Little information is available concerning the mechanism responsible for tetraploidy in such cultures. It is possible that cell fusion with subsequent homosynkaryosis (*9, 10*) may be responsible for a proportion of tetraploid cells. However, tetraploidy may also result from failure of cytoplasmic cleavage with subsequent fusion of two daughter nuclei or from endoreduplication (*6*).

It would be of interest to carry out similar studies with a variety of chromosomally marked strains to determine the frequency of somatic segregation as a function of specific genomes and autosomes. Should it prove to be of widespread occurrence, it could form the basis of a beginning formal genetic analysis in man. Cytogenetic studies would have to be combined with biochemical or antigenic analysis of clones from individuals heterozygous for markers that are expressed in cell culture. In view of the widespread occurrence of enzyme polymorphism in man (*10*), it is probable that a variety of suitable markers could be exploited for such a genetic analysis. The zymogram technique (*10*) should permit the identification of heterozygous clones and each of the two types of homozygous diploid segregants. Replicate plating of such segregants could permit the assignment of other loci to the relevant linkage groups, since all loci linked to the segregated chromosomes would become homozygous. Such an analysis cannot be carried out efficiently, however, without methods for the selection of recombinant diploid clones. Selective techniques are especially essential in view of the eventual "senescence" of such cultures (*7*); in our laboratory, more than 100 mass cultures and over 200 clones from a variety of human diploid cultures have ceased to replicate, with a maximum life-span of about 65 cell doublings (*11*).

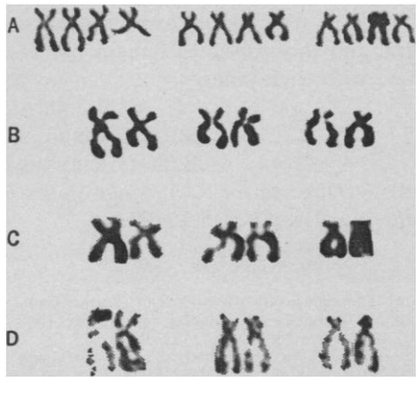

Fig. 2. Partial karyotypes (group E, or chromosomes Nos. 16 to 18) of representative cells from a clone of tetraploid skin fibroblasts from a woman heterozygous for a morphologic variant of chromosome No. 16. (A) Tetraploid; (B) diploid nonrecombinant; (C) diploid recombinant 16/16; and (D) diploid recombinant, 16?q+/16?q+.

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References and Notes

1. G. Pontecorvo, *Trends in Genetic Analysis* (Columbia Univ. Press, New York, 1958).
2. An abstract of portions of this work appeared in *Proc. Int. Congr. Genet. 12th* (1968), pp. 1 and 215.
3. A. J. Therkelsen, L. U. Lamm, K. Henningsen, *Hereditas* **57**, 149 (1967).
4. G. M. Martin, *Exp. Mol. Pathol.* **3**, 622 (1964); ———, *Proc. Soc. Exp. Biol. Med.* **116**, 167 (1964); R. Loder and G. M. Martin, *J. Invest. Dermatol.* **47**, 603 (1966); G. M. Martin and A. Tuan, *Proc. Soc. Exp. Biol. Med.* **123**, 138 (1966); G. M. Martin, C. A. Sprague, W. B. Dunham, *Lab. Invest.* **15**, 692 (1966).
5. *Chicago Conference: Standardization in Human Cytogenetics*, D. Bergsma, J. L. Hamerton, H. T. Klinger, Eds. (National Foundation-March of Dimes, New York, 1966).
6. S. Ohno, *In Vitro* (American Tissue Culture Symposium on Phenotypic Expression, 1966), vol. 2, p. 46.
7. L. Hayflick, *Exp. Cell Res.* **37**, 614 (1965).
8. E. Glass, *Chromosoma* **7**, 655 (1956).
9. H. Harris, J. F. Watkins, C. E. Ford, G. I. Schoefl, *J. Cell Sci.* **1**, 1 (1966); B. Ephrussi, *In Vitro* (American Tissue Culture Symposium on Phenotypic Expression, 1966), vol. 2, p. 40.
10. H. Harris, *Proc. Roy. Soc. London Ser. B* **164**, 298 (1966).
11. G. M. Martin, C. A. Sprague, C. J. Epstein, in preparation.
12. Supported in part by NIH grants AM 04826, 1 LO1 GM 13543, and GM 00100. The technical assistance of Mary Ann Derr and Ada Tuan is acknowledged. A skin biopsy was provided by Dr. A. J. Therkelsen.

3 June 1969

Fungal Endogenous Rhythms Expressed by Spiral Figures

Abstract. *Culture zonations in two fungi,* Nectria cinnabarina *and* Penicillium diversum, *are expressions of endogenous rhythms. These culture zonations may take the form of either concentric rings or Archimedes' spirals. The rhythm in* N. cinnabarina *is noncircadian. The rhythm in* P. diversum *is relatively insensitive to temperature and has a period of approximately 24 hours. The lack of a demonstrable mechanism for phase shifting suggests that this rhythm may also be noncircadian.*

Spirals of biological origin are common and almost always logarithmic (*1*). A notable exception is the web of some spiders which is often an Archimedes' spiral. Archimedes' spirals occur in the fungi *Nectria cinnabarina* and *Penicillium diversum*. These spirals are the visible expression of endogenous rhythms.

Nectria cinnabarina was cultured on media containing 48 g/liter of Bacto-potato dextrose broth (PDB) and 20 g/liter of Bacto-agar (Difco). *Penicillium diversum* was cultured on 12 g/liter of PDB and 20 g/liter of Bacto-agar. Cultures were initiated from a single germinated spore placed in the center of a plate. Sporulation of *N. cinnabarina* was induced by growing the cultures under continuous illumination from a Growlux fluorescent lamp at 4.8×10^3 erg cm^{-2} sec^{-1}. Since growth of the *P. diversum* isolate was somewhat inhibited by light, the cultures were maintained in continuous darkness, where sporulation occurred. All cultures were incubated in constant temperature chambers.

Both *N. cinnabarina* and *P. diversum*, under the conditions described above, produce concentric rings of spores similar to many fungi that exhibit zonation phenomena (*2*). Culture zonations of both fungi are produced with stable periodicities under constant environmental conditions. The rhythm of *N. cinnabarina* is noncircadian. The period of the rhythm ranges from 6 to 16 hours, depending upon the temperature. A Q_{10} of 2.73 has been determined for the period of the *N. cinnabarina*

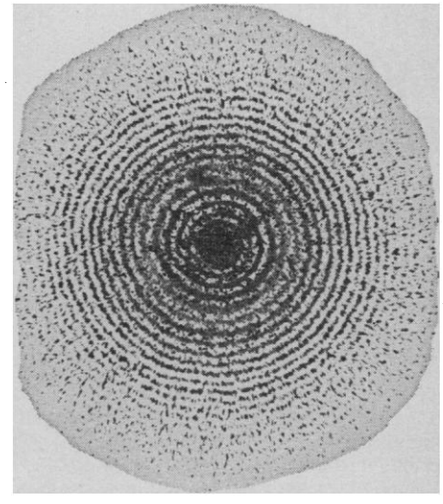

Fig. 1. Culture zonation in *Nectria cinnabarina* in the form of a single Archimedean spiral.

rhythm from 19° to 28°C. Above 28°C the period is so short that sporulation bands are essentially continuous. Growth is severely restricted below 19°C. The rhythm of *N. cinnabarina* is free-running—once the rhythm is evident, no further environmental cues are required to maintain the oscillation. The period of the rhythm is the same in either constant light or dark.

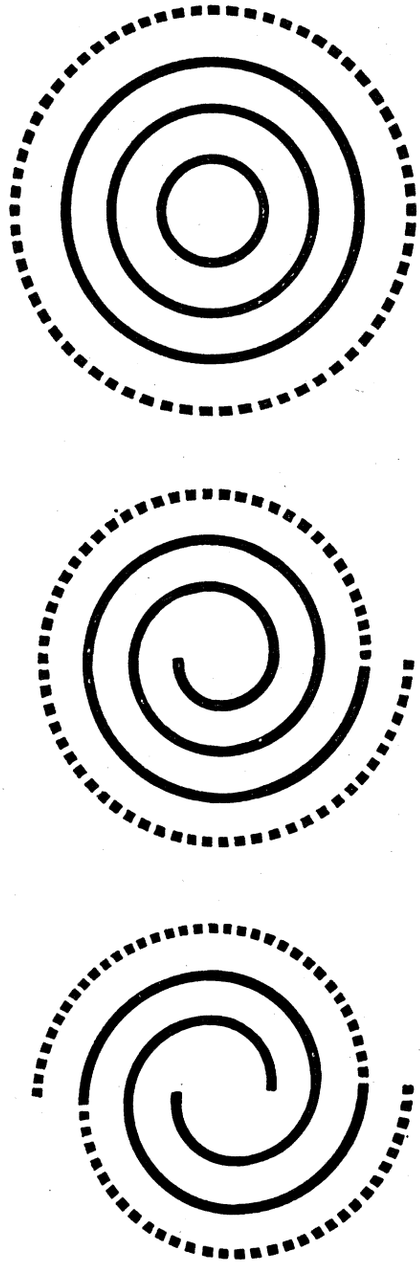

Fig. 2. Culture zonation in *Nectria cinnabrina*; concentric rings (top), single spiral (center), and double spiral (bottom). In each case the broken lines represent the sporulation band that develops during one period of the rhythm. The rate of development of each of the spirals in the double spiral is one-half that of the single spiral, but the net effect is the formation of spiral through 360° in each case.

Cultures grown in the dark produce few spores, but the zonations are evident as alternating rings of sparse and dense hyphae. Spore rings, produced in the light, become less distinct after approximately 14 rings have been produced.

Approximately 40 percent of the cultures of *N. cinnabarina* produce a spiral pattern of zonation rather than concentric rings (Fig. 1). Because the radial distance between any two adjacent bands is equal, the spiral is Archimedean.

Infrequently two spirals were observed to form within the same culture, one turning within the other. The same relationship holds with the double spiral in that the distance between adjacent bands is equal. The distance between adjacent bands of the same spiral is doubled because any two bands of the same spiral are separated by an interposing band of the alternate spiral.

Since the colony diameter enlarges at a constant rate, the number of sporulation bands encountered on a radial transect is an expression of the period of the rhythm regardless of whether the zonation pattern is concentric rings, a single spiral, or a double spiral. The single spiral turns through 360° in the same time interval required to produce one ring of spores in comparable cultures. On the other hand, in those cultures that produce double spirals, each individual spiral turns through 360° in twice the time required for the formation of the same arc in a single spiral or for one ring (Fig. 2).

Spiral zonation is not peculiar to *N. cinnabarina*. Both concentric rings and single spirals (Fig. 3) are observed in the zonation patterns of *Penicillium diversum* cultures. As in *N. cinnabarina*, the spirals of *P. diversum* are Archimedean and may be of either clockwise or counterclockwise configuration. The zonation pattern of *P. diversum*, whether concentric or spiral, is formed rhythmically, has a period of approximately 24 hours, and is nearly temperature-independent. A Q_{10} value of 1.15 has been determined for the period of the rhythm between 23° and 31°C. Zonation, as rings or spirals, is produced in darkness or in constant light. As yet no mechanism for phase shifting has been found. The rhythm of *P. diversum* must be tentatively regarded as noncircadian.

These rhythms provide a contrast in the character of endogenous rhythms.

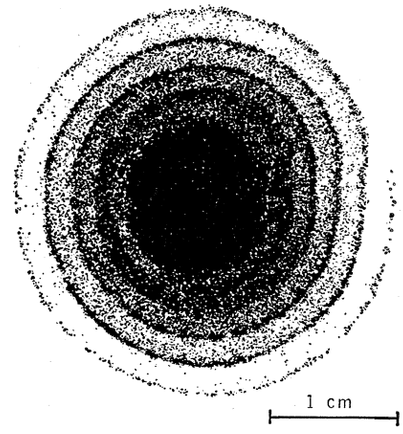

Fig. 3. Archimedean spiral produced by culture zonation in *Penicillium diversum*.

As noted by Cumming and Wagner (*3*): "Fungi may provide a physiological link between organisms which do and those which do not exhibit classic circadian phenomena." The zonation rhythm of *N. cinnabarina* is noncircadian. The rhythm in *P. diversum*, although not clearly circadian, has some properties of circadian rhythms (*4*). Both rhythms have the same expression, producing zonation either as concentric rings or as Archimedean spirals. Normally, spiral growth would not be considered a rhythmic process because the configuration forms a continuum. The spirals described in these fungi grow at a predictable rate in terms of radians per hour, and therefore they can be considered rhythmic.

The mechanism for the formation of spirals is unknown. It is known, however, that discontinuity in rings does not occur. Apparently a spiral forms when one side of a colony becomes out of phase with the other. The continuum of zonal lines is then expressed as a spiral and the two sides remain out of phase with each other.

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References and Notes

1. D. Thompson, *On Growth and Form* (Cambridge Univ. Press, London, 1942), pp. 748–873.
2. S. Jerebzoff, in *The Fungi*, G. Ainsworth and A. Sussman, Eds. (Academic Press, New York, 1965), vol. 1, pp. 625–645; ——— and E. Lambert, *Compt. Rend.* **266**, 1269 (1968); M. Sargent, W. Briggs, D. Woodward, *Plant Physiol.* **41**, 1343 (1966).
3. B. Cumming and E. Wagner, *Annu. Rev. Plant Physiol.* **19**, 381 (1968).
4. C. Pittendrigh, *Cold Spring Harbor Symp. Quant. Biol.* **25**, 159 (1961).
5. Supported by NSF grant GB-7199 and NIH grant AI-06950-2.

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