of brain. After L-dopa administration, the concentration was 0.68 ± 0.02 μg per gram of brain. Therefore, an increase in the release and metabolism of serotonin may be due to displacement by dopamine.

The possibility of a similar decrease of brain serotonin occurring in patients treated with L-dopa must be considered highly likely and may be pertinent to the therapeutic effects or the side effects, or both, observed (*2*).

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Somatic Cell Mating and Segregation in Chimeric Frogs

Abstract. *Both pentaploid and haploid cells were observed in a short-term culture of bone marrow of a diploid-triploid frog chimera. Apparently, diploid and triploid marrow cells fused to form "hybrid" pentaploid cells, which subsequently gave rise by somatic reduction to haploid daughter cells. The hybrid marrow cells and their haploid segregants are presumably at a selective disadvantage, as neither type of cell has been detected in the circulating blood of chimeric frogs.*

The finding by Barski *et al.* (*1*) of hybridization of mammalian cells in culture has been substantiated and extended (*2*). Somatic cell mating in vitro has been demonstrated between cells of different strains of a species (for example, the Swiss and C3H mice), of different genera (for example, the mouse and rat), and even of different orders (for example, the mouse and man). Several loci of both parental genomes function and interact in the hybrid somatic cell. After hybridization, there is typically a reduction in the chromosome number of the hybrid cell over an extended period of time.

Notwithstanding the many successes achieved, hybrid formation in vitro is an infrequent event. The fusion of somatic cells in vivo occurs even less frequently. Somatic hybridization and segregation of two different cell types in the living organism was first detected by Stone *et al.* in a twin bull (*3*). As shown by Owen (*4*), the blood cell population of each twin is a mosaic of cells of two different genetic constitutions. Primordial blood cells are reciprocally exchanged between the twins through vascular anastomoses in embryonic life, and the translocated red cell precursors become established and perpetuate themselves in the hemopoietic tissues of the respective hosts. Thus, each member of a dizygotic pair possesses not only its own antigenically distinct kind of erythrocyte but also the antigenic type of its former twin.

In the chimeric bull studied by Stone *et al.* (*3*), a new hybrid type of diploid erythrocyte arose which was identified by its unique antigenic properties. Evidently donor and host erythrocytoblasts fused, and subsequent chromosome reduction in the tetraploid cell line resulted in the establishment of the diploid recombinant cells. The new diploid recombinants comprised 96 percent of the blood cell population of the bull (*5*).

We present here an apparent instance of somatic cell hybridization in a "twin" frog. We simulated experimentally the natural twinning in cattle by joining two frog embryos in parabiosis at a very early stage of development, before any differentiation of the blood cells. A diploid embryo was united side-to-side with a triploid embryo in the region of the gill primordium to ensure vascular communication. The differences in chromosomal complements permit the unequivocal demonstration of the mutual exchange of primordial blood elements. In later life (Fig. 1), each parabiont has blood-forming tissue capable of producing two kinds of blood cells, its own kind and that of its partner. Chromosome preparations of cultured bone marrow tissue as well as of peripheral blood cells of the chimeric frogs invariably contain both diploid ($2n = 26$) and triploid ($3n = 39$) metaphase plates (*6*).

In a system that involves the coexistence of two cell types in the same organism, selection would appear to be inevitable. In our frog chimeras, we demonstrated (*7*) that the composition of the blood cell mosaicism does not change appreciably during larval development. During the 2-month larval period, each paired larva contains approximately equal amounts of each cell type in the peripheral blood. The donor-type blood cells apparently are able to compete successfully with the resident cells. In other words, there seems to be no selective advantage to the host's own cell type, and similar equilibria are established in both partners. However, with the transition to juvenile life, the donor-type blood cells are at a selective disadvantage (*8*). As the larva undergoes metamorphosis, the incidence of the donor-type cells in the circulating blood falls from approximately 50 percent to as low as 8 percent.

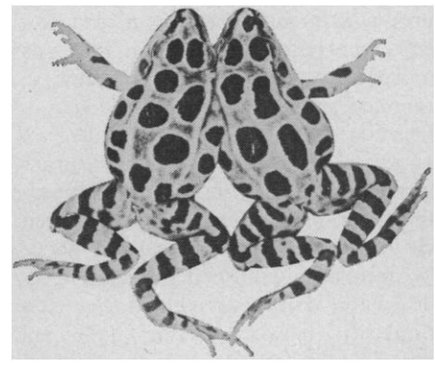

Fig. 1. Postmetamorphic diploid (left) and triploid (right) parabiotic frogs that had been united together at the tail bud stage of embryonic development [stage 17, defined by Shumway (*12*)] approximately 60 hours after fertilization. The copartners are blood cell chimeras; each contains a mixed population of diploid and triploid blood cells.

The morphological and physiological changes at metamorphosis apparently upset the equilibrium between the two hemopoietic tissues resulting in a selective advantage of the host blood cells. The metamorphic changes may also furnish the appropriate setting for somatic cell mating of the two different blood stem cells. We found no indication of cell fusions in cultures of bone marrow from 21 of 22 parabionts. In one parabiont, however, we found strong presumptive evidence that somatic cell hybridization does occur, albeit at low frequency. Examination of 365 metaphase plates from a short-term marrow culture revealed the following distribution of chromosome complements: 217 were diploid ($2n = 26$), 133 were triploid ($3n = 39$), 2 were pentaploid ($5n = 65$), 3 were haploid ($n = 13$), 6 were hypodiploid (< 26), and 4 were hypotriploid

(< 39). The pentaploid cell (Fig. 2) is presumed to have originated by fusion of a diploid cell and a triploid cell. Moreover, the short-term conditions of culture (96 hours) would seem to exclude the in vitro origin of the pentaploid cells.

The rare, and probably accidental, nuclear fusion of the two different hemopoietic stem cells in vivo is not amenable to direct observation. The event has to be inferred. Somatic hybridization, however, is more credible than the formation of the pentaploid cell by faulty divisions of either the diploid cell or the triploid cell. Indeed, pentaploid nuclei in cultured marrow cells of control diploid or control triploid frogs have not been observed.

We may speculate on the fate of the hybrid marrow cells. The hybrid hemocytoblasts in the bone marrow may either perpetuate themselves, leaving the pentaploid condition unaffected, or become reduced to a lower chromosome count. The finding of haploid cells in the marrow suggests that the hybrid cells are not stable and segregate by reduction to the haploid state. Somatic reduction of chromosomes is known to occur in both plants and animals. Huskins *et al.* (*9*) described a reductional type of mitosis in the root tips of onion bulbs. The chromosomes in the haploid nuclei can be identified as a complete haploid set. This indicates an orderly movement of an intact haploid set into daughter cells, rather than an uncontrolled random separation of homologs. The studies of Sinha (*10*) and Teplitz *et al.* (*11*) also reveal a systematic movement of haploid genomes during reductional mitoses of polyploid cells. In the latter investigation, embryonic diploid cells of the mink (*Mustela vision*) and cattle (*Bos taurus*) were hybridized in vitro (*11*). The tetraploid hybrid cells subsequently segregated into four diploid daughter cells, each of which possessed a complete haploid set of chromosomes of each parental type. Admittedly, the haploid cells in our parabiotic frogs could have been derived from reduced diploid cells, but the presence of pentaploid and haploid cells in the same tissue would appear to be more than coincidental.

We doubt that the pentaploid cells and their haploid derivatives enter the bloodstream, or even survive in the marrow milieu which evidently favors the host blood cells of long residence. No hybrid cells or haploid cells have been found in cultures of peripheral blood, which adds weight to the supposition that the pentaploid cells are at a selective disadvantage in the living organism. In population genetics, it is axiomatic that a hybrid organism is less fit than either parental species in the absence of a disturbed ecological niche or a "hybrid habitat." If we are permitted to compare hybrid cells to hybrid organisms, then the hybrid cell is destined for elimination in the absence of a "hybrid niche" in the individual organism. It should be recalled that the success of somatic cell mating in vitro depends wholly upon the investigator devising an artificial means

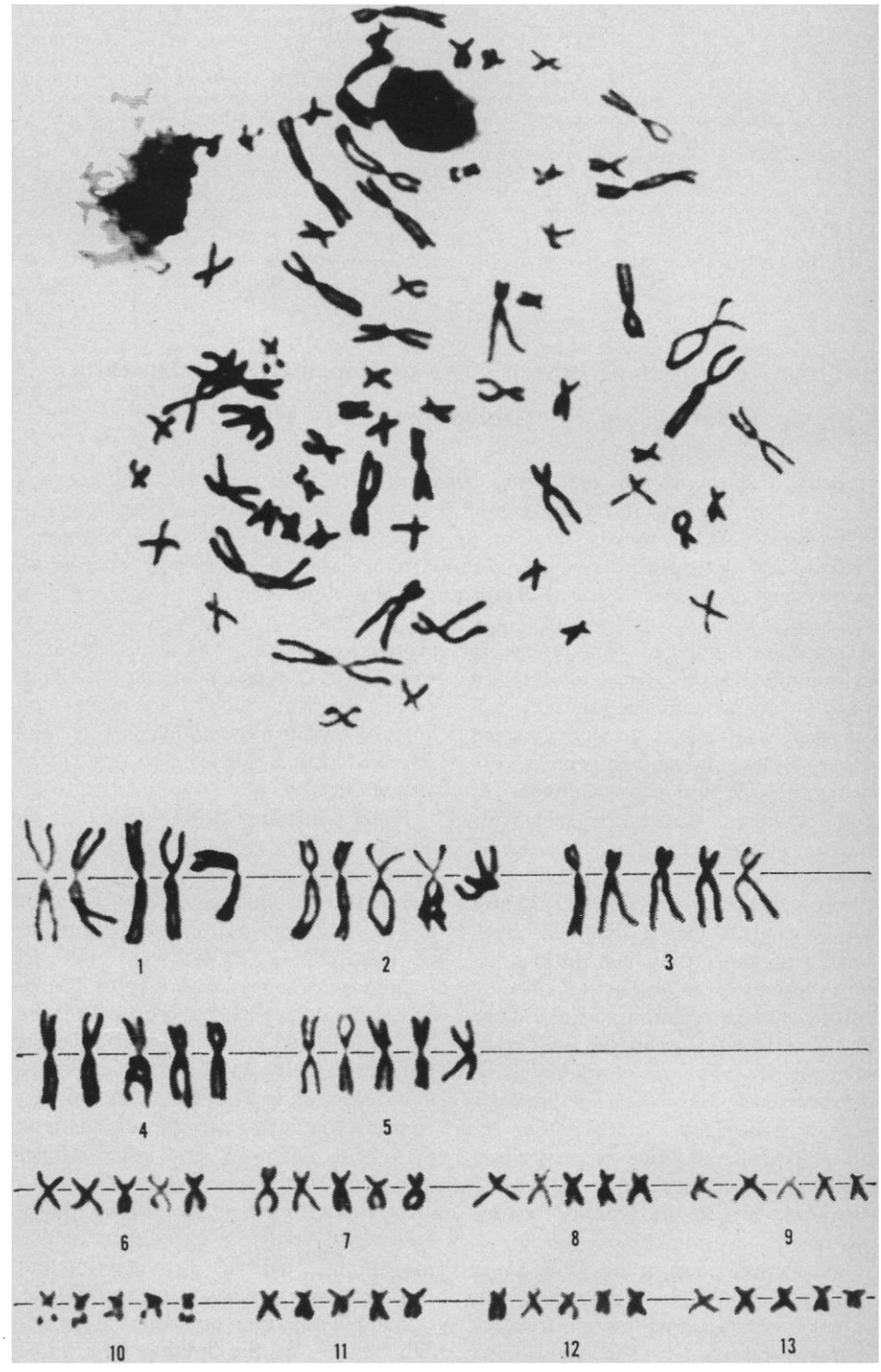

Fig. 2. Pentaploid hybrid metaphase plate from a marrow cell of a diploid-triploid chimeric parabiont. Karyotype of hybrid cell shows 65 chromosomes resulting from the fusion of a diploid ($2n = 26$) and a triploid ($3n = 39$) cell. The appearance of the hybrid spread does not suggest the mere superposition of diploid and triploid nuclei.

of conferring a selective advantage to the hybrid cells in a mixed culture. The environment of the living organism is not likely to change so radically as to favor an accidentally produced hybrid somatic cell.

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Immune Responses of Inbred Mice to Repeated Low Doses of Antigen: Relationship to Histocompatibility (H-2) Type

Abstract. *Immunization of inbred strains of mice with repeated minute doses (0.1 to 1.0 microgram) of hapten-protein conjugates demonstrated wide differences in the magnitude of their antibody responses, which were related to the histocompatibility (H-2) type of the strains. Immunization with a single high dose (100 micrograms) of antigen failed to demonstrate these differences.*

There are significant differences in the immune responsiveness of different mouse strains to complex multideterminant antigens, such as heterologous red cells, proteins, and hapten-protein conjugates (*1*). When poor-responder or good-responder animals were selectively bred, in a few generations homogeneously low or high levels of immune responsiveness were attained (*2*). These findings suggest that a relatively small number of genes may control the immune responses to a variety of antigenically complex immunogens. When substances with a restricted degree of heterogeneity, such as synthetic amino acid polymers, were used as immunogens, it was often observed that the ability to make immune responses was under control of single autosomal dominant genes (*3*). In the immune responsiveness to a homologous series of multibranched polypeptides this single gene was found to be closely linked to the major histocompatibility locus (H-2) of the species (*4*). The experiments described herein indicate that the magnitude of the antibody responses of mice to repeated injections of small doses of a complex immunogen, such as hapten-protein conjugates, was also associated with the H-2 type of the strains. This association with the H-2 locus was not observed when the animals were immunized with a single large dose (100 μg) of antigen.

Benzylpenicilloyl (BPO) conjugates of bovine gamma globulin ($BPO_{25}BGG$), hen's ovomucoid (BPO_4OM), bovine pancreatic ribonuclease (BPO_4RNAse) and a dinitrophenyl (DNP) conjugate of BGG ($DNP_{42}BGG$) were used as immunogens. Repeated 0.1-μg or 1.0-μg doses, mixed with 0.1 mg or 1.0 mg of an $Al(OH)_3$ gel adjuvant, were injected intraperitoneally into groups of three mice every 4 to 5 weeks. The animals were bled serially from the orbital sinus at various intervals after the antigen injections, over a period of 20 weeks after the primary immunization. Hapten-specific antibody titers were determined by passive hemagglutination (HA) or hapten-reacted human red cells (*5*) and by passive cutaneous anaphylaxis (PCA) in the mouse. The PCA tests in the mouse were done with sensitization periods of 2 hours and 48 hours, in order to assay respectively IgG_1 and reaginic antibodies (*6*). These methods have been described extensively elsewhere (*5*, *6*).

The results of immunization of mice of different strains with a single high dose (100 μg) of antigen are shown in Table 1, A columns. Primary antibody responses were elicited in all strains. There were moderate variations in the magnitude and duration of the antibody responses among the strains, but these were not related to their H-2 type. By contrast, immunization with repeated 1.0-μg doses of $BPO_{25}BGG$ plus 1.0 mg of $Al(OH)_3$ revealed marked differences among the strains. No significant antibody responses were detectable at 14 or 28 days after the first antigen injection. However, striking differences were seen in the magnitude of the secondary antibody responses evoked by a second injection of antigen given 4 weeks later. The antibody titers measured 7 days after the secondary dose are shown in Table 1, B columns. There was a clear relationship between the H-2 type of the strains and the magnitude of their secondary responses. No antibodies were detectable in H-2^b, H-2^d, or H-2^q strains or in SWR mice (*7*). On the other hand, quite vigorous antibody responses were seen in A/He mice (H-2^a) and in six out of nine H-2^k strains. Three other H-2^k strains (C57BR/cdj, MA/J, RF/J) and SJL mice (H-2^s) made weaker, although easily detectable, responses.

Eight strains of mice were also tested with repeated 0.1-μg or 1.0-μg doses of BPO_4OM, BPO_4RNAse, $DNP_{42}BGG$, or $BPO_{25}BGG$. When repeated 1.0-μg doses were used, H-2^a and H-2^k strains were the better-responding strains to each of the four different immunogens, as shown in Table 2. There was evidence, however, that certain strains responded better to certain immunogens. Table 2 shows that SJL mice responded moderately well to three of the antigens, but failed to respond to BPO_4RNAse; similarly, SWR mice also responded to three of the antigens, but failed to respond to $BPO_{25}BGG$. The two H-2^b strains tested (C57BL/6J and C57L/J) failed to respond significantly to any of the four immunogens. Similar patterns of immune responsiveness were seen when repeated 0.1-μg doses were used, although the antibody titers were lower in all strains.

Several antiserums were analyzed for the presence of antibodies specific for determinants of the carrier protein molecules, by using solutions of native BGG, OM, or ribonuclease to elicit PCA reactions. Both IgG_1 and reaginic