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VOLUME 3

D. J. D. HOCKENHULL

PROGRESS IN INDUSTRIAL MICROBIOLOGY

VOLUME III

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PREFACE

VOLUME III of *Progress in Industrial Microbiology* again attempts to cover those areas of microbiology that are of interest to the industry. The concept behind this effort is that the applied worker has limited time and opportunity for surveying the literature and in particular for finding where the information is to be procured. In fact, a good deal of the purpose of these volumes is to act as 'guides to the academic literature for those in industry'. To a secondary extent, they deal with the application of this information. The good industrial worker, however, is not generally lacking in the ability to do this for himself. A further function of the series is to provide a means of publishing more technical material not usually suitable for the usual microbiological or biochemical publications. It must be realized, however, that there are limitations on the extent to which this can be done. First, much industrial practice is confidential and its publication is restricted. Second, much is either a matter of 'oral tradition' or is scattered between a number of departments so that its collection into comprehensive surveys has never been made. Third, so much is a matter of expediency that papers on the subject would lack the consistency of theme necessary to a review.

At the same time, it is realized by the Editor that it is difficult for the average graduate or academic researcher to envisage how large-scale industrial processes are carried out. (This is most apparent when new graduate intake is interviewed.) As far as possible, therefore, the articles endeavour to satisfy this need. It is hoped that reviews on the 'philosophy' and organization of industrial microbiology can be obtained for later volumes.

D. J. D. HOCKENHULL

RECENT RESEARCH ON THE YEASTS

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RECENT RESEARCH ON THE YEASTS

1. INTRODUCTION

There appears to have been in recent years a quickening of interest in the yeasts. For biochemical research in a number of fields the yeasts are tools convenient to handle and for genetical work they may offer possibilities of reasonably quick results which may in some cases be of economic importance. The increasingly scientific approach to perhaps the oldest fermentation industry, brewing, has also tended towards a greater interest than ever before in this group of micro-organisms. A further reason may be in the realization that yeast is a valuable food, and with an ever-increasing world population, this easy source of nutrient from carbohydrate may become a valuable adjunct to the diet.

The past few years have seen the appearance of a number of textbooks and monographs devoted to yeasts. Some such as those edited by Cook (1958)[1] and Roman (1957),[2] that written by Ingram (1955),[3] and the Society of Chemical Industry Monograph (1958)[4] have a broad coverage, while more specialized contributions have come from Lodder and Kreger-van Rij (1952)[5] on taxonomy (now out of print), White (1954)[6] on yeast technology, Lindegren (1949)[7] on genetics and cytology, Lund (1954)[8] on ecology, and Vogel (1949)[9] on beer yeast. Of the textbooks appearing in the last decade that edited by A. H. Cook (1958)[1] is at present the most comprehensive and reliable source of information. The contributors to that volume cover their respective topics very adequately up to the latest possible date, which means in most cases work reported to 1956 and, in several instances, early 1957. For the purpose of this review, therefore, the term 'recent' is defined broadly as the period since the contributions to Cook's book were written, but some overlap is inevitable in the interests of clarity and continuity.

Many papers which have come to the writer's notice are not referred to here as an attempt has been made to avoid compiling a catalogue. Some of them are dealing specifically with techniques and analytical methods. A number of biochemical papers dealing particularly with such topics as the yeast nucleic acids are not mentioned but the reader is referred to an excellent brief exposition by Cook (1959).[10] In the sphere of industrial microbiology and in that part of it concerned with fermentation, a number of subjects are of great present interest; it is these that receive particular emphasis.

2. CLASSIFICATION

Progress in work on the yeasts has been assisted in the last few years by the general acceptance of the system of classification of Lodder and Kreger-van Rij[5] which has introduced some law and order into a previously somewhat chaotic field of microbiology. The studies of systematics and taxonomy generate strong opinions, and it is not difficult for the specialist to disapprove

of cavalier treatment of the group of organisms in which he is interested. Reference to the chapter by Lodder, Slooff, and Kreger-van Rij in Cook (1958)[1] will indicate the efforts made over the past century to improve classification of the yeasts and the position is now better than it has ever been.

There are of course anomalies; for example, a frequenter of earlier textbooks *Oidium lactis*, alias *Geotrichum candidum*, has now disappeared as it is really *Oospora lactis* and therefore not a yeast at all. This puzzles some of the older brewers and yeast manufacturers who maintain that, whatever its name, it is still here! The same frequency of shifting nomenclature applies for example to *Torula utilis*, later *Torulopsis utilis*, and now (at the time of writing) *Candida utilis*. Other organisms met with in brewing but which are not what they were are *Mycoderma* and *Dematium*; their demise or renaming was inevitable because that particular labelling was too wide and vague. Changes in classification are not, however, made whimsically and the process is bound to continue with increasing knowledge. In the genus *Torulopsis*, for example, under certain conditions spores may be produced and it appears that a number of *Torulopsis* species are really imperfect forms of species of other genera such as *Saccharomyces*, *Debaryomyces*, and *Hansenula*. So long as it is realized that no system of classification is perfect, Lodder and Kreger-van Rij's scheme is the best at present available, although other workers have made valuable contributions regarding particular groups. The reader is referred for further details to the chapter in Cook[1] already mentioned. It is unfortunate that Lodder and Kreger-van Rij's book is now out of print and its reappearance in the near future is unlikely. A simpler version of their system has appeared in a British journal (1954, 1955)[11, 12] and more recently articles on the topic, with illustrations, come from de Becze(1959).[13]

From the point of view of the practical brewer, his interest in classification is not as between families and genera but rather as between different strains of two species, *Sacch. cerevisiae* and *Sacch. carlsbergensis*. In this regard, Streutker (1959)[14] has typed a number of strains on the basis of their bios requirements, nitrogen metabolism, and hydrogen sulphide production. Thorne (1958)[15] has taken fermentation efficiency as a basis for classification of strains and has measured this property in more than 400. He realizes that the system, being an artificial one, and indeed based on a variable character, has its limitations, but it has an appeal to the brewer. Thorne's (1954)[16] definition of efficiency is the ratio of fermentation velocity to nitrogen content of the yeast (fermentation velocity being expressed as the quantity in millilitres of carbon dioxide at n.t.p. evolved per hour per gram of moist yeast from a standard synthetic medium at 20° C).

In the context of the relationship between fermentation velocity and nitrogen content, Curtis and Clark (1956)[17] found no relationship between the nitrogen of the yeast crop skimmed from the fermentation and the degree of attenuation at rack. Therefore when considering variable attenuations for the same strain of yeast (so far as those flocculent and non-flocculent strains examined by them were concerned) variations in fermentation velocity of the yeast did not appear to be a factor of importance. The nitrogen content of poorly attenuative yeasts of high generation number was found to be no lower than that of more strongly attenuative yeasts of lower generation

number! In brewery fermentations, the rate of fermentation at the point when the yeast count was constant was quite unrelated to the final degree of attenuation.

Characterization and identification of yeasts by a rapid but exact method is claimed by Kockova-Kratochvilova et al. (1959).[18] Two mixtures of sugars in 5 per cent yeast water are prepared, one containing raffinose, sucrose, galactose, and arabinose, and the other containing lactose, maltose, glucose, and fructose. These are inoculated with the unidentified yeast, incubated for 7 days at 25° C, and the medium chromatographed on paper. It is contended that the chromatograms reveal not only which sugar has been used but also how the mechanism of the sugar fermentation proceeds, whether digestion has been anaerobic or oxidative, and so forth. These results are supplemented by fermentation tests of the maltose and sucrose. The organisms fall into four fermentation types.

3. CYTOLOGY

3.1. Structure of the Cell Wall

In the last few years progress in the study of the yeast cell has quickened, especially in the investigation of the cell wall. The importance of this structure cannot be exaggerated, for apart from the fact that it is a barrier through which metabolites and metabolic products must pass, it plays a most important role in flocculence, fining behaviour, and the action of germicides, antibiotics, and surface active agents, including such widely used materials as the cationic quaternary ammonium compounds.

Earlier workers isolated cell walls by 'chemical' methods, i.e. by autolysis, or acid and alkali extraction. More recently Northcote and Horne (1952),[19] among others, employed mechanical means of breaking up the cells and confirmed the presence of glucan and mannan in the walls; they showed that these components were in distinct layers, a fact which became evident when the lipid contents were extracted. The mannan is associated as a complex with protein. The use of enzymes in earlier work on certain fractions of yeast constituents (e.g. Giaja and Kraut, Eichhorn and Rubenhauer) suggested to Eddy (1958)[20] this mode of approach to the degradation of the cell walls. He found that enzymes such as trypsin, papain, and a malt enzyme dissolved up to about 50 per cent, but that an enzyme prepared from the snail *Helix pomatia* caused complete dissolution of the yeast cell wall. Of great significance was the fact that with the malt and snail enzymes the speed of the reaction was directly proportional to the phosphorus content of the wall.

Products of the cell walls dissolved by the malt and snail enzymes were shown chromatographically to be glucose and N-acetylglucosamine, but with trypsin and papain no dialysable products were found. In all cases the non-dialysable substances included mannan and glucan. When papain was the enzyme used, an initial phase of rapid reaction led almost to the complete loss of negative charge associated with phosphate ions. Practically the whole of the dissolved phosphate was recovered as a complex with mannan and protein. Eddy concludes from his observations that the major constituents

of the cell wall consist of an insoluble glucan matrix to the extent of about 50 per cent, attached to mannan (about 20 per cent), and to soluble glucan (about 10 per cent) by a protein cement of about 7 per cent. This is expressed pictorially in Figure 1.

It is concluded from the magnitude of the charge density at the inner and outer surfaces of the wall that a relatively large portion of each is probably occupied by mannan and a small proportion by protein.

Kessler and Nickerson (1959)[21] have also worked on the composition of the cell wall and have reported on the isolation and characterization of the glucomannan–protein complexes, and recent evidence on the structure of yeast glucan comes from Peat et al. (1958).[22, 23]

At the time of writing, a paper has appeared by Korn and Northcote (1960)[24] which carries information on the yeast cell wall a stage further. They have separated three fractions: A, soluble in ethylenediamine and

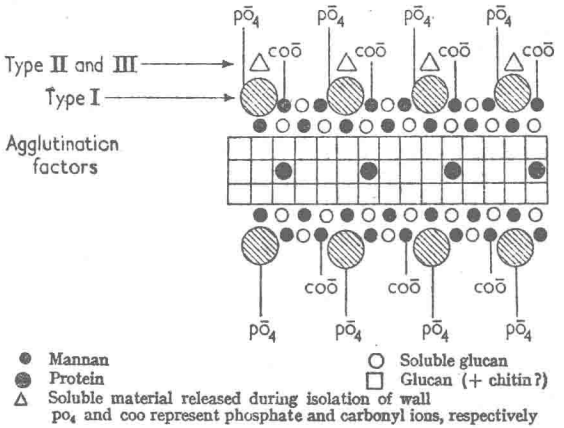

Figure 1. Diagramatic representation of the structure of the cell wall of typical yeasts. From Cook[10]

water; B, soluble in ethylenediamine but insoluble in water; and C, insoluble in both ethylenediamine and water. Fraction A seems to be a mannan–protein complex, and fraction B is possibly a glucan–mannan–protein complex. Glucosamine is present in all three fractions and this amino sugar may provide the 'link' between the carbohydrate and protein. Not more than about 9 per cent of the total glucosamine of the cell wall is estimated to occur as chitin. Yeast mannan was found to have a molecular weight of $5{\cdot}9 \times 10^4$ and to contain 1·4 per cent of glucosamine and 1·5 per cent of protein. Yeast glucan contained 6 per cent of glucosamine and 0·8 per cent of protein.

Eddy's work followed on from previous investigations by Eddy and Rudin (1958)[25] on the identification of charged groups on the surface of the yeast cell wall. They found that for various strains of *Sacch. cerevisiae* and *Sacch. carlsbergensis* ('top' and 'bottom' brewing yeasts, respectively) the electrophoretic mobilities were of an order indicating that the portion of the cell surface occupied by ions must be rather small. Furthermore, intact cells and isolated cell walls behaved similarly on electrophoresis and in a way which

varied with the strain of yeast. Hence it was concluded that it is wall composition that determines the electrophoretic properties. Eddy and Rudin found that they could distinguish two general effects (I and II) of change of pH on electrophoretic mobility characterizing certain yeast strains, although in some instances these effects occurred together. In type I, mobility was practically independent of pH and corresponded to a negative charge. Such charges were lacking from yeasts grown in phosphate-deficient media and the mobility of the isolated cell wall was apparently directly related to the cell wall phosphorus content. Hence it is considered that the negative charge can be attributed to combined phosphate forming part of the fixed structure of the wall. In type II, the mobility varied continuously between pH values 3 and 6, with change of charge from positive to negative round about pH 4. In this instance, the charged groups are attributed to protein, because apparently material of this nature was entirely removed with the groups themselves when cell walls were treated in alkaline solution.

The connection considered to exist between the charge in the cell wall and phenomena in brewing such as flocculence is obviously of practical interest. Eddy and Rudin (1958)[26] suggest an explanation based on the observation described above, demonstrating the two distinct main components due to phosphate groups and protein material.

An incidental feature of the work by Eddy and his collaborators is the fate of the residual protoplasts after removal of the cell wall by the enzyme from *Helix pomatia*. It is found (Eddy and Williamson (1959)[27]) that the protoplasts, while incapable of reverting to whole cells, can give rise to a superficial membrane which somewhat resembles a normal yeast cell wall. This result is at variance with that of Nečas (1956)[28] but a possible explanation of this is offered by the British workers. The composition of the new wall differs appreciably from that of a normal wall; the nitrogen content is much higher, but instead of amino acid residues the nitrogenous material is predominantly

TABLE I

Comparison of Cell Walls Produced by the Protoplast with Those from the Parent Yeast[27]

	Normal cell wall	Protoplast wall[e]
Nitrogen (per cent dry wt)	1·0	2·2–2·7
Phosphorus (per cent dry wt)	0·19	0·03
N-acetylglucosamine[a] (per cent dry wt)	0·9	27–33
Invertase[b] (μmole sucrose/mg wall/hr)	6·0	<0·03
Amino acids	+++	±
Mannose	+	+
Glucose[c]	++	++
Anthrone-positive carbohydrate[d] (per cent dry wt)	55	35–45

(a) Determined after hydrolysis for 72 hr with two successive portions of snail enzyme.
(b) Measured at 25° C in presence of 0·01M acetate buffer, pH 5·3. Activity of normal walls was shown to be twenty times greater than could be accounted for by the proportion of whole cells present.
(c) Found after hydrolysis with sulphuric acid (70 per cent v/v) for 2 hr.
(d) Calculated as glucose equivalent.
(e) Values refer to three independent preparations.

N-acetylglucosamine (see Table I). It has been found possible to induce the protoplasts to sporulate and thence on germination to give whole normal walled cells again.

By ultrasonic treatment, Eddy (1959)[28a] has been able to isolate fragile parts of the yeast cell, including the primary vacuole and various accessory vacuoles, besides a distinct spheroidal vesicle. Furthermore, the refractile bodies were isolated and found to be associated with an amount of DNA representing the whole of that found in the protoplast. It is thought that these bodies derive from the cell nucleus, which apparently contains only 1 per cent of DNA by weight but several times that amount of RNA.

3.2. Sporulation and Related Phenomena

A number of papers on cytology of yeast have appeared from Indian workers. Royan and Bhattacharya (1956)[29] state that by washing and fixing and then hydrolysing with N-hydrochloric acid prior to electron microscopy, and then by repeated acid hydrolysis, they achieved progressive clearing which facilitated cell studies. In later papers, Royan and Subramaniam (1956),[30] Royan (1956),[31,32] and Narayana (1956)[33, 34] give results of their further cytological studies with various microscopieal and staining techniques and are concerned with the vacuole and the nucleus. These have been followed by papers from Royan (1956, 1958)[35–38], Thyagarajan (1958),[39–41] Aswathanarayana (1958),[42] and Aswathanarayana and Subramaniam (1958)[43] on similar problems and on spore formation and germination.

In an endeavour to induce more successful sporulation in bottom fermentation yeasts, with a view to hybridization, Oppenoorth (1957),[44] noting that spore formation occurred more readily in winter and spring, therefore tested the effect of light of different wavelengths. A relationship to light sensitivity was found in a few cases, in the red region. Alternating temperatures also stimulated sporulation, but in no case was sporulation induced where it had hitherto failed.

Another contribution on sporulation has come from Ganesan, Holter, and Roberts (1958)[45] who set out to see what actually happens when yeasts are put into a sporulating medium. The general assumption that meiosis results from a lack of nutrients was not considered by these workers to be borne out by facts and they focused interest on the stages before sporulation occurs. Using a technique in which they put cells into Fowell's medium and then transferred to droplets of wort, they found that the critical exposure time to Fowell's medium was 13–17 hr. After this, 50 per cent of the cells produced asci while the remainder started vegetative growth. It was also evident that, once induced, meiosis will not be hindered by sudden removal from the sporulation medium. It was calculated that the length of the first meiotic prophase is about 3 hr and the bi-nucleate stage about 1 hr.

Ganesan (1959)[46] has also recently studied the cytology of a hybrid of *Sacch. italicus* and *Sacch. carlsbergensis*. Elegant staining technique and a photographic record enabled the stages of division and sporulation to be followed.

The difficulty of achieving easy sporulation with many sporogenous yeasts was at one time a drawback to their study and utilization, as for example in hybridization. The use of media such as acetate agar has proved of great

value in this regard. A further series of papers on sporulation has recently come from Miller and his colleagues (1956, 1957, 1959).[47–49] While many yeasts sporulate readily on such media as acetate, for growth they require an abundant carbon source, and while a nitrogen source is also necesssary for growth, even small quantities of it can inhibit spore formation. A number of inhibitory substances have been tested, some after growth and others after sporulation. Thus glucose in relatively high concentration (0·33–1·0 per cent) assisted growth but deterred good spore formation, in that fewer 3- and 4-spored asci occurred. Fluoride and iodoacetate were inhibitory to growth with either glucose or acetate, but sporulation was less affected. Ethanol also inhibited sporulation, but as with glucose, transference of the cells after 24 hr to buffer solutions achieved spore formation. Substances such as acetoin, 2:3-butylene glycol, and ethylene glycol had no effect either way, but fluoroacetate inhibited sporulation, but not growth. With acetate as sole carbon source, urethane affected spore formation more than growth, while the opposite effect was noted with azide, malachite green, cyanide, and dinitrophenol. The effect of different sugars on sporulation varies and also depends on their concentration. Fructose, mannose, and galactose give higher yields of asci than glucose, but with xylose, arabinose, and lactose, no asci are found. As high concentrations of glucose appear to inhibit sporulation, an investigation was made to assess the relationship of preliminary stages of carbohydrate metabolism to sporulation. Both growth and sporulation were carried out in presence of phthalate buffer at pH 5·0. With glucose, fructose, and mannose, spore formation of baker's yeast was best at 0·05 per cent sugar. There was a decline in ascus production at higher concentrations. On the other hand, with galactose there was no appreciable difference. With the last-named, but not with the other three, added acetate increased sporulation. The same occurred with dihydroxyacetone. Phosphate increased the number of 3- and 4-spored asci in galactose, but arsenate did not, although when added to the presporulation medium it reduced spore formation. Pyruvate gave good sporulation with no ill-effect at higher concentrations. For growth, as opposed to spore formation, galactose was the best carbohydrate, although it also affected the shape and retarded fission of the cells. In the latest paper (1959)[49] from this team, an investigation of the respiration of growing and sporulating cells is discussed.

Instead of the usual sodium acetate medium for inducing sporulation, McClary, Nulty, and Miller (1959)[50] find that for strains of *Sacch. cerevisiae* the potassium salt is more effective.

4. METABOLISM AND ENZYME ACTION

A paper from Slonimski (1958)[4] draws attention to the chief discrepancies between generally accepted theory and the experimental facts of respiration and growth of yeasts. He discusses the relationship between fermentation and respiration from the point of view of the inhibition by glucose fermentation of the synthesis of cytochrome required for terminal respiratory processes. He suggests that when baker's yeast is inoculated into a glucose medium the chain of events is: high glucose concentration→high aerobic

fermentation → slow differential rate of synthesis of respiratory enzymes → low respiration → low Pasteur effect → higher aerobic fermentation → slower rate of synthesis of respiratory enzymes → low respiration, and so on until a seeming equilibrium is reached; then, as the concentration of glucose decreases, the sequence is in the opposite direction: low glucose concentration → low aerobic fermentation → high differential rate of synthesis of respiratory enzymes → high respiration → high Pasteur effect → lower aerobic fermentation, etc. All the time the metabolic properties of the cell are determined by the relative importance of the two antagonistic mechanisms: the Pasteur effect and the counter-Pasteur effect.

In conjunction with Slonimski's paper a contribution by Holzer (1958)[4] to the same Monograph should be read. It also is concerned with the regulation of carbohydrate metabolism in the yeast cell and discusses the occurrence of aerobic fermentation in connection with growth and the inhibition of glucose uptake under transition from anaerobic to aerobic conditions (Pasteur effect). Holzer suggests that the supply of *ortho*phosphate can control the change-over from fermentation to respiration.

Among papers on enzyme action and adaptation is an account by Strittnatter (1957)[51] of experiments in connection with the utilization of various saccharides by yeast. The adaptation of the organism to the use of galactose and other sugars is believed to be due to the formation of enzymes which convert these sugars into intermediates of glucose and ribose metabolism which then proceed along the usual pathways of oxidation or fermentation. However, the possibility of alternative pathways needed pursuing; for example, it had been observed that a specified strain of *Sacch. cerevisiae*, after growth on galactose, oxidized glucose more rapidly than this strain normally did. This increased oxidative activity was confirmed while the anaerobic fermentation rate was unchanged, but there was, as might be expected, increased growth of yeast. The higher oxidative metabolism included enhanced respiratory quotient with glucose and other oxidizable substrates, more cytochrome, and much increased cytochrome oxidase activity. The observed differences are associated with an inhibitory effect of glucose on the the formation of oxidative enzymes when it is present to the extent of more than 0·05 per cent. The differences were influenced by the composition of the basal medium, particularly when the amount of a yeast autolysate was reduced. From this fact it is deduced that in the ordinary course the inhibitory effect of glucose may involve a depletion of those constituents necessary for the formation of oxidative enzymes. The inhibitory effect of glucose could, furthermore, be connected with a regulating mechanism within the cell, since in the presence of readily fermented glucose, the *constitutive* enzyme systems supply the energy requirements of the yeast without needing the aid of *adaptive* oxidative enzyme systems.

White (1956)[52] refers to enzyme adaptations which he has followed in fermentations of sucrose, D-glucose, invert sugar, beet molasses, and maltose. Some of these adaptations were expected, such as a very high maltose-fermenting activity developed in yeast grown in a maltose solution. In fact, yeasts have been produced giving a greater gas production in maltose than in glucose, which appears to support the view that maltose may be fermented

directly, without preliminary hydrolysis to glucose. Less expected was the finding that the degree of ability to ferment maltose and glucose proved to be a function of the stage of harvesting the yeast crop, as well as being influenced by the nature of the substrate. The invertase content of the yeast was affected similarly.

These findings can be compared with those of Davies (1956)[53] with *Sacch. fragilis*. He found that in a synthetic medium, so long as glucose was present (in association with sucrose) invertase activity was low. It was also low in the presence of lactose and galactose. With excess sucrose, a low invertase activity is attributed to the likely presence of small quantities of invert sugar. It is considered that the effect of low concentrations of invert sugar accord with Hestrin and Lindegren's belief that biosynthesis of carbohydrases continues until the rate of hexose production by hydrolysis of oligosaccharides exceeds the rate of hexose utilization; when this point is reached the accumulating hexose inhibits further enzyme formation.

In 1955, Hopkins[54] reported that small amounts of β-amylase were present in a number of top and bottom yeasts from British breweries. It subsequently transpired (Hopkins, 1956)[55] that the presence of the enzyme was fortuitous and that it is not secreted by the yeast. It is a fairly common practice to 'dress' defective fermentations with wheat flour and it is this which is the source of the β-amylase; it is adsorbed from the fermenting wort on to the yeast cell surfaces, from which it is not easily washed. Hopkins suggests that this may explain cases where maltotetraose has been found in the brewery laboratory to be fermentable by the yeast in use.

That maltases from different yeasts differ in character seems to be indicated by Cook and Phillips (1957).[56] They found that both maltose and maltotriose were readily attacked by enzymic preparations from *Sacch. cerevisiae* and yet with similar extracts from *Sacch. uvarum* only maltose was hydrolysed. As the enzyme from the *Sacch. cerevisiae* was considered, as a result of electrophoresis, chromatography, and fractional precipitation, to be a single entity, it would seem that the different yeasts have different maltases. A later paper by Phillips (1959)[57] on the purification of a maltase presumably from the same strain of *Sacch. cerevisiae* does not appear to alter this view. Harris, MacWilliam, and Phillips (1957)[58] have also shown that in addition to this *Sacch. cerevisiae* maltase hydrolysing maltotriose and maltose, the maltase from a superattenuating strain of *Sacch. cerevisiae* also breaks down most of the wort dextrins.

Hopkins and Kulka (1957)[59] in discussing the enzymatic breakdown of polysaccharides, refer to the activity of *Sacch. diastaticus* which secretes an enzyme glucamylase, capable of liberating glucose directly by splitting α-1:4-linkages, by-passing the α-1:6-linkage. *Sacch. diastaticus* apparently also secretes a maltase which, in conjunction with glucamylase, speeds up the fermentation of dextrins. A single-spore hybrid (DS) of *Sacch. diastaticus* showed poor fermentation of dextrin and maltose, but another single-spore hybrid (M+) from this yeast fermented maltose rapidly but not dextrin; in other words it secreted maltase but not glucamylase. The enzyme from M+ debranched β-limit-dextrin, whose subsequent breakdown by the enzyme of DS was accelerated. It is pointed out that the difference between

slow and fast dextrin fermentation is due not to maltase itself but to the debranching enzyme. In *Sacch. diastaticus* there may be a strong linkage between the debrancher and maltase genes.

This glucamylase is an adaptive enzyme: in a glucose–peptone medium and in absence of a polysaccharide, *Sacch. diastaticus* secreted less than 1 per cent of the normal amount of glucamylase, and yeast DS none. In the presence of starch, however, glucamylase was secreted in the usual amounts by both yeasts—much more by *Sacch. diastaticus*. When transferred to amylopectin the yeasts fermented half of it, *Sacch. diastaticus* in one day and DS more slowly. Further fermentation followed in both cases but very slowly.

Rothstein and his co-workers (1958, 1959)[60, 61] have made valuable contributions to the study of the geography, as it were, of the enzymes in the yeast cell responsible for carbohydrate dissimulation. Since Willstätter deduced the location of invertase to be on the cell surface, increasing evidence has been forthcoming that other enzymes are similarly situated. Rothstein[60] considers that there is a 'carrier' in the cell wall which combines with the sugar and transports it across the wall to the inside of the cell. In further studies[61] in which cells were carefully treated in such a manner as to permit the draining away of the soluble cytoplasmic contents, it was found that the remaining structures could ferment sugars in the same way as do intact cells. It is therefore considered that fermentative activity springs from the region immediately beneath the cell surface.

The transport of metabolites into the yeast cell has occupied the attention of Conway for some time; ten years ago (Conway and Downey, 1950)[62] he postulated an 'outer metabolic region' identified with the cell wall, and references to his more recent work will be found elsewhere (Cook).[1] A later paper (1958)[4] condenses much of this material and may be summarized thus: *the metabolites required by the living cell are actively transported during fermentation by a carrier mechanism and not simply by passive diffusion.* Conway in this paper discusses the active transport of glucose, potassium (also as a general cationic carrier), magnesium, and so forth. Latterly, the carrier transport mechanism for the penetration of sugars into the cell has been studied by Burger, Hejmová, and Kleinzeller (1959)[63] who have shown the mutual interference with, or inhibition of, influx and efflux of various sugars. There are indications that the transport mechanism is not the same for different yeasts, nor for different sugars.

5. GENETICS

The comparative ease of manipulation of the cells and spores of yeasts and the rapid rate of growth make them convenient tools for the geneticist, and there are certain commercial possibilities which render the study attractive.

Two general papers on genetics in relation to brewing which have appeared in the last four years merit particular attention because of their authoritative origins. Lindegren (1956)[64] pointed out the following directions in which genetics might help the brewer.

The reassortment of genes resulting from hybridization is an example; given a sufficient number of trials and not too many factors, any desired combination of these factors can be obtained.

Gene mutation is also important. Mutation of a particular gene occurs spontaneously with a relatively low frequency, usually about once in ten million individuals (not so rare, therefore, in the case of micro-organisms), but irradiation can increase the rate of change. Unfortunately many of the induced changes seem to be in the negative direction, i.e. an irradiated strain ceases to be able to do something it did before.

Another suggested possibility is loss of mitochondria through mis-division during budding. One effect of this might be the inability to utilize oxygen, so that the cells would be forced to a completely fermentative (anaerobic) utilization of carbohydrate; this might be advantageous in that oxidative dissimulation means a loss of alcohol production.

As the hereditary characteristics of yeasts are under genetical control, it should therefore be possible for the geneticist to help the brewer. Size of cell, sensitivity to toxic agents and to high concentrations of alcohol, sugar, etc., resistance to autolysis, and removal of specific amino acids affecting flavour,

TABLE II

Genetically Controlled Characters in Saccharomyces[4, 64]

Cell type	Resistance to	Ability to synthesize	Content of
Size	Autolysis	Amino acids	Respiratory enzymes
Flocculation	Alcohol concentration	Purines, pyrimidines	
Colour	Salt concentration	B-vitamins	Specific carbohydrases
Growth rate	Sugar concentration		
	Cation concentration		

are examples given by Lindegren of factors which might be susceptible to control (Table II).

Gilliland (1958)[4] has also contributed views on what industry may gain from the genetical study of yeasts. As regards brewing, he considers that 'even if success is achieved in producing hybrids of brewing yeasts, it is doubtful if they will be an improvement on the strains which have emerged by the continual selection which has taken place in the brewing process over many years'. This pessimism to which perhaps all would not subscribe is compensated to some extent by the statement that in other industries, genetics has been, and is being, of practical benefit; for example, in the production of alcohol and in yeast manufacture. In the latter case, such factors as yield, rate of gas production (these two characters seem to be inversely proportional)(Winge, 1959[65]), and vitamin content can be of importance, and it is known that the use of hybrids is now well established.

Apart from hybrids, polyploid yeasts can be produced; that is yeasts which, instead of being diploid (possessing two sets of chromosomes) and haploid (or having one set of chromosomes in the spore form), may be triploid or tetraploid (having three or four sets of chromosomes, respectively). These polyploid yeasts are large and ferment faster than the normal diploid