showed greater amounts of biotin, folic acid, inositol, nicotinic acid, pantothenic acid, pyridoxin, riboflavin and aneurin in the former. The ratio of such factors in I as compared to II varied from a maximum of 91·5 × for pantothenic acid to a minimum of 2·3 × for inositol.

2. A solution of factors of the B complex corresponding to the quantities found in I stimulated the growth of yeast more than did II but not as much as did I, the relative proliferation-promoting potencies as compared with II being 25·9 for I and 4·5 for the B factor solution. It is concluded that factors of the vitamin B complex account for a part, but not all, of the proliferation-promoting effect in yeast-growth assays of products from damaged yeast cells.

3. Combined equal samples of the intercellular fluids, cell-residue wash waters, and residue autolysates from damaged cells (*A* I) and from undamaged cells (*A* II) were assayed for proliferation-promoting effect on yeast. Greater growth stimulation was obtained with *A* I than with *A* II. This confirms previously reported evidence that certain proliferation-promoting factors are replaced within the damaged cells as they are lost to the intercellular fluids by diffusion from the cells throughout the course of cell damage.

4. The total yield of factors of the B complex from intercellular fluids, residue wash waters, and residue autolysates was considerably greater from damaged than from undamaged cells in the case of biotin (1·6 ×), folic acid (1·9 ×) and pantothenic acid (1·9 ×) indicating that replacement within the living injured cells accompanies loss of these factors to the intercellular fluids. It is suggested that the instances in which lesser yields were obtained may possibly be explained by photochemical destruction in the irradiated (damaged-cell) preparations.

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REFERENCES

Cook, E. S. & Cronin, A. G. (1941). *Stud. Inst. Divi Thomae*, **3**, 203.
Cook, E. S. & Cronin, A. G. (1942). *Nature, Lond.*, **150**, 93.
Davidson, J. N. (1940). *Biochem. J.* **34**, 1537.
Hennessy, D. J. (1941). *Industr. Engng Chem.* (Anal ed.), **13**, 216.
Hennessy, D. J. & Cerecedo, L. R. (1939). *J. Amer. chem. Soc.* **61**, 179.
Hodson, A. Z. & Norris, L. C. (1939). *J. biol. Chem.* **131**, 621.
Karrer, P., Salomon, H., Schöpp, K., Schlittler, E. & Fritsche, H. (1934). *Helv. chim. Acta*, **17**, 1010.
Landy, M. & Dicken, D. M. (1941). *Proc. Soc. exp. Biol., N.Y.*, **46**, 449.
Loofbourow, J. R. (1942*a*). *Biochem. J.* **36**, 631.
Loofbourow, J. R. (1942*b*). *Biochem. J.* **36**, 737.
Loofbourow, J. R. (1942*c*). *Nature, Lond.*, **150**, 349.
Loofbourow, J. R., Cook, E. S. & Stimson, M. M. (1938). *Nature, Lond.*, **142**, 573.
Loofbourow, J. R., Webb, A. M., Loofbourow, D. G. & Abramowitz, R. K. (1942). *Biochem. J.* **36**, 513.
Melnick, D. (1941). *Industr. Engng Chem.* (Anal. ed.), **13**, 879.
Melnick, D. & Field, H. (1940*a*). *J. biol. Chem.* **134**, 1.
Melnick, D. & Field, H. (1940*b*). *J. biol. Chem.* **135**, 53.
Rogosa, M. (1944). *J. Bact.* **47**, 159.
Uber, F. M. & Verbrugge, F. (1940*a*). *J. biol. Chem.* **134**, 273.
Uber, F. M. & Verbrugge, F. (1940*b*). *J. biol. Chem.* **136**, 81.
Williams, R. J. (1941). Univ. Texas Publ. no. 4137.

Intercellular Hormones

7. RELEASE OF AMINO-ACIDS BY DAMAGED LIVING YEAST CELLS

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It has been demonstrated that factors of the vitamin B complex account in part for the proliferation-promoting activity ('wound hormone' effect) of products from yeast cells slowly damaged by sublethal ultra-violet radiation, and that the living, damaged cells appear to elaborate considerable quantities of certain of these factors (notably biotin and pantothenic acid) during the course of injury (Webb & Loofbourow, 1947).

Davidson (1940) found the amino-N content of suspension fluids from yeast cells damaged by ultra-violet radiation to be approximately 7 times that of suspension fluids from non-irradiated cells. This suggested that amino-acids may be involved in the

greater proliferation-promoting effect of the former, since it is well known that amino-acids are accessory growth factors for yeast (Mitchell & Williams, 1940). Many workers have concluded that polypeptides or amino-acids are involved in the increased proliferation of cells following tissue damage; the literature on this subject has been reviewed recently by Davidson (1943). Cook & Cronin (1941) found that addition to the culture media of various amino-acids suppressed to some extent the proliferation-promoting effect of products from ultra-violet irradiated yeast cells. In view of such results, it seemed of interest to investigate further the role of amino-acids in the proliferation-promoting effect of intercellular fluids from suspensions of cells subjected to controlled ultra-violet injury.

METHODS

The cellular products employed in these experiments were released by undamaged (control) and damaged (ultra-violet injured) yeast cells (*S. cerevisiae*, F.B. strain) into distilled water suspension media. The preparation methods are described in detail elsewhere (Loofbourow, 1942*a*; Webb & Loofbourow, 1947).

The total N content of the intercellular fluids was determined by the micro-Kjeldahl procedure, non-protein-N by the same procedure after deproteinization with sodium tungstate, and amino-N by formol titration and Folin's colorimetric method.

Analyses for specific amino-acids were made as follows: arginine was determined by a modified Sakaguchi reaction (Jorpes & Thoren, 1932), tyrosine and tryptophane by the Lugg method as modified by Brand & Kossel (1939), threonine and alanine by the method of Block & Bolling (1939*a*, *b*; Block, Bolling & Webb, 1940), and histidine by the diazo reaction (Koessler & Hanke, 1919) using Corning no. 502 filter for comparison with the colour developed in histidine reference solutions.

The proliferation-promoting effects of the suspension fluids from irradiated (I) and control cells (II), and of amino-acids (III), were determined by growth tests in Reader's (1927) medium with yeast (*S. cerevisiae*, F.B. strain) cultured in rotor tubes according to previously described techniques (Loofbourow, Webb, Loofbourow & Abramowitz, 1942).

RESULTS AND CONCLUSIONS

Nitrogen determinations were made upon four preparations each from damaged and undamaged cells, with results similar to those shown for preparation 627 in Table 1. Davidson's data (1940), recomputed to units of mg./ml., are included in Table 1 for comparison. Davidson's values are in all instances higher than ours. In particular, he found appreciable quantities of protein nitrogen in his preparations, whereas ours contained negligible amounts as indicated both by lack of precipitate with phosphotungstic acid, trichloroacetic acid or sodium tungstate and by the fact that our non-protein-N values were close to those for the total N. His conditions of preparation differed from ours, however, in that he employed: (i) twice the yeast concentration used in our experiments (as a result of which higher total solid and N contents would be expected in his preparations), (ii) a different ultra-violet source and time of irradiation, and (iii) 0·9 % NaCl as the suspension medium instead of distilled water.

Table 1. *Nitrogen determinations on suspension fluids from control and irradiated cells*

	Davidson's preparation			Preparation 627		
	I, Irr.* (mg./ml.)	II, NI* (mg./ml.)	Ratio I : II	I, Irr.* (mg./ml.)	II, NI* (mg./ml.)	Ratio I : II
1. Solid content	11·64	2·80	4·1	4·12	0·192	21·5
2. Total N	1·049	0·126	8·3	0·273	0·021	13·0
3. N.P.N.	0·962	0·105	9·2	0·272	†	—
4. Protein-N (by difference)	0·087	0·021	4·1	0·001	†	—
5. Amino-N	0·633	0·090	7·0	0·233	0·0167	13·9

* Irr. refers to cell-free suspension fluids from cells damaged by irradiation; NI to cell-free suspension fluids from non-irradiated cells.

† N.P.N. (non-protein nitrogen) was the same as total N in this material within the limits of experimental error.

Using the factor 6·25 to convert from amino-N to weight of amino-acids present, the indicated amino-acid content of preparation 627 I is 1·46 mg./ml. On this basis, assays for certain individual amino-acids other than alanine indicated their presence in this preparation in the following proportions relative to the total amino-acid content: arginine 1·7 %, histidine 2·3 %, threonine 4·9 %, tryptophan 0·7 %, and tyrosine 1·7 %. These values agree within experimental error with the distribution of amino-acids in yeast protein (Block & Bolling, 1940). In so far as these particular amino-acids are concerned, there was, therefore, no evidence for the release of amino-acids by the irradiated cells through any mechanism other than that of protein breakdown. Alanine, on the other hand, appeared to be present in I in considerably higher proportions than in yeast protein,

but the data were so variable as to be considered unreliable.

In the growth tests, various combinations of amino-acids in total concentrations varying from approximately 0·1–40 times the amino-acid content of I were tested in Reader's medium. The concentration of yeast in the cultures was determined turbidimetrically at various times; in a typical test, readings were made at 4, 9, 20, 24, 28, 33, 44, 50, 67 and 75 hr. Significant increases in the yeast crops, as compared with controls, were obtained only when the amino-acid concentration in the cultures was 30 or more times as great as that resulting from additions of I in amounts just sufficient to cause significant crop increases. These results confirm earlier conclusions (Cook & Cronin, 1941; Loofbourow, 1942*b*) that amino-acids do not account entirely for the proliferation-promoting activity of damaged-cell products under the conditions of our experiments.

The determination of the precise contribution of the amino-acid fraction to the 'wound-hormone' effect is complicated by the well-known fact that the increase in yeast crop obtained on addition of amino-acids varies with the presence or absence of other substances in the medium. This is illustrated by the results in Table 2. In the series of experiments of medium): aneurin hydrochloride, 0·25; riboflavin, 0·5; pyridoxin, 0·5; calcium pantothenate, 0·125; biotin (free base), 0·000125; choline, 0·75; and nicotinamide, 2·5.

Addition of 0·313 mg. of III/ml. of final culture to yeast grown in Reader's medium resulted in increases in the 28 and 44 hr. yeast crops of 0·048 and 0·040 mg./ml. (wet weight of yeast) respectively (Table 2). When, however, the same amount of III was added to cultures grown in Reader's medium supplemented with B factors, the increases in 28 and 44 hr. crops were respectively 1·57 and 1·72 mg./ml. Thus, amino-acids probably play a more significant quantitative role in the 'wound hormone' effect under the conditions of our investigations than is indicated by experiments in which amino-acids alone are tested for growth-stimulating effect in Reader's medium.

Table 2 also shows that the addition to yeast cultures, grown in Reader's medium supplemented with B factors, of 0·159 mg. of I‡/ml. of final culture *plus* 0·313 mg. of III resulted in greater increased 28 and 44 hr. crops than addition of 0·313 mg. of III alone. The increased 28 and 44 hr. crops were respectively 2·87 and 3·07 in the former case as against 1·57 and 1·62 in the latter. This confirms earlier evidence (Cook & Cronin, 1941; Loofbourow, 1942*b*)

Table 2. *Increased growth resulting from addition of amino-acids and damaged-cell products to yeast cultures*

Basal medium	Added material (mg./ml. of culture)	Yeast crop (mg./ml. wet weight) 28 hr.	Yeast crop (mg./ml. wet weight) 44 hr.	Increased yeast crop resulting from added material 28 hr.	Increased yeast crop resulting from added material 44 hr.
(1) Reader's	(*a*) None	0·152	0·157	—	—
	(*b*) 0·313 mg. amino-acids (III)*	0·200	0·197	0·048	0·040
	(*c*) 0·159 mg. I†	4·03	4·43	3·88	4·27
(2) Reader's + B factors (see text)	(*a*) None	3·33	3·70	—	—
	(*b*) 0·313 mg. amino-acids (III)*	4·90	5·42	1·57	1·72
	(*c*) 0·313 mg. amino-acids (III)* + 0·159 mg. I†	6·20	6·77	2·87	3·07

* III refers to equal parts by weight of arginine, asparagine, aspartic acid, glutamic acid and leucine.
† I refers to cell-free suspension fluid from yeast cells damaged by ultra-violet radiation.

which this is a typical example, the amino-acid mixture (III) consisted of equal parts by weight of arginine, asparagine, aspartic acid, glutamic acid and leucine. Only these amino-acids and β-alanine appear from our experiments to be appreciably effective in increasing the growth of our strain of yeast, confirming previous results of Cook & Cronin (1941). β-Alanine was omitted from the amino-acid mixture employed because it acts as a critical exogenous growth factor for *S. cerevisiae*, F.B. strain, in lieu of pantothenic acid in media unsupplemented with B factors (Rogosa, 1944).

The basal media employed were (1) Reader's unsupplemented, (2) Reader's supplemented with the following (concentrations in μg./ml. of culture that cell-free suspension fluids from irradiated yeast contain proliferation-promoting substances other than B factors and amino-acids. The additional effect of I is believed to be attributable to nucleotides, as will be discussed in a subsequent communication.

SUMMARY

1. Cell-free suspension fluids from damaged (ultra-violet injured) cells (I) had appreciably higher content of amino-N than fluids from control suspensions (II).

2. Amino-acids (III) in concentrations equivalent to or greater than that in I stimulated the growth of

‡ I.e. by addition of a volume of I containing 0·159 mg. solids.

yeast in unsupplemented Reader's medium and in Reader's medium supplemented with B factors.

3. Addition of I to Reader's medium stimulated the growth of yeast more than addition of III in all concentrations employed. Addition of I and III to Reader's medium supplemented with B factors stimulated the growth of yeast more than addition of III alone.

4. It is concluded that amino-acids account for a part, but not all, of the proliferation-promoting effect of I.

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REFERENCES

Block, R. J. & Bolling, D. (1939*a*). *Proc. Soc. exp. Biol., N.Y.*, **40**, 710.
Block, R. J. & Bolling, D. (1939*b*). *J. biol. Chem.* **130**, 365.
Block, R. J. & Bolling, D. (1940). *The Determination of Amino Acids.* Minneapolis, Minn., U.S.A.: Burgess Publishing Co.
Block, R. J., Bolling, D. & Webb, M. (1940). *J. biol. Chem.* **133**, xiv.
Brand, E. & Kossel, B. (1939). *J. biol. Chem.* **131**, 489.
Cook, E. S. & Cronin, A. G. (1941). *Stud. Inst. Divi Thomae*, **3**, 203.
Davidson, J. N. (1940). *Biochem. J.* **34**, 1537.
Davidson, J. N. (1943). *Edin. med. J.* **50**, 70.
Jorpes, E. & Thoren, S. (1932). *Biochem. J.* **26**, 1504.
Koessler, K. K. & Hanke, M. T. (1919). *J. biol. Chem.* **39**, 497.
Loofbourow, J. R. (1942*a*). *Biochem. J.* **36**, 631.
Loofbourow, J. R. (1942*b*). *Biochem. J.* **36**, 737.
Loofbourow, J. R., Webb, A. M., Loofbourow, D. G. & Abramowitz, R. K. (1942). *Biochem. J.* **36**, 513.
Mitchell, H. K. & Williams, R. J. (1940). *Biochem. J.* **34**, 1532.
Reader, V. (1927). *Biochem. J.* **21**, 901.
Rogosa, M. (1944). *J. Bact.* **47**, 159.
Webb, A. M. & Loofbourow, J. R. (1947). *Biochem. J.* **41**, 114.

Intercellular Hormones

8. RELEASE OF NUCLEOTIDES AND NUCLEOSIDES BY DAMAGED LIVING CELLS

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The chemical mechanism whereby the rate of proliferation of normal cells is increased in the neighbourhood of damaged cells (sometimes referred to as the wound-hormone phenomenon) involves several parameters, including: (1) the nature of the cells involved, (2) the method, extent, and duration of cell damage, and (3) the nature of the intercellular *milieu*. In preceding communications, we have presented evidence that in the special case of yeast cells damaged by prolonged sublethal dosages of ultraviolet radiation in a distilled water suspension medium, members of the vitamin B complex, amino-acids and nucleotides are released in considerable quantities into the suspension medium without appreciable cytolysis (Loofbourow, 1942*a*, *b*, 1947; Webb & Loofbourow, 1947).

With regard to the vitamin B complex ('B factors'), it appears that the living, damaged yeast cells elaborate biotin, pantothenic acid and folic acid in appreciable quantities during the course of cellular damage (Webb & Loofbourow, 1947). The strain of yeast we have employed in these experiments (*S. cerevisiae*, F.B. strain) is, according to Rogosa (1944), incapable of synthesizing pantothenic acid in normal growing cultures in sufficient quantities to support growth; hence it appears that the synthetic processes in damaged living cells may differ materially from those in normal cells.

The increased content of 'B factors' and amino-acids in the suspension fluids from damaged cells (I) as compared with those from control cells (II) accounts in large measure, but not entirely, for the increased proliferation-promoting effect of the former (Cook & Cronin, 1941; Webb & Loofbourow, 1947; Loofbourow, 1947). When, however, adenine nucleotides are added to yeast cultures in combination with B factors and amino-acids in appropriate concentrations, the proliferation-promoting effect of I can be duplicated in its entirety (Loofbourow, 1942*b*). This fact, together with various spectroscopic and chemical evidence for the presence of

* Deceased.