Kaplan, N. O. (1955). In *Methods in Enzymology*, vol. 2, p. 660–663. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
Keller, E. B. (1951). *Fed. Proc.* **10**, 206.
Keller, E. B. & Zamecnik, P. C. (1956). *J. biol. Chem.* **221**, 45.
Kornberg, A. & Pricer, W. E. (1951). *J. biol. Chem.* **191**, 535.
Korner, A. (1959). *Exp. Cell Res.* **18**, 594.
Lamborg, M. R. & Zamecnik, P. C. (1960). *Biochim. biophys. Acta*, **42**, 206.
McLean, J. R., Cohn, G. L., Brandt, I. K. & Simpson, M. V. (1958). *J. biol. Chem.* **233**, 657.
Mager, J. (1960). *Biochim. biophys. Acta*, **38**, 150.
Mejbaum, W. (1939). *Hoppe-Seyl. Z.* **258**, 117.
Novikoff, A. B. (1959). In *Analytical Cytology*, p. 69–168. Ed. by Mellors, R. C. New York: McGraw-Hill Book Co. Inc.
Peterson, E. A. & Greenberg, D. M. (1952). *J. biol. Chem.* **194**, 359.
Reis, P. J., Coote, J. L. & Work, T. S. (1959). *Nature, Lond.*, **184**, 165.
Rendi, R. (1959*a*). *Exp. Cell Res.* **17**, 585.
Rendi, R. (1959*b*). *Exp. Cell Res.* **18**, 187.
Rutman, R. J., Cantarow, A. & Paschkis, K. E. (1954). *Cancer Res.* **14**, 115.
Sanger, F. (1945). *Biochem. J.* **39**, 507.
Schneider, W. C. (1945). *J. biol. Chem.* **161**, 293.
Schneider, W. C. (1948). *J. biol. Chem.* **176**, 259.
Schneider, W. C. (1959). *Advanc. Enzymol.* **21**, 1.
Schneider, W. C. & Potter, V. R. (1943). *J. biol. Chem.* **149**, 217.
Siekevitz, P. (1952). *J. biol. Chem.* **195**, 549.
Siekevitz, P. & Watson, M. L. (1956). *J. biophys. biochem. Cytol.* **2**, 653.
Siekevitz, P. & Watson, M. L. (1957). *Biochim. biophys. Acta*, **25**, 274.
Simkin, J. L. (1958). *Biochem. J.* **70**, 305.
Simkin, J. L. & Work, T. S. (1957*a*). *Biochem. J.* **65**, 307.
Simkin, J. L. & Work, T. S. (1957*b*). *Biochem. J.* **67**, 617.
Sisakyan, N. M. & Filippovich, I. I. (1957). *Biokhimiya*, **22**, 375.
Slater, E. C. (1953). *Biochem. J.* **53**, 521.
Slater, E. C. & Holton, F. A. (1953). *Biochem. J.* **55**, 530.
Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1957). *Manometric Techniques*, p. 274, 3rd ed. Minneapolis: Burgess Publishing Co.
Van Slyke, D. D. (1929). *J. biol. Chem.* **83**, 425.
Van Slyke, D. D., Dillon, R. T., MacFadyen, D. A. & Hamilton, P. (1941). *J. biol. Chem.* **141**, 627.
Veall, N. (1948). *Brit. J. Radiol. N.S.*, **21**, 347.
Watson, M. L. & Siekevitz, P. (1956). *J. biophys. biochem. Cytol.* **2**, 639.
Zamecnik, P. C. & Keller, E. B. (1954). *J. biol. Chem.* **209**, 337.

Biochem. J. (1961) **80**, 21

The Regulation of Pathways of Glucose Catabolism in Maize Roots

BY V. S. BUTT AND H. BEEVERS
Department of Botany, Oxford University, and Department of Biological Sciences, Purdue University, Lafayette, Indiana, U.S.A.

(*Received* 28 *November* 1960)

The operation of the pentose phosphate pathway in glucose catabolism, as demonstrated by low C-6/C-1 ratios (the yield of $^{14}CO_2$ from [6-^{14}C]-glucose relative to that from [1-^{14}C]glucose), can now be regarded as a feature of many plant tissues (Gibbs, 1959). On the other hand, the internal and external conditions which determine the extent of its activity have received little attention. In the work described here, the action of oxidizing agents on the yield of $^{14}CO_2$, when maize root-tips were provided with glucose specifically labelled in various carbon atoms, has been examined and compared with nitrite as a likely natural electron acceptor, and with 2:4-dinitrophenol as an uncoupling agent.

Maize root-tips, in common with other young developing plant tissues (Gibbs & Beevers, 1955), show a C-6/C-1 ratio of unity (Beevers & Gibbs, 1954), suggesting that hexose breakdown proceeds primarily through the classical pathways of glycolysis and the tricarboxylic acid cycle. However, Beevers (1956) has shown that such tissues break down labelled gluconate, ribose, xylose and sedoheptulose in air and under nitrogen to give products with the radiochemical distribution expected from the operation of the pentose phosphate pathway, so that they must possess the necessary enzymic equipment, which appears to be inoperative when glucose is the substrate at this stage. Any induction of the alternative oxidation pathway should therefore be recognized readily by a decrease in the natural C-6/C-1 ratio.

The stimulation of the oxygen consumption and carbon dioxide production of plant tissues by artificial hydrogen acceptors, especially by methylene blue (Turner, 1940), has been recognized for some time (James, 1953), and recent work with carrot disks (ap Rees & Beevers, 1960) has shown

that this is accompanied by a vigorous increase in the release of C-1 of glucose. In work with carrot tissue (ap Rees & Beevers, 1960), liver slices (Cahill, Hastings, Ashmore & Zottu, 1958), erythrocytes (Brin & Yonemoto, 1958), corneal epithelium (Kinoshita, 1957) and ascites-tumour cells (Wenner, 1959), the effects of such reagents have been interpreted as showing that the supply of triphosphopyridine nucleotide is a major factor in controlling the activity of the pentose phosphate pathway. A similar demonstration was sought in young maize roots, by treating them with three oxidizing agents under both aerobic and anaerobic conditions.

The possibility that natural oxidants for reduced triphosphopyridine nucleotide present within tissues elicit the participation of the pentose phosphate pathway has been raised. Thus the assimilation of ammonia by yeast cells (Holzer & Witt, 1960) and possibly the synthesis of fatty acids in lactating rat mammary tissue (Abraham, Cady & Chaikoff, 1957; McLean, 1960) are activities which may be coupled with the pathway. The reduction of inorganic nitrogen in plant tissues suggests another likely activity, and the changes induced in the pattern of metabolism during nitrite assimilation by maize roots have now been examined.

A preliminary account of this work has been presented (Butt & Beevers, 1960).

EXPERIMENTAL

Maize grain (hybrid variety Wf 9 × 38–11) was soaked under water overnight for 16 hr., and allowed to germinate between moist absorbent paper for 24 hr. at 30°. The roots were then up to 2 cm. long; the first 1 cm. segment from the apex was cut as exactly as possible. The segments were washed thoroughly with deionized water and used within 2 hr. after excision. In each vessel 20 root-tips were generally used; the mean fresh weight for six samples was 0·217 ± 0·009 g.

Uniformly labelled glucose, [1-^{14}C]-, [2-^{14}C]- and [6-^{14}C]-glucose were commercial products. The sample of [3:4-$^{14}C_2$]-glucose had been prepared by exposing castor beans to $^{14}CO_2$ and contained at least 90% of the radioactivity in C-3 and C-4 (ap Rees & Beevers, 1960).

Methods. The root-tips were incubated in Warburg vessels with KH_2PO_4–Na_2HPO_4 (0·067 M) or water at 25°. Measurements of gas exchange were carried out by standard manometric techniques. Production of CO_2 was determined by the direct method of Warburg; the flask constants were adjusted to allow for CO_2 retention in the buffers used (Umbreit, Burris & Stauffer, 1957). In experiments with labelled substrate, 2 μmoles of glucose, containing 0·05–0·006 μc of ^{14}C, were supplied in a total volume of 2·5 ml.

The $^{14}CO_2$ released by the tissues was absorbed by KOH in the centre wells. At intervals, usually after incubation for 3 and 6 hr., the KOH was removed quantitatively and replaced by fresh alkali. The carbonate present in each sample of KOH was precipitated as $BaCO_3$ and the ^{14}C content measured by counting in a windowless gas-flow counter. C-6/C-1 ratios are calculated as described by Beevers & Gibbs (1954).

Glucose uptake was estimated in replicate vessels or in separate experiments by taking out samples (0·1–0·2 ml.) at suitable intervals and determining their radioactivity after drying gently on a nickel planchet.

RESULTS

Effect of artificial electron acceptors

Rate of respiration. After the root-tips had been excised, their respiratory rate showed a steady decline over some hours. As the gas measurements were started within 2 hr. after excision, they reflect this decline. In one experiment 20 root-tips absorbed 155, 108, 102, 97, 99 and 97 μl. of O_2 in successive periods of 1 hr., during which CO_2 production fell from 122 to 93 μl., so that the R.Q. showed little change with an average of 0·95 ± 0·01.

The effects of the three artificial electron acceptors, methylene blue, phenazine methosulphate and triphenyltetrazolium chloride at concentrations giving maximum stimulations are shown in Table 1. Methylene blue (2 mM) stimulated O_2 uptake and CO_2 output equally without any effect on the R.Q.; the stimulations, though small, were reproduced in three experiments. Phenazine methosulphate, at 1 mM and at lower concentrations, increased the rate of CO_2 production rather more than that of O_2 uptake so that the R.Q. was in all cases increased to nearly 1·0. Triphenyltetrazolium chloride, applied at pH 7·3 to increase its

Table 1. *Effect of artificial electron acceptors on the respiration of maize root-tips*

Twenty maize root-tips were incubated in KH_2PO_4–Na_2HPO_4 buffer at 25° for 3 hr. Total volume, 2·5 ml.

Additions	pH	O_2 uptake (μl./hr.)	CO_2 production (μl./hr.)	R.Q.
None	6·0	107	83	0·77
2 mM-Methylene blue	6·0	117	91	0·78
None	6·0	144	109	0·76
1 mM-Phenazine methosulphate	6·0	171	163	0·95
None	7·3	124	105	0·85
8 mM-Triphenyltetrazolium chloride	7·3	99	164	1·67

effectiveness (Marrè & Arrigoni, 1954), gave a heavy red precipitate in the tissue. At 8 mM, as shown in Table 1, O_2 uptake was lowered but the CO_2 output was considerably stimulated, so that the R.Q. rose as high as 1·67; higher concentrations reduced the O_2 consumption still further. Over a further period of 3 hr., these differences were sustained with each reagent; the R.Q. values remained almost constant despite falls in the rates of gas exchange.

Glucose uptake. During successive periods of 3 hr. the rate of glucose uptake into untreated roots remained constant, although a large proportion of the applied glucose was absorbed. Glucose uptake was considerably lowered by phenazine methosulphate and triphenyltetrazolium chloride and less so by methylene blue (Table 2).

Output of $^{14}CO_2$. When only a small fraction of the glucose absorbed by the untreated roots was released as CO_2, the C-6/C-1 ratio was close to 1 in this material (Table 3). The result of adding each of the artificial electron acceptors was a striking (two- to three-fold) stimulation in the rate of $^{14}CO_2$ release from [1-^{14}C]glucose, and this in spite of the fact that glucose uptake was curtailed. The release of C-1 in the presence of phenazine methosulphate and triphenyltetrazolium chloride was such that it represented an almost complete utilization of the [1-^{14}C]glucose which had been absorbed. In addition these reagents appreciably suppressed the release of C-6 as CO_2 so that the resulting C-6/C-1 ratios were extremely low (Table 3). In the presence

Table 2. *Effect of artificial electron acceptors on glucose uptake*

Twenty maize root-tips were incubated with 2 μmoles of uniformly labelled [^{14}C]glucose in buffer, as in Table 1. Total volume, 2·5 ml. The residual glucose was measured after 3 hr. and 6 hr.

Additions	pH	Uptake (%) 3 hr.	6 hr.
None	6·0	28	60
2 mM-Methylene blue	6·0	20	38
None	6·0	31	73
1 mM-Phenazine methosulphate	6·0	25	25
None	7·3	18	40
8 mM-Triphenyltetrazolium chloride	7·3	5	6

Table 3. *Effect of artificial electron acceptors on the release of $^{14}CO_2$ from* [1-^{14}C]- *and* [6-^{14}C]-*glucose*

Conditions were as in Table 2.

Additions	Period (hr.)	Conversion of applied glucose into $^{14}CO_2$ (%) [1-^{14}C]	[6-^{14}C]	C-6/C-1
None	0–3	2·5	2·2	0·88
	3–6	4·9	4·5	0·92
2 mM-Methylene blue	0–3	7·5	2·1	0·28
	3–6	10·6	4·2	0·39
None	0–3	1·4	1·4	1·00
	3–6	3·5	3·0	0·86
1 mM-Phenazine methosulphate	0–3	8·5	1·0	0·12
	3–6	12·1	2·8	0·23
None	0–3·5	1·0	0·9	0·90
	3·5–7	2·7	2·1	0·78
8 mM-Triphenyltetrazolium chloride	0–3·5	2·3	0·3	0·13
	3·5–7	4·4	0·9	0·20

Table 4. *Effect of artificial electron acceptors on the release of $^{14}CO_2$ from specifically labelled glucose*

Thirty maize root-tips were incubated under the conditions in Table 2.

Additions	Period (hr.)	O_2 uptake (μl./hr.)	Glucose uptake (%)	Conversion of applied glucose into $^{14}CO_2$ (%) [1-^{14}C]	[2-^{14}C]	[3:4-$^{14}C_2$]	[6-^{14}C]
None	0–3	166	31	2·0	0·7	5·1	1·8
	3–6	163	31	3·6	2·6	10·2	3·2
2 mM-Methylene blue	0–3	193	21	4·4	1·8	6·1	1·5
	3–6	177	14	5·3	3·9	8·9	2·2
1 mM-Phenazine methosulphate	0–3	218	18	10·8	4·4	3·0	0·8
	3–6	205	13	13·3	8·3	7·6	2·3

of methylene blue, the release of C-6 was only slightly affected so that the C-6/C-1 ratios were not so severely decreased. When the $^{14}CO_2$ release from [2-^{14}C]glucose was measured (Table 4) it became evident that phenazine methosulphate and methylene blue also had striking stimulatory effects on the release of C-2 as CO_2. During the 3–6 hr. period in the phenazine methosulphate experiments, the release of C-2 was almost as great as in the preceding 3 hr., although that of C-1 was declining as it reached completion. This increase in release of C-2 contrasts with the severe decrease in $^{14}CO_2$ production from [3:4-$^{14}C_2$]glucose with phenazine methosulphate; methylene blue had little effect on the release of C-3:4.

Effect of artificial electron acceptors under nitrogen

Both methylene blue and tetrazolium were reduced when they were incubated with maize roots under N_2; triphenyltetrazolium chloride gave a heavy red precipitate in the tissue and methylene blue was decolorized both in the tissue and in the ambient solution. The reduction was accompanied by a stimulation of CO_2 production (Table 5). This was sustained while oxidizing agent was available, but, with 2 mM-methylene blue, fermentation returned to the control rate after 4 hr., when the dye had been completely decolorized.

Neither of the oxidants, at the concentrations employed, affected seriously the release of $^{14}CO_2$ from [3:4-$^{14}C_2$]glucose nor the very slight and barely significant release of C-6. With each, however, the rate of release of C-1 was clearly increased and the threefold increase observed with 10 mM-methylene blue was sufficient to account for the extra CO_2 production which it induced. The lower stimulation of C-1 release with 2 mM-methylene blue might be anticipated from the fact that reduction was complete before the end of the incubation period. The glucose uptake was sufficient in all cases to avoid any limitation of radiocarbon supply.

In these experiments, the [3:4-$^{14}C_2$]glucose was applied in greater concentration than the other sugars but this does not affect their quantitative interpretation, since parallel experiments showed that the proportions of applied glucose absorbed and of $^{14}CO_2$ produced were unaffected by this difference.

Effect of 2:4-dinitrophenol

The pattern of radiocarbon release with the artificial electron acceptors was contrasted with the effect of an uncoupling agent, 2:4-dinitrophenol. Beevers (1953) observed that dinitrophenol stimulation of O_2 uptake may be accompanied by a greater stimulation of CO_2 production with a consequent rise in R.Q. At 0·05 mM, O_2 uptake was found here to be stimulated by 15% and CO_2 production by 70% over 3 hr., so that the R.Q.

Table 5. *Effect of artificial electron acceptors on fermentation and the conversion of specifically labelled glucose into $^{14}CO_2$ by maize root-tips under nitrogen*

Fifty maize root-tips were incubated in KH_2PO_4–Na_2HPO_4 buffer (0·067 M) at 25° under N_2. [1-^{14}C]Glucose or [6-^{14}C]glucose (2 μmoles), or 3·3 μmoles of [3:4-$^{14}C_2$]glucose were added. Total volume, 2·5 ml.; incubation period, 4 hr.

Additions	pH	CO_2 production (μl./hr.)	Glucose uptake (%)	Conversion of applied glucose into $^{14}CO_2$ (%) [1-^{14}C]	[3:4-$^{14}C_2$]	[6-^{14}C]
None	6·0	115	21	0·5	8·4	0·0
10 mM-Methylene blue	6·0	130	20	1·8	7·7	0·0
None	6·0	145	—	1·6	13·3	0·1
2 mM-Methylene blue	6·0	167	—	2·4	11·9	0·1
None	7·3	124	11	0·4	8·2	0·1
8 mM-Triphenyltetrazolium chloride	7·3	166	38	0·8	8·2	0·1

Table 6. *Effect of 2:4-dinitrophenol on the release of $^{14}CO_2$ from specifically labelled glucose*

Thirty maize root-tips were incubated under the conditions in Table 2, at pH 6·0.

Additions	Period (hr.)	Glucose uptake (%)	Conversion of applied glucose into $^{14}CO_2$ (%) [1-^{14}C]	[2-^{14}C]	[3:4-$^{14}C_2$]	[6-^{14}C]
None	0–3	31	2·0	0·7	5·1	1·8
	3–6	31	8·6	2·6	10·2	8·2
0·05 mM-Dinitrophenol	0–3	19	1·5	2·5	9·8	1·0
	3–6	26	5·4	6·0	15·6	3·6

rose from 0·73 to 1·08; in the subsequent 3 hr., both stimulation and R.Q. declined.

Despite the reduction in absorption of [^{14}C]-glucose (Table 6), the release of C-3:4 and of C-2 was considerably increased. By contrast, the release of C-1 and C-6 was less than that in the controls; a slight but significant fall in the C-6/C-1 ratio was observed.

Effect of sodium nitrite

The assimilation and reduction of nitrite in plant tissues is accompanied by a stimulation of CO_2 production with relatively little effect on the O_2 uptake (Willis & Yemm, 1955; Syrett, 1955). Similar effects of nitrite assimilation on the metabolism of maize root-tips were observed when nitrite was supplied in unbuffered solution. With 5 mM-$NaNO_2$, the O_2 uptakes of control and nitrite-treated roots were 121 and 123 μl./hr. Outputs of CO_2 were 122 and 145 μl./hr. to give R.Q. values of 0·93 and 1·18 respectively over 3 hr. The stimulation of CO_2 output declined in the subsequent 3 hr. period. The uptake of glucose was virtually unaffected by treatment with nitrite.

Table 7 shows that nitrite induced very different effects on the contributions of the various carbons of glucose to the respired CO_2. Thus while the release of C-6 was reduced, that of C-2 and particularly of C-1 was strikingly increased.

DISCUSSION

The three artificial electron acceptors employed here show a useful range of biochemical and metabolic activity. Phenazine methosulphate oxidizes reduced pyridine nucleotide directly and non-enzymically, but methylene blue and triphenyltetrazolium chloride are effective only through diaphorase action. Further, although the first two are each auto-oxidizable after reduction, the red formazan precipitate derived from tetrazolium reduction cannot be reoxidized by air. These differences are reflected in their metabolic effects, for, although each induced a stimulation of carbon dioxide production, methylene blue and phenazine methosulphate acted as alternative electron carriers to oxygen and in fact stimulated oxygen uptake; triphenyltetrazolium chloride decreased oxygen consumption by the tissue. However, treatment with each reagent strikingly reduced the C-6/C-1 ratio primarily by increasing the contribution of C-1 to the respired carbon dioxide. This suggests that the operation of the pentose phosphate pathway was increased more than glucose catabolism through glycolysis and the tricarboxylic acid cycle.

Support for this interpretation comes from the fact that the release of C-2 as well as that of C-1 was increased by treatment with methylene blue and phenazine methosulphate whereas that of C-3:4 was actually depressed. In the presence of phenazine methosulphate, the contributions to respired carbon dioxide were C-1 > C-2 > C-3; precisely the reverse of those predicted from a sequence comprising only the Embden–Meyerhof–Parnas pathway and the tricarboxylic acid cycle. These results are those predicted if the glucose units formed during the passage through the pentose phosphate pathway (with the original C-2 now primarily in position 1) were recycled in this sequence. The reduction in the fraction of glucose catabolized through the Embden–Meyerhoff–Parnas sequence as a result of removal of restraint at the oxidative steps of the pentose phosphate pathway would decrease still further the contribution of C-3:4 to the respired carbon dioxide in short-term experiments.

It is clear of course from the data on glucose uptake and release of $^{14}CO_2$ that the effects of the three oxidants are not identical, and are not limited to facilitating the oxidation of reduced TPN (Smith, 1952; Novikoff, 1959; Stenlid, 1950; Gaur & Beevers, 1959). Nevertheless it seems reasonable to conclude that the positive action of each in increasing the contribution of C-1 and C-2 to respired carbon dioxide is due to this common feature, and this implies that the participation of the pentose phosphate pathway in this tissue is limited by the capacity for oxidation of reduced TPN.

The effects of nitrite on the release of individual carbons as carbon dioxide (Table 7) are strikingly

Table 7. *Effect of sodium nitrite on the release of $^{14}CO_2$ from specifically labelled glucose*

Thirty maize root-tips were incubated in water at 25° with 2 μmoles of labelled glucose. Total volume, 2·5 ml.

Additions	Period (hr.)	Glucose uptake (%)	Conversion of applied glucose into $^{14}CO_2$ (%) [1-^{14}C]	[2-^{14}C]	[6-^{14}C]	C-6/C-1
None	0–3	30	3·2	1·1	4·0	1·25
	3–6	43	6·0	3·3	6·5	1·08
5 mM-Sodium nitrite	0–3	33	7·2	3·1	2·6	0·36
	3–6	38	19·3	10·0	5·7	0·30

similar to those induced by the oxidants, particularly by methylene blue. This suggests that the carbon dioxide burst accompanying nitrite treatment arises from decarboxylation in the pentose phosphate pathway, which, we presume is stimulated as reduced TPN is reoxidized during nitrite reduction. Such a reduction accompanied by the same effects on carbon dioxide output has been demonstrated in barley roots (Yemm & Willis, 1956) and *Ankistrodesmus* (Kessler, 1953) and the requisite enzymes have been isolated from higher plants (Nason, Abraham & Averbach, 1954). The sustained release of C-6 and the unimpaired uptake of glucose in the presence of nitrite suggests that glycolysis and the tricarboxylic acid cycle are not adversely affected.

The effect of stimulation of glycolysis and the acid cycle is shown in the response to dinitrophenol (Table 6). Results from pyruvate feeding experiments (Neal & Beevers, 1960) suggest that the release of C-3:4 should be immediately accelerated with C-2 and C-1:6 successively less affected. In spite of the reduction of glucose uptake due to treatment with dinitrophenol the anticipated stimulatory effects on the release of C-3:4 and of C-2 are indeed observed. The conclusion from these results is similar to that drawn from experiments with carrot, which, however, differs from the tissue used here in that the pentose phosphate pathway contributes noticeably to the respiration of the untreated material (ap Rees & Beevers, 1960). The results with dinitrophenol form a useful contrast with the effects of the artificial oxidants and nitrite.

Finally, an interesting problem is raised by the pattern of $^{14}CO_2$ release in the controls in Tables 4 and 7. Although $^{14}CO_2$ production from [1-^{14}C]- and [6-^{14}C]-glucose were about equal, that from [2-^{14}C]glucose was less than either; according to the foregoing arguments, it should be greater if the pentose phosphate pathway is inoperative. In each case, the release of C-3:4 was greatest. Humphreys & Duggar (1959) have reported similar observations. The results suggest that in this tissue, although the C-6/C-1 ratio is unity, some glucose breakdown was occurring by the pentose phosphate sequence. A likely explanation of this effect is that the excess of C-1 produced by this sequence is balanced by the release of C-6 during pentosan formation from glucose (Slater & Beevers, 1958). Equilibration between C-1 and C-6 of glucose (Shibko & Edelman, 1957) would also tend to increase the ratios towards unity (Axelrod & Beevers, 1956). These results emphasize again the desirability of providing supporting evidence from the respiration of glucose specifically labelled in other carbon atoms to sustain even qualitative deductions about pathways of breakdown.

SUMMARY

1. The effects of artificial electron acceptors on the respiration of maize root-tips and on their release of $^{14}CO_2$ from specifically labelled glucose in air and under nitrogen have been investigated.

2. Methylene blue, phenazine methosulphate and triphenyltetrazolium chloride each stimulated the production of carbon dioxide, with preferential release of $^{14}CO_2$ from [1-^{14}C]glucose. In air, $^{14}CO_2$ release from [2-^{14}C]glucose was also stimulated.

3. By comparison, 2:4-dinitrophenol increased the release of $^{14}CO_2$ from [3:4-^{14}C]- and, rather less, from [2-^{14}C]-glucose, but decreased that from [1-^{14}C]glucose.

4. Treatment with nitrite gave effects similar to those with the artificial electron acceptors.

5. It is suggested that the activity of the pentose phosphate pathway in this tissue is limited by the supply of oxidized triphosphopyridine nucleotide, and that nitrite reduction is coupled with its operation.

This work was carried out while one of us (V.S.B.) was guest at Purdue University during leave from Oxford, and was supported financially by the Atomic Energy Commission [Contract AT (11–1)–330], to whom we express our appreciation.

REFERENCES

Abraham, S., Cady, P. & Chaikoff, I. L. (1957). *J. biol. Chem.* **224**, 955.
ap Rees, T. & Beevers, H. (1960). *Plant Physiol.* **35**, 830.
Axelrod, B. & Beevers, H. (1956). *Annu. Rev. Pl. Physiol.* **29**, 318.
Beevers, H. (1953). *Amer. J. Bot.* **40**, 91.
Beevers, H. (1956). *Plant Physiol.* **31**, 339.
Beevers, H. & Gibbs, M. (1954). *Plant Physiol.* **29**, 322.
Brin, M. & Yonemoto, R. H. (1958). *J. biol. Chem.* **230**, 307.
Butt, V. S. & Beevers, H. (1960). *Biochem. J.* **76**, 51P.
Cahill, G. F., jun., Hastings, A. B., Ashmore, J. & Zottu, S. (1958). *J. biol. Chem.* **230**, 125.
Gaur, B. K. & Beevers, H. (1959). *Plant Physiol.* **34**, 427.
Gibbs, M. (1959). *Annu. Rev. Pl. Physiol.* **10**, 329.
Gibbs, M. & Beevers, H. (1955). *Plant Physiol.* **30**, 343.
Holzer, H. & Witt, I. (1960). *Biochim. biophys. Acta*, **38**, 163.
Humphreys, T. E. & Duggar, W. M., jun. (1959). *Plant Physiol.* **34**, 580.
James, W. O. (1953). *Plant Respiration*, p. 187. Oxford: The Clarendon Press.
Kessler, E. (1953). *Flora, Jena*, **140**, 1.
Kinoshita, J. H. (1957). *J. biol. Chem.* **228**, 247.
McLean, P. (1960). *Biochim. biophys. Acta*, **37**, 296.
Marrè, E. & Arrigoni, O. (1954). *Nuovo G. bot. ital.* **56**, 21.
Nason, A., Abraham, R. G. & Averbach, B. C. (1954). *Biochim. biophys. Acta*, **15**, 159.
Neal, G. E. & Beevers, H. (1960). *Biochem. J.* **74**, 409.

Novikoff, A. B. (1959). In *Subcellular Particles*, p. 14. Ed. by Hayashi, T. New York: Ronald Press Co.
Shibko, S. & Edelman, J. (1957). *Biochim. biophys. Acta*, **25**, 642.
Slater, W. G. & Beevers, H. (1958). *Plant Physiol.* **33**, 146.
Smith, G. F. (1952). *Plant Physiol.* **27**, 445.
Stenlid, G. (1950). *Physiol. Plant.* **3**, 197.
Syrett, P. J. (1955). *Physiol. Plant.* **8**, 924.
Turner, J. S. (1940). *Aust. J. exp. Biol. med. Sci.* **18**, 273.
Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1957). *Manometric Techniques*, p. 30. Minneapolis: Burgess Publishing Co.
Wenner, C. E. (1959). *J. biol. Chem.* **234**, 2472.
Willis, A. J. & Yemm, E. W. (1955). *New Phytol.* **54**, 163.
Yemm, E. W. & Willis, A. J. (1956). *New Phytol.* **55**, 229.

Biochem. J. (1961) **80**, 27

The Effect of Pituitary Growth Hormone on Phospholipid Synthesis

BY R. S. LEAL* AND A. L. GREENBAUM
Department of Biochemistry, University College London, Gower Street, London, W.C. 1

(*Received* 5 *December* 1960)

Amongst the many metabolic effects described in rats treated with growth hormone are the marked modifications in fat metabolism such as an increased rate of fatty acid oxidation (Greenbaum & McLean, 1953*a*) and a decreased rate of fatty acid synthesis (Greenbaum & Glascock, 1957). Phospholipid synthesis is also affected, although previous studies on the effect of the hormone on the rate of phospholipid synthesis in the livers of rats have led to some discordant results. Greenbaum, Graymore & Slater (1957) have reported a substantial increase in the rate of incorporation of injected [^{32}P]orthophosphate into the liver phospholipids of growth-hormone-treated animals, whereas Greenbaum & Glascock (1957) found a threefold decrease in the rate of incorporation of ^{14}C from [1-^{14}C]acetate into phospholipids of similarly treated animals. It appeared desirable to investigate this discrepancy more closely and to attempt to obtain further information on the nature of the action of the hormone which results in the lower incorporation rate of ^{14}C.

To this end the decreased rate of ^{14}C incorporation into phospholipid has been examined in more detail in an attempt to establish at which steps in the pathway of biosynthesis of phospholipids this decrease occurred and how far it is explicable in terms of isotope dilution caused by the appearance of larger quantities of intermediates of the synthetic pathway in the livers of growth-hormone-treated animals. By use of [^{14}C]acetate and [^{14}C]-palmitate as precursors it has been established that one of the spans affected by the hormone is acetate → long-chain acyl-coenzyme A. A second factor leading to the decrease in radioactive labelling is tentatively identified as a dilution at the level of diglyceride, which appears to be present in greater quantities in the livers of growth-hormone-treated rats than in control livers.

* Present address: Laboratorio de Radioquimica, C.E.E.N. Faculdade de Ciencias, Lisbon.

METHODS

Animals. Adult female rats of the hooded Norwegian strain aged about 3–4 months and weighing 170–200 g. were fed *ad lib.* on diet 41 of Bruce & Parkes (1946).

Growth hormone. A twice-recrystallized preparation of anterior-pituitary growth hormone prepared from ox pituitaries by the method of Wilhelmi (1955) was used. Rats were injected with 1 mg. of the hormone (in 1 ml. of water) and killed 6 hr. later. This time was chosen as it had been shown by Greenbaum & Glascock (1957) that there is a considerable inhibition of lipogenesis in the livers of growth-hormone-treated rats at this time-interval after the injection. Controls were injected with 1 ml. of 0·9% sodium chloride soln. After killing, the livers were rapidly removed, rinsed and placed in ice-cold Ringer bicarbonate (Umbreit, Burris & Stauffer, 1945). In all experiments in which incorporation of the two precursors has been compared, the liver slices used were always taken from the same liver.

Incubation and isolation procedures. For some of the experiments on the incorporation of acetate and palmitate into fatty acids, phospholipids and neutral fats, 400 mg. of slices were cut from the livers with a Stadie–Riggs (1944) microtome and incubated in 5 ml. of Krebs–Ringer bicarbonate in the presence of either [1-^{14}C]acetate or [1-^{14}C]palmitate at a final concentration of 0·1 μc/ml. The [1-^{14}C]acetate had a specific activity of 4 mc/m-mole and the [1-^{14}C]palmitate 2 mc/m-mole. Both substances were obtained from The Radiochemical Centre, Amersham, Bucks. No carrier was added. The flasks were gassed with $CO_2 + O_2$ (5:95) and incubated at 37° for 3 hr. At the end of this time the slices were collected by centrifuging, washed twice with ice-cold 0·9% sodium chloride soln. and then suspended and homogenized in 5 ml. of 5% trichloroacetic acid in an all-glass Potter–Elvehjem (1936) homogenizer. After centrifuging, the residue was again suspended in 5 ml. of 5% trichloroacetic acid and the extracts were discarded.