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.

The investigations reported here were supported by a grant from E. I. du Pont de Nemours and Co. I wish to thank Miss Constance A. Yeats and Miss Edythe Baum for assistance in the amino-acid and nitrogen determinations.

REFERENCES

- Block, R. J. & Bolling, D. (1939a). *Proc. Soc. exp. Biol., N.Y.*, **40**, 710.
 Block, R. J. & Bolling, D. (1939b). *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. (1942a). *Biochem. J.* **36**, 631.
 Loofbourow, J. R. (1942b). *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

By J. R. LOOFBOUROW, S. OPPENHEIM-ERRERA,* DOROTHEA G. LOOFBOUROW
AND CONSTANCE A. YEATS

(Received 26 June 1946)

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 ultra-violet 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, 1942a, 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

* Deceased.

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, 1942b). This fact, together with various spectroscopic and chemical evidence for the presence of

nucleotides and nucleosides in I (Loofbourow, Cook & Stimson, 1938; Davidson, 1940) indicates that nucleotides or substances related thereto are involved in the proliferation-promoting effect of I.

The present investigations afford further evidence as to: (1) the nature of the nucleotides and nucleosides present in I, (2) the role of such substances in the proliferation-promoting activity of I, and (3) the possibility of synthesis of such substances within the living damaged cells during the course of injury.

METHODS

To prepare cell-free suspension fluids from damaged and undamaged cells, washed, starch-free, moist yeast (*S. cerevisiae*, F.B. strain) was suspended at 100 g. (wet weight)/l. of suspension in distilled water, and divided into 2 l. samples. For the preparations from damaged cells, the 2 l. samples were irradiated with full ultra-violet from a quartz mercury arc for 8 hr. periods. During these periods, the undamaged-cell samples were maintained under the same conditions of temperature, stirring, etc. Following irradiation, the cell-free suspension fluids were obtained from irradiated (I) and non-irradiated (II) cells by centrifugation and Seitz filtration. Further details of the preparation methods are given in earlier communications (Loofbourow, Webb, Loofbourow & Abramowitz, 1942; Loofbourow, 1942b).

In all, six preparations each of I and II were employed in the investigations reported here. Three of the preparations were autoclaved at a pressure of 15 lb./sq.in. for 20 min. at pH about 6 following Seitz filtration; three were not autoclaved. There was no indication that autoclaving caused appreciable degradation of the nucleotides present, but this question requires further investigation.

The above method of preparing suspension fluids from damaged yeast cells yields a sparkling-clear, straw-coloured solution, substantially free from protein. There is inappreciable cytolysis of the damaged cells, the substances which enter the intercellular fluids apparently doing so by diffusion through the

intact cell membranes, made more permeable as a result of cellular damage. The damaged cells are alive during most of the irradiation period, as determined by the methylene blue staining reaction, and are capable of carrying on synthetic processes, as indicated by their tendency to form buds (Loofbourow, 1942a). The synthetic processes which take place during the course of irradiation under these conditions must, of course, be supported by substances already present in the cells, since the suspending medium is water.

Amino-N was determined by formol titration and Folin's colorimetric procedure. All other N determinations were made by the micro-Kjeldahl procedure: non-protein-N (N.P.N.) after precipitation of protein with sodium tungstate; nucleotide and nucleoside and free purine-N after separation of these fractions according to the method of Kerr & Blish (1932; Kerr, 1940).

Free and bound P were determined by the method of Fiske & Subbarow (1925).

The proliferation-promoting potencies of the various samples were assayed on yeast (*S. cerevisiae*, F.B. strain) grown in rotor tubes according to previously described procedures (Loofbourow *et al.* 1942). Two media were used: (1) Reader's (1927), unsupplemented, and (2) the same, supplemented with the following growth factors (concentrations in $\mu\text{g./ml.}$ of medium): aneurin hydrochloride, 0.25; riboflavin, 0.5; pyridoxin, 0.5; calcium pantothenate, 0.125; biotin (free base), 0.000125; choline, 0.75; nicotinamide, 2.5; arginine, 625; asparagine, 625; aspartic acid 625; glutamic acid, 625; and leucine, 625.

Ultra-violet absorption spectra were determined with a Hilger quartz spectrograph and Spekker photometer.

RESULTS

The results of analyses for nucleotide-N and nucleoside- and free purine-N of the cell-free suspension fluids of preparation 627 are shown in Table 1. Analyses of other preparations gave similar results; e.g. the cell-free suspension fluids from irradiated

Table 1. Analyses of cell-free suspension fluids from irradiated and non-irradiated cells

	Davidson (1940)			Preparation 627		
	I, Irr.* (mg./ml.)	II, NI* (mg./ml.)	I/II	I, Irr.* (mg./ml.)	II, NI* (mg./ml.)	I/II
1. Solid content	11.64	2.80	4.1	4.12	0.192	21.5
2. Nucleotide-N	0.118	0.004	29.5	0.0155	0.00045	34.4
3. Nucleoside and free purine-N	0.094	0.014	6.7	0.0214	0.0040	5.4
4. Amino-N	0.633	0.090	7.0	0.233	0.0167	13.9
5. Totals 2, 3 and 4	0.845	0.108	—	0.270	0.0212	—
6. N.P.N.	0.962	0.105	9.2	0.272	0.021	12.9
7. Ultra-violet absorption in 0.1 N-HCl:						
(a) λ_{max}	—	—	—	2570 A.	2650 A.	—
(b) $1/x \log_{10} I_0/I_x$	—	—	—	8.8	1.9	4.6

* Irr. refers to suspension fluids from irradiated cells. NI refers to suspension fluids from non-irradiated cells.

and non-irradiated cells in preparation 456 had nucleotide-N values of 0.0159 and 0.00047 mg./ml. respectively.

In comparing our results with those of Davidson (1940), which have been recomputed from his data to terms of mg./ml. and included in Table I, it should be noted that his preparations were obtained from yeast suspensions twice as concentrated as ours, so that his values per ml. of suspension fluid would be expected to be larger than ours. Furthermore, his conditions of irradiation were different, and the strain of *S. cerevisiae* he employed may not have been identical with the one we used. Comparison of our 627 preparation with his on the basis of the ratios (I/II) of the analyses of suspension fluids from irradiated cells (I) to those of suspension fluids from non-irradiated cells (II) shows considerable similarity in so far as nucleotide and nucleoside-N are concerned. The nucleotide-N ratios (I/II) were particularly high, being 29.5 for Davidson's preparation, 34.4 for our preparation 627, and 39.5 for our preparation 456 (not shown in the table).

Davidson found greater quantities of nucleotide-N than of nucleoside and free purine-N in I. The reverse was true in all of our preparations. This may indicate that there was greater degradation of nucleotides to nucleosides and free purines under our conditions of irradiation and fractionation.

In endeavouring to isolate the nucleotide fraction, we were led by the following considerations to undertake the separation of adenosine triphosphate (ATP) from the preparations: (1) early investigations (Loofbourow *et al.* 1938; Cook, Loofbourow & Stimson, 1939) indicated that the ultra-violet absorption of I was attributable primarily to adenine and possibly to some extent to guanine; (2) Davidson (1940) isolated adenine as the picrate and sulphate from his preparations of I; small quantities of guanine were also found to be present; (3) in growth tests (Loofbourow, 1942*b*), the proliferation-promoting effect of I was duplicated with combinations of amino-acids, B factors, and ATP; neither muscle adenylic acid nor adenosine was found to be as effective as ATP; (4) the ratios of purine-N to organic P for preparation 225 I ranged from 0.8 to 0.9, suggesting the presence of nucleotides of high P content, such as ATP (N/P=0.75) or adenosine diphosphate (N/P=1.13). In view of these considerations, an endeavour was made to isolate the barium salt of ATP from I by the method of Kerr (1941), even though it appeared unlikely that ATP would be present in I in appreciable quantities following the usual 8 hr. irradiation period at room temperature.

In the ATP preparations, all manipulations following irradiation, including centrifugation and Seitz filtration to free the suspensions from cells, were carried out in a cold room at 4° or with the pre-

parations cooled by an ice-bath. In two preparations, the yield of ATP was negligible. Control preparations from freshly killed rabbit muscle gave satisfactory yields of ATP in both instances. Ultra-violet absorption spectra indicated that almost the entire purine fraction remained in the supernatants of the yeast preparations following precipitation of ATP by barium acetate and ethanol.

In further treatment of supernatants obtained by the above procedure or slight modifications thereof, the nucleotide fraction was precipitated with mercuric acetate, resuspended, freed from mercury and precipitated repeatedly from water solution by addition of acetone.

Fig. 1 shows the similarity of the ultra-violet absorption spectrum of one such preparation (N_1) to a typical absorption spectrum of an adenine nucleo-

Fig. 1. Ultra-violet absorption spectrum of nucleotide (N_1) prepared by Hg-Ba-acetone precipitation from cell-free fluid from irradiated cells, compared with the absorption of BaATP prepared from rabbit muscle. The concentration of the nucleotide solution was adjusted to give a maximum density, $\log_{10} I_0/I_x$, of 2 to facilitate comparison of the shapes of the curves.

tide (BaATP from rabbit muscle). Fig. 2 compares the absorption of N_1 with that of a later preparation by the same method (N_2). Variability in spectra to the extent indicated by this comparison was characteristic of various preparations made by this method; such variability is probably attributable to differences in the purity of the various preparations.

Fig. 2 also shows that changes in pH had little effect on the ultra-violet absorption of the acetone-precipitated nucleotide fraction, a characteristic which would be expected if the absorption were attributable to adenine (Holiday, 1930; Loofbrouwer & Stimson, 1940; Stimson & Reuter, 1945).

Fig. 2. Ultra-violet absorption spectra of nucleotides N_1 and N_2 prepared by Hg-Ba-acetone precipitation from cell-free fluids from irradiated cells. The concentration of N_1 was adjusted to give a maximum optical density, $\log_{10} I_0/I_x$, of 2. The two curves showing effect of pH on N_2 are for identical concentrations of N_2 , this concentration being chosen so as to give a maximum optical density of about 1.8 to facilitate comparison of the shapes of the curves with that of N_1 . In determining the spectra of N_2 , acid or alkali was added to the comparison cell in the same concentration as employed in the solution undergoing measurement.

Two of the acetone-precipitated nucleotide preparations (N_1 and N_2) were tested for proliferation-promoting activity in Reader's medium, unsupplemented and supplemented with B factors and amino-acids. The results are summarized in Table 2. In all concentrations employed, the nucleotide fractions stimulated the growth of yeast, both in the unsupplemented and the supplemented Reader's media. Addition of the nucleotides to the supplemented medium in concentrations such that their absorption

at 260 A. after dilution by the medium was equivalent to that of $3.7 \times 10^{-6} - 1.85 \times 10^{-5}$ mol. of adenine/l., resulted in yeast crops closely similar to those obtained by addition to unsupplemented Reader's medium of 0.05 ml. of preparation 627 I/ml. of culture (corresponding in ultra-violet absorption at 2600 A. to 3.24×10^{-5} mol. of adenine/l. of culture). This indicates that the nucleotides and related compounds in I account for the additional proliferation-promoting effect of I above that attributable to amino-acids and B factors.

Two further preparations of nucleotide were made following the procedure described above except that the nucleotide was precipitated by addition of acetone to a water solution containing the barium salt. One of these preparations was tested for growth activity with results similar to those shown for preparations N_1 and N_2 in Table 2. The spectra of both preparations were characteristic of adenine derivatives, as in Fig. 1. The ratios of purine-N to bound P for these two preparations were respectively 1.0 and 1.6.

The yields by the Hg-Ba-acetone precipitation method were of the order of 10–20 % of the theoretical purine content of the starting material as determined spectrographically. Because of the low yield, no further attempt was made to isolate the nucleotide in this manner. Instead, preliminary studies were made of the nucleoside and nucleotide fractions isolated for N determinations by uranium and copper salt precipitation according to the method of Kerr & Blish (1932; Kerr, 1940).

Adenine was estimated by the method of Hitchings (1933) after isolation as the picrate from the nucleotide fraction. Guanine was estimated by a quantitative colorimetric procedure based on the diazo reaction. The results indicated that at least 85 % of the purine content of the nucleotide fraction was adenine, the remainder probably being guanine. Satisfactory quantitative data were not obtained for the nucleoside fractions; adenine and guanine also appeared to be present therein, but the possibility of other purines or pyrimidines being present in substantial quantities was not excluded.

Positive tests for ribose were obtained with both the nucleotide and nucleoside materials. Tests for deoxyribose were negative.

The spectrum of the nucleotide fraction at acid pH (approximately 0.1 N-HCl) was similar to that of adenine and its nucleotide and nucleoside derivatives (Fig. 3). The spectrum of the nucleoside fraction at similar pH had a maximum at 2550 A. rather than at 2600–2610 A., the latter being characteristic of adenine.

The nucleotide and nucleoside fractions obtained by the Kerr & Blish procedure accounted for nearly all of the purine content of I as indicated by the N balance sheet (Table 1) and by comparison of the

Table 2. *Proliferation-promoting effect of nucleotide fractions prepared by Hg-Ba-acetone precipitation*Fractions N_1 and N_2 are derived from the cell-free fluids from irradiated cells (see text).

Medium	Addition to medium per ml. of culture	Equivalent adenine in culture (mol./l.)†	Yeast crop (mg./ml. wet weight)		Increased crop resulting from added material	
			24 hr.	34½ hr.	24 hr.	34½ hr.
Reader's	None	—	0.25	0.25	—	—
	0.1 ml. Nucleotide N_1	3.7×10^{-5}	1.07	1.66	0.82	1.41
	0.05 ml. Nucleotide N_1	1.85×10^{-5}	0.63	0.95	0.38	0.70
	0.02 ml. Nucleotide N_1	7.4×10^{-6}	0.40	0.71	0.15	0.46
	0.01 ml. Nucleotide N_1	3.7×10^{-6}	0.31	0.50	0.06	0.25
	0.05 ml. Nucleotide N_2	1.85×10^{-5}	0.53	1.39	0.28	1.14
	0.05 ml. Prep. 627 I†	3.24×10^{-5}	3.44	5.40	3.19	5.15
Reader's + B factors + amino-acids*	0.05 ml. Prep. 627 NI†	6.9×10^{-6}	0.62	0.71	0.37	0.46
	None	—	3.02	4.35	—	—
	0.05 ml. Nucleotide N_1	1.85×10^{-5}	3.70	6.30	0.68	1.95
	0.01 ml. Nucleotide N_1	3.7×10^{-6}	3.25	5.40	0.23	1.05
	0.05 ml. Nucleotide N_2	1.85×10^{-5}	3.21	6.14	0.19	1.79

* Reader's medium supplemented with the following growth factors (concentrations in $\mu\text{g./ml.}$ of medium): aneurin hydrochloride, 0.25; riboflavin, 0.5; pyridoxin, 0.5; calcium pantothenate, 0.125; biotin (free base), 0.000125; choline, 0.75; nicotinamide, 2.5; arginine, 625; asparagine, 625; aspartic acid, 625; glutamic acid, 625; and leucine, 625.

† Irr. refers to cell-free suspension fluid from irradiated cells. NI refers to cell-free suspension fluid from non-irradiated cells.

‡ The solutions of N_1 and N_2 were made up at such concentrations as to have optical densities ($1/x \log_{10} I_0/I_x$) of 5.0 at $\lambda=2600 \text{ A.}$, equivalent to adenine concentrations of $3.7 \times 10^{-4} \text{ mol./l.}$ if all absorption at this wave-length were attributable to adenine. The optical densities at 2600 A. of preparations 627 I and 627 NI were respectively 8.8 and 1.9, equivalent to adenine concentrations of 6.48×10^{-4} and $1.38 \times 10^{-4} \text{ mol./l.}$ respectively, assuming all absorption at 2600 A. to be attributable to adenine.

Table 3. *Proliferation-promoting effect in Reader's medium of nucleotide and nucleoside fractions prepared as copper salts*

Addition to medium	Equivalent adenine in cultures (mol./l.)*	Yeast crop (mg./ml. wet weight)		Increased crop resulting from added material	
		24 hr.	39½ hr.	24 hr.	39½ hr.
None	—	0.28	0.40	—	—
Nucleotide fraction	4.42×10^{-5}	0.43	0.66	0.15	0.26
	2.21×10^{-5}	0.37	0.51	0.09	0.11
	1.11×10^{-5}	0.37	0.48	0.09	0.08
	5.55×10^{-6}	0.35	0.40	0.07	0.00
Nucleoside fraction	1.18×10^{-5}	0.53	0.82	0.25	0.42
	5.90×10^{-6}	0.42	0.51	0.14	0.11
	2.95×10^{-6}	0.35	0.43	0.07	0.03
	1.48×10^{-6}	0.35	0.42	0.07	0.02

* The nucleotide and nucleoside solutions were added to the cultures in such concentrations as to have ultra-violet absorption at 2600 A. corresponding to the absorption of adenine at the molar concentrations shown.

summation of the spectra of these fractions with the spectrum of I (Fig. 3). In plotting Fig. 3, the optical densities of the nucleotide and nucleoside solutions were divided by the number of ml. of starting material (I) per ml. of each solution, so as to obtain data representing the contribution of the absorption of each of these fractions to the absorption of the original material. From data as to the content of tyrosine, tryptophan, and absorbing B factors in I (Loofbourow, 1946; Webb & Loofbourow, 1946) it was computed that such substances might account for an optical density of about 0.6 at 2560 A. The net optical density of I at 2560 A. after

subtracting the absorption of such materials is 8.25, as compared with 7.9 for the nucleotide-nucleoside summation curve.

Growth tests of the Cu-free nucleotide and nucleoside fractions in Reader's medium are summarized in Table 3. Both fractions had appreciable activity at the higher concentrations employed. The activity of the nucleoside was apparently somewhat greater than that of the nucleotide. Neither fraction appeared to be as active in Reader's medium as the Hg-Ba-acetone preparations at concentrations corresponding to equivalent ultra-violet absorption at 2600 A.

The total absorption at 2600 A. of the cell-free intercellular fluids and residue cells was determined

alkali. The absorption at 2600 A. was in all instances greater in the case of the total intracellular and extra-

Fig. 3. Ultra-violet absorption spectrum of the cell-free fluid from irradiated cells (prep. 627I) in comparison with the spectra of the nucleotide and nucleoside fractions thereof obtained by the Kerr & Blish (1932), Kerr (1940) procedure. The absorption coefficients, $1/x \log_{10} I_0/I_x$, of the two fractions are shown for concentrations in which each ml. of the nucleotide or nucleoside solution corresponds to 1 ml. of the cell-free preparation (627I) from which it was prepared.

in several preparations from irradiated and non-irradiated yeast after release of the contents of the cell residues by autolysis or by digestion with strong

cellular materials from irradiated cells (IA) than in the case of like materials from non-irradiated cells (IIA). The ratio of absorption (IA/IIA) at 2600 A. in

a typical preparation was 1.9. If the absorption at 2600 Å. is assumed to be a measure of the purine-pyrimidine content of the preparations, these data indicate that such materials were synthesized by the living, damaged cells during the course of injury. The data further indicated that only about 10% of the total absorption at 2600 Å. in irradiated cell preparations is accounted for by the absorption of the cell-free suspension fluids; thus most of the increase in total absorption of irradiated as compared with non-irradiated preparations is attributable to substances which accumulate within the cells and are not released into the intercellular fluids.

DISCUSSION

The results reported here afford direct evidence of what has long been suggested by less direct experiments: nucleotides and nucleosides account for that part of the proliferation-promoting effect of cell-free fluids from suspensions of yeast cells slowly damaged by ultra-violet radiation which is not accounted for by amino-acids and B factors.

The exact nature of the nucleotides and nucleosides responsible for the effect remains to be established. The evidence indicates that adenine derivatives play a major role, that guanine derivatives and possibly other purine and pyrimidine derivatives may also be involved, and that the nucleotides and nucleosides are of the ribose rather than the deoxyribose type.

Recent investigations of Englert (1945) have shown that hydrolysis products of ribonucleic acid stimulate the growth of yeast and have led her to suggest that nucleosides released in this way (in particular adenosine and guanosine) account in part for the proliferation-promoting activity of damaged-cell preparations. From preliminary growth experiments other than those reported here, we are inclined to believe, however, that the most active components in the damaged-cell preparations are nucleotides rather than nucleosides.

The absorption spectra of the total extracellular and intracellular material indicate that nucleotide-like substances are synthesized, as well as released into the intercellular fluids, by cells slowly damaged by ultra-violet irradiation. This is consistent with the increase in ultra-violet absorption at 2600 Å. which has been noted in the cytoplasm of cells irradiated and observed periodically on the stage of a quartz microscope (Loofbourow & Joyce, 1941) and with the increased yield of ribonucleic acid previously found for irradiated as compared with non-irradiated yeast (Loofbourow *et al.* 1941; Englert, 1942, 1945). Mitchell (1942) reported a similar increase in the content of ribonucleic-acid-like substances in the cytoplasm of living cells exposed to Roentgen radiation or gamma rays.

It is perhaps not surprising that the synthetic processes in living cells should undergo some degree of alteration, quantitatively or qualitatively, when the cells are subjected to various damaging agents under circumstances which do not lead to rapid cell death, thus leading to increased quantities of particular substances within the cell and (in the case of substances capable of diffusing through the cell membrane) in the intercellular *milieu*.

After a careful review of the wound-hormone problem, Davidson (1943) appears to favour the hypothesis that the only substances set free by damaged cells are breakdown products of the cellular protoplasm, and that there is no synthesis of liberated components within living, injured cells during the injury period. We are inclined to disagree with Davidson on this point since: (1) data with regard to B factors (Webb & Loofbourow, 1947) indicate rather clearly an increased production, as well as release, of some of these factors during the course of injury; (2) the increased extracellular and intracellular absorption at 2600 Å. in the case of damaged as compared with undamaged cells is presumptive evidence for more rapid synthesis of purine-pyrimidine-like materials in the injured cells; (3) it is difficult to conceive of a mechanism whereby a dynamic chemical system, like that which occurs in living cells, should maintain a *status quo* despite the drastic changes in conditions imposed by exposure to lethal radiation.

When we first undertook a study of the 'wound hormone' problem, some years ago, we expected to find, as Davidson suggests, that dead-cell disintegration products accounted entirely for the phenomenon of increased growth of normal cells in the presence of products from damaged cells. The early observation that products from slowly injured cells had greater proliferation-promoting activity than products from quickly killed cells (Loofbourow, Dwyer & Lane, 1940) suggested, however, that in addition to disintegration of dead cells, changes in the metabolism and related synthetic processes of living, injured cells might be involved in the phenomenon. While the hypothesis that cell death and disintegration are the only factors involved in the 'wound hormone' phenomenon is an attractive one because of its simplicity, we believe, in view of our experiments, that such an hypothesis is an oversimplification in instances in which the damaged cells continue to live for an appreciable period of time.

SUMMARY

1. An investigation was made of the role of nucleotides and nucleosides in the increased proliferation-promoting activity of cell-free suspension fluids from cells slowly damaged by lethal radiation (I) as compared with similar fluids from undamaged cells (II).

The nucleotide-N and nucleoside- and free purine-N contents of I were respectively about 34 and 5 times those of II. Nucleotides isolated from I, when combined with amino-acids and B-factors found in I, duplicated the proliferation-promoting effect of I.

2. The nucleotide and nucleoside fractions were found to consist principally of adenine and guanine derivatives.

3. Spectroscopic evidence indicated a synthesis of materials absorbing at 2600 A. within the living, injured cells during the course of injury.

The investigations reported here were supported in part by a grant from E. I. du Pont de Nemours and Co. Present address of Dorothea G. Loofbourow, The Massachusetts General Hospital, Boston, and of Constance A. Yeats, U.S. Naval Air Base, Pensacola, Florida.

REFERENCES

- Cook, E. S. & Cronin, A. G. (1941). *Stud. Inst. Divi Thomae*, **3**, 203.
- Cook, E. S., Loofbourow, J. R. & Stimson, M. M. (1939). *Att. X. Congr. Int. Chim.* **5**, 26.
- Davidson, J. N. (1940). *Biochem. J.* **34**, 1537.
- Davidson, J. N. (1943). *Edinb. med. J.* **50**, 70.
- Englert, M. E. (1942). *Rep. Third Conf. Inst. Divi Thomae*. Cincinnati.
- Englert, M. E. (1945). *Stud. Inst. Divi Thomae*, **4**, 59.
- Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
- Kerr, S. (1940). *J. biol. Chem.* **132**, 147.
- Kerr, S. (1941). *J. biol. Chem.* **139**, 121.
- Kerr, S. & Blish, M. E. (1932). *J. biol. Chem.* **98**, 193.
- Hitchings, G. H. (1933). Dissertation, Harvard University.
- Holiday, E. R. (1930). *Biochem. J.* **24**, 619.
- Loofbourow, J. R. (1942a). *Biochem. J.* **36**, 631.
- Loofbourow, J. R. (1942b). *Biochem. J.* **36**, 737.
- Loofbourow, J. R. (1947). *Biochem. J.* **41**, 119.
- Loofbourow, J. R., Cook, E. S. & Stimson, M. M. (1938). *Nature, Lond.*, **142**, 573.
- Loofbourow, J. R., Dwyer, C. M. & Lane, M. M. (1940). *Biochem. J.* **34**, 432.
- Loofbourow, J. R., Englert, M. E. & Dwyer, C. M. (1941). *Nature, Lond.*, **148**, 113.
- Loofbourow, J. R. & Joyce, Lois (1941). *Nature, Lond.*, **148**, 166.
- Loofbourow, J. R. & Stimson, M. M. (1940). *J. chem. Soc.* p. 844.
- Loofbourow, J. R., Webb, A. M., Loofbourow, D. G. & Abramowitz, R. K. (1942). *Biochem. J.* **36**, 513.
- Mitchell, J. F. (1942). *Brit. J. exp. Path.* **23**, 285.
- Reader, V. (1927). *Biochem. J.* **21**, 901.
- Rogosa, M. (1944). *J. Bact.* **47**, 159.
- Stimson, M. M. & Reuter, M. A. (1945). *J. Amer. chem. Soc.* **67**, 2191.
- Webb, A. M. & Loofbourow, J. R. (1947). *Biochem. J.* **41**, 114.

The Quantitative Separation on the Micro-scale of Phenylarsenious Acid, Phenylarsonic Acid, Arsenious Acid and Arsenic Acid in Simple Solution and in Urine

By T. B. B. CRAWFORD

Departments of Biochemistry and Pharmacology, University of Edinburgh

(Received 26 August 1946)

It has been generally assumed that aromatic arsonic acids undergo reduction *in vivo* to the corresponding arsenoxides before producing their parasitocidal or toxic actions. All evidence for this is, however, of an indirect nature (Voegtlin & Smith, 1920; Yorke & Murgatroyd, 1930; Murgatroyd, Russell & Yorke, 1934; Thuret, 1938). It has also been suggested that arsenoxides are oxidized before excretion in the urine (Chance, Crawford & Levvy, 1945). Evidence as to whether or not inorganic arsenic derivatives are formed from organic arsenicals in the body is scanty and inconclusive. Definite information regarding the metabolism of organic arsenicals has awaited the development of suitable microchemical methods.

To investigate the changes undergone in the body by phenylarsenious acid and phenylarsonic acid, a

method was required whereby each constituent of a mixture of phenylarsenious acid, phenylarsonic acid, arsenious acid and arsenic acid when present as their salts in biological material could be determined on the micro-scale. It was considered that such a method could be developed incorporating the procedure previously described (Crawford & Levvy, 1946) for the separation of the trivalent and pentavalent arsenic compounds, provided that extraction of the organic but not the inorganic arsenicals from aqueous solution could be effected by an immiscible solvent. The elaboration of a suitable procedure along these lines for pure solutions containing the four arsenicals, and its application, with some modifications, to urine, is described below.