More precise characterization of possible DDT molecular association phenomena could be obtained from studies of solution behavior in which nuclear magnetic resonance spectrometry (*13, 15*) is used and from correlations of colligative properties with charge-transfer characteristics of appropriate molecular complexes of pesticides.

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References and Notes

1. Abbreviations are: DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; DDD, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane; DDMS, 1-chloro-2,2-bis(*p*-chlorophenyl)ethane; DDA, 1,1-bis(*p*-chlorophenyl)acetic acid.
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Silent Hemoglobin Alpha Genes in Apes: Potential Source of Thalassemia

Abstract. *Small quantities of unusual hemoglobins were found in 1 of 37 chimpanzees and 2 of 6 gorillas. In each genus these hemoglobins contain unique α chains that differ from the ordinary by eight to nine scattered amino acid changes. The unusual chains arise from a hitherto undetected* hemoglobin $^3\alpha$ *locus. No* $^3\alpha$ *products are found in most apes; accordingly,* $^3\alpha$ *is considered synthetically inactive in all but a few reversion mutants. Indirect evidence that the inactive* $^3\alpha$ *locus is juxtaposed to an active α locus together with the supposition that* $^3\alpha$ *exists in man provides a setting wherein thalassemia might be produced by nonhomologous recombination between two loci.*

Silent genes, that is, genetic loci without demonstrable products in most individuals of a species, have not been heretofore identified in higher organisms. In this report we provide reasons for believing that a silent locus, termed *hemoglobin* $^3\alpha$, exists in great apes and probably also in man. In most individuals $^3\alpha$ seems to be inactive and produces no evident product; however, in a few mutants the locus is active and produces an unusual α chain.

During an electrophoretic survey of adult hemoglobins from great apes, three exceptional animals were encountered. One of 37 (*1*) chimpanzees (*Pan troglodytes*) and 1 of 5 (*1*) unrelated lowland gorillas (*Gorilla gorilla gorilla*) exhibited not only hemoglobins A and A_2 but also small quantities (2.4 to 3.4 percent, Fig. 1 legend) of an unusual form of hemoglobin A and still smaller quantities (0.04 to 0.1 percent) of an unusual A_2. Both components differed from the usual by a net gain of about four electrostatic changes per hemoglobin molecule. Identical amounts of these unusual components were also found in the son of the variant gorilla (*2*). Electrophoresis of isolated (*3*) concentrates of the principal unusual components, designated hemoglobin Hyzoo in the chimpanzee and hemoglobin Wazoo (*4*) in the gorilla, are shown in Fig. 1 (*5*).

Parallel variation of both hemoglobin A ($\alpha_2\beta_2$) and A_2 ($\alpha_2\delta_2$) in all affected individuals suggested that an unusual α chain was present in these animals. This was corroborated by column chromatographic separation of constitutive hemoglobin chains (*6*). Chromatographic behavior and amino acid composition of β chains from both Hyzoo and Wazoo were identical to A-β. In contrast, the α chains of Hyzoo and Wazoo each showed net gains of about two electrostatic changes when compared with A-α from variant animals. After whole chain analysis, both variant α chains were distinctly unusual in the proportions of particular amino acids among the total of 141 residues present (*7*).

The differences between variant α and A-α sequences were further dissected through amino acid analysis of purified tryptic peptides (*7*). The net number of various residues realized from the sum of tryptic peptides exactly matched those obtained by whole chain analysis, thereby suggesting that characterization of variant chains is reasonably complete. A synopsis of differences is shown in Fig. 2. A remarkable feature—pivotal to our later interpretation—is the similarity between chimpanzee (*Pan*) Hyzoo-α and *Gorilla* Wazoo-α. These chains share a presumed constellation of eight scattered amino acid differences, outlined in Fig. 2, with respect to the A-α sequences characteristic of each genus.

The extent and diffuse distribution of differences shown in Fig. 2 make it most unlikely that either Hyzoo-α or Wazoo-α, let alone both, have arisen simply as allelic mutations at the locus for A-α. Detectable hemoglobin mutants differ from wild-type alleles, either through changes in one nucleotide or, in a few instances, through deletion of short runs of nucleotides in multiples of three (*8*). Aside from a few instances of within-locus recombinants between two separate nucleotide changes, multiple scattered changes are not found among uncommon variants. Multiple scattered differences may, however, develop between common alleles (*9*) when these have been maintained by natural selection for millions of generations. In this connection both Hyzoo and Wazoo are distinctly uncommon; nothing like them was detected in other surveys involving samples from substantial numbers of great apes (*10*). As persistently rare alleles at the locus for A-α these variants would, by Fisher's prediction (*11*), be lost long before they could accumulate step by step the pattern of change shown in Fig. 2. Accordingly, Hyzoo-α and Wazoo-α can only be regarded as the products of an α locus that is separate from the locus for A-α. It is likely that this additional α locus has a common ancestry in the two species, that is, it arose from a single gene duplication in some common ancestor of apes. Although six of the eight positions wherein Hyzoo-α and Wazoo-α are seemingly alike and different from A-α

have not been definitively placed by exact sequence analysis, the pattern of differences shown in Fig. 2 is nonetheless sufficient to support belief in a common origin. In the context of later interpretation a commonality of ancestry is all that matters; whether present day Hyzoo-α and Wazoo-α differ as shown (Fig. 2) by two changes or in fact by, say four changes, is immaterial.

In man there appear to be two generally indistinguishable α loci in some (*12*), but not necessarily all (*13*), populations. Where there are two active α loci, each locus is thought to produce about one-half and each allele about one-fourth of all α chains. An analogous state of affairs may exist in chimpanzees where a healthy individual was found to have about 25 percent of an electrophoretically fast moving α chain variant (*10, 14*). We designate the synthetically vigorous α loci as $^1\alpha$ and $^2\alpha$, but in so doing recognize that only one of these loci may be active in some men and perhaps in all gorillas. The synthetically impoverished (*5*) locus responsible for Hyzoo-α and Wazoo-α is designated $^3\alpha$. Terminology follows that adopted for the several *hemoglobin* γ loci of man (*15*) and allows molecular formulas to be written in an unambiguous fashion, for example, hemoglobin A: mixture of $^1\alpha_2{}^A{\cdot}\beta_2{}^A$ and $^2\alpha_2{}^A{\cdot}\beta_2{}^A$; hemoglobin Hyzoo: $^3\alpha_2{}^{Hyzoo}{\cdot}\beta_2{}^A$.

The hemoglobin $^3\alpha$ locus, like $^1\alpha$ and $^2\alpha$, presumably arose through gene duplication via the successive processes of nonhomologous meiotic pairing and recombination. The antiquity of the events producing $^3\alpha$ may be judged first by the appearance of $^3\alpha$ chains in each of two genera and second by the minimum of nine to ten nucleotide changes calculable from genetic code for the amino acid differences (Fig. 2) between A-α and the α chains of *Pan* Hyzoo and *Gorrilla* Wazoo. The first observation indicates that the age of $^3\alpha$ antedates the separation of evolutionary lines leading to chimpanzee and gorilla, whereas the second finding suggests $^3\alpha$ has had a much longer history. The nine to ten nucleotide changes exceed the minimum of four nucleotide differences existing between the α genes of man and *Rhesus* monkey (*8*) and, in addition, approximate the minimum of 9 to 14 nucleotide differences existing between hemoglobin β and δ genes in several primates (*14*). The latter two loci probably arose before the ancestors of apes and New World monkeys had diverged from one another (*14, 16*).

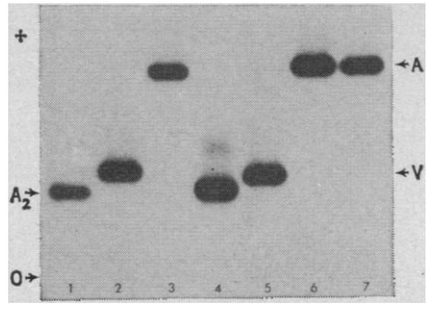

Fig. 1. Amido schwarz stain of *p*H 8.6 EBT [EDTA, boric acid, tris (*9*)] starch-gel electrophoresis of chromatographically isolated (*3*) concentrates of great ape hemoglobins: 1, chimpanzee hemoglobin A_2; 2, chimpanzee hemoglobin Hyzoo; 3, chimpanzee hemoglobin A; 4, gorilla A_2; 5, gorilla Wazoo; 6, gorilla A; 7, whole hemolyzate from gorilla. All chimpanzee samples are from animal No. 9, in whom Hyzoo forms 3.4 ± 0.4 percent ($N = 3$) of total hemoglobin. All gorilla samples come from Tomoka in whom hemoglobin Wazoo forms 2.5 percent of the total (compare 2.4 percent in his father, Nikumba). The proportion of A_2 is 1.8 percent in No. 9 and 2.0 percent in Tomoka. *V* refers to variant hemoglobin.

In light of such comparisons the $^3\alpha$ locus seems quite old and one that man is entitled to by right of descent.

If the $^3\alpha$ locus is indeed ancient and widespread how has it remained hidden from view, and how has it become visible in uncommon individuals from two different genera? There are three alternative explanations. First, the $^3\alpha$ locus may only occur in a few individuals. Second, $^3\alpha$ may be present and active in all, but its product in most may lie electrophoretically and chromatographically buried within the envelope of hemoglobin A. Under these conditions sparse quantities of $^3\alpha$ chain might easily have escaped detection during separation and analysis of A-α peptides. Third, $^3\alpha$ may be present but synthetically inactive in most individuals because long ago, in some common ancestor of apes, it underwent, for example, a nonsense or a profound missense mutation that has only been overcome in a few back mutants. As we shall indicate, the first explanation seems decidedly improbable, the second explanation can be eliminated, and thus the third explanation is favored.

The first explanation, limitation of $^3\alpha$ locus to a few individuals, is statistically unattractive. It requires the preservation for millions of generations of what would now be a rare locus in chimpanzees and, at best, an uncommon locus in gorillas. Just as noted for the case of rare alleles, the chance for loss of a rare locus is enormous in each species (*11*). In the specific case of a rare locus the opportunity for loss is compounded by risk of extinction during obligate mispairing that must occur in each carrier during each meiotic division.

It is anticipated from the second explanation that uncommon variant animals are probably heterozygotes, that is, $^3\alpha^A/^3\alpha^{Hyzoo}$ or $^3\alpha^A/^3\alpha^{Wazoo}$. If this is so then it is supposed that Hyzoo and Wazoo have become electrophoretically visible through single but separate $^3\alpha$ mutations producing net gains of about four electrostatic charges per hemoglobin molecule (Fig. 1). The only remotely appropriate candidates (Fig. 2) for such charge changes are aspartic acid to lysine (Asp; Lys) mutations at $^3\alpha^{64}$ (*17*). Under the terms of this explanation hypothetical wild-type $^3\alpha^A$ alleles produce $^3\alpha^{64\ Asp}$; and their products are buried in the mass of hemoglobin A; but the variant $^3\alpha^{Hyzoo}$ and $^3\alpha^{Wazoo}$ alleles are concordant mutants that produce $^3\alpha^{64\ Lys}$ and are thereby electrophoretically visible. The undoing of this hypothesis comes from starch-gel electrophoretic analysis at *p*H 7.1 (*18*), where the imidazole group of histidine is more positively charged than at *p*H 8.6. Differences in histidine content (Fig. 2) between A-α and the α of Hyzoo and Wazoo that are nearly silent at *p*H 8.6 are expressed at *p*H 7.1. The same should be true for *p*H 7.1 electrophoretic comparisons involving products of hypothetical $^3\alpha^A$. In an explanation that accounts for Hyzoo and Wazoo visibility through the occurrence of single but separate $^3\alpha^{64}$ mutations, it is expected that other residues, including those involving differences in histidine, will remain unchanged. As already noted, most known differences between normal and mutant alleles involve single, not multiple, residues (*8*). Despite such expectations, based on differences between the histidine content of A-α and hypothetical $^3\alpha^A$, no new products appear after *p*H 7.1 electrophoresis of hemolyzates and hemoglobins from a number of different chimpanzees and gorillas. Failure to uncover buried products occurs despite the unexpected (*19*) finding that Hyzoo and Wazoo are slightly less mobile than A at *p*H 7.1. Thus a buried $^3\alpha^A$ product—if it existed and differed from both Hyzoo and Wazoo only at α^{64}—should be distinctly less mobile than A at *p*H 7.1 and easily discernible. For such

Position / Chain	23	64	65[b]	67	70[c]	102	105[d]	106[d]	110[e]	112[f]
A-α	Glu[a] Asp	Asp	Ala	Thr	Val	Ser	Leu	Leu	Ala	His
Pan Hyzoo-α	Glu	Lys	Glx	Ser	Leu	Asn	Val	Phe	Ser	Asx
Gorilla Wazoo-α	Asp	Lys	Glx	Thr	Leu	Asn	Val	Phe	Ser	Asx

Fig. 2. Minimum amino acid differences between hemoglobin A-α chains of man—*Pan-Gorilla* (*8*) and the variant α chains of hemoglobin Hyzoo from chimpanzee *Pan*, animal No. 9, and hemoglobin Wazoo from *Gorilla*, animal Nikumba. Positions where all chains are similar are omitted. Results in Hyzoo-α and Wazoo-α are based on amino acid compositions of tryptic peptides (*7*). Positions 65–90 derive from tryptic peptides α-9b; positions 105–127 are from peptide α-12b. Distinction between acids and amides depends on peptide electrophoresis. Position assignments are inferred from homology with human A-α sequence. The superscript letters indicate the following: (a) Glu in man and *Pan* A-α, Asp in *Gorilla* A-α (*8*); (b) positions 65, 69, 71, 79, 82, or 88; (c) position 70 or 73; (d) differences involve any two of positions 105, 106, 109, 113, and 125; (e) positions 110, 111, 115, 120, or 123; (f) positions 112 or 122. Boxes denote the residues where Hyzoo-α and Wazoo-α are alike and different from A-α. Abbreviations: Ala, alanine; Asn, asparagine; Asp. aspartic acid; Glu, glutamic acid; His, histidine; Lys, lysine; Phe, phenylalanine; Ser, serine; Thr, threonine; Val, valine; x, not ascertained whether acid or amide.

reasons we discount the possibility of an active $^3\alpha$ locus with a hidden product and favor the third explanation, namely, a $^3\alpha$ locus that is usually synthetically inactive. Consequently, hemoglobins Hyzoo and Wazoo are each regarded as the product of a mutation whose effect is to reverse a regulatory mutation, for example, a nonsense or a profound missense mutation for which $^3\alpha$ is usually homozygous. Although our detection of such concordance of reversal in separate genera seems remarkable we do not know whether the same kind of back mutation has been operative in each genus. For example, a nonsense mutation might be reversed either by back mutation at $^3\alpha$ or by a suppressor mutation in a transfer RNA that translates the nonsensical codon of $^3\alpha$. Thus concordance of reversal may be less exact and a little less remarkable than it first seems.

In terms of thalassemia the significance of an inactive $^3\alpha$ locus depends on the assumption that an array of two or more nearly identical and genetically juxtaposed loci—for example, $^1\alpha$, $^2\alpha$, $^3\alpha$—favors recurrent meiotic mispairing (*20*). In fact, two findings suggest that meiotic mispairing between adjacent α loci may have occurred in *Gorilla* during comparatively recent times. First, *Gorilla* is unique among primates, and in particular among apes, in possession of A-$\alpha^{23\ \mathrm{Asp}}$ (*8*). This individuality presumably reflects an $\alpha^{23\ \mathrm{Glu}\rightarrow\mathrm{Asp}}$ mutation (Glu, glutamic acid) in the comparatively short evolutionary period since taxonomic separation of great apes. Second, $\alpha^{23\ \mathrm{Asp}}$ occurs in both the A-α and Wazoo-α of *Gorilla* (Fig. 2). Although such concordance at α^{23} may reflect isologous mutations, there has been relatively little evolutionary time for a pair of these to develop. It seems more likely that a single Glu → Asp mutation occurred at α^{23} in one α locus followed by its insertion into another and neighboring α locus through nonhomologous crossing over. It is notable that $^3\alpha$ is left silent in the process. Accordingly—if our reconstructions are correct—the synthetic silence of $^3\alpha$ does not depend on information contained in the early portion of its message. By extension we presume that $^3\alpha$ silence depends on information following specification of α^{23}. It may thus be expected that a portion of new intergenic recombinants beginning as $^1\alpha$ or $^2\alpha$ and ending as $^3\alpha$ will be silent. Where only $^1\alpha$ or $^2\alpha$ are present, heterozygotes for a nonhomologous recombinant fusion of two loci, for example, $^1\alpha$-$^3\alpha$, will have only one synthetically active α locus. Homozygotes will have none. Such hypothetical $^1\alpha$-$^3\alpha$/$^1\alpha$-$^3\alpha$ homozygotes for a fusion gene might be a source of a lethal form of α thalassemia associated with hydrops fetalis. Infants with this condition have no demonstrable α chain synthesis (*21*). This postulated origin of α thalassemia is in some ways analogous to the β-δ fusion recombinant producing hemoglobin Lepore (*22*) and associated in homozygotes with β thalassemia. Finally, α thalassemia might also develop even if the discounted notion of an active but synthetically impoverished $^3\alpha$ locus, discussed earlier, is correct. In this situation we suspect that only small quantities of hemoglobin, possibly similar to Hyzoo and Wazoo and perhaps indistinguishable from hemoglobin A_2, would be produced by a $^1\alpha$-$^3\alpha$ fusion locus. Whatever the case, we reiterate that the significance of $^3\alpha$ as a source of thalassemia lies within its supposed tight juxtaposition to an active α locus. In this setting the frequency with which meiotic mispairing leads to loss of α synthesis is likely to be common (*20*) and may greatly exceed the frequency with which point mutations lead to the same condition. The virtue of the admittedly elaborate $^3\alpha$ model thus lies with its potential rate of appearance: a feature that may help to account for the steadily increasing evidence of heterogeneity among thalassemics. In all such interpretations, the crucial unknown is the question of $^3\alpha$ existence in man. While we do not know whether or not $^3\alpha$ endures in ourselves, it nonetheless seems likely that a locus that has apparently persisted for perhaps 40 million years or more in our remote ancestors will have survived the last several million since our taxonomic divergence from the ape stem line.

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References and Notes

1. Chimpanzee samples derive from the generosity of J. Moore, Baltimore Zoo: four animals; Dr. A. Eldadah, Johns Hopkins University School of Hygiene: nine animals including the Hyzoo individual (No. 9) since transferred and bled for us by Dr. C. Gibbs, Patuxent, Md.; Delta Regional Primate Center, Covington, La.: 24 animals. Gorillas examined include Jacky, Hercules, and Sylvia from the Baltimore Zoo; and Femelle, Nikumba, and Tomoka from Smithsonian's Washington Zoo.
2. Hematocrits, hemoglobin concentrations, erythrocyte concentrations, erythrocyte morphology, and A_2 percentages in the variant apes were unremarkable. Accordingly, there is no reason to suspect that the variant hemoglobins in themselves produce disease.
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are hyphenates with the suffix *zoo* being used to denote the usual source and to emphasize that the variant occurs in a nonhuman subject. The prefix denotes the place of first sampling. Thus Wazoo is found in gorillas at the *Wa*shington *Zoo* and Hyzoo was found in a chimpanzee in the Johns Hopkins University School of *Hy*giene *zoo*logical collection.

5. The observed quantities of hemoglobin Hyzoo and Wazoo present in whole hemolyzates are little more than the 1.6 to 2.0 percent of A_2 present in chimpanzees and gorillas, and, moreover, considerably less than the amounts of variant hemoglobin found in almost any of a variety of human hemoglobin heterozygotes (*14*). The source of such scant quantities of Hyzoo and Wazoo seems to lie with impoverished synthesis rather than with premature destruction of abundantly produced hemoglobin. Estimates of the specific activity of purified α and β chains from the gorilla Tomoka, after incubation in vitro of bone marrow aspirates (obtained by Dr. S. Charache) with 1 mc of [^{3}H]leucine at 35°C for 100 minutes, indicate that both chains of hemoglobin Wazoo—like those of hemoglobin A from the same animal—are synthesized by marrow approximately in proportion to their final concentration in peripheral blood.
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17. An Asp → Lys change is a two-nucleotide change. The presumption that the hypothetical $^3\alpha^A$ bears $\alpha^{64\,Asp}$ is a convenient fiction; it might just as well, for example, be $\alpha^{64\,Glu}$ in one or both genera. It must, however, be either Asp or Glu if the hypothesis of a buried $^3\alpha^A$ product is to remain tenable.
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19. The relative order of cathodic mobilities at *p*H 7.1 was expected, on the basis of amino acid composition (*7*), to be $A_2 > \text{Hyzoo} = \text{Wazoo} > A$. The observed order, $A_2 > A > \text{Hyzoo} = \text{Wazoo}$, is attributed to conformational dependent differences between A and the variant hemoglobins in ionization of histidine residues.
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23. Supported in part by PHS grant HD-02508-04 and PHS career development award K3-GM-6308-03 to S.H.B.

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Functional Sequences Modulated by Morphological Transitions in Human Lymphoid Cells Grown in vitro

Abstract. *Immunoglobulin-producing cells undergo a series of morphological transitions; each configuration displays specific functional attributes. The life cycle of immunocytes may be visualized as a series of functional compartments expressed by morphological sequences.*

A long-term culture of human lymphoid cells derived from a patient with lymphoma was established in 1966 (*1*). These cells now have been maintained in monolayer cultures for 5 years. Reticuloid fusiform cells and lymphocytoid and plasmacytoid round cells are predominant. Binucleate cells and transitional forms are a frequent finding (Fig. 1). Indirect immunofluorescent studies have demonstrated that these cells synthesize gamma globulin. Cells growing on Leighton cover slips were rinsed in saline and fixed in acetone for 10 minutes. The cells were incubated with unlabeled goat antiserum to human gamma globulin for 30 minutes at room temperature. The cells were then washed twice with a buffer solution (*p*H 7.2) and incubated for 30 minutes with fluorescein tagged rabbit antiserum to goat gamma globulin. The cells, without counterstaining, were examined under an ultraviolet microscope. Positive apple-green fluorescence was easily distinguishable from autofluorescence.

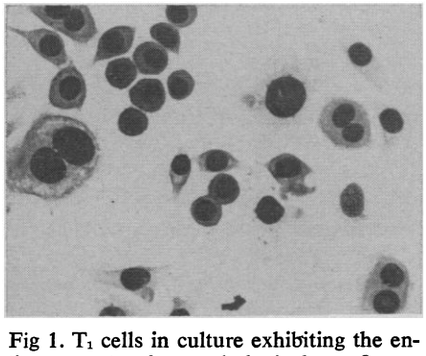

Fig 1. T_1 cells in culture exhibiting the entire gamut of morphological configurations (Wright's stain; × 480).

Time-lapse photographic studies were performed with a culture system (*2*). Because of the prolonged doubling time of these cells (52 ± 2 hours), one picture was taken every 30 minutes for 165 hours. Cell pedigrees were generated from enlargements of the negatives for morphologic analysis. Generation times were measured from one cell division to the next daughter-cell division. The median generation time was 36 hours.

Rare cells, still metabolically active as evidenced by mobility and changes in shape, failed to divide during the experiment. Most of the cells undergo changes in shape, from round to fusiform and often back to round. Each of these changes lasts for several hours which allows for morphologic definition. When spindle-shaped cells become round prior to mitosis, they do so very rapidly within a single half-hour interval. Upon division, a fusiform cell can give rise to one elongated and one round daughter cell (Fig. 2) or more commonly, to two fusiform cells. Round cells may also give rise to a morphologically mixed population; sometimes they produce only smaller round cells. Apparently these smaller round cells are terminal because, after a brief period of rapid movement, they become immobile and never divide. Occasionally a cell that remained round for numerous hours will adopt a fusiform shape for a few hours and then start to divide vigorously.

An unusual finding is that two daughter cells may come into close contact and fuse, and a single binucleated cell will emerge (Fig. 3). After several hours this cell may either dissociate into a rapidly mobile round cell and a static spindle-shaped form, or it will divide giving four round daughter cells.

This fusion of cells is different from the mechanisms of emperipolesis (*3*), peripolesis (*4*), and uropodapsis (*5*) because the fusion is long-lived, distinguishable cell boundaries disappear, and the process may sometimes con-

Table 1. All of the cells in the examined fields were arbitrarily assigned to a morphological category according to the prevalent feature and classified as fluorescent or nonfluorescent.

Cell types	Morphological distribution		
	Fluorescent cells	Nonfluorescent cells	Fluorescent cells (%)
Round	183	45	80.5
Intermediate forms	114	93	65.5
Fusiform	32	265	10.7
Total	329	403	44.6