

Y E A S T S

A News Letter For Persons Interested in Yeast

November 1954

Volume III, Number 2

Editor for ~~1953, 1954~~

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The Editor takes pleasure in thanking all those who have contributed to this issue. Without this gratifying support the News Letter cannot fulfill its purpose. The Editors would like to invite others to send in contributions for future issues. It is planned to publish the next issue of the News Letter during the spring of 1955. It would be appreciated if anyone would notify the Editor of additional people in our field who would like to receive the Yeast News Letter.

Cost of Operation. Contributions to help finance the News Letter are voluntary. Present funds on hand are very meager. It would be most helpful if each of those sufficiently interested would contribute a quarter. Many thanks to those who have recently contributed.

The Editors

I. Note from Dr. Earl G. La Roe, National Distillers Products Corporation, 1275 Seaton Road, Cincinnati 37, Ohio.

Dr. La Roe writes that the Research Division of the National Distillers Products Corporation is studying the production of hydrogen sulfide by a variety of yeast strains. He would welcome correspondence contacts with other workers who might be interested or who have engaged in research along the same lines. The published investigations on the phenomenon are not extensive.

II. Dr. F. M. Clark, our Associate Editor for the Illinois area, has sent in the following information.

Clifford H. Spotholz and Z. John Ordal of the Department of Food Technology, University of Illinois at Urbana, have been investigating the effect of certain environmental conditions on the production of Rhodotorula gracilis cells. This organism has been of interest because of its ability to produce fat. The environmental conditions were studied in a fermentor assembly provided with automatic controls for both pH and temperature. The following results have been obtained: (1) conditions for a rapid growth rate were established, giving a generation time almost 30% less than that previously reported, (2) the optimum temperature for rate of growth was found to be 4°C higher than commonly used, (3) the variation of pH within a wide range (pH 3.5 to pH 6.5) had only a slight effect on growth rate, (4) efficiency of glucose utilization was high (greater than 65%, which is higher than even the theoretical efficiency of fermentative yeasts) and (5) residual sugar in the medium was reduced to practically zero.

These and other experiments have indicated that this organism has attributes which might make it suitable for use as a primary food or feed yeast. Thus, its metabolic pathway was found to be non-fermentative, it is capable of assimilating xylose, and the flavor of the toasted yeast cells is very acceptable.

Dr. S. Spiegelman, Department of Bacteriology, University of Illinois is anxious to obtain some good cellobiose fermenting yeasts. He has obtained some strains which are supposed to ferment, but these were found to be rather slow fermenters of cellobiose.

III. Institute of Microbiology, Rutgers University, New Brunswick, N. J. Communicated by Professor Walter J. Nickerson.

Recent publications on yeast physiology from the Institute of Microbiology by Dr. Nickerson and co-workers are as follows:

Nickerson, W. J., and Romano, A. H. 1952. Enzymatic reduction of cystine by coenzyme I (DPNH). Science 115, 676-678.

Nickerson, W. J., and Chung, C. W. 1952. Reversal of fluoride inhibition of yeast growth with glucose-1-phosphate. Am. J. Botany 39, 669-679.

- Nickerson, W. J., and Mankowski, Z. 1953. A polysaccharide medium of known composition favoring chlamyospore formation in Candida albicans. J. Infect. Diseases 92, 20-25.
- Nickerson, W. J. 1953. Reduction of inorganic substances by yeasts. I. Extracellular reduction of sulfite by species of Candida. J. Infect. Diseases 93, 43-56.
- Nickerson, W. J., and Mankowski, Z. 1953. Role of nutrition in the maintenance of the yeast-shape in Candida. Am. J. Botany 40, 584-592.
- Nickerson, W. J., and Merkel, J. R. 1953. A light activation phenomenon in the enzymatic and nonenzymatic reduction of tetrazolium salts. Proc. Nat. Acad. Sci. 39, 901-905.
- Merkel, J. R., and Nickerson, W. J. 1953. Release of mitochondria from yeast cells by the action of metal-chelating agents. Proc. Nat. Acad. Sci. 39, 1008-1013.
- Nickerson, W. J., and Chung, C. W. 1954. Genetic block in the cellular division mechanism of a morphological mutant of a yeast. Am. J. Botany 41, 114-120.
- Nickerson, W. J. 1954. An enzymatic locus participating in cellular division of a yeast. J. Gen. Physiol. 37, 483-494.
- Romano, A. H., and Nickerson, W. J. 1954. Cystine reductase of pea seeds and yeasts. J. Biol. Chem. 208, 409-416.
- Chung, C. W., and Nickerson, W. J. 1954. Polysaccharide syntheses in growing yeasts. J. Biol. Chem. 208, 395-407.

Visiting post-doctorate fellows this year are Dr. A. H. Rose from the University of Birmingham, England. Dr. Rose obtained his Ph.D. from the Department of Applied Biochemistry at Birmingham in June, 1954 with a thesis, "Growth of a yeast and of a lactic acid bacterium in mixed culture, with particular reference to their nutritional requirements." He was awarded one of the newly established King George VI Fellowships to study here and is engaged on a problem of the mechanisms of sulfite and nitrate reduction by yeasts. Dr. Warren Silver, who obtained his degree in June, 1953 in biology at the Johns Hopkins University is here on a Public Health Service post-doctorate fellowship and is studying polysaccharide syntheses in yeasts.

In other work on yeasts in this laboratory, mechanisms of heavy metal uptake are being analyzed while investigation of the enzymatic blocks in division-less mutants continues.

IV. Biological Research Laboratory, Southern Illinois University, Carbondale, Illinois. Director Carl C. Lindgren.

The activities of the Biological Research Laboratory at Southern Illinois University are organized as follows:

- A. Yeast Genetics - Mrs. Lindegren, John Craig, Robert Drysdale and Ernest Shult.
- B. Biochemical Genetics - Dr. Ogur and Ralph St. John.
- C. Yeast Cytology - Dr. McClary and Marion Williams.
- D. Radiobiology - David Pittman and Paul Pedigo.

In addition to the above, the staff of the laboratory includes two part-time dishwashers, a part-time artist and a full-time secretary. Dr. Lindegren is director of the laboratory. There are seven graduate students: David Pittman, Robert Drysdale, Arturo Brenes-Pomales, Paul Pedigo, John Paul Hughes, John Craig and Ralph St. John.

The laboratory is now housed in a new \$1,500,000 building. There are seven staff laboratories 10 x 20 feet each; a small library; a departmental office; a 10 x 20 room containing the sterilizing equipment; a 20 x 20 room for making up media and washing dishes; a 20 x 60 undergraduate laboratory, and a 20 x 40 graduate laboratory connected by a 10 x 20 stock room. In addition, there is a suite of three radiobiology laboratories of about 600 sq. ft., two very large storage rooms, a cold room, an incubator room and two small laboratories. The laboratory is supported by the University and by grants from different agencies. In the past six years, these grants have totaled \$214,600.00 exclusive of the University support which has been approximately equal. During the period, 69 scientific reports have originated from the laboratory.

Since the last report the following papers have been published or accepted:

Ogur, Maurice, Lindegren, Gertrude and Lindegren, Carl C. A simple screening test for genetic studies of respiration deficiency in yeast. *Jour. of Bact.* 68, 391 (1954).

Ogur, Maurice. The rapid estimation of ploidy in cultures of Saccharomyces. *Jour. of Bact.* (accepted).

Ogur, Maurice. Respiration in a polyploid series in Saccharomyces. *Arch. of Biochem. and Biophys.* (accepted).

Sarachek, Alvin. X-ray inactivation of Saccharomyces during the budding cycle. *Experientia* Vol. X/9, 377 (1954).

Sheffner, A. L. and McClary, Dan O. The relationship between the oxidation and fermentation of galactose in the course of adaptation by Saccharomyces cerevisiae. *Arch. of Biochem. and Biophys.* 52, 74 (1954).

Lindegren, Carl C. and Shult, Ernest E. A general theory of crossing-over. *Jour. Of Genetics*.

V. United States Department of Agriculture, Northern Utilization Research Branch, Peoria, Ill. Communicated by Dr. L. J. Wickerham.

The search for mating types of haploid yeasts in nature is continuing. Mating types for Candida guilliermondii have been found. Sporulation is poor, amounting to a maximum of about 4 percent. The spores are hat-shaped and healthy asci rupture at maturity. Some 20 to 25 percent of stock cultures of this species mated with one or the other sex. Mating types for Lodder and van Rij's new species C. melibiosi also have been found and they mate with mating types of C. guilliermondii. The Dutch workers separated C. melibiosi from C. guilliermondii on the basis of melibiose fermentation. Several strains of C. melibiosi were obtained from the Centraalbureau. Strains of C. melibiosi and of C. guilliermondii were found to ferment melibiose, and the fermentation of both "species" was very slow. Correspondence with Miss Slooff of the CBS revealed that Dr. Lodder had also found, after changing to the Durham fermentation tubes for fermentation tests, that both "species" ferment melibiose, and Dr. Lodder concurs in our conclusion that C. melibiosi is a synonym of C. guilliermondii.

C. guilliermondii var. membranaefaciens, created by Lodder and van Rij, also exists as haploid mating types. It is the same as Candida chalmersi and Etchells and Bell's Endomycopsis ohmeri. All of these "species" produce moderately large numbers of ascospores when the opposite sexes are mated. So far as we are aware Etchell's is the only name indicating the perfect form of the species, and if this is true, the valid name is Endomycopsis ohmeri. E. ohmeri, by virtue of marked differences in physiology and morphology from C. guilliermondii, and by the fact that the two species will not mate with each other, is a distinct and autonomous species, and not a variety of C. guilliermondii.

Mating types of C. guilliermondii and E. ohmeri are available from this laboratory. To date, sexual forms have been found for seven species or varieties currently classified in the genus Candida and for two currently classified in the genus Torulopsis.

Taxonomic study of a new genus of yeasts, for which the name Dekkeromyces will be proposed, is underway, and the genetic phase is nearly complete. The genus will contain the species now known as Saccharomyces fragilis, S. lactis, S. marxianus, Zygosaccharomyces ashbyi and Z. dobzhanskii, and several other as yet undescribed species. Most of the species hybridize readily. Haploid species may be hybridized with diploid species, homothallic species with heterothallic species, and even the crossing of a homothallic, diploid species with a heterothallic haploid species is readily accomplished in the following simple procedure.

Actively growing cells of the two species are mixed together on the surface of a malt extract sporulation slant. The mixed culture is serially transferred on this medium four times at 10 day intervals at 25° C. At the end of this series of transfers the culture consists of both hybrids and parent cultures.

The parents should be selected on properties which will permit easy isolation of their hybrids. One of the easiest is by pigmentation. In this genus, the ability to produce a pink or red pigment is shared to varying degrees by nearly all species. Hybrids of weakly chromogenic parents may produce pink colonies on a medium on which their parents produce none, and thus the hybrids may be easily selected for further study. Another way to isolate hybrids is to select parents that differ in their ability to assimilate two carbon sources, A and B. By passing the mixture of hybrids and parents through a series of shaken flasks containing a chemically defined basal medium and A or B as the only source of carbon, in the order A,A,A,B,B,B,A, both parents are usually eliminated and only the hybrids assimilating both A and B are able to grow throughout the series. It is imperative that a very minute amount of inoculum be used from flask to flask, since the process is essentially a means of diluting each non-growing parent in turn to the point where it no longer exists in the culture.

For production of pigmented hybrids, parent strains NRRL Y-1140, S. lactis, a heterothallic haploid yeast, may be crossed with NRRL Y-1598, Z. ashbyi, a homothallic, predominantly diploid yeast. For novelty, either sex of S. lactis, Y-1140 or Y-1205, may be mated with the nonascosporogenous strain NRRL Y-2007, S. fragilis, reputedly a good pectinase producer, and some of the hybrids they produce are sporogenous.

Saccharomyces fragilis may be hybridized with Z. dobzhanskii and the hybrids isolated by serial transfers through lactose and maltose shake flasks as just described. The hybrids ferment both lactose and maltose, thus being the first ascosporogenous yeasts to possess this property.

During the past several months I have lectured on "Evolution of the Genus Hansenula" at bacteriology departments of the University of Illinois, Purdue University, and the University of Wisconsin.

The editor is happy to announce to our readers that Dr. Wick-erham was married last May to Laura MacMasters, an accomplished musician who likes the north country and mountains as much as Wick does. I would like to take this opportunity to speak for all of us and wish the Wick-erhams lots of luck and happiness.

H.J. P.

VI. U. S. Department of Agriculture, Food Fermentation Laboratory, North Carolina State College, Raleigh, North Carolina. Communicated by John L. Etchells and T. A. Bell.

In cooperation with the North Carolina Agricultural Experiment Station our investigations on the isolation and identification of yeasts from commercial cucumber fermentations has continued with interest directed at yeast populations and variation in species with relation to

different salt (NaCl) concentrations. We are also working with three commercial pickle plants in an attempt to control the gaseous fermentation caused by yeasts. In two plants the sorbic acid is used in the process of making salt stock whereas the other plant is attempting to control gaseous fermentation in the making of "Overnight" dills. Early observations with the use of sorbic acid indicate that the yeast populations are greatly reduced and also some of the lactic acid bacteria.

Publications:

Peterson, Bell, Etchells, and Smart. 1954. Procedure for Determining Carotenoid Pigments in Yeasts. Jour. of Bact. 67, 708.

Etchells, Costilow, Bell, and Demain. 1954. Control of Molds During the Enumeration and Isolation of Yeasts from Soil and Plant Material. Jour. of Applied Microbiology 2, 296.

Costilow, Etchells, and Blumer. Yeasts from Commercial Meat Brines. 1954. Jour. of Applied Microbiology 2, 300.

Hall, Etchells, Jones, and Lewis. 1954. Microbiological and Chemical Studies of Sweet Potato Vine Silage. Jour. of Dairy Science. (in press).

Single copies of "Morphology and Pigmentation of Certain Yeasts from Brines and the Cucumber Plant", by Etchells, Bell, and Jones. Farlowia 4, 265 (1953). Illus., 15 color plates, 109 black and white plates, are still available to students, teachers, and research workers as long as the supply lasts. Address request to: Box 5578, Raleigh, N. C.

VII. Illinois Institute of Technology, Chicago 16, Illinois. Associate Editor Leslie L. Hedrick has sent in the following news items.

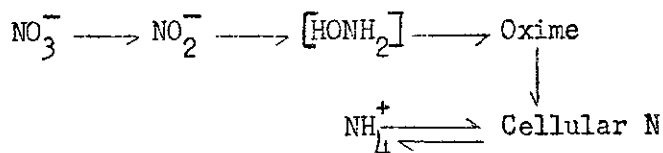
William Chesbro has been studying the sedimentation of yeasts, Hansenula subpelliculosa and Rhodotorula pilimanae, in the presence of influenza PR8 virus. The results obtained so far are summarized below.

"Under the conditions of the experiment, a stock virus having a titer of 1-512 with chicken erythrocytes will give a titer of 1-256 with either species of yeast used. The saline control and normal chorioallantoic fluid was negative at dilutions of 1-32.

Several differences have been observed between viral hemagglutination and yeast sedimentation. 1. There is no microscopic appearance of agglutination when yeast treated cells are used. 2. Prepared yeast cells in the presence of the virus form a pellet or a heavy ring. The saline controls form a broad shield on the inner curvature of the bottom of the test tube. 3. There is a nonspecific reaction with low dilutions of normal chorioallantoic fluid. Furthermore, yeast cells starved for two to three weeks before treatment give similar sedimentation patterns in viral and normal chorioallantoic fluid. 4. Urinary mucoprotein (Tamm and Horsfall, Proc. Soc. Exp. Biol. and Med., 74, 108 (1950))

does not interfere with the reaction when indicator virus (Stone, Aust. J. Exp. Biol. and Med. Sci., 27, 337 (1949)) is used with yeast cells. 5. Yeast cells exposed to a 1-32 dilution of the virus for 3 days at 4°C give positive titers with another aliquot of virus and form negative patterns with saline."

Selwyn Simon is continuing his work on the nitrogen metabolism of Hansenula anomala. The evidence from his preliminary experimental data suggests that nitrate enters into the cellular metabolism through the following basic pathways.



Clyde Doughty is working upon yeast agglutination using radioactive polysaccharide antigens in relation to yeast cell wall permeability.

Jules Corbett is continuing his studies upon the yeast agglutination.

The following persons are starting their research upon yeasts. Clare Forney - the nutrition of some pathogenic Candida yeasts; Betty Burkhardt - the carbon assimilation and classification of species of Kloeckera; Valerie Krol - some factors responsible for cell growth in selected Candida and Hansenula forms.

Harry Domnas is continuing his study of the adaptive formation of uricase in Torulopsis utilis. A. H. Roush has been awarded a research grant by the National Cancer Institute, U. S. Public Health Service, for a project entitled, "A Study of Enzymes of Purine Metabolism in Yeasts". A preliminary report on "Yeast Adenase", concerned with the adaptive formation and some of the properties of this new enzyme, was published in the June, 1954 Archives of Biochemistry and Biophysics.

L. R. Hedrick is continuing work upon some of the fundamental factors involved in yeast sedimentation and agglutination.

Your attention is called to an interesting abstract in Chemical Abstracts, 10829, September 25, 1954, "Viable protein bodies (filterable form) of S. cerevisiae. I. The regeneration of destroyed yeast cells in the sterile filtrate." T. Keleti, et al., Hung. Acad. Sci., Budapest. Acta Physiol. Acad. Sci. Hung., 5, 213-40 (1954) German.

In our laboratories, working with living H. californica that had been disrupted with glass beads in a sonic disintegrator, we were not able to get any growth on a malt yeast extract agar from a Seitz filtrate after incubation for two to three weeks at 25°C. The total number of cells disrupted by the sonic device in five hours before filtration was 9.5×10^6 .

During the latter part of August, my wife, son and I spent two or three weeks camping and tramping in the Grand Teton Mountain area and in one of our favorite spots, the Bear Tooth Mountain Plateau area, northeast of Yellowstone Park.

VIII. Department of Viticulture, University of California at Davis. Dr. J. G. B. Castor has sent in the following information.

Departments of Chemistry and Viticulture, University of California, Davis.

R. E. Kepner, J. G. B. Castor and A. Dinsmoor Webb. Conversion of α -amino n-butyric acid to propanol during alcoholic fermentation. Arch. Biochem. Biophysics 51 (1), 88-93 (1954).

Department of Viticulture.

J. G. B. Castor. Fermentation products and flavor profiles of yeasts (a progress report). Wines and Vines 35 (8), 29-31 (1954).

Departments of Viticulture and Agricultural Engineering.

J. G. B. Castor and F. C. Jacob. A study of the thermal death time of yeasts. In connection with work on TDT of yeasts by radio-frequency heating, a study of TDT of two strains of Saccharomyces cerevisiae with heat alone has been made. Rapid heating equipment employing small volumes of yeast suspension, intended to minimize "come-up" heating time, was employed. The results indicate that a log plot of carefully corrected TDT data between 55 and 75°C is not a straight line. In preparation for publication.

IX. Commonwealth Scientific and Industrial Research Organization. Oenological Investigations. Waite Institute, Adelaide, South Australia.

Dr. Bryce C. Rankine has sent the Editor three recent papers dealing with yeast investigations at the above institute.

Rankine, B. C. 1953. Quantitative Differences in Products of Fermentation by Different Strains of Wine Yeasts. Australian Jour. of App. Sci. 4, (4), Pages 590-602.

Fornachon, J. C. M. 1953. The Accumulation of Acetaldehyde by Suspensions of Yeasts. Australian Jour. of App. Sci. 6, (2), Pages 222-233.

Rankine, B. C. 1954. Fermentation by Pairs of Wine Yeasts. Australian Jour. of App. Sci. 5, (3), Pages 298-304.

X. University of São Paulo, Brazil.

Dr. El Tabey Shehata from Egypt is spending a year at São Paulo on a Rockefeller Foundation Fellowship to study the relationship between yeasts, various Brazilian species of Drosophila and some fermenting fruits on which Drosophila feeds. The project is carried out in cooperation with Dr. Brito da Cunha. Dr. Shehata has isolated about 300 yeasts, the identification of which has been nearly completed. A high percentage of the yeasts isolated proved to be apiculates. Altogether 29 different yeast species were isolated.

Besides this work Dr. Shehata has carried out a project with Dr. Camargo of the Instituto Zymotecnico concerning the microbiological flora involved in the fermentation of "Pinga" (A beverage made by distilling fermented sugar cane). About 100 yeasts and 20 bacteria were isolated. Dr. Shehata expects to return to Egypt about April 1, 1955.

XI. Hartley Botanical Laboratories, The University of Liverpool, England.

Dr. John H. Burnett writes that he has accepted a position at the University of Liverpool as Lecturer and is setting up work and research activities. His principal fields of interest are cytology, nucleic acid metabolism of yeasts and yeast nutrition of Drosophila. His graduate student, Miss B. Allsopp, will study yeast nutrition of Drosophila.

XII. The University of Tennessee, Knoxville. The Department of Bacteriology. Communicated by Dr. J. Orvin Mundt.

Mr. George Appleton, together with Dr. R. Kieber, is conducting a study at the University of Tennessee (Knoxville) of the sterol content of a number of representative fungi. Results to date indicate a continuous pattern of sterol content among individual groups of yeasts and molds. The Saccharomycetes possess the highest sterol content among the yeasts, and the Penicillia among the molds. Continuing investigations are an attempt to enhance the sterol content of yeasts.

XIII. News Item.

Dr. Hatfield has returned to California State Polytechnical College, San Luis Obispo, California, after spending 20 months at Camp Detrick, Maryland, in charge of the F.O.D. Assessment Branch laboratories, which included research in Bacteriology, Virology, Animal Pathology, and Methods Research units.

XIV. University of California, Department of Food Technology, Davis, California. Communicated by H. J. Phaff.

1. We have spent considerable time in expanding the replica plating procedure for the determination of carbon assimilation patterns

in yeasts (cf. Jour. Bact. 68, 28, 1954) to include nearly all the compounds recommended by Wickerham. The period of observation has been extended to about 17 days in order to detect more clearly latent utilization of compounds by certain yeasts. The results have been quite satisfactory so far and no evidence of interference between neighboring colonies has been found with the exception of sucrose and raffinose for a few yeasts. The results as a whole check well with those obtained by the liquid medium as used by Wickerham. We believe that it is preferable to use the free acid form of organic acids, rather than their salts, since in some cases the salt is not utilized, whereas the free acid is assimilated. The reverse situation has not been observed.

2. Dr. Elisa P. Knapp from the University of Sao Paulo, Brazil, who is spending a year in our laboratory under a Rockefeller Foundation fellowship, has isolated about 120 yeasts from slime flux of Quercus kelloggii (black oak) and certain mushrooms. These yeasts are being identified at present. The purpose of the survey is to study the microbiological composition of some of the food sources of wild species of Drosophila.

3. An extensive survey has been made of the yeasts found in the alimentary canal of six species of Drosophila in the Yosemite region of California. About 300 cultures have been isolated, the identification of which is nearly completed. Many yeasts were isolated which according to the keys of Lodder and van Rij would fall in S. cerevisiae. However, many differences in carbon assimilation patterns of these predominantly haploid yeasts were found as compared to typical bakers yeasts. They will be described therefore as new species. Other common species found include Hansenula angusta, a group of yeasts related to Zygosaccharomyces marxianus, Zygosaccharomyces drosophilae Shehata and Mrak, many species of Candida and various miscellaneous organisms. In addition systematic studies were conducted regarding differential attraction of species of Drosophila to bait inoculated with pure cultures of different species of yeasts.

The projects described under 2) and 3) were done in cooperation with Th. Dobzhansky, Columbia University, New York and Hampton L. Carson, Washington University, St. Louis, Missouri.

It is planned to publish the yeast Drosophila project in Ecology as a series of papers.

4. Two more papers have been published on the pectic enzyme produced exo-cellularly by Saccharomyces fragilis.

Demain, A. L., and Phaff, H. J. Hydrolysis of the Oligogalacturonides and Pectic Acid by Yeast Polygalacturonase. Jour. of Biol. Chem. 210, 381, 1954.

Demain, A. L., and Phaff, H. J. Composition and Action of Yeast Polygalacturonase. Nature 174, 515, 1954.

Dr. Demain is now holding a position in the Research Department of Merck and Company, Danville, Penna.

National Meeting Society of American Bacteriologists

New York - May 8 - 13, 1954.

The editor would like to call attention to the section "Round Table Discussions 1955 Meeting" on page 2 of the November News Letter of the Society. If a sufficient number of microbiologists interested in yeast problems attend this meeting, it might be worthwhile to organize a round table discussion on mutual problems. Persons interested in such an informal meeting are invited to write to me so that a meeting room may be reserved in time. Topics on taxonomy, ecology, genetics, cytology and others might be discussed.

Herman J. Phaff
Editor