in the members of one set of heterosexual twins with chimer-

Table 1. Distribution of chromosome counts in five animals, of the genera *Callithrix* (or *Hapale*) *jacchus*, or common marmoset, and *Leontocebus rosalia*, or lion marmoset (*16*).

Animal	Sex	44	44 XX	44 XY	88 XXX	Total cells karyotyped	Total cells analyzed
M_1, *C. jacchus*	M		2	5		5	7
M_2, *C. jacchus*	M		11	49		13	60
M_3, *C. jacchus*	M	3	0	26		2	29
M_4, *C. jacchus*	M	4	0	44		1	48
M_6, *L. rosalia*	F	1	29	14	2	8	46

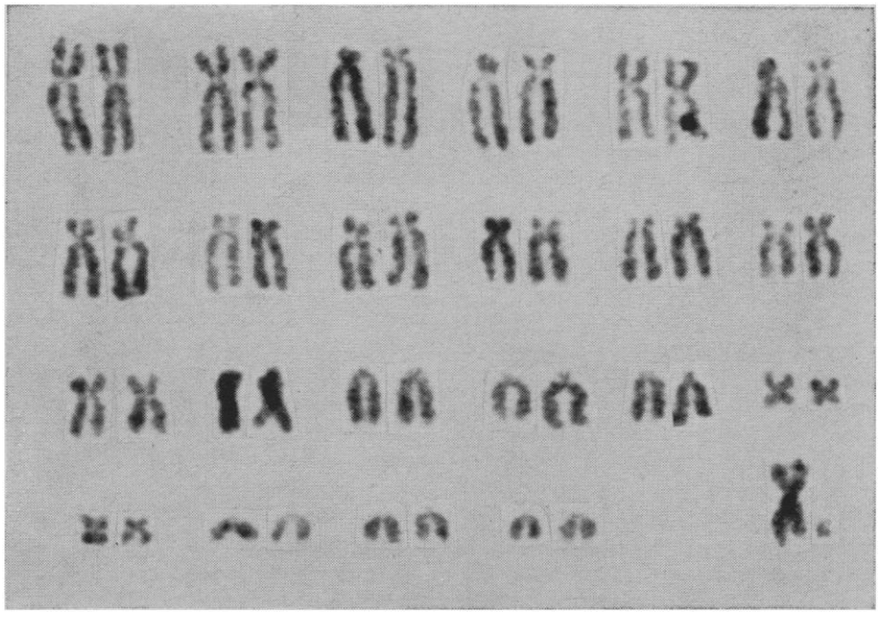

Fig. 1. Karyotype of male cell in male *Callithrix jacchus*, M_2: bone marrow, acetoorcein. The chromosomes are paired and arranged according to size, with the sex chromosomes last. (About × 2310)

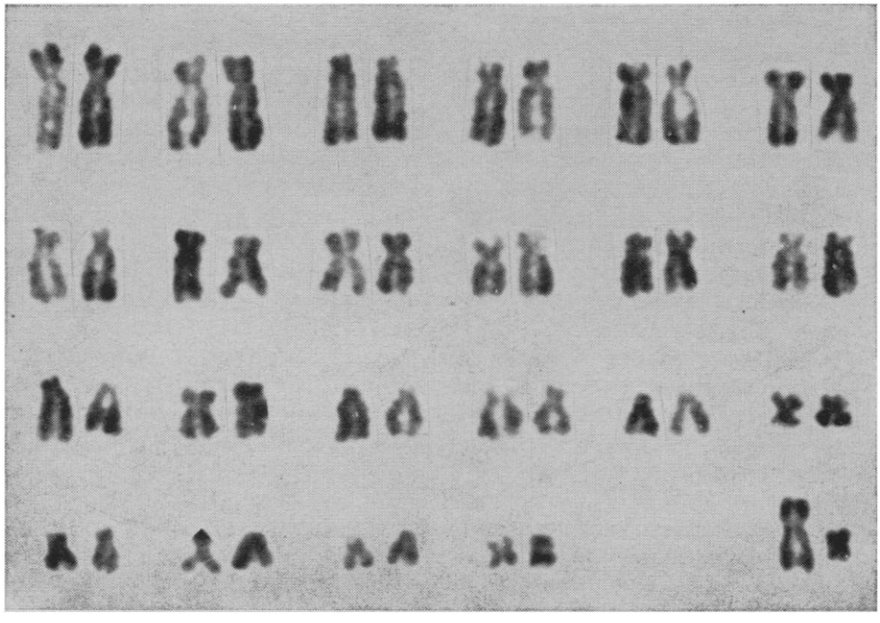

Fig. 2. Karyotype of male cell in female *Leontocebus rosalia*, M_6: bone marrow, acetoorcein. (About × 2310)

ism the percentages of agglutinable cells, drumsticks, and different-sexed white blood cells in peripheral leukocyte cultures were nearly identical. The number of granulocytes in the marmosets studied is too low to make a similar study feasible. To date, apparently no studies have been published concerning blood grouping of marmosets. While the quantitative aspects of chimerism can probably be assessed by this means, the very fact that blood chimerism occurs in most marmosets would seem to make blood grouping more difficult in the absence of breeding records.

The study reported here supports the inference made previously on the basis of anatomic study that prenatal exchange of blood occurs between fraternal marmoset twins. It was possible to detect a chimeric population of marrow elements by using the sex chromosomes as distinguishing markers, as suggested by Woodruff *et al.* (*14*). An admixture of male cells was demonstrated in at least one female animal (M_6); the genital organs of this marmoset were found to be normal on gross and microscopic examination. It can also reasonably be inferred that the female twin partners of M_1 and M_2 had marrow from the male twin, although the female partners were not available for study. Despite the inadequacy of our knowledge of the time of exchange, of the quantitative aspects, and of whether circulating androgens of sufficient amounts to produce the freemartin effect were also exchanged, the finding of male cells in a female marmoset with normal genital organs is further support of our hypothesis that the steroid ring-A aromatizing enzyme of the primate placenta may be the factor that prevents female sterilization (*9*). Further quantitative studies and an investigation of comparative fetal sex differentiation are needed to clarify these aspects of the problem.

In one set of human heterosexual twins with blood chimerism an apparent chimerism of secretor status was reported by Ueno *et al.* (*5*), and these authors inferred that cells other than hemopoietic precursor cells may circulate in embryonic life and become implanted in the co-twin. In possible contrast to these observations, Woodruff and Lennox (*15*) were unable to find cells containing sex chromatin in the buccal mucosa of the male partner of the twins previously referred to (*14*). The marrow chimerism that has

now been demonstrated in marmosets indicates that this animal may be suitable as a subject for further investigation along these lines.

Two members of the Callithricidae were studied earlier (*16, 17*), the silky marmoset *(Callithrix chrysoleucos)* and the red-mantled temarin [*Tamarinus* (or *Leontocebus*) *illigeri*]. Members of *Callithrix* and of *Tamarinus* were found to have a complement of 46 chromosomes; this finding is in accord with the finding for the genera (*C. jacchus* and *L. rosalia)* investigated in our study. While the X chromosome is a large metacentric element in all four of these genera of marmosets, differences exist with respect to the Y chromosome. The Y chromosome of the two genera studied by Bender and Mettler (*16*) was the smallest element and metacentric in structure. The best karyotypes of our *Callithrix jacchus* (Fig. 1) indicate that the Y chromosome of this genus is a minute acrocentric element. Most commonly, however, the preparations show only a small speck of chromatin material; the Y is distinctly the smallest member. In *Leontocebus rosalia* the Y chromosome, by contrast, is metacentric and cannot be clearly distinguished from the smallest pair of autosomes (Fig. 2). Structural differences in the autosomes in the genera of our study can also be seen in Figs. 1 and 2, but these cannot be compared with the autosomes of the animals that were reported by Bender and Mettler (*16*), in the absence of photographic representation of the karyotypes of their test animals.

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Ribonucleotides of RNA: Separation by Chromatography on Sheets of Diethylaminoethylcellulose

Abstract. The four main ribonucleotides of RNA can be separated on diethylaminoethylcellulose paper sheets, with results comparable to column chromatography, by irrigation with 0.05*M* formic acid for 2 to 3 hours, and then reversing the direction of irrigation with 4*M* formic acid.

No single developing system has been reported for the successful separation of the four main mononucleotides which are obtained from an alkaline digest of RNA. The migratory behavior of various ribonucleoside mono-, di-, and triphosphates on an ion-exchange cellulose was reported by Randerath, who used several developing systems (*1*). In addition, the migration of various oligonucleotides was explored by Bollum (*2*). Smillie developed a procedure for separating the ribonucleotides of adenylic, uridylic, guanylic, and cytidylic acids (AMP, UMP, GMP, and CMP, respectively) by using a secondary migration in a direction opposite to the first one (*3*). The use of paper impregnated with ion-exchange resin precludes the possibility of observing the ultraviolet-absorbing compounds directly (*3*). For this reason, the use of diethylaminoethylcellulose paper is more desirable, and the present report describes its use for successful separation of the above mononucleotides from one another and from such contaminants as orthophosphate and ribonucleosides.

Sheets of DEAE-cellulose (18 by 22 inches, Whatman DE-20) were suspended in chromatography tanks and supported by Kurtz-Miramon glass frames for descending chromatography (*4*). Commercially available reagent-grade formic acid (97 to 98 percent) was used to prepare the developing solutions.

The DEAE-cellulose sheets were converted to the formate form by irrigating, on a frame, with 1*M* formic acid for an interval that was two to three times that required for the front of the solvent to travel the length of the paper. The papers were then irrigated with water until the formic acid on the paper was less than 0.003*M*, as detected by taste. The papers were dried at 25°C. The migration of aqueous solutions on papers washed with formic acid was three times faster than the migration on sheets of the untreated Whatman DE-20; about 2½ hours were required for the front of aqueous solutions to advance the length (22 inches) of the washed paper. The positions of the nucleotides appeared as dark areas when the paper was illuminated with ultraviolet light of short wavelength (*5*).

Samples of ribonucleotides from the hydrolysis of RNA with KOH could be satisfactorily applied to the paper in amounts up to 0.1 ml per inch at the origin if they were neutralized with $HClO_4$ and the salt had been removed by cooling to 0°C. For analysis of larger samples of hydrolyzate, the nucleotides were absorbed on acid-washed Norit and subsequently eluted with 10 percent aqueous pyridine (*6*). This step also removed much orthophosphate. The capacity of the paper was such that 0.1 mg of each of the four ribonucleotides could be separated from one another.

Samples were applied so that the origin was 9 inches from the upper end of the paper. Irrigation with 0.05*M* formic acid in a descending manner was continued until the cytidylic acid had moved about 8 to 9 inches; at this

Table 1. Results of three paper chromatograms and one column chromatogram of ribonucleotides. RNA was hydrolyzed by 0.1*M* KOH, 18 hours at 37°C, acidified with $HClO_4$, and mixed with acid-washed Norit. The charcoal was washed with water and extracted with 10 percent aqueous pyridine. The pyridine was removed by drying. Samples were dissolved in 0.01*M* NH_4OH and applied either to column or paper. Column chromatography on Dowex-1 was done according to the method of Cohn and Bollum (*7*).

Ribonucleotide	Total counts recovered (%)			
	Paper			Column
CMP	27.6,	27.5,	27.4	28.4
AMP	21.4,	21.8,	20.8	21.4
GMP	27.4,	26.6,	28.0	27.9
UMP	23.6,	23.0,	22.5	22.6