182. THE EFFECT OF ULTRAVIOLET LIGHT ON LIVING YEAST CELLS

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In recent communications, Loofbourow *et al.* [1938; 1939; 1940] state that living yeast cells, injured by ultraviolet light, liberate, possibly as a specific response to injury, substances which powerfully stimulate the growth of yeast. These substances they call "proliferation-promoting intercellular hormones" or wound hormones. They suggest, mainly on the basis of spectrographic evidence, that such substances are adenine-guanine nucleotide complexes.

Similar substances with the power of stimulating yeast growth were also obtained by ultraviolet irradiation of living cells of newts and of chick and rat embryos. Such substances from chick embryos are claimed to accelerate the growth of chick fibroblasts *in vitro*.

Loofbourow's conclusions concerning the chemical nature of the growth-promoting substances are based mainly on qualitative tests and on spectrographic evidence. It therefore seems desirable to carry out quantitative chemical analyses of the products released by yeast cells injured by ultraviolet irradiation.

150 g. fresh D.C.L. bakers' yeast (*Saccharomyces cerevisiae*) were washed twice on the centrifuge with 0·9 % NaCl solution and were then suspended in 750 ml. of the same solution. The suspension was divided into three equal portions. One portion (*a*) was autoclaved at 120° for 20 min. Such treatment destroyed cell structure. The second portion (*b*) was exposed for 6 hr. at 13 cm. distance to ultraviolet light from a quartz mercury vapour lamp (3·5 amp., burner volts 150) by being repeatedly run through the rack of quartz tubes described by Stiven [1930] at such a rate that the temperature of the suspension never exceeded 40°. A third portion (*c*) was kept for the same time and at the same temperature as a control but without exposure to ultraviolet light.

The effect of irradiation was followed by withdrawing a drop of the suspension from time to time, treating it with methylene blue and examining microscopically. Living yeast cells are not stained by methylene blue while injured and dead cells stain deeply [Richards, 1932]. At the outset only 1 % of the cells stained, but at the end of irradiation 60 % stained. The cells were shrunken. In the control suspension 10 % of the cells stained at the end of the period. In both cases the total number of cells, counted in a haemocytometer chamber, was unaltered.

The three suspensions were finally centrifuged and the clear supernatant fluid collected. The extract from the autoclaved suspension (*a*) was deep yellow in colour, that from the irradiated cells (*b*) yellow, while the extract from the control (*c*) was colourless. All showed a blue fluorescence in ultraviolet light, the control being only slightly fluorescent. All extracts were kept at 0° and analysed immediately, as they were, especially in the case of the extract from irradiated cells, excellent media for bacterial growth. Extract (*b*), which had *p*H 5·8, gave a positive biuret test but no precipitate on boiling or with nitric acid. It gave a precipitate with tungstic acid. Extract (*c*) from the control

gave a negative response to the biuret test and no precipitate with tungstic acid. Extract (*a*) from autoclaved cells was heavily loaded with protein.

In the three extracts total N was estimated by the micro-Kjeldahl procedure, amino-N by the manometric method of Van Slyke, non-protein-N after deproteinization with tungstic acid, and nucleotide, nucleoside, and free purine-N by the method of Kerr & Blish [1932] as modified by Kerr [1940].

Several separate batches of yeast were treated in this way at different times and the results of a typical experiment are shown in Table 1.

Table 1. *Analyses of centrifuged extracts from autoclaved, irradiated and control cells*

Extract from ...	(*a*) Autoclaved cells mg./100 ml.	(*b*) Irradiated cells mg./100 ml.	(*c*) Control cells mg./100 ml.
Dry wt. (excluding NaCl)	1705	1164	280
Total N	148·4	104·9	12·6
Non-protein-N	72·8	96·2	10·5
Protein-N (by difference)	75·6	8·7	2·1
Amino-N (non-protein)	57·4	63·3	9·0
Nucleotide-N	11·8	11·8	0·4
Nucleoside- and free purine-N	2·9	9·4	1·4

Irradiation sets free a large amount of nitrogenous material. A very small amount is in the form of protein while a high proportion is present as amino-N. Much more N than in the case of the control is present in the form of nucleotide, nucleoside and purine: Table 1 (*b*).

Complete destruction of the cells yields an extract with much protein. The non-protein-N is chiefly amino-N: Table 1 (*a*).

After treating the extract from irradiated cells with 10 % sulphuric acid to hydrolyse nucleic acid derivatives after the method of Jones [1920], adenine was isolated both as the picrate and the sulphate. Small amounts of guanine were also found. Thus it is probable that the nucleotide and nucleoside fractions are chiefly adenine derivatives with small amounts of guanine present.

The growth-promoting powers of the three extracts were tested by seeding Reader's medium [Reader, 1927] with a dilute suspension of yeast cells. Varying amounts of the extracts were added to the media and the cultures grown at 21° in Erlenmeyer flasks as recommended by Williams & Saunders [1934] and by Narayanan [1930]. The crop was estimated after 20 hr. by counting the cells in a haemocytometer. At all concentrations extract (*b*) from irradiated cells had six times the growth-promoting power of the extract from the control. The irradiated suspension had six times as many cells injured as the control, suggesting a correlation between cell injury and liberation of growth-promoting material. The extract (*a*) from autoclaved cells stimulated growth as powerfully as extract (*b*) but, since the extract from autoclaved cells contained more solid matter, weight for weight the material from the irradiated cells was more potent.

Extracts from irradiated and autoclaved cells had greater growth-promoting power than either inositol or aneurin.

Discussion

A large number of substances can accelerate yeast growth, for example, such components of the bios group as inositol, aneurin, biotin, pantothenic acid and adermin. Other substances with similar properties are found in liver extracts

[Williams *et al.* 1940]. Amino-acids such as leucine [Miller, 1936], alanine, aspartic acid, glutamic acid, lysine and arginine stimulate yeast growth, and mixtures of these substances have a more pronounced effect than equivalent amounts of the acids alone [Nielsen & Hartelius, 1938; 1939]. Lysine and arginine are known to be plentiful in the proteins of yeast [Kraut & Schlottmann, 1937].

Such being the case, it is not surprising that rupture of yeast cells by autoclaving should liberate substances with growth-promoting properties towards yeast. Irradiation of yeast, however, yields a surprisingly potent extract without destruction of the cells. This extract has even more non-protein nitrogenous matter than the extract from autoclaved cells. The slow death of the yeast cell does seem to cause liberation of substances into the surrounding medium but it is impossible to say whether or not this is a specific response to injury and an attempt to compensate for the death of some cells by accelerated growth of others.

Among the nitrogenous materials are nucleotides and nucleosides containing adenine and it is highly probable that their presence accounts for the spectrographic findings of Loofbourow *et al.* But it seems premature to conclude that these adenine nucleotide complexes are necessarily the active principles in the extract from irradiated yeast which are responsible for stimulating the growth of yeast.

SUMMARY

Exposure of living yeast cells to ultraviolet light results, without disintegration of the cells, in the liberation of large amounts of nitrogenous material into the surrounding medium. This material has the property of stimulating yeast growth to a marked extent. Although the medium contains adenine nucleotide derivatives, these are not necessarily the growth-promoting principles.

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