Cytological Evidence Opposing the Theory of Brachymeiosis in the Ascomycetes

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Recently a collection of the discomycete, *Patella melaloma*, was obtained by the writer from burnt-over ground in this area. The fungus has been maintained in culture, where it readily produces its fruiting structures or apothecia. The species is of particular interest because it is one of several discomycetes studied by Gwynne-Vaughan (*1*), the most outstanding contemporary proponent of the theory of double fertilization (two nuclear fusions) and brachymeiosis (two reductional divisions) in the ascomycetes.

Probably the majority of geneticists and most cytologists working on the ascomycetes have doubted for some time that these two processes occur. Thus far, however, no convincing evidence has been presented either to prove or to disprove the theory for those species in which these phenomena are said to exist. Most of the species of ascomycetes in which double fertilization and brachymeiosis have been reported to occur have proved unfavorable for genetical study. In those heterothallic species which might be used for genetical study such difficulties are experienced as inability to obtain a satisfactory percentage of ascospore germinations, or failure of the ascospores to occur in an orderly sequence in the ascus, or failure of the fungus to fruit well in culture. All the cytological evidence bearing on this subject has thus far been in the form of drawings and descriptions of sectioned material stained with the use of techniques which are frequently inadequate for revealing the chromosome numbers of the nuclei at various phases in the life cycle.

With the use of the propiono-carmine staining technique, recently employed with excellent results by Wheeler *et al.* (*2*) in a cytological study of ascus development in *Glomerella*, the writer has been able to observe the three successive nuclear divisions in the ascus of *Patella melaloma* and to determine with certainty the number of chromosomes present during each division. One of the most important features of this technique is that it leaves the spindle fibers unstained and that the chromosomes stand out clearly in the nuclear vacuole. Photographs demonstrating the numbers of chromosomes in all three divisions in the ascus have been obtained and will be included in a paper to be published later.

Gwynne-Vaughan (*1*), in her study of this species, reported that the nuclei fused in pairs in the ascogonium to produce diploid nuclei. These diploid nuclei were believed to pair among themselves, migrate into the ascogenous hyphae, and finally fuse in pairs in the young asci. Thus each ascus was believed to contain a tetraploid nucleus. She stated that the tetraploid number of 8 chromosomes (4 pairs) was observed at metaphase of the first division in the ascus. These chromosomes were believed to pass in groups of 4 (the diploid number) to opposite poles of the spindle. In this way the first reductional division was completed. Each daughter nucleus was then observed to divide mitotically, 4 chromosomes passing to opposite spindle poles. Then in the third division, each of the 4 nuclei was believed to undergo a second reductional division with 2 chromosomes (the haploid number) passing to opposite spindle poles. Therefore each of the 8 ascospores was supposed to receive a nucleus with only 2 chromosomes.

The writer is able to confirm Gwynne-Vaughan's report that there are 4 pairs of chromosomes present at the beginning of the first nuclear division in the ascus, and that a complement of 4 passes to each pole as the division is completed. In the second division also, 4 chromosomes appear and divide, a complement of 4 passing into each daughter nucleus. But the third division, instead of being reductional as Gwynne-Vaughan described it, is similar to the second division, and 4 chromosomes, rather than 2, pass into the daughter nuclei. The 8 ascospore nuclei, therefore, contain 4 chromosomes each. It is obvious from this brief description that the chromosome number is reduced only in the first division. The diploid number of chromosomes is 8 and the haploid number is 4. The third division is nonreductional. It is therefore obvious that brachymeiosis does not occur in this fungus. It is equally obvious that there can be no double fertilization in the life cycle.

The writer intends to extend these observations to other species in which double fertilization and brachymeiosis have been reported. The propiono-carmine technique is recommended as an efficient method for obtaining chromosome counts in the ascomycetes.

References

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Inability of Thymine and Adenine to Substitute for Pteroylglutamic Acid in the Folic Acid-deficient Rat

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Although it has long been recognized that thymine can replace folic acid for growth of microorganisms (*8*), it has not been emphasized sufficiently that both thymine and purine bases are required for this purpose (*5, 6*). If an analogous situation exists in animals, it might be expected that thymine alone would be ineffective in replacing folic acid in a synthetic type of diet which does not supply purine bases, and this has indeed been shown true for both the rat and the chick (*1, 3, 4*). Experi-

[1] The authors are greatly indebted to Dr. E. E. Snell of the University of Wisconsin for his helpful suggestions and criticism. They also wish to thank Alice M. Bergdahl for assistance in obtaining the hematologic data.

ments wholly analogous to the bacterial growth tests, i.e., in which a dietary source of both thymine and purine bases has been supplied in an effort to duplicate the growth and hematopoietic effects of folic acid, have not been previously reported. We have therefore extended our previous work to include such experiments.

Sulfasuxidine (succinyl sulfathiazole). These data are pertinent in discerning the possible mode of action of pteroylglutamic acid in animal metabolism, since it has been postulated by Rogers and Shive (*5*) and Spies *et al.* (*2, 7*) that folic acid operates in the enzymatic synthesis of purines and pyrimidines.

TABLE 1

EXPERIMENT 1. EFFECT ON RATS OF THYMINE, ADENINE, AND ADENOSINE AS SUPPLEMENTS TO A PURIFIED DIET CONTAINING 2% SUCCINYL SULFATHIAZOLE (FOLIC ACID-DEFICIENT DIET)

Supplements	Initial weight in g of 28-day-old rats	Final weight in g of 63-day-old rats	Weight gain in g	R.B.C. (cells/mm³ × 10⁻⁶)	Hemoglobin g/100 ml blood	Hematocrit (vol. %)	W.B.C. (cells/mm³ × 10⁻³)	% Granulocytes	Total granulocytes (cells/mm³ × 10⁻³)	Ratio of animals surviving
None	67	133	66	8.53	17.1	43.0	8.64	1.6	0.14	10/10
Thymine (150 mg/100 g)	66	141	75	7.91	17.9	41.3	7.02	1.7	0.12	10/10
Thymine (150 mg/100 g) + adenine sulfate (150 mg/100 g)	66	135	69	8.78	17.9	45.5	7.88	2.0	0.16	10/10
Thymine (150 mg/100 g) + adenosine (150 mg/100 g)	68	132	64	8.42	17.5	42.9	7.16	1.4	0.10	10/10
Folic acid (50 μg/100 g)	65	185	120	8.37	17.2	41.6	11.78	8.7	1.02	10/10

TABLE 2

EXPERIMENT 2. HEMATOPOIETIC EFFECT OF THYMONUCLEIC ACID ON FOLIC ACID-DEFICIENT RATS FED 2% SUCCINYL SULFATHIAZOLE

Supplement from 63rd day to 77th day	Weight in g at 63 days	Weight in g at 77 days	R. B. C. (cells/mm³) × 10⁻⁶		Hemoglobin g/100 ml		W. B. C. (cells/mm³) × 10⁻³		Granulocytes (cells/mm³) × 10⁻³		Ratio of animals surviving
			63rd day	77th day	63rd day	77th day	63rd day	77th day	63rd day	77th day	77th day
2.1 μg folic acid	138	158	8.7	7.5	16.5	14.7	5.3	7.4	0.11	0.67	8/8
150 mg of thymonucleic acid ≅ 15 mg of thymine	131	123	8.6	6.8	15.7	13.2	4.8	2.0	0.08	0.03	8/8
5 μg folic acid	140	179	8.7	7.5	16.4	15.2	5.6	9.7	0.12	1.43	10/10

Twenty-eight-day-old littermate rats and the basal diet containing 2% succinyl sulfathiazole as previously described (*4*) were used in each of the two experiments reported here. In experiment 1, using ten rats, the supplements were incorporated in the basal diet and fed ad lib. for a 5-week preventive period. In experiment 2 the supplements were fed daily for a 2-week curative period after a depletion of 5 weeks. The results are shown in Tables 1 and 2.

The data seem conclusive in demonstrating that neither thymine combined with adenine sulfate or adenosine, as in experiment 1, nor thymine and adenine supplied as thymus nucleic acid, as in experiment 2, show physiological activity in replacing folic acid for growth and hematopoiesis in the rat fed a purified diet containing

The possibility exists that folic acid may serve essential functions in the animal body other than hematopoiesis; and whereas thymine and adenine may replace folic acid for these, they do not do so for hematopoiesis, which is the limiting function in the experiments described here.

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