period. Trehalose can serve as an energy source, since Bücher & Klingenburg (1958) found a pronounced fall in concentration after 2 hr. flight in *Locusta*, and a similar result was found in *Phormia* (Evans & Dethier, 1957). Before the time of moulting, chitin is required for the formation of the new cuticle, and it seems possible that the *N*-acetylglucosamine units in it may be ultimately derived from the trehalose carbohydrate store.

SUMMARY

1. An extract prepared from the fat body of *Schistocerca gregaria* catalyses the formation of trehalose from glucose in the presence of adenosine triphosphate and uridine diphosphate glucose.

2. Glucose 6-phosphate and trehalose phosphate were indicated as intermediates in this reaction.

3. The extract hydrolyses trehalose phosphate at a greater rate than any other sugar phosphate tested.

4. The presence was shown of phosphoglucosyltransferase, nucleoside diphosphate-kinase and uridine diphosphoglucose-pyrophosphorylase activities in the fat body.

5. A scheme for the biosynthesis of trehalose from glucose in the fat body of *Schistocerca* is presented.

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REFERENCES

- Baar, S. & Bull, J. P. (1953). *Nature, Lond.*, **172**, 414.
 Baer, E. (1952). In *Biochemical Preparations*, vol. 2, p. 31.
 Ed. by Ball, E. G. New York: John Wiley and Sons Inc.
 Bellamy, D. (1958). *Biochem. J.* **70**, 580.
 Bücher, T. & Klingenburg, M. (1958). *Angew. Chem.* **70**, 552.

- Cabib, E. & Leloir, L. F. (1958). *J. biol. Chem.* **231**, 259.
 Candy, D. J. & Kilby, B. A. (1959). *Nature, Lond.*, **183**, 1594.
 Candy, D. J. & Kilby, B. A. (1960). *Biochem. J.* **74**, 19r.
 Carey, F. G. & Wyatt, G. R. (1960). *Biochem. biophys. Acta*, **41**, 178.
 Evans, D. R. & Dethier, V. G. (1957). *J. insect Physiol.* **1**, 3.
 Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
 Friedman, S. (1960). *Arch. Biochem. Biophys.* **87**, 252.
 Hanes, C. S. & Isherwood, W. A. (1949). *Nature, Lond.*, **164**, 1107.
 Harrop, F. E. G. (1958). *Nature, Lond.*, **182**, 876.
 Hartley, P. (1925). *Brit. J. exp. Path.* **6**, 180.
 Howden, G. F. & Hunter-Jones, P. (1958). *Nature, Lond.*, **182**, 1527.
 Howden, G. F. & Kilby, B. A. (1956). *Chem. & Ind.* p. 1453.
 Howden, G. F. & Kilby, B. A. (1960). *J. insect Physiol.* **4**, 258.
 Kalf, G. F. & Rieder, S. V. (1958). *J. biol. Chem.* **230**, 691.
 Keilin, D. & Hartree, E. F. (1938). *Proc. Roy. Soc. B*, **124**, 397.
 Kerly, M. & Leaback, D. H. (1957). *Biochem. J.* **67**, 245.
 Kilby, B. A. & Neville, B. E. (1957). *J. exp. Biol.* **34**, 276.
 Leloir, L. F. & Cabib, E. (1953). *J. Amer. chem. Soc.* **75**, 5445.
 Leloir, L. F. & Cardini, C. E. (1957). In *Methods in Enzymology*, vol. 3, p. 840. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
 Markham, R. & Smith, J. D. (1949). *Nature, Lond.*, **163**, 250.
 Markham, R. & Smith, J. D. (1951). *Biochem. J.* **49**, 401.
 Mejbaum, W. (1939). *Hoppe-Seyl. Z.* **258**, 117.
 Paladini, A. C. & Leloir, L. F. (1952). *Biochem. J.* **51**, 426.
 Robison, R. & Morgan, W. T. J. (1928). *Biochem. J.* **22**, 1277.
 Sato, T. & Tsumura, N. (1954). *J. agric. chem. Soc. Japan*, **28**, 624.
 Treherne, J. E. (1958a). *J. exp. Biol.* **35**, 297.
 Treherne, J. E. (1958b). *J. exp. Biol.* **35**, 611.
 Trevelyan, W. E., Procter, D. P. & Harrison, J. S. (1950). *Nature, Lond.*, **166**, 444.
 Wyatt, G. R. & Kalf, G. F. (1956). *Fed. Proc.* **15**, 388.
 Wyatt, G. R. & Kalf, G. F. (1957). *J. gen. Physiol.* **40**, 833.
 Zebe, E. C. & McShan, W. H. (1959). *Biochim. biophys. Acta*, **31**, 513.

Biochem. J. (1961) **78**, 536

Pathways of Glucose Metabolism in Ox Retina

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In retina, metabolism of glucose is much faster in a medium buffered with bicarbonate than in one buffered with phosphate (Laser, 1937; Craig & Beecher, 1943), but the mechanism of this effect has never been satisfactorily explained. It has been suggested (Hopkinson & Kerly, 1959) that it

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may be concerned with oxidation of triphosphopyridine nucleotide during assimilation of carbon dioxide by pyruvate and the maintenance of a high ratio of oxidized to reduced triphosphopyridine nucleotide. If this is so, and if the availability of oxidized triphosphopyridine nucleotide limits oxidation of glucose 6-phosphate, then the ratio of glucose metabolized by the hexose monophosphate

shunt pathway to that metabolized by the glycolytic route would be greater in a medium buffered with bicarbonate than in one buffered with phosphate. This hypothesis has been tested by the use of $[1-^{14}\text{C}]$ - and $[6-^{14}\text{C}]$ -glucose (see Korkes, 1956). Estimations were made of the specific activity of the carbon dioxide and of the lactate formed by ox retina incubated in media containing the labelled glucose and buffered either with phosphate or with bicarbonate.

Futterman & Kinoshita (1959) and Cohen & Noell (1959), in experiments published whilst this work was in progress, have also measured the activity of carbon dioxide and lactate produced during incubation of retina in a medium containing labelled glucose and buffered with phosphate, whereas Holt, Lüth, Hallman, Holt & Voelker (1959) have carried out similar experiments with a medium buffered with bicarbonate. In agreement with the findings of these authors, the results of the experiments to be described (already briefly reported, Kerly & Rahman, 1960) show that the ratio of $^{14}\text{CO}_2$ formed during incubation with $[1-^{14}\text{C}]$ glucose to that formed with $[6-^{14}\text{C}]$ glucose was near unity in both buffers, but that the difference in the isotope content of the lactate produced from the two glucose samples was greater than that found by Futterman & Kinoshita. This, together with the low values found for the specific activity of the carbon dioxide, has led to the conclusion that the hexose monophosphate shunt may be of more importance for glucose metabolism in retina than has been suggested previously.

METHODS

Estimation of respiratory carbon dioxide. In order to measure the specific activity of respiratory CO_2 produced in a medium buffered with bicarbonate and in equilibrium with a gas phase containing CO_2 , it was necessary to determine not only the isotope content of the CO_2 formed during incubation but also the amount produced; this was done by estimating the total amount of CO_2 in the system before and after incubation. The CO_2 was absorbed in alkali, then precipitated as BaCO_3 , weighed and counted. It was essential to exclude all contact with the atmosphere until precipitation was achieved; after this stage absorption of CO_2 from the atmosphere was negligible. Warburg flasks with a single side arm and modified as described by Chernick, Masoro & Chaikoff (1950) were used for incubation. The centre well, which extended for about 1 cm. through the bottom of the flask, was sealed with a firmly fitted rubber vaccine cap. In this way the volume of the flask remained constant and gas changes could be determined. Carbon dioxide-free NaOH was stored in a Woulfe bottle with the lower outlet sealed with a vaccine cap so that alkali could be withdrawn by a syringe and injected into the Warburg flask already attached to its manometer.

After absorption of CO_2 the alkali was removed from the

flask with a syringe and injected through a rubber cap into a small flask previously well flushed with O_2 to remove all atmospheric CO_2 . The centre well in the Warburg flask was washed six to eight times with warm CO_2 -free water (injected and removed with a syringe), and the washings were added to the alkali. During the process of removal of alkali and washing the centre well, care was needed to prevent manometer fluid being blown out or sucked back into the flask.

In experiments where a phosphate medium was used, alkali and washings were collected in 25 ml. conical flasks containing 1 ml. of saturated BaCl_2 with a trace of NH_4Cl . For the bicarbonate experiments, where larger amounts of CO_2 were concerned, alkali and washings were injected into a 25 ml. graduated flask and, after the contents had been made up to volume, four 5 ml. samples were withdrawn and injected into conical flasks containing BaCl_2 as before.

The precipitate of BaCO_3 was collected on paper supported on sintered glass in an apparatus similar to that described by MacKenzie & Dean (1948), except that it was made of glass. Flasks and precipitates were washed with warm CO_2 -free water and with alcohol, and the precipitates were dried under an infrared lamp by suction with hot air. After a few minutes, when the precipitate was completely dry, the filter paper and precipitate were removed and placed on the appropriate weighed planchet in a desiccator, later to be weighed and counted. Before counting, 0.3–0.5 ml. of artist's pastel fixative (diluted 1:5) was added to the planchets, which were again dried under the infrared lamp. A curve was constructed to relate the number of counts to the thickness of the fixative.

The amount of CO_2 produced in the experiments where the incubation medium contained phosphate was calculated directly from the weight of BaCO_3 . In experiments in which a bicarbonate-containing medium was used in equilibrium with a gas mixture $\text{O}_2 + \text{CO}_2$ (95:5), it was necessary to measure the total CO_2 in the system both at the beginning and at the end of the incubation period. Two flasks of almost equal volume were used with pieces of retina of equal weight so that the CO_2 content was the same in each at the beginning of the incubation period. Acid was tipped into the control flask at the beginning and into the second flask at the end of the experimental period. The difference in weight of the BaCO_3 produced from the two flasks corresponded to the respiratory CO_2 formed during the experiment. ^{14}C was present only in BaCO_3 from the second flask and accounted for the whole of the isotope present in the CO_2 .

This procedure for estimation of CO_2 was checked by addition of acid to a known amount of NaHCO_3 in a flask gassed with O_2 . In 31 experiments 9.98 ± 0.16 mg. of BaCO_3 was produced compared with the expected 9.95 mg. In a control experiment carried out without tissue, the calculated amount of BaCO_3 from medium and gas phase (allowing for the measured CO_2 content of the gas mixture, 3.9%) was 16.03 mg. and the amount estimated was 16.10 mg.

Determination of activity of barium carbonate. Activity was assayed in a gas-flow counter (Tracerlab SC 16). A self-absorption curve was constructed and the observed values were corrected for activity at infinite thinness.

Estimation and determination of specific activity of lactic acid. Trichloroacetic acid was added to the incubation medium to give a final concn. of 10%, and the precipitated

proteins were removed by centrifuging. The precipitate was washed twice with water and the washings were added to the supernatant fluid. Lactic acid was adsorbed on a Dowex-1 (Cl^- form, X 10, 80–100 mesh) column and eluted as described by Kinoshita, Masurat & Helfant (1955). Experiment showed that recovery was almost 100% and that extraction of the protein-free solution with ether before adsorption was not necessary. A sample of the eluate was used for estimation of lactic acid (Barker & Summerson, 1941) and, after addition of 4.3 mg. of lithium lactate to act as carrier, lactic acid was oxidized and the resulting aldehyde absorbed in dimedone (5:5-dimethyl-1:3-cyclohexanedione, Eastman) according to the method of Brin & Olson (1952). After it had stood overnight, the dimedone-acetaldehyde complex was removed by filtration through the apparatus used for separation of BaCO_3 , weighed and counted. The dimedone complex was not washed with alcohol or dried with hot air as it is soluble in alcohol and melts under the infrared lamp. A self-absorption curve was constructed for dimedone-acetaldehyde collected from several experiments and observed values were corrected for activity at infinite thinness. The yield of dimedone complex was only 50–60% of the theoretical value which, if allowance is made for loss on oxidation and aeration, does not differ greatly from the 72% reported by Gaffney, Williams & McKennis (1954) for absorption of acetaldehyde in dimedone. This method does not detect ^{14}C in the carboxyl group of lactic acid but, as the labelled carbon was added as $[1\text{-}^{14}\text{C}]$ - or $[6\text{-}^{14}\text{C}]$ -glucose, glycolysis would produce lactic acid labelled in the methyl group and no ^{14}C would be lost on oxidation to acetaldehyde.

Determination of the specific activity of glucose. $[^{14}\text{C}]$ -Glucose, obtained from The Radiochemical Centre, Amersham, Bucks, was diluted with unlabelled glucose to give an activity of approximately $1.5\mu\text{C}/\text{m-mole}$. The specific activity was determined by a modification of the persulphate oxidation method described by Abraham & Hassid (1957). Incubation medium (2 ml.) containing $20\mu\text{moles}$ of glucose, together with the oxidizing reagents, was placed in a 50 ml. conical flask connected to a 25 ml. volumetric flask. Both flasks were flushed with O_2 to remove all traces of CO_2 and 1 ml. of CO_2 -free NaOH was injected by syringe into the volumetric flask. The whole apparatus was evacuated and the glucose oxidized. After absorption of the CO_2 produced was complete, the contents of the volumetric flask were made up to volume with CO_2 -free water, and 5 ml. samples were removed for estimation and counting of CO_2 as already described. Four determinations gave activities of 749 ± 18 and 567 ± 5 counts/min./ μmole for $[1\text{-}^{14}\text{C}]$ glucose and $[6\text{-}^{14}\text{C}]$ glucose respectively.

Incubation procedure. Ox eyes were brought from the slaughterhouse on ice; the retinae were dissected and placed in the appropriate ice-cold oxygenated Ringer solution. A piece of retina weighing about 400 mg. was drained, weighed on a torsion balance and placed in an incubation flask containing 0.5 ml. of 50% (w/v) trichloroacetic acid in the side arm and, in the main compartment, 2 ml. of Ringer solution buffered either with phosphate or with bicarbonate and containing $20\mu\text{moles}$ of labelled glucose. The Ringer solutions were made by diluting 10 ml. of 0.1M-sodium phosphate, pH 7.4, or 16 ml. of 0.154 M- NaHCO_3 (gassed with CO_2 to pH 7.4) to 100 ml. with stock saline solution. This latter was made by mixing 0.154 M-solutions of NaCl (100 parts), KCl (4 parts), CaCl_2 (3 parts),

KH_2PO_4 (1 part) and MgSO_4 (1 part) (see Umbreit, Burris & Stauffer, 1957). The flasks were attached to their manometers and shaken in a constant-temperature bath at 38° . In the experiments in which phosphate buffer was used the flasks were gassed with O_2 for 10 min.; they were then removed from the bath, 0.5 ml. of saturated NaOH was injected into the centre wells and the flasks were replaced in the bath for a further 5 min. equilibration (15 min. in all). Uptake of O_2 was recorded for periods of up to 1 hr., and the reaction was stopped by tipping in the trichloroacetic acid from the side arm. Alkali containing the respiratory CO_2 was removed from the centre well, and its CO_2 content, together with the lactic acid content of the medium, estimated and counted as described above.

In the experiments in which retina was incubated in bicarbonate buffer a control experiment was carried out at the same time. Two flasks of almost equal volume were used, containing the same weight of tissue. Both were gassed with a mixture of $\text{O}_2 + \text{CO}_2$ (approx. 95:5) for 10 min. and a further 5 min. was allowed for attainment of temperature equilibrium. The manometer taps were then opened momentarily, and the fluid level was adjusted so that at atmospheric pressure the quantity of gas should be the same in both flasks. Acid was tipped into the main compartment of the control flask to stop oxidation and to liberate all the CO_2 into the gas phase. The retina in the other flask was allowed to respire for 30 min. but gas changes were not recorded; acid was then tipped in from the side arm. Alkali was injected into the centre wells of both flasks and shaking was continued for 2 hr. to ensure complete absorption of CO_2 . Lactic acid in the medium and CO_2 in the alkali were then estimated and counted as already described.

Wet weight/dry weight ratio. The wet weight and dry weight (after heating to 105°) were determined on a number of retinae. The ratio did not differ appreciably from 10:1 and this value was used in calculating dry weights for the incubation experiments.

RESULTS AND DISCUSSION

The values recorded for oxygen uptake, carbon dioxide output and lactic acid production, both in preliminary experiments carried out while testing the methods to be used and also in the experiments with labelled glucose, are shown in Table 1. In the experiments in which Ringer phosphate was used oxygen uptake was linear and, as carbon dioxide output was measured on the same piece of tissue, the respiratory quotient could be calculated. In experiments with the bicarbonate medium only carbon dioxide output was determined, but if the respiratory quotient is assumed to be unity also in these conditions (Hopkinson & Kerly, 1959) then the value for $-Q_{\text{O}_2}$ would have been identical with that for Q_{CO_2} . The values recorded both for oxygen uptake and lactic acid production were rather lower than those recorded previously (Hopkinson & Kerly, 1959), possibly because the eyes were stored for a longer period after death of the cattle, but the effect of bicarbonate in pro-

ducing a higher rate of both respiration and glycolysis is clearly shown.

The specific activities of the carbon dioxide and lactic acid produced during incubation with labelled glucose are shown in Table 2. In the first series of experiments in the phosphate medium the full incubation period, including 15 min. equilibration, was 60 min., but for the experiments in the bicarbonate medium the incubation period was decreased to 45 min., and, so that results might be more directly comparable, two further experiments were carried out in phosphate medium with the shorter incubation period. Gas exchange could not be measured during the equilibration period, and in all experiments the values for specific activity of respiratory carbon dioxide refer to gas collected after this period; but amounts of carbon dioxide formed have been calculated for the full incubation period for comparison with lactic acid production, which was necessarily determined for the whole incubation time. No allowance has been made for the initial lactic acid content of the retina, but, as the retinae after dissection were placed in Ringer solution and transferred to fresh medium for incubation, the initial lactic acid level was likely to

have been low [see Hopkinson & Kerly (1959), who, using the same technique, found only 1.0–1.5 μ moles of lactic acid/50 mg. dry wt. after incubation of retina in glucose-free media].

The ratio of $^{14}\text{CO}_2$ produced during incubation in a medium containing $[1-^{14}\text{C}]$ glucose to that produced when the medium contained $[6-^{14}\text{C}]$ glucose did not differ appreciably from unity when the medium was buffered either with phosphate or with bicarbonate. On the other hand, during incubation in both media lactic acid formed from $[1-^{14}\text{C}]$ glucose contained less isotope than that formed from $[6-^{14}\text{C}]$ glucose. If lactic acid were formed only by glycolysis it would be expected to have half the specific activity of the starting glucose (i.e. 375 counts/min./ μ mole) whether the glucose were labelled in carbon atom 1 or 6, but, if some glucose were metabolized by the shunt pathway, lactic acid formed by this route would be labelled only if the isotope had been present initially as $[6-^{14}\text{C}]$ glucose. In this case the specific activity of the lactic acid, produced by both routes, should be greater than half that of the starting glucose whereas that formed from $[1-^{14}\text{C}]$ glucose should be less. In most experiments this was so. In both

Table 1. *Metabolism of retina*

Ox retina (approx. 500 mg. wet wt.) was incubated at 38° in 2 ml. of Ringer solution, pH 7.4, containing 20 μ moles of glucose and buffered either with phosphate (gas phase O_2) or with bicarbonate (gas phase $\text{O}_2 + \text{CO}_2$, 95:5). Equilibration for 15 min. was allowed before measurement of O_2 uptake and CO_2 output was started; total incubation periods are as shown. Rate of lactic acid formation was calculated from lactic acid produced during the whole incubation period. (Values are means $\pm$ S.E.M., with numbers of experiments shown in parentheses.)

Buffer	Incubation period (min.)	$-Q_{\text{O}_2}$	Q_{CO_2}	R.Q.	Lactic acid (μ moles/g. dry wt./hr.)
Phosphate	75	6.18 ± 0.13 (65)	6.43 ± 0.14 (65)	1.04 ± 0.02	293 ± 16.5 (22)
Bicarbonate	45	—	13.36 ± 0.62 (42)	—	535 ± 21.7 (12)

Table 2. *Specific activity of respiratory carbon dioxide and lactic acid formed on incubation of ox retina with labelled glucose*

Retina was incubated at 38° in 2 ml. of Ringer solution, pH 7.4, containing 20 μ moles of $[1-^{14}\text{C}]$ - or $[6-^{14}\text{C}]$ -glucose and buffered either with phosphate (gas phase O_2) or with bicarbonate (gas phase $\text{O}_2 + \text{CO}_2$, 95:5). Total incubation times include 15 min. equilibrium period during which CO_2 output was not measured; values shown are calculated for the whole incubation period. Values (mean $\pm$ S.E.M.) are corrected for an initial specific activity of 750 counts/min./ μ mole of glucose.

No. of expts.	Glucose used	Carbon dioxide formed		Lactic acid formed	
		(μ moles)	(counts/min./ μ mole)	(μ moles)	(counts/min./ μ mole)
(a) In phosphate: incubation for 60 min.					
4	[1- 14 C]	15.9 $\pm$ 1.1	43 $\pm$ 2.6	18.5 $\pm$ 0.8	284 $\pm$ 19
3	[6- 14 C]	12.4 $\pm$ 0	41 $\pm$ 4.0	12.9 $\pm$ 0.1	384 $\pm$ 27
(b) In phosphate: incubation for 45 min.					
2	[1- 14 C]	10.5 $\pm$ 0.3	37 $\pm$ 2.0	11.9 $\pm$ 1.3	320 $\pm$ 6
2	[6- 14 C]	9.8 $\pm$ 1.6	33 $\pm$ 4.0	10.8 $\pm$ 0.8	455 $\pm$ 4
(c) In bicarbonate: incubation for 45 min.					
6	[1- 14 C]	26.6 $\pm$ 1.0	18 $\pm$ 1.6	19.1 $\pm$ 0.1	402 $\pm$ 29
6	[6- 14 C]	24.4 $\pm$ 0.3	14 $\pm$ 1.1	17.9 $\pm$ 1.2	534 $\pm$ 25

phosphate and bicarbonate media the ratio of activity found in lactic acid from [1-¹⁴C]glucose to that from [6-¹⁴C]glucose was 0.75, corresponding to metabolism of 1 glucose molecule in 4 by the shunt pathway (Kinoshita *et al.* 1955). In contrast the specific activity of the carbon dioxide was in all experiments, and especially in those in which the medium contained bicarbonate, much lower than would have been expected had all the carbon dioxide been derived from complete oxidation of glucose (i.e. one-sixth of the activity of the starting glucose, 125 counts/min./μmole). This low activity could have been due to dilution of carbon dioxide produced from glucose by that formed from endogenous substrate, or to assimilation of respiratory carbon dioxide. This latter reaction may well be significant in retina, which is known to assimilate carbon dioxide actively (Crane & Ball, 1951). This very low specific activity makes the ratio of the activity of carbon dioxide produced from glucose labelled at carbon atoms 1 and 6 an unreliable guide to the pathways of glucose metabolism.

The results of these experiments do not throw light on the reason for the increased rate of glucose metabolism of retina incubated in media containing bicarbonate, but the specific activity of the lactic acid produced from [1-¹⁴C]- and from [6-¹⁴C]-glucose suggests that, although in retina hexose monophosphate oxidation has not the importance it has in corneal epithelium (Kinoshita *et al.* 1955) or in lens (Kinoshita, 1955), some glucose is normally metabolized by this route and that in aerobic conditions the shunt pathway is not suppressed completely as has been suggested (Futterman & Kinoshita, 1959; Cohen & Noell, 1959). de Berardinis (1959), who estimated gluconic acid phosphate and lactic acid dehydrogenases, has also concluded that the shunt pathway exists in retina.

SUMMARY

1. The specific activity of carbon dioxide produced during incubation of ox retina with labelled glucose in media buffered with either phosphate or with bicarbonate was lower, whereas that of the lactic acid was higher, than that to be expected from the activity of the glucose.

2. The ratio of ¹⁴CO₂ produced from [1-¹⁴C]-glucose to that from [6-¹⁴C]glucose was near unity whether the incubation medium was buffered by phosphate or by bicarbonate, but the specific activity of the lactic acid produced from [1-¹⁴C]-glucose was less than that produced from [6-¹⁴C]-glucose, the ratio in both media being approximately 0.75.

3. It is concluded that the activity of the lactic acid gave more reliable evidence about the pathway of glucose metabolism than did that of the carbon dioxide and that one glucose molecule in four was metabolized by the hexose monophosphate shunt pathway.

REFERENCES

- Abraham, S. & Hassid, W. Z. (1957). In *Methods of Enzymology*, vol. 4, p. 549. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.* **138**, 535.
- Brin, M. & Olson, R. E. (1952). *J. biol. Chem.* **199**, 475.
- Chernick, S. S., Masoro, E. J. & Chaikoff, I. L. (1950). *Proc. Soc. exp. Biol., N.Y.*, **73**, 348.
- Cohen, L. H. & Noell, W. K. (1959). *Fed. Proc.* **18**, 28.
- Craig, F. N. & Beecher, H. K. (1943). *J. gen. Physiol.* **26**, 467.
- Crane, R. K. & Ball, E. G. (1951). *J. biol. Chem.* **188**, 819.
- de Berardinis, E. (1959). *Ophthalmologia*, **138**, 419.
- Futterman, S. & Kinoshita, J. H. (1959). *J. biol. Chem.* **234**, 723.
- Gaffney, G. W., Williams, W. A. & McKennis, H., jun. (1954). *Analyt. Chem.* **26**, 588.
- Holt, C. V., Lüth, H., Hallman, I., Holt, L. V. & Voelker, I. (1959). *Biochem. Z.* **331**, 239.
- Hopkinson, L. & Kerly, M. (1959). *Biochem. J.* **72**, 22.
- Kerly, M. & Rahman, M. Ataur (1960). *Biochem. J.* **74**, 16r.
- Kinoshita, J. H. (1955). *Arch. Ophthalm., Chicago*, **54**, 360.
- Kinoshita, J. H., Masurat, T. & Helfant, M. (1955). *Science*, **122**, 72.
- Korkes, S. (1956). *Annu. Rev. Biochem.* **25**, 703.
- Laser, H. (1937). *Biochem. J.* **31**, 1671.
- MacKenzie, A. J. & Dean, L. A. (1948). *Analyt. Chem.* **20**, 559.
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1957). *Manometric Techniques*, 3rd ed. Minneapolis: Burgess Publishing Co.