Unicellular Chemistry. The Part Played by External Influences in Determining the Chemical Character and Biological Behaviour of Unicellular Organisms.

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WHEN opening a discussion on " Unicellular Chemistry " on May 3rd, 1934, I expressed the view that too frequently insufficient regard is paid to facts concerning the cell itself in speculations as to the course of biochemical change which it brings about when in contact with various media. The theme of the cell itself, rather than what it can do, was chosen with a view to stressing the important part which questions of nutrition, conditions of growth, condition of cell membrane, and mutation play in determining the subsequent activity of unicellular organisms. These factors are frequently overlooked by those studying extra-cellular biological changes brought about by such organisms; consequently, no little confusion arises, particularly on questions of vitamin potency and of enzymic activity, and much recorded work is valueless. It was felt that the gist of observation, extending over a number of years, bearing upon these and other questions concerning the cell itself would be of interest to other workers in the same field.

Unusual opportunities are afforded by the research organisation of the Distillers Company Limited for studying questions of this kind in connexion with yeast manufacture on a large scale. For example, one is able to study the influence of the medium in which the cell grows, the influence of temperature, of aeration, and of many other factors upon the chemical and biological character of the cells themselves over some fifty million multiplications and through many generations on a large scale under rigidly controlled conditions. In this way it is possible to study factors which in large measure seem to determine the chemical and biological behaviour of the final cell.

It is well known that certain moulds, when grown from the same pure culture but on different media, show marked differences in their outward appearance and in their manner of growth. It may be urged that moulds are not unicellular organisms; but some are, for example, the *Mucors*, although others grow in chains and clusters, but the same applies to yeasts and to bacteria, which, under some conditions, also grow in chains and clusters. It is largely a matter of nutrition and of conditions of growth, as indeed seems to be the case where other cell differences are concerned, which, although not so easy to demonstrate and appreciate, are no less profound. The late Professor R. Chodat, when cultivating *Phoma* on different media, found that variation of the proportion of carbohydrate in the medium exercises an effect on the growth, the colour, and the morphology of the organisms. Increased sugar promotes more active growth and intensity of colour, whilst diminished quantities of sugar reduce mycelial growth. Further, variation of the nitrogen content of the same medium also markedly influences the growth, the colour, and the morphology; the growth being decreased by increased nitrogen, the colour intensity increased and the conidial growth restricted by shortage of nitrogen. The colour differences and the growth differences in these cases are not caused by a change of p_H due to growth products, nor to the selective use in part only of the nutrient salts contained in the medium on which growth takes place, although there are, of course, instances of this kind, and also cases where the type of growth and power of developing colour and other obvious signs of difference are brought about by changes of temperature, through the influence of light, and through other cultural conditions. The following photographs of preparations of *Isaria* by C. E. Grover afford interesting examples of this kind. Fig. 1 shows growth with carbohydrate deficiency; Fig. 2, growth in the dark with carbohydrate in plenty; and Fig. 3, growth with carbohydrate in plenty when exposed to the light on one side only, providing a good example of heliotropism.

Great importance is attached to evidence brought forward by H. B. Hutchinson and T. B. Bright as a result of observations over a number of years, that differences, although

less obvious than in the above examples of *Phoma* and of *Isaria*, are frequently shown by individual strains of yeast derived from a single original mother-cell. Although the medium on which these strains are grown is identical in all cases, the resulting growths—giant colonies—from single cells exhibit marked variations in size, colour, general configuration, and enzymic activity, reflecting to some extent the character of the individual cells of which the growths are composed. In the absence of any evidence of actual conjugation of the cells, one has to admit some sudden and unexpected jump, something equivalent to the idea of mutation, carrying with it changes in appearance and properties of the resulting cell; an interesting feature being that these variations from the original cell are in some cases heritable.

An illustration of this kind, due to genetic rather than to physiological factors, is furnished by the following series of photographs (Fig. 4) of giant colonies of yeast from the work of H. B. Hutchinson and T. B. Bright. In this series each giant colony was derived from a single cell taken from one and the same culture and grown on the same medium. That shown as A is regarded as similar in all respects to the original culture from which the series was derived and may be taken as representing the parent stock; B and C are obviously different, whilst the colony shown as D, although apparently similar in appearance to A, is composed of cells the enzymic activity of which is markedly greater than that of the original mother-cell from which the colony was derived.

Equally striking examples are also obtained when similar giant colonies, all derived from the same culture from the same cell, are grown on media of different compositions. In these cases, too, the enzymic and other properties of the variants are found to have changed in degree of intensity of activity. A further example of this is given in Fig. 5, which represents two colonies derived from the same culture, but grown (A) on a normal gelatin medium and (B) on the same medium to which have been added the products of the growth of (A). Marked difference is found between yeast colonies grown in these circumstances.

In unicellular organisms metabolic changes are governed primarily by the activity of the enzymes contained within the cell and by the condition or permeability of the cell wall, which regulates and promotes relatively enormous transfers of material. One knows that within a certain range of acidity or alkalinity the cell wall may change markedly and as a consequence osmosis may proceed quite differently; for example, the free entry of nutrient materials may be hindered or facilitated either generally or selectively, or enzymes of the cell may become more active or less active as a consequence of change in the penetrability of the membrane.

Far too little regard is paid to the important part played by the surface condition of micro-organisms as determining chemical change. One has grown accustomed to the rapid and remarkable chemical changes brought about at the surface of inorganic catalysts and one has learned to appreciate the necessity of having these active surfaces of the right kind and under rigidly controlled conditions. With micro-organisms the ratio of surface to substance is extremely high and for the most part enzymic changes take place at the surface of the organisms. Yet these questions of membrane condition are often overlooked in considerations of the mechanism of enzyme reactions. Again, a change of condition of the membrane may be sufficient to cause an agglutination of otherwise single free-moving cells so that they form mass groupings, thus giving an entirely different outward appearance, apart from colour. Further, the membrane may be the seat of base exchange, as indeed seems to be the case with starch grains.

The enzymic activity of the cell content shows an interesting gradation from quite simple to highly complex functions; for example, in the case of the action of yeast on sugars, one has the following series showing increasing enzymic versatility:

Enzymes.	Organism.	Acts upon
No saccharide splitting. Growth only	*Hansenula belgica*	No fermentation of hexoses
No saccharide splitting, merely fermenting	*Sacc. apiculatus*	Only the simple, closely related hexoses (glucose, fructose, and mannose)
Invertase	*Torula dattila*	*Saccharose*, glucose, fructose, mannose

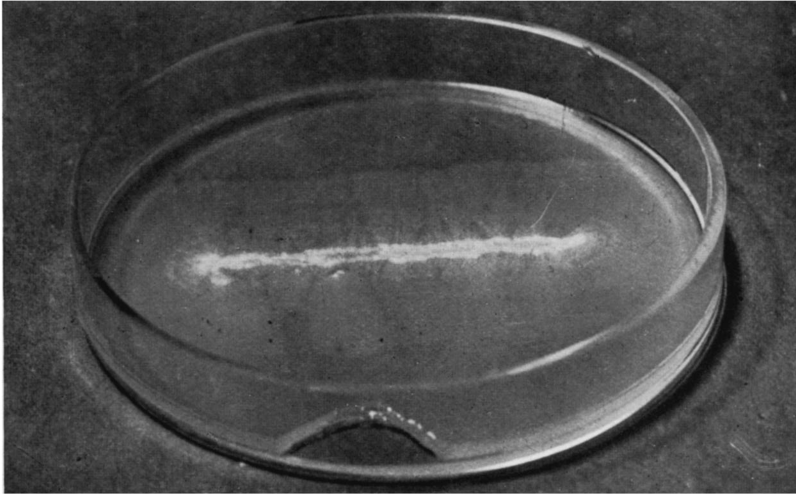


Fig. 1.

Fig. 2.

Fig. 3

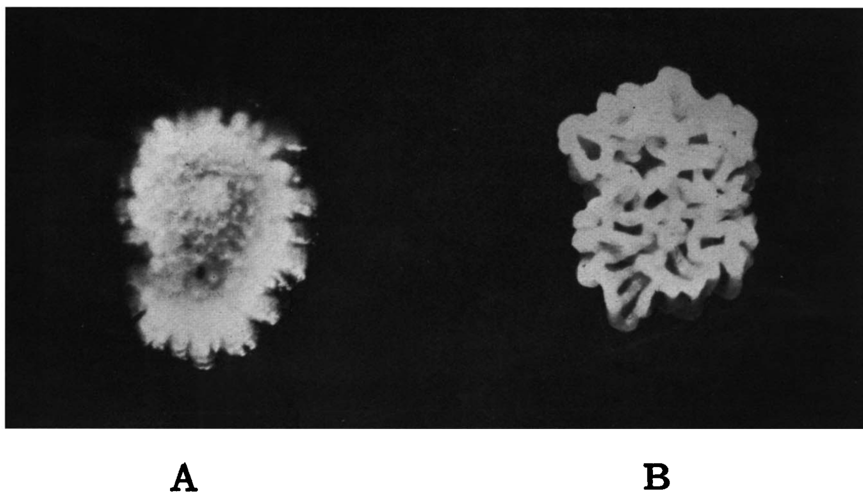
Isaria.

[*To face p.* 202.

A B

FIG. 4.

C D



A B

FIG. 5.

Enzymes.	Organism.	Acts upon
Invertase and maltase	*Sacc. cerevisiae*	*Maltose*, saccharose, glucose, fructose, and mannose
Invertase, maltase, and melibiase	*Sacc. Carlsbergensis*	Raffinose, maltose, saccharose, glucose, fructose, and mannose
Invertase, lactase, and inulinase	*Sacc. fragilis*	*Lactose*, *inulin*, saccharose, glucose, fructose, galactose, mannose, and raffinose
Invertase, maltase, melibiase, trehalase, and dextrinase	*Sacc. Logos*	*Trehalose*, *dextrin*, saccharose, maltose, glucose, fructose, galactose, mannose, and raffinose

Although *S. Logos* in the above table is seen to be able to hydrolyse and ferment dextrin, yeasts do not contain amylases and therefore cannot attack starch or polysaccharides. The moulds are far more catholic than yeasts in these respects and are, indeed, veritable portmanteaux of enzymes, fully equipped to deal with almost anything. They are able to break down polysaccharides, starches, and protein; in many cases they also possess fat-splitting lipases, and can, of course, deal with all the sugars which the various yeasts are able to hydrolyse.

There is no little confusion about the enzymic properties of unicellular organisms; some, depending upon the collection from which they are drawn, show different activity from others which are supposedly identical. Reference will, however, be made to this later and some light will be thrown on the probable cause of these divergencies.

Of the enzymes associated with single cell organisms, some appear to act at the surface, others are regarded as diffusing out from the cell, although there is, indeed, very little evidence of this, whilst others are far less accessible and only come into play when the cell is ruptured. A simple example of a marked difference of this kind is to be found with ordinary yeast, in which the enzyme invertase is extremely active towards a cane sugar solution, resolving it to glucose and fructose without detriment to the cell structure, whereas, under similar conditions, the maltase of the yeast cell remains inactive towards maltose, being unable to get at it; maltose is only resolved to glucose when the yeast cell is ruptured and the enzyme freed.

A further point of interest here is that a yeast having a low invertase activity may, through appropriate propagation, for example, in a medium rich in cane sugar, become extremely invertase-active, particularly if the propagation is carried through a number of generations. The following results, taken from the work of E. R. Dawson, provide an example of this kind and show also that in a similar way a yeast of low maltase activity may be brought to a relatively high degree of maltase activity by appropriate propagation in a maltose-rich medium. It is not suggested, however, that this is the normal or the only way of influencing the enzymic activity of a yeast, but it serves to illustrate the comparative ease with which enzymic activity may be attained through external influences. It should be understood, when considering the following figures, that the units for invertase activity are larger than those for maltase activity and that in developing increased maltase activity different methods and different media are employed from those adopted when developing increased invertase activity, so one should not compare the figures for the one with those for the other; they show, however, marked increase in the activity of the enzymes concerned as the propagation proceeds.

Yeast.	Invertase activity.	Maltase activity.
Seed used	1·8	0·9
After 5 multiplications	3·0	1·8
,, 25 ,,	5·9	2·5
,, 125 ,,	6·6	—
,, 650 ,,	7·9	—

It seems that, by calling these enzymes into play through environmental influence, either they become more active or their quantity in the cell is increased. Cases have been observed where the initial enzymic activity of the cells is so low and ultimately becomes so high as to suggest that the cell is able to provide itself with such enzymic powers as will enable it to live under widely different conditions on widely different media. Clearly, yeast brought up on one medium may behave differently from a yeast brought

up on another. It is doubtful whether in biological work sufficient regard is paid to these strong environmental influences on yeast and on other unicellular organisms.

Attempts to produce in a yeast some new enzymic power different from those initially possessed have so far failed, except, perhaps, in the case of enzymes capable of resolving galactose. For example, one has not yet succeeded in detecting maltase activity in *S. Marxianus* or in *S. exiguus*. This may be due to the right conditions not having been found or to the propagation not having been carried sufficiently far under the most favourable conditions. However, some measure of success in this direction has attended the work of Armstrong, Slator, and Harden, who, employing a yeast which initially possessed no measurable power of fermenting galactose, eventually induced it to show such activity by bringing it up in the presence of galactose. Whether this is actually a case of creating new power for new cells, or whether it is a general calling into play of otherwise dormant powers, is difficult to say: the case seems clear in so far as new cells are concerned, although the latter explanation seems the more probable in view of the relationship between yeasts and moulds and the diverse enzymic powers which the latter show. It will suffice, however, to stress here the importance in biochemical work of knowing the past history of the biological materials with which one works; it is insufficient to work merely with a specific organism—if the work is to be of value, one has to know a great deal about the past history of the organism since it was at the single cell culture stage.

With regard to oxidative–reductive systems of unicellular organisms, frequently referred to as the oxidase–reductase system, there seem to be two lines of thought, with but little experimental evidence upon which to base any definite ideas. In the one case, the assumption is made that there are two definite and independent systems at work, the one oxidative, the other reductive, linked by the cytochrome of the cell; cytochrome being widely distributed in the cell in an oxidised form or in a reduced form. The other view is based upon the idea of the functioning only of a reductive system of the cell itself through the activation of hydrogen. This involves the conception of some cell substance activating hydrogen in certain circumstances, causing a transfer of hydrogen to a hydrogen acceptor, or causing a transfer of oxygen from an oxygen donator, and in that way being responsible for both types of change generally referred to as biological reduction and biological oxidation; terms which seem necessary in order to distinguish these changes, which, in the presence of the living cell, proceed easily, from the more usual reduction and oxidation processes of chemistry, which proceed with comparative difficulty. The latter hypothesis, namely, that of hydrogen activation, has the merit of being simpler than that calling for independent oxidative and reductive systems and is supported by rather more evidence and, moreover, seems to meet equally well all the cases which have been studied. Recently, too, W. G. Bennett has shown that the existence of an oxidative system, in addition to the reductive system postulated in the former scheme, is extremely doubtful. He has shown that a yeast may be prepared, having the normal oxygen absorption powers, which will not exhibit the indophenol oxidase reaction. If, however, a very minute trace of copper be added, something of the order of a few parts per million, then the indophenol oxidase reaction becomes evident in some cases, but not in all.

One is tempted to ask whether quite a number of these biochemical activities are not due to traces of inorganic substances and whether, indeed, some biological processes themselves are not dependent upon the presence of mere traces of inorganic agents acting directly or indirectly through subtle combinations with organic materials. As an example of this, one calls to mind the part played by magnesium in the chlorophyll system.

When examining the vitamin value or vitamin activity of unicellular organisms, a similar state of affairs is found to exist as with the enzymic activity. Here, again, although nothing very definite is known, there is a considerable body of evidence showing that vitamin potency may be dependent to a marked extent upon the vitamin potency of the medium in which the organism has been grown. There are cases where bacteria are believed to fabricate their own vitamins: there are cases, for example, that of *Sacc. Logos*, where, as shown by J. C. Drummond and Whitmarsh, the balance of evidence points to the probability that this yeast is able to synthesise its own vitamin **B.** Generally speaking, however, one may say the vegetable kingdom is the chief source of vitamins;

in plants they are fabricated and passed on to the animal kingdom to play their part in cell metabolism. It is interesting, therefore, to find amongst yeasts cases both of vitamin fabrication and of vitamin acquisition.

In so far as vitamin B is concerned, yeasts seem to be in the main dependent upon the medium in which they are grown for their vitamin B potency. In any case, a yeast of low vitamin B value may be changed to one of high vitamin B value by growing it through appropriate stages in a special extract of grain material. Whether the same applies to other cases of vitamin activity is not certain, but one knows of entirely different circumstances in which vitamin potency may be changed. There are, however, other, less direct, ways of changing vitamin potency; for example, a unicellular organism propagated under one set of conditions may have a very low ergosterol content, whilst if propagated in the same kind of medium but under different conditions of nutrition, it may be very rich in ergosterol, which is the precursor of vitamin D, being changed to that vitamin when brought under the influence of ultra-violet irradiation. In this particular instance, the essential points of difference seem to be an abnormal condition of oxygenation and an abnormal condition of nitrogen feeding. The ergosterol content of yeast prepared in accordance with the procedure worked out by Bennett may be raised from, say, 0·4% calculated on dry matter, to well over 1%, say 1·5%, calculated on the same basis. This appears to be a case of synthesis brought about by changed nutritional conditions and is of considerable interest when taken in conjunction with the observations in the case of vitamin B.

Coming to the question of the protein content of the cell, one may well consider how this complex molecule gets within the confines of the cell wall. A considerable amount of work has been done on the proteolytic enzymes of bacteria and of yeast, but generally from the point of view of understanding the degradation of the protein complex, not from the point of view of its upbuilding. There is, however, much uncertainty regarding the evidence brought forward, owing to the difficulty of interpreting the results and also from the almost complete disregard by many workers of the important influence which the concentrations of salts and of sugar exert on the cell membrane; likewise, the degree of acidity or alkalinity of the medium employed; all, or any, of which may promote or hinder osmosis. The opinion is fairly widely held that protein is broken down in two stages, first to amino-acids by some extra-cellular proteolytic enzyme; the second stage, namely, the subsequent resolution of the amino-acids, by some endocellular oxidation process. That is to say, in the case of yeast activity, the protein is first resolved to amino-acids by an extra-cellular enzyme and the amino-acids are then resolved further by an endocellular agent.

Examining changes of this kind in the reverse order, from the point of view of the cell itself, what it does, what it consists of, and from what it builds up its protein, helps one to accept or reject some of the current ideas. It is known that yeast cells contain 6—10% of nitrogen, calculated on dry matter, and that this is present in protein built up from simple inorganic or organic nitrogenous matter through enzymic agency. It is difficult to regard this process as taking place first by means of an endocellular enzyme building up the amino-acids and then, subsequently, an extra-cellular enzymic process completing the protein complex. In the case of yeast it seems the more likely that this synthesis takes place in some way through the agency of the cell wall: at any rate, a small amount of nitrogen is always excreted during protein synthesis by yeast. The protein is generally located within the cell, but under some conditions of growth, regarded as quite abnormal, protein may be formed on the exterior surface of the cells themselves.

The general mechanism of the synthesis of yeast protein seems to be one probably between the carbohydrate (glycogen) reserve and simple ammonia, amino- or amide groupings. Different kinds of nitrogen are made use of differently and it is known that a source of carbon is always simultaneously necessary. Under conditions where nitrogen is present at the same time as ammoniacal salts, as amino-acids and as polypeptide nitrogen, it has been found that about 97% of the ammoniacal nitrogen is utilised, about 60% of the amino-acids, and about 30% of the polypeptide nitrogen. If the nitrogen supply is in the form of amino-acids, its utilisation is associated with the utilisation of sugar, and

free oxygen is unnecessary. If, however, the nitrogen supply is in the form of inorganic salts, as urea, or as acid amides, then alcohol or organic acids are needed at the same time, together with a liberal supply of free oxygen, such as is provided during intensive aeration. So far as is known, the more complex forms of organic nitrogen, with the exception of simple polypeptides, are not utilised in any circumstances.

Under proper control, and given the right conditions—and they are very exacting—one may readily convert nitrogen of the air in the form of ammonium salts into protein on a large scale; in fact, it is the basis of industrial yeast production at the present day.

For example, in the manufacture of 10,000 kg. of fresh yeast, representing, say, 2700 kg. of dry matter, there may be used about 250 kg. of inorganic nitrogen in the form of ammonium salts of one kind or another, from which are recovered about 1300 kg. of dry full-protein. This, of course, does not represent the total quantity of protein contained in the total quantity of yeast cell substances, because, in addition to the 1300 kg. of full-protein referred to, a certain amount is also formed from organic sources of nitrogen present in the fermenting mash. It is necessary to bear in mind that these high conversions of inorganic nitrogen in the form of ammonia into organic forms of nitrogen as full-protein are only possible with certain races of yeast, and even then it is largely dependent upon the previous upbringing of the yeast used as seed for the fermentation.

Processes of this kind not only are of considerable scientific and industrial interest, but might be of great economic importance to the country, particularly in time of scarcity. Reference has been made to these facts elsewhere, but it may be pointed out that a yeast factory producing, say, 200 tons of bakers' yeast per week is producing about 27 tons per week of dry protein and 4 tons of fat. Put into terms of a better-known form of protein—namely, beef—and making allowances for bones, hide and other non-edible parts, this means the conversion of nitrogen from the air, through ammonia, into protein at the rate of some 500 bullocks per week at a cost not widely different from the cost of raising beef in this country on the basis of its food value.

Much attention has been given to the study of the fixation of nitrogen by soil organisms since the early observations of Jodin that mineral salts as well as carbohydrates play an important rôle. But little is known regarding the factors which regulate the nitrogen intake of organisms, or how yeast, which contains so large a proportion of protein within its cell wall, builds up complex nitrogen-containing compounds from simple ammonia.

Of the other well-known constituents of the cell, namely, carbohydrate and fat, it is known that the cell stores within the confines of its membrane a reserve of carbohydrate as glycogen accompanied by mannan, the latter probably constituting the main portion of the cell membrane. Glycogen seems to be the reserve from which is drawn the carbon necessary, not merely for the fabrication of the cell wall, but also, as has already been mentioned, for the synthesis of protein and for the building up of fat and ergosterol. This central store of carbohydrate is very labile, being readily disintegrated when the cell is introduced into media rich in sugar, but is gradually built up again, thus replenishing the store as the amount of sugar available declines. When a heavy demand is made upon this reserve of carbohydrate for any one purpose, for example, in protein synthesis, less remains for fat or ergosterol formation. Therefore, in growing yeast, for instance, to contain a high proportion of ergosterol, care must be taken to keep the nitrogen supply at a minimum.

With this carbohydrate store is also associated the storage of inorganic constituents such as phosphate and potassium. Large utilisation of the carbohydrate store for protein synthesis is thus inimical to the storage of potassium. Far more complex processes seem to be involved when one comes to the question of the storage of phosphates within the cell. Phosphates and magnesium salts are utilised in proportion to their concentration, whether owing to mass-action or through osmosis is not known. These questions are extremely complicated, the influences at work are interdependent and their balance easily disturbed. Comparatively slight changes of environment may lead to marked changes in the physical properties, the chemical constituents, and the chemical activity of the cells concerned.

There are, of course, many other important constituents of the cell; for example,

sulphur for cystine and glutathione formation, and iron for cytochrome formation; these are present only in small quantities. Zinc is often found in yeast in unexpected amounts, but this may arise from assimilation of zinc contained in the mash materials and be without any functional significance.

In conclusion, however, reference may be made to " bios," as several investigators are endeavouring to ascertain the nature of this mysterious growth-promoting factor. Very little is known definitely, but from observation on single cell behaviour, it is recognised, in a general way, that " bios " influences cell multiplication, promoting budding rather than an increase in the size of the cells. Whereas carbohydrate and nitrogen, together with the necessary inorganic salts, seem to influence cell size rather than cell multiplication, nitrogen seems also to influence the general appearance of the cell protoplasm regardless of the size of the cells concerned.

It is only possible within the scope of this communication to refer to some of the more important aspects of cell fabrication and of cell properties. The whole question of the chemistry of unicellular organisms, particularly of yeast, calls for most careful study. A better understanding of the processes involved will throw much needed light on the more complex problems and the larger issues of animal and vegetable nutrition. The feeling grows that chemistry would profit from a closer study of biological phenomena and that biochemistry is in need of more chemistry.