

AN INTRODUCTION TO THE BIOLOGY OF YEASTS

BY

M. INGRAM, M.A., Ph.D.

*Low Temperature Research Station, University
of Cambridge and Department of Scientific
and Industrial Research*

U E
I-17

PREFACE

THIS book is intended to tell the student, or the worker beginning in this field, briefly what the yeasts are and what they do, because I found a great lack of such a book when I began to study the yeasts myself some years ago. The only book, in English, dealing with the yeasts as a group, was then Tanner (published in 1920), itself a translation of an older work by Guilliermond.

The plan of the book is, after an introductory chapter describing the general nature of yeasts, to pass from the simpler to the more complex metabolic processes, and from them to the processes of growth and reproduction, closing with an account of the behaviour of the yeast colony, and its place in nature. Because of current interest in the nutritive properties of yeasts, their composition has been treated at more length than might otherwise have been desirable.

It has been necessary to choose what to omit, from so short a book, and its emphasis is physiological, partly because our knowledge of the physiology of yeasts has been revolutionized during the past few years. This is less true of their morphology, of which fairly satisfactory general descriptions are already available. The taxonomy and morphology have recently been the subject of a most authoritative monograph by Lodder and Kreger-van Rij (1952), but their new nomenclature has not been followed in the text, since that would make many of the quoted species unrecognizable to a reader unfamiliar with such complications. In the index, however, the various names are cross-referred to the synonym currently accepted by Lodder and Kreger-van Rij, so as to correlate the various observations; although the identity of the several organisms may sometimes be questionable. I am grateful to Mr. James A. Barnett for his help there. The practical application of yeasts in fermentation, etc., has been covered in many other books, notably Jørgensen's *Micro-organisms and Fermentation*, so only the basic principles need be mentioned here. Recently, too, the genetics and cytology have been dealt with in some detail (Lindegren, 1949), so a general account suffices in this book. References have to be few, and aim simply to guide the reader to recent reviews or papers of particular interest.

The encouragement and advice of friends have helped me to write the book, and I should like to express my thanks to them. I am

specifically indebted to Mr. T. B. Bright and the Distillers Co., Ltd., for Plates I, V, and VI; to Dr. J. R. Northcote and the Cambridge University Press for Plate II; to Dr. K. M. Brandt for Plates III and IV; to Prof. Ö. Winge for Plates VIII to XI and for Fig. 4; and to Dr. T. Mann for Figs. 8 and 21.

M. L.

CONTENTS

<i>Preface</i>	PAGE V
CHAPTER I	
GENERAL MORPHOLOGY AND CYTOLOGY	I
General morphology—Internal structure: Capsular material; The cell-wall; The protoplast; The nucleus—Haploid and diploid phases	
CHAPTER II	
THE CHEMICAL COMPOSITION OF YEAST CELLS	II
Nitrogen compounds: Nucleoproteins; Proteins; Amino-acids—Carbohydrates: Structural polysaccharides; Cytoplasmic polysaccharides; Other carbohydrates—Fats—Accessory substances: Vitamins; Inorganic constituents	
CHAPTER III	
FERMENTATION	25
Fermentation of monosaccharides; Glucose: Historical and introductory; Glucose: The Embden-Meyerhof-Parnas scheme; Fructose and Mannose; Galactose; Other simple sugars—Fermentation of multisaccharides: Maltose; Trehalose; Sucrose; Lactose; Melibiose; Raffinose; Melaxitose; Polysaccharides; "Direct" fermentation—Fermentations in general: The effect of salts; The effect of vitamins; Reactions of various yeasts	
CHAPTER IV	
RESPIRATION AND THE PASTEUR EFFECT	74
The Pasteur effect—The mechanism of respiration: Hydrogen transference; Oxidation-reduction potentials; The fate of the carbon atoms; The mechanism of the Pasteur effect; The mechanism of endogenous respiration—Accessory factors—Respiration of multisaccharides	
CHAPTER V	
CARBON ASSIMILATION	100
The effect of air on assimilation; Sources of carbon—Synthesis of carbohydrate: The effect of conditions on carbohydrate synthesis; The mechanism of assimilation—Fat synthesis: Aeration; Carbon source; Age of cells; The mechanism of fat-formation	
CHAPTER VI	
NITROGEN METABOLISM	115
Degradation reactions—Assimilation of nitrogen: Ammonia; Nitrate; Nitrogen; Amino-acids; Amides, etc.; Amines; Miscellaneous compounds—Mechanisms of assimilation: Conditions affecting assimilation	

CHAPTER VII

	PAGE
SULPHUR METABOLISM	127

CHAPTER VIII

SPECIAL ASPECTS OF YEAST METABOLISM	129
---	-----

Alcohol tolerance—Reactions incidental to fermentation: Acid and ester production; Phytochemical reduction; Reactions related to acetaldehyde—Adaptation to ferment sugars: Adaptation in general—Biochemical organization of yeast cells

CHAPTER IX

GROWTH	151
------------------	-----

Chemical requirements for growth: Sources of carbon, nitrogen, and sulphur; Mineral requirements; Bios factors—Kinetics of growth: The adaptation phase; The lag phase; The logarithmic phase; Phase of negative acceleration; The stationary phase; The death phase

CHAPTER X

REPRODUCTION	180
------------------------	-----

Vegetative reproduction: Budding; Pseudomycelium; Appareil sporifère; Arthrospores—Sexual processes: Conjugation; The ascus—Sporulation: Liberation of spores from the ascus; Ascospores as resistant cells; Germination of the ascospores—Physiological requirements for sporulation: Pre-sporulation media; Requirements in the sporulation medium

CHAPTER XI

GENETICS AND VARIATION	203
----------------------------------	-----

Genetics: Techniques for hybridization; Mendelian segregation of characters of yeasts; Cytoplasmic factors—Variation: Spontaneous variations; Induced variations; Training of yeasts

CHAPTER XII

THE YEAST COLONY IN PRACTICE	222
--	-----

Liquid cultures—Solid media

CHAPTER XIII

ECOLOGY OF YEASTS	230
-----------------------------	-----

Air—Water: Brines—Soil—Miscellaneous plant materials: Flowers; Honey; Fruits; Plant pathogens—Insects—Higher animals: Milk; Animal pathogens—Yeast associations: Symbiosis; Succession

References	239
----------------------	-----

Index	247
-----------------	-----

PLATES

(Between pages 194 and 195)

PLATE

- I. Vegetative reproduction in *Saccharomyces cerevisiae*—Multipolar budding
- II. Electron micrographs of smashed *Saccharomyces cerevisiae*
- III. Living baking yeast photographed in ultraviolet light showing state of aggregation of volutin granules
- IV. Living baking yeast photographed in ultraviolet light showing dissolution of volutin granules
- V. Artificial hybridization of two ascospores—fusion and first budding
- VI. Artificial hybridization of two ascospores—formation of diploid colony
- VII. *Trichosporon behrendii*, showing true mycelium (elongated terminal cells), pseudomycelium (budding tips) and typical budding cells
- VIII. Simple segregation controlling morphological and colony characters in *Saccharomyces italicus*
- IX. Giant colonies of *Saccharomyces cerevisiae* "Mo," from each of three sister ascospores from each of two asci
- X. Giant colonies of *Saccharomyces cerevisiae* Hansen Rasse II showing range of colony form within a single species
- XI. Giant colonies of *Saccharomyces unisporus* showing "mutant" sectors
- XII. *Cladosporium herbarum*, showing yeast-like and fungal forms

INSET

FIG. 8. Diagrammatic presentation of the arrangement of reactions in the Embden-Meyerhof-Parnas scheme facing p. 46

TABLES

TABLE	PAGE
I. Stains to show the structure of yeast cells	8
II. Approximate composition of yeast	11
III. Approximate percentages of amino-acids in yeast "protein"	13
IV. Extracellular amylose production by various yeasts	15
V. Composition of fat of yeasts	18
VI. Uptake of thiamine by bakers' yeast	19
VII. Uptake of biotin in relation to synthetic ability	19
VIII. Vitamin-content of yeasts	20
IX. Nutrilite relations of <i>Tor. utilis</i> grown on fruit juice	21
X. Effect of aeration in increasing the vitamin-content of yeast	22
XI. Composition of dry, salt-free, autolysed brewers' yeast extract \	23
XII. Approximate mineral composition of yeast ash	23
XIII. Proportion of yeast protein in the form of some enzymes	46
XIV. Effect of temperature on rate of fermentation	67
XV. Rate of fermentation at 25°C by yeasts of various ages	67
XVI. Decline in the fermenting power of baking yeasts during storage	69
XVII. Fermentation reactions of ascospore-forming yeasts	71
XVIII. Fermentation reactions of anascosporogenous yeasts	72
XIX. Increased respiration of yeasts caused by the presence of respirable substrates	74
XX. Increase in respiration of yeasts due to the presence of respirable carbohydrates	75

TABLES

ix

PAGE

TABLE

XXI. Approximate molar concentration of glucose at which respiration is maximal and half-maximal . . .	77
XXII. Relations between aerobic and anaerobic respiration in various yeasts	82
XXIII. Differences between "top" and "bottom" yeasts . . .	84
XXIV. Effect of cyanide on respiration of yeasts	88
XXV. Oxidation-reduction potentials attained in suspensions of various yeasts in 3 per cent glucose in phosphate buffer of pH 5.4	89
XXVI. Increase in cell material and supernatant residue after fermentation of glucose by <i>Sacch. cerevisiae</i>	100
XXVII. Some sources of carbon for assimilation by yeasts . .	103
XXVIII. Effect of phosphate on utilization of glucose by baking yeast on 22 per cent glucose plus 0.3 per cent yeast extract	107
XXIX. Effect of concentration of yeast extract on utilization of glucose from 10 per cent solution	108
XXX. Fat-contents, and conversion factors, for several yeasts, etc.	112
XXXI. Inhibition of yeast action by alcohols	130
XXXII. Chief products of alcoholic fermentation, as percentage of sugar fermented	131
XXXIII. Substances which can be phytochemically reduced by fermenting yeast	133
XXXIV. Adaptive and constitutive fermentations by yeasts . .	137
XXXV. Effect of prior feeding with glucose on the rate of adaptation of washed cells to galactose	141
XXXVI. Competition between adaptive enzymes	142
XXXVII. Decrease of the mean generation time in the training of <i>Sacch. cerevisiae</i> to grow on carbon sources	144

TABLES

x

TABLE	PAGE
XXXVIII. Fermentation and "assimilation" reactions of <i>Hansenula</i> species	152
XXXIX. Growth on amino-acids as sole nitrogen source	154
XL. Effect of nitrogen deficiency on growth of <i>Sacch. cerevisiae</i>	156
XLI. Constituents of several synthetic media for yeasts	157
XLII. Concentrations of trace metals required for maximum yield when growing yeasts	159
XLIII. Inhibition by some trace elements of growth of yeast in a synthetic medium	159
XLIV. Stimulatory concentrations of bios factors	160
XLV. Uses of yeasts in assaying vitamins	166
XLVI. Comparison of quoted temperature limits for budding and sporulation.	187
XLVII. Ascospore characteristics	193
XLVIII. Resistance of yeast spores and vegetative cells to moist heat	195
XLIX. Percentage of spores obtained on wood blocks with different nitrogen sources in the pre-sporulation medium	198
L. Sporulation with various carbohydrates in the sporulation medium	201

CHAPTER I

GENERAL MORPHOLOGY AND CYTOLOGY

EVERYBODY knows the use of yeast, to make bread and brew liquor. Man has used it so from time immemorial in complete ignorance of the nature of yeast itself. Thus its name was originally a mere description of what it did: the English "yeast" derives from Greek ζέω—I boil (or the French "levure" from the Latin "levare"—to raise), and the term meant simply the sediment which formed in a brew, say of beer, which was known by experience to be able to reproduce the same kind of change if added to fresh sugary liquor.

Nobody knew more until 1680, when Leeuwenhoek looked at some of this yeast with his newly invented microscope. He found it to consist of very small spherical or oval bodies, in extremely large numbers. After this, nearly 150 years passed before it was shown that these bodies are isolated cells which reproduce by budding; and it was Pasteur, as late as 1860, who rounded off the matter by his classical demonstration that, in the absence of living yeasts, fermentation does not take place. Since that time, many different kinds of fermentation have been recognized, associated with slightly different kinds of yeasts (or sometimes bacteria). It has been recognized, too, that the yeast cell with its thick wall and sessile habit belongs to the plant kingdom; and is a fungus, because of its dependence on external food, and because of other relations to be detailed later. In this way, a conception of the yeasts grew up, as a unicellular group of fungi, reproducing by budding, and characterized by ability to cause fermentations.

General Morphology

The view of the yeast as a unicellular organism may be natural to the worker in the fermentation industry, for in the yeasts he uses the unicellular form predominates, especially in the actively growing state in which commercial yeasts are generally maintained. It is, however, much too restricted a view. Even an ordinary baking or brewing yeast, *Saccharomyces cerevisiae*, if grown for a time on a solid medium, produces a colony with a rhizoidal margin reminiscent of a fungal colony, and in these rhizoidal filaments the yeast cells are united in chains (*pseudomycelium*). In the related genus *Endomycopsis*, it

2 AN INTRODUCTION TO THE BIOLOGY OF YEASTS

is the rule for the yeast-like cells to cohere in chains; and there is an indefinite series of gradations between this habit and the normal fungal mycelium. The medical worker is, indeed, concerned mostly with yeasts in which the mycelial forms predominate, at least in culture. The botanist thinks of the yeasts as fungi belonging to the lower

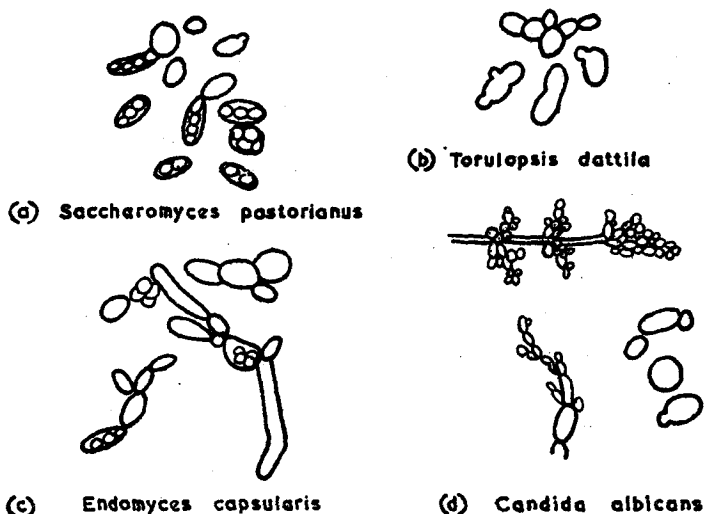


Fig. 1. The relationship of the main groups of yeasts

	Ascospores present	Ascospores absent
Pseudomycelium absent .	<i>a</i>	<i>b</i>
Pseudomycelium present .	<i>c</i>	<i>d</i>

The letters *a*, *b*, *c*, *d*, refer to the diagrams in Fig. 1.

Ascomycetes; although in fact the yeast-like form is found among other distantly related fungi—thus, *Mucor*, growing in jam, ferments it with the production of yeast-like cells in the anaerobic medium, and the basidiospores of *Ustilago* multiply by budding if sugary solutions are available. It is plain that the yeast-like form is largely a reaction to rapid multiplication in a favourable liquid medium; and the yeasts as a group can be only vaguely demarcated as fungi in which the unicellular form, multiplying by budding, is prominent.

Many of the typical yeasts, however, form endogenous spores,

which clearly resemble those of the lower Ascomycetous fungi. Consideration of these relations will be resumed later, but we may here distinguish four groups of yeasts, according as pseudomycelium is prevalent or not, and spores are present or not, as in Fig. 1.

These groups are, of course, difficult to define precisely: it has already been mentioned that some pseudomycelium can be produced by most yeasts; and it will be clear later that the power of forming spores is easily lost when a yeast is being cultivated, so that probably many yeasts regarded as without spores really belong in the groups with them. Nevertheless, on a broad view the four groups are clearly distinguishable: the first may be typified by the *Saccharomycetaceae* (including the fermentation yeasts); the second by the *Torulae* (more correctly *Torulopsidoideae*); the third by the *Endomycetaceae*; and the fourth by the *Mycotoruloideae*. The last group, particularly, is very vaguely limited, merging gradually into the *Fungi Imperfecti*.

The typical yeast cell is isolated, spherical to oval, or sometimes rather elongated especially in old cultures: Rahn regarded the average yeast cell as an ellipsoid of size $7.2 \times 5.6 \mu$, area $118 \mu^2$, volume $118 \mu^3$, and weight 1.3×10^{-10} g. A few yeasts have cells of highly characteristic shape, e.g. lemon-shaped in the "apiculate" yeasts, or "ogivally" pointed in *Brettanomyces* (cf. Fig. 34). Some species multiply by budding, others by transverse fission, and others by processes intermediate between the two. Old cells may become surrounded by a thick wall, and are "durable-cells" ("Dauerzellen") which bud again on germination—these structures correspond to the chlamydospores of related fungi. Some species form special spores. The details of these processes will be described later; for the present we shall consider the structure of a typical cell.

Internal Structure

The yeast cell has the structure of plant cells. There is a well-defined wall, quite thick in old cells, which encloses a protoplast containing a "nucleus" and "vacuole" and many other visible inclusions. The whole may be embedded in a rather vaguely defined layer of capsular material.

The finer points of this structure are imperfectly known, which is not surprising, because the cells are so small. When it comes to the smaller inclusions, with dimensions of fractions of a micron, one cannot expect to define their structure clearly by ordinary microscopy with visible light, and published diagrams should therefore be regarded

4 AN INTRODUCTION TO THE BIOLOGY OF YEASTS

with caution. The photographs of Plate I illustrate the resolution that can be expected without special techniques. Fig. 2 indicates the nature of some of the structures visible in Plate I. The nature of the visible structures has been variously interpreted in the past, as will become evident below.

Capsular Material. Sometimes yeasts produce a mucilaginous material outside the cells. It may take the form of a vague mass in which the yeast cells remain closely embedded, or it may take the form of a more or less well-defined capsule, especially definite in the pathogenic yeast *Cryptococcus* (= *Torulopsis*) *neoformans*. Hansen first observed such a structure in slowly dried brewing yeast. With species of *Zygosaccharomyces* it is produced especially on cultivation in media with high concentrations of sugar. On solid media the production of the capsules makes the colony soft, slimy and relatively translucent.

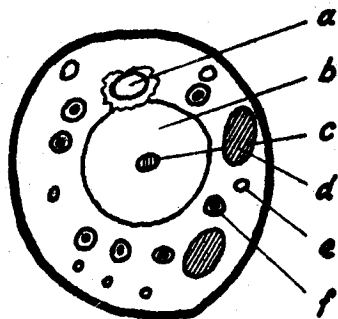


Fig. 2. Diagram, simplified from Wager and Penistone, of the chief visible structures in the yeast cell

- (a) Nucleus of Guilliermond, nucleolus or centrosome of other observers.
- (b) Vacuole of Guilliermond, or nuclear vacuole.
- (c) Volutin granule ("dancing body") in the vacuole.
- (d) Glycogen deposits.
- (e) Fat globules.
- (f) Basophilic granules, or "mitochondria."

The capsular material may be polysaccharide in nature: in *Tor. neoformans* and *Tor. rotundata* it consists of a pentosan plus amylose (Aschner *et al.*, 1945). The so-called

"yeast gums" probably originate in part from ill-defined capsular material of this sort.

The appearance of the capsule in microscopic preparations may be greatly changed by bad fixation: heating, for example, leaves vague granular haloes in place of the capsular material, from which the cells readily break away leaving an empty network as described by Hansen.

The Cell-wall. This is very thin initially, and remains elastic while the cell is growing. Later it becomes thicker and relatively rigid. The chemical nature of the wall has been doubtful, for various authors reported a variety of substances in it, and their different findings have still to be satisfactorily correlated. Components recognized histologically were pectic substances, hemicellulose, cellulose, yeast gum, and chitin, and overall chemical analysis related the wall substance to "fungal cellulose," which contains nitrogen; but recent analytical

studies (Northcote and Horne, 1952) give quite a different picture. Mechanical disintegration of the cells, and centrifuging, separated first a glycogen fraction; then a wall fraction, composing 15 per cent of the original fresh weight, consisting of mannan and glucan (about 75 per cent) associated with protein (13 per cent), fats (8 per cent), and ash (c. 3 per cent). The electron microscope reveals two distinct layers when the fat is removed (Plate II), and the structure is believed to be

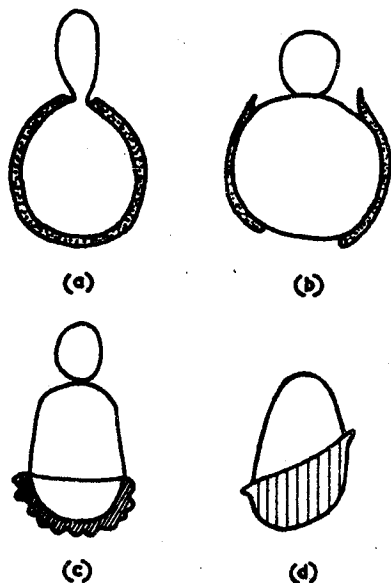


Fig. 3. Germination of "durable-cells" of *Sacch. cerevisiae* ((a) and (b)), and of ascospores of *Schwanniomycetes* (c) and *Sacch. guttulatus* (d), showing rupture of the outer wall layer

glucan-lipid: mannan-protein, with some glycogen granules adhering internally. The composition is likely to depend on the age of the cell, in a manner not easy to determine because cells of various ages are mixed together. In "durable-cells" the outer layer becomes thick and hard, and is ruptured when the cell begins to bud (Fig. 3).

The Protoplast. Relatively devoid of structure in the young cell, and solid and dense, it becomes progressively filled with various visible inclusions as the cell ages.

The cytoplasm of actively dividing cells appears homogeneous in normal light, but in resting cells small granules, up to about fifty, are visible in it. They stain with basic dyes (e.g. Gram stain) and so were

called basophilic granules by Guilliermond, who thought they were composed of protein because in autolysing acetone-treated yeast the granules disappear while nitrogenous compounds appear in the solution outside the cells. The granules are removed by washing with acetic acid, and the staining disappears. They coalesce when treated with alcohol, or heated, or the cell is highly compressed, and they are highly refractile, suggesting that they contain fat. Caspersson and Brandt, by ultraviolet microscopy, showed that they contain nucleoprotein, and believed they consist of volutin. Lindegren denies this, and calls the granules "mitochondria." They behave in very different ways according to the physiological state of the cell (Plates III and IV). It is clear from the u.v. absorption that in actively dividing cells the granular material is more evenly dispersed throughout the cytoplasm, which is why it seems homogeneous, but there is, in fact, a concentration of the u.v. absorbing material near the vacuole, and it is suggested that it may be synthesized there.

In older, well-nourished cells, granules of *glycogen* accumulate, refractile, but less so than fat globules. This is the reserve carbohydrate of yeasts, the normal form in which food is stored (Lindegren, 1945); it disappears during starvation. Some apiculate, and some lactose-fermenting, yeasts do not store glycogen.

Fat appears in the cytoplasm of cells fed with sugar but little nitrogen, in the form of refractile globules, which can be dissolved out with ether, and stain with the usual fat stains. They are not normally conspicuous in fermentation yeasts, but in a few other species they coalesce gradually and nearly fill the cell.

Pigments give colour to some yeasts, usually brown, yellow, or red. Those of the *Rhodotorulaceae* are carotenoids (Mrak and Phaff, 1948). In pigmented strains of *Sacch. cerevisiae*, the pigment is a quinoid prosthetic group carried on a polypeptide. The red pigments formed by *Tor. pulcherrima*, *Sacch. lactis*, and by other species grown on a biotin-deficient medium with added methionine, are believed to be the same, and contain much iron; the pink pigment of adenine-deficient mustard-gas induced mutants is different (van der Walt, 1952). In other genera (e.g. *Geotrichoides*, *Pichia*, *Torulopsis*, *Zygosaccharomyces*) the nature of the pigments is not generally known.

Vacuole. There is usually one large central vacuole, though there may be others near the poles of elongated cells. Granules in the vacuoles show greater Brownian movements ("dancing bodies"), suggesting that the vacuoles are sacs of fluid less viscous than the

surrounding cytoplasm. They were believed to be analogous to the vacuoles of plant cells, but this is now questioned (see below).

Volutin is a cytological conception based on staining reactions, and the term has unfortunately been applied to two different things. Guilliermond used it for granules appearing in the vacuoles, regarded it as a proteinaceous food reserve, and identified it with metachromatin. Caspersson and Brandt, on the other hand, regarded as volutin the cytoplasmic granules visible in ultraviolet light (Plates III and IV) which were shown to consist of yeast nucleic acid; and, observing no u.v. absorption in the vacuole, they believed the staining reactions of the granules there to be caused by ester sulphates. Lindegren prefers to retain the term volutin for the vacuolar granules, from which basic dyes are not removed by acid washing, and he thinks that they probably consist of metaphosphate. The cytoplasmic granules he calls "mitochondria" (see Lindegren, 1949). This confusion has arisen because the classical nuclear stains, like iron haematoxylin, react with yeast nucleic acid because of its similarity in composition to the desoxyribose nucleic acid of normal nuclei (Pollister and Mirsky, 1944):

Volutin	Yeast nucleic acid	D-ribose, uracil
Normal nuclear chromatin	Chromonucleic acid	D-desoxyribose, thymine

The Nucleus. This is of special interest in the yeast cell, as intermediate between the orthodox nuclei of other Fungi and the rudimentary nuclear structures in bacteria (Delamater, 1950). According to Guilliermond, the nucleus is a small and relatively dense body situated at one side of the central vacuole; it divides into two on budding, and the daughter-nucleus passes into the bud cell. Guilliermond claimed to distinguish, within a nuclear membrane, a nucleolus and a chromatinic network as in other plant cells; but his interpretation has been doubted, and there is still no generally accepted method for demonstrating the internal structure microscopically. Badian thought he could demonstrate a haploid number of two chromosomes in *Sacch. cerevisiae*, by fixing in osmic vapour, staining with methylene blue, and de-staining with eosin.

Lindegren has lately contended that the nucleus as understood above cannot be the real nucleus, because it is too dense. By vital staining with 0.01 per cent methylene or toluidine blue he has observed