preparations, devoid of most of these contaminants, the presence of wishbone or Y-shaped molecules was a characteristic recurrent phenomenon. The gross morphology of the s-IgA molecule resembled serum IgG, but the linear dimension of the s-IgA molecule was larger and it was less electron-transparent than IgG, indicating a greater thickness, compatible with the molecular model for s-IgA postulated above. Tomasi has published optical rotary dispersion data (*14*) suggesting a close similarity between serum IgG and serum IgA as well as s-IgA.

Normal, as well as paraproteinemic, serums contain IgA with sedimentation coefficients ranging from 7 to 17*S* (*12*, *15*). The excellent resolving capacity of the tall agarose columns used in our study permitted a partial separation of high-polymer serum IgA from 7*S* IgA. The predominant structure in the high-polymer IgA (11 to 13*S*) preparations was made up of four filamentous subunits joined at a central point of high contrast; Ballieux *et al.* (*16*) suggested that the monomers in high-polymer myeloma IgA were joined at their Fc parts. Others (*6*, *17*) have been unable to recover any significant amount of intact Fc or F'c material after proteolytic degradation of 7*S* serum IgA and 11*S* myeloma IgA. The two-dimensional profiles of the subunits in the high-polymer IgA molecules examined in the electron microscope revealed no contribution of Fc parts. It is possible, however, that the contrast-rich, rather compact, center of the molecule represents a close-packed Fc region visible only when the molecule is embedded sideways.

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References and Notes

1. T. B. Tomasi, Jr., E. M. Tan, A. Solomon, R. A. Pendergast, *J. Exp. Med.* **121**, 101 (1965).
2. L.-Å. Hansson, *Int. Arch. Allergy Appl. Immunol.* **18**, 241 (1961).
3. M. A. South, M. D. Cooper, F. A. Wollheim, R. Hong, R. A. Good, *J. Exp. Med.* **123**, 615 (1966).
4. L.-Å. Hansson and B. G. Johansson, *Nobel Symp.* **3**, 141 (1967).
5. J. J. Cebra and P. A. Small, *Biochemistry* **6**, 503 (1967).
6. T. B. Tomasi, Jr., and D. S. Czerwinski, in *Immunological Deficiency Disease in Man*, R. A. Good, P. Miescher, R. T. Smith, Eds. (Univ. of Florida Press, Gainesville, 1968), p. 270.
7. R. W. Newcomb, D. Normansell, D. R. Stanworth, *J. Immunol.* **101**, 905 (1968).
8. Colostrum samples were centrifuged at 100,000*g* for 1 hour, and the fat layer and pellet were removed. The clarified samples were subjected to gel filtration on tall (2.5 by 400 cm) Bio-Gel A-15m (Bio-Rad) columns equilibrated with 0.01*M* phosphate buffer, *p*H 7.2, containing 0.2*M* NaCl and 2 percent butanol. The eluted fractions were analyzed by immunodiffusion performed on glass slides in 1 percent agarose (Bio-Rad) gel. Single radial immunodiffusion was used for estimation of IgA, IgM, and IgG. The various eluted fractions were titrated for poliovirus-neutralizing antibody activity by incubation of equal volumes of virus (150 plaque-forming units) at varying dilutions of the fractions for 4 hours at room temperature and 20 hours more at 4°C. The mixtures were assayed without dilution on HeLa cell monolayers by the plaque-inhibition method.
9. J. J. Cebra and J. B. Robbins, *J. Immunol.* **97**, 12 (1966).
10. T. B. Tomasi and J. Bienenstock, *Adv. Immunol.* **9**, 1 (1968).
11. R. E. Ballieux, J. W. Imhof, G. H. Niehaus, *Nature* **189**, 768 (1961); R. Rask-Nielsen, J. F. Heremans, H. E. Christensen, R. Djurtoft, *Proc. Soc. Exp. Biol. Med.* **107**, 632 (1961).
12. R. E. Ballieux, *Structure Analysis and Classification of* β_{2A}*-Paraproteins* (Schotaans and Jens, Utrecht, 1963); J. F. Heremans, *Les globulines sériques du système gamma* (Arscia, Brussels, 1960).
13. S-E. Svehag, B. Chesebro, B. Bloth, *Science* **158**, 933 (1967); B. Chesebro, B. Bloth, S-E. Svehag, *J. Exp. Med.* **127**, 399 (1968); R. C. Valentine and N. M. Green, *J. Mol. Biol.* **27**, 615 (1967); B. Bloth, B. Chesebro, S-E. Svehag, *J. Exp. Med.* **127**, 749 (1968); G. M. Forte, A. V. Nichols, R. M. Glaeser, *Chem. Phys. Lipids* **2**, 396 (1968); B. Bloth and S-E. Svehag, in preparation.
14. T. B. Tomasi, thesis, Rockefeller University, New York.
15. J. P. Vaerman, H. H. Fudenberg, C. Vaerman, W. J. Mandy, *Immunochemistry* **2**, 263 (1965).
16. R. E. Ballieux, J. W. Stoop, B. J. M. Zegers, *Scand. J. Haematol.* **5**, 179 (1968).
17. G. Cederblad, B. G. Johansson, L. Rymo, *Acta Chem. Scand.* **20**, 2349 (1966).
18. Supported by the Swedish Medical Research Council (Projects No. B69-11X-2427-02 and B68-16X-744-03B) and by the foundation "Konung Gustaf V's 80-årsfond." We thank Misses Y. Karlsson and K. Winberg for assistance and Drs. L-Å. Hansson and R. Norberg for two s-IgA preparations.

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L-Dopa: Effect on Concentrations of Dopamine, Norepinephrine, and Serotonin in Brains of Mice

Abstract. *Large doses of* L-*dopa given to mice produced marked increases in brain dopamine, no change in norepinephrine, and a remarkable decrease in brain serotonin. This reduction apparently results from a release or displacement, or both, of serotonin from its storage sites.*

Large daily doses (2 to 8 g) of L-dopa (dihydroxyphenylalanine) are used for the relief of akinesia, rigidity, and tremor in Parkinson patients (*1*). We investigated the problem of how these large amounts of the precursor of dopamine (DA) affect the concentrations of norepinephrine (NE) and serotonin (5HT) in the brain.

White male mice (ICR) were given varying doses (100, 200, and 400 mg per kilogram of body weight) of a warmed L-dopa solution (10 mg/ml). Concentrations of the amines in the brain were determined at peak action time (30 minutes after administration of L-dopa, intraperitoneally). At peak action time, the behavioral effects are proportional to the doses of L-dopa, and symptoms include increasing alertness and irritability; at very high doses, fighting and jumping are noted. There was an increase in the concentration of dopamine proportional to the dose of L-dopa, but no significant effect on norepinephrine concentrations occurred (Table 1). Apparently the step from dopamine to stored norepinephrine is not easily achieved. The concentrations of brain serotonin decreased markedly in proportion to the dose of L-dopa administered. These data suggest that dopa and dopamine may cause changes in the metabolism and storage of serotonin in the brain.

We also determined the concentrations of indoleacetic acid (HIAA) in the brains of normal mice and mice treated with L-dopa (400 mg/kg; intraperitoneally) 1 hour before they were killed. The concentration of HIAA in controls was 0.37 ± 0.01 μg per gram

Table 1. Effect of L-dopa on concentrations of biogenic amines in the brains of male mice (ICR). The mice were killed at peak action time (30 minutes after the intraperitoneal administration of L-dopa).

L-Dopa (mg/kg)	No. of determinations	DA (μg/g of brain)	Percentage of change	NE (μg/g of brain)	Percentage of change	5HT (μg/g of brain)	Percentage of change
Controls	17	1.01 ± 0.02	0	0.45 ± 0.01	0	0.54 ± 0.01	0
100	12	3.34 ± 0.37	332	0.48 ± 0.01	7	0.44 ± 0.01	−18
200	12	6.22 ± 0.74	617	0.47 ± 0.01	5	0.30 ± 0.02	−45
400	15	11.85 ± 0.90	1177	0.43 ± 0.01	−5	0.20 ± 0.01	−63

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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References

1. G. C. Cotzias, P. S. Papavasiliou, R. Gellene, *N. Engl. J. Med.* **280**, 337 (1969).
2. M. D. Yahr, R. C. Duvoisin, M. J. Sechear, R. E. Barett, M. M. Hoehn, *Arch. Neurol.* **21**, 343 (1969).

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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.

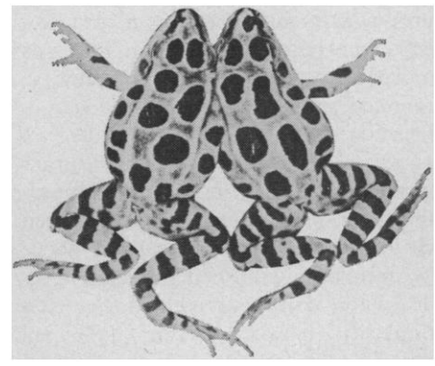

Fig. 1. Postmetamorphic diploid (left) and triploid (right) parabiotic frogs that had been united together at the tail bud stage of embryonic development [stage 17, defined by Shumway (*12*)] approximately 60 hours after fertilization. The copartners are blood cell chimeras; each contains a mixed population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