- Cookson, G. H. & Rimington, C. (1954). *Biochem. J.* **57**, 476.
Documenta Geigy Scientific Tables (1956). 5th ed., p. 105.
 Basle: J. R. Geigy, S.A.
- Dresel, E. I. B. & Falk, J. E. (1956a). *Biochem. J.* **63**, 72.
 Dresel, E. I. B. & Falk, J. E. (1956b). *Biochem. J.* **63**, 80.
 Elsdon, S. R. (1954). In *Symp. Soc. gen. Microbiol.: Autotrophic Micro-organisms*, **4**, 202.
- Falk, J. E. & Benson, A. (1953). *Biochem. J.* **55**, 101.
 Falk, J. E., Dresel, E. I. B., Benson, A. & Knight, B. C. (1956). *Biochem. J.* **63**, 87.
- Gibson, K. D. (1958). *Biochim. biophys. Acta*, **28**, 451.
 Gibson, K. D., Neuberger, A. & Scott, J. J. (1955). *Biochem. J.* **61**, 618.
- Granick, S. & Mauzerall, D. (1958). *J. biol. Chem.* **232**, 1119.
- Heath, H. & Hoare, D. S. (1958). *4th Int. Congr. Biochem., Vienna*, p. 123.
- Hoare, D. S. & Heath, H. (1958a). *Nature, Lond.*, **181**, 1592.
- Hoare, D. S. & Heath, H. (1958b). *Biochem. J.* **69**, 51P.
- Kikuchi, G., Shemin, D. & Bachmann, B. J. (1958). *Biochim. biophys. Acta*, **28**, 219.
- Lascelles, J. (1955). In *Ciba Foundation Conf.: The Biosynthesis of Porphyrins and Porphyrin Metabolism*, p. 265. Ed. by Wolstenholme, G. E. W. London: J. and A. Churchill Ltd.
- Lascelles, J. (1956). *Biochem. J.* **62**, 78.
- Lockwood, W. H. & Rimington, C. (1957). *Biochem. J.* **67**, 8P.
- McIlwain, H. (1948). *J. gen. Microbiol.* **2**, 288.
- Mauzerall, D. & Granick, S. (1958). *J. biol. Chem.* **232**, 1141.
- Rimington, C. (1957). *Annu. Rev. Biochem.* **26**, 561.
- Rimington, C. & Sveinsson, S. L. (1950). *Scand. J. clin. Lab. Invest.* **2**, 209.
- Robinson, R. (1955). *Structural Relations of Natural Products*, p. 25. Oxford: University Press.
- Shemin, D., Russell, C. S. & Abramsky, T. (1955). *J. biol. Chem.* **215**, 613.
- van Niel, C. B. (1944). *Bact. Rev.* **8**, 1.

The Effect of Monoiodoacetate on the Aerobic Metabolism of Ox Retina *in vitro*

By LEONORA HOPKINSON AND MARGARET KERLY

Department of Biochemistry, University College, Gower Street, London, W.C. 1

(Received 6 August 1958)

Noell (1951) and Schubert & Bornschein (1951) have shown that, in a number of species, injection of sublethal doses of monoiodoacetate abolishes the retinogram (the change in potential across the retina caused by light) and destroys the visual cells. Noell found that the changes in the retinogram differed from those caused by anoxia and he suggested that the damage was due to inhibition of glycolytic reactions and that oxidative processes were unaffected. This suggestion is supported by the experiments of Graymore & Tansley (1959), in which the anaerobic glycolysis of rat retina fell to 20% of the normal value within 10 min. of injection of iodoacetate, and by Oguchi's (1938) report that respiration of rabbit and frog retina was not inhibited after injection of iodoacetate. Noell (1958) has also reported experiments which confirm Oguchi's finding, although Auricchio (1950) found that addition of iodoacetate *in vitro* did depress oxygen uptake of rabbit retina.

The lack of effect of iodoacetate on oxygen uptake of retina cannot be regarded as established, for respiration in this tissue is dependent on the composition of the incubation medium and is much faster in a bicarbonate than in a phosphate buffer (Laser, 1937; Craig & Beecher, 1943a). Krebs-Ringer phosphate solution was used for the experiments reported by Noell (1958), but Oguchi (1938)

does not state the composition of the Ringer solution which he used. Auricchio (1950) used a Ringer phosphate solution but with air as the gas phase and so introduced some carbon dioxide into his system. Reports of the effect of iodoacetate on oxygen uptake of other tissues are conflicting. Lundsgaard (1930), Stannard (1937) and Saslow (1937) found that iodoacetate had no effect on carbohydrate oxidation in frog muscle, whereas Meyerhof & Boyland (1931), who also used frog muscle, and Krebs (1931) and Quastel & Wheatley (1932), working with brain and other tissues from a variety of species, observed a decrease either in oxygen uptake or in respiratory quotient. Barker, Shorr & Malam (1939) found that the effect varied with the tissue used (cat and dog); with testis oxygen uptake was always inhibited to a greater extent than glycolysis, but with heart and brain, at concentrations of iodoacetate which inhibited anaerobic glycolysis nearly completely, the respiratory quotient was not depressed and glucose was oxidized, although in a number of experiments the amount of oxygen used was reduced.

In the experiments described below iodoacetate has been added to ox retina incubated in bicarbonate and in phosphate buffer. In bicarbonate there was a pronounced inhibitory effect but in phosphate inhibition was small and only just significant.

METHODS

Ox eyes were brought from the slaughter house on ice, and the retinae dissected and placed in ice-cold, oxygenated Ringer solution. When a sufficient number had been dissected a whole retina, or in some experiments a part of a retina, was placed in a Warburg flask containing the appropriate Ringer solution (see below), gassed with O_2 or a mixture containing $O_2 + CO_2$ (95:5) and incubated at 37° for 40 or 60 min. At the end of this time portions of the medium were removed for estimation of lactic acid (Barker & Summerson, 1941) and glucose (Somogyi, 1945) or pyruvate (Umbreit, Burris & Stauffer, 1945). The remaining contents of the flask were poured into a small weighed centrifuge tube, and the tissue was spun down, dried and weighed. The glycogen content (Good, Kramer & Somogyi, 1933) and the ratio of wet weight to dry weight was determined on a number of retinae before incubation.

Incubation in Ringer phosphate solution. Krebs-Ringer phosphate solution was prepared according to the formula given by Umbreit *et al.* (1945), by mixing 100 ml. of 0.154M-NaCl, 4 ml. of 0.154M-KCl, 3 ml. of 0.11M- $CaCl_2$, 1 ml. of 0.154M- KH_2PO_4 , 1 ml. of 0.154M- $MgSO_4$ and 12 ml. of 0.1M- Na_2HPO_4 containing 0.02N-HCl; glucose or pyruvate and iodoacetate were added as required and the medium was gassed with O_2 . In the first series of experiments small pieces of retina (10–20 mg. dry wt.) were incubated in 1.5 ml. of medium for 60 min., but in a second series whole retinae, or larger pieces (30–75 mg. dry wt.) were incubated in 3 ml. of medium for 40 min. only. There was no significant difference in the amount of O_2 used during the first 40 min. of incubation in experiments in the first series and in those in the second series so the results have been treated as a single group. In Table 1 values for uptake of O_2 at 40 min. refer to experiments from both groups, all other 40 min. values to experiments from the second group and those for 60 min. incubation to experiments from the first group.

Incubation in Ringer bicarbonate solution. A number of methods have been devised for measurement of uptake of O_2 in a bicarbonate-containing medium. Krebs's (1951) method, which makes use of the CO_2 buffers recommended by Pardee (1949), was found to be satisfactory for measurement of respiration of brain slices but was not suitable for retina. This tissue has a high rate of both respiration and aerobic glycolysis and the CO_2 produced was not absorbed sufficiently rapidly to allow accurate readings. Warburg's indirect method (1924a) was therefore used. A small piece of retina (15–20 mg. dry wt.) was placed in 1.5 ml. of medium in one flask and a larger piece (50–75 mg.) in 6.0 ml. in the other. Krebs-Ringer bicarbonate solution was prepared as described by Umbreit *et al.* (1945) and the appropriate amounts of glucose or pyruvate and iodoacetate were added. The solution was equilibrated with a gas mixture containing $O_2 + CO_2$ (95:5) immediately before use and the same gas mixture was passed through the flasks after they had been placed in the water bath at 37°. A period of 15 min. was allowed for gassing and temperature equilibration; pressure changes were then recorded for 25 min. During this time the rate of gas production was linear so that the total consumption of O_2 for the whole 40 min. incubation period could be calculated and compared with the lactic acid production and glucose (or pyruvate) utilization. Respiratory CO_2 was calculated by

subtraction of the CO_2 equivalent of the lactic acid produced from the total CO_2 liberated.

RESULTS

Incubation in phosphate buffer. Addition of glucose greatly increased lactic acid production but not oxygen uptake during the first 40 min. incubation period (Table 1). Without glucose in the medium the rate of oxygen consumption fell off when incubation was continued for a further 20 min., but when glucose was added consumption continued at the same rate for 60 min. Although during the first 40 min. of incubation no extra oxygen was used in the presence of glucose, it is likely that some glucose was oxidized for the amount utilized was greater than that required for lactic acid production and, at glucose concentrations of 5 and 15 mM, this excess was approximately equivalent to the oxygen used. During this period, when the rate of oxygen uptake was not limited by lack of endogenous substrate, addition of glucose caused a reduction in the amount of oxygen used, but not of lactic acid produced, an effect similar to that observed by Crabtree (1929) for mouse tumours. Noell (1958) has also reported that glucose at a concentration of 20 mM causes an inhibition of 10 % in oxygen uptake of adult rabbit retina incubated in Ringer phosphate solution. Racker (1956) found a much greater effect of added glucose on the oxygen uptake of ascites-tumour cells. With this preparation endogenous respiration does not fall off during incubation for periods of 60 min., and, although at low glucose concentrations Racker found a small increase in oxygen uptake, at concentrations of 5 mM and above he found that oxygen uptake was reduced to 50–60 % of the endogenous value. In the experiments shown in Table 1, although an analysis of variance between the values for glucose concentrations of 0, 1, 5 and 15 mM showed no significant difference [F (variance ratio) < 1.0], the amount of oxygen used fell continuously as the glucose concentration was increased and at a concentration of 25 mM it was significantly less than at any other concentration, being 70 % of the endogenous level.

Addition of iodoacetate to Ringer phosphate solution to give a concentration of 0.1 mM, sufficient to reduce lactic acid formation, caused no significant decrease in oxygen uptake. When the concentration was raised to 1.0 mM the decrease was significant at glucose concentrations of 0 and 15 mM ($P < 0.01$). At a glucose concentration of 5 mM, where the values for individual experiments varied widely, the fall was not significant ($P > 0.20$) and the low value observed at a glucose concentration of 25 mM was not further reduced by addition of iodoacetate. Glucose utilization, like lactic acid production, was

Table 1. Effect of addition of glucose and monoiodoacetate on the oxygen consumption, lactic acid production and glucose utilization of *ox retina*

Retina was incubated for 40 or 60 min. in Krebs-Ringer phosphate solution equilibrated with O_2 at 37° . Results are given as means $\pm$ s.e.m., with the number of experiments in each group shown in parentheses.

Concn. of glucose (mM)	Concn. of monoiodo-acetate (mM)	O ₂ uptake (μ moles/g. dry wt.)		Lactic acid production (μ moles/g. dry wt.)		Glucose utilization (μ moles/g. dry wt.)		Glucose equivalent* of O ₂ uptake and lactic acid production (μ moles/g. dry wt.)	
		40 min.	60 min.	40 min.	60 min.	40 min.	60 min.	40 min.	60 min.
0	0	280 $\pm$ 17 (12)	307 $\pm$ 21 (5)	39 $\pm$ 6 (8)	32 $\pm$ 10 (5)	—	—	—	—
	0.1	284 $\pm$ 16 (10)	359 $\pm$ 24 (4)	23 $\pm$ 5 (6)	45 $\pm$ 18 (4)	—	—	—	—
	1.0	214 $\pm$ 16 (13)	224 $\pm$ 29 (5)	20 $\pm$ 4 (8)	26 $\pm$ 10 (5)	—	—	—	—
1	0	273 $\pm$ 17 (4)	416 $\pm$ 28 (4)	—	129 $\pm$ 23 (4)	—	80 $\pm$ 13 (4)	—	—
	5	260 $\pm$ 14 (13)	351 $\pm$ 13 (6)	272 $\pm$ 43 (7)	403 $\pm$ 38 (6)	192 $\pm$ 15 (4)	221 $\pm$ 13 (6)	179	260
15	0.1	208 $\pm$ 17 (7)	—	169 $\pm$ 27 (7)	—	181 $\pm$ 22 (6)	—	120	—
	1.0	219 $\pm$ 21 (12)	269 $\pm$ 27 (4)	57 $\pm$ 15 (8)	66 $\pm$ 4 (5)	109 $\pm$ 27 (6)	108 $\pm$ 9 (3)	66	78
	0	245 $\pm$ 11 (21)	364 $\pm$ 20 (10)	245 $\pm$ 17 (11)	416 $\pm$ 40 (10)	194 $\pm$ 29 (9)	291 $\pm$ 28 (10)	176	269
25	0.1	219 $\pm$ 19 (10)	379 $\pm$ 33 (3)	163 $\pm$ 23 (7)	293 $\pm$ 47 (3)	125 $\pm$ 52 (2)	156 $\pm$ 30 (3)	119	210
	1.0	198 $\pm$ 10 (11)	283 $\pm$ 11 (3)	52 $\pm$ 11 (8)	93 $\pm$ 7 (3)	119 $\pm$ 15 (4)	74 $\pm$ 19 (3)	59	94
	0	192 $\pm$ 17 (8)	277 $\pm$ 0 (2)	362 $\pm$ 37 (6)	495 $\pm$ 88 (2)	—	—	—	—
0.1	0.1	187 $\pm$ 22 (6)	—	197 $\pm$ 27 (6)	—	—	—	—	—
	1.0	207 $\pm$ 21 (9)	—	74 $\pm$ 17 (8)	—	—	—	—	—

* Glucose equivalent was calculated from mean values for O₂ uptake and lactic acid production.

inhibited by addition of iodoacetate, but in these conditions, where phosphate esters were likely to have been formed, the amount of glucose oxidized cannot be calculated by subtraction of the amount required for lactic acid formation from the total amount used.

Incubation in bicarbonate buffer. In this medium both oxygen consumption and lactic acid production were greater than during incubation in phosphate buffer (Table 2). When no glucose was added oxygen uptake was nearly twice as great and in the presence of glucose the value was nearly doubled again. There was no significant fall in oxygen uptake at high concentrations of glucose. When differences were large compared with the standard errors of the means statistical tests have not been applied. Endogenous lactic acid formation was not increased above the amount formed in a phosphate buffer, but when glucose was added to the medium production was much greater than in a phosphate buffer.

When no glucose was added the respiratory quotient was not significantly different from 1.0, indicating that carbohydrate was oxidized. The glycogen content of the retina before incubation was found to be 0.73 ± 0.11 mg./g. wet wt. (equivalent to 40μ moles of glucose/g. dry wt.), somewhat lower than the value of 0.92 mg./g. found by Crane & Ball (1951b) and only sufficient to account for about half the endogenous oxygen uptake in bicarbonate medium. It is possible that lactic acid, produced during dissection and not removed by the preliminary soaking in cold Ringer solution, was the substrate for oxidation.

In contrast with its effect on retina incubated in Ringer phosphate solution, iodoacetate markedly inhibited oxygen uptake during incubation in bicarbonate buffer containing glucose. At an iodoacetate concentration of 0.1 mM oxygen consumption fell to about 60 % of the control value (Table 2), but an increase in concentration to 1.0 mM brought about only a small, and not significant, further decrease (for all concentrations of glucose, $P > 0.1$). Lactic acid formation was inhibited in a similar manner in both buffers, that is to a small extent at the lower and almost completely at the higher concentration. Glucose utilization was determined in only a few experiments. In the presence of iodoacetate it was reduced but, as in the phosphate experiments, the amount oxidized cannot be determined since phosphate esters may have been formed. The low respiratory quotient observed in the presence of iodoacetate could have been caused by oxidation of non-carbohydrate substrates or by an increased assimilation of carbon dioxide. The latter effect was observed by Crane & Ball (1951b) when iodoacetate was added to retina incubated under anaerobic conditions.

Table 2. *Effect of addition of glucose and monoiodoacetate on the oxygen consumption, lactic acid production and glucose utilization of ox retina*

Retina was incubated for 40 min. in Krebs-Ringer bicarbonate solution equilibrated with $O_2 + CO_2$ (95:5) at 37°. Respiratory quotients were calculated from total CO_2 less CO_2 equivalent of lactic acid production. Results are given as means $\pm$ s.e.m., with the number of experiments in each group shown in parentheses.

Concn. of glucose (mm)	Concn. of monoiodoacetate (mm)	O_2 uptake (μ moles/g. dry wt.)	Respiratory quotient	Lactic acid production (μ moles/g. dry wt.)	Glucose utilization (μ moles/g. dry wt.)	Glucose equivalent* of O_2 uptake and lactic acid production (μ moles/g. dry wt.)
0	0	494 $\pm$ 51 (12)	0.97 $\pm$ 0.04 (11)	40 $\pm$ 4 (11)	—	—
	0.1	428 $\pm$ 57 (5)	0.92 $\pm$ 0.04 (5)	49 $\pm$ 9 (5)	—	—
	1.0	357 $\pm$ 81 (7)	1.01 $\pm$ 0.07 (7)	28 $\pm$ 5 (7)	—	—
5	0	955 $\pm$ 70 (13)	0.98 $\pm$ 0.02 (13)	515 $\pm$ 36 (13)	381 $\pm$ 43 (3)	418
	0.1	545 $\pm$ 52 (5)	0.62 $\pm$ 0.14 (4)	306 $\pm$ 37 (4)	—	—
	1.0	414 $\pm$ 54 (9)	0.89 $\pm$ 0.13 (9)	68 $\pm$ 17 (9)	113 $\pm$ 12 (3)	101
15	0	872 $\pm$ 56 (14)	0.88 $\pm$ 0.09 (13)	424 $\pm$ 77 (13)	199 $\pm$ 28 (7)	357
	0.1	465 $\pm$ 31 (7)	0.61 $\pm$ 0.07 (6)	352 $\pm$ 34 (6)	—	—
	1.0	379 $\pm$ 47 (10)	0.67 $\pm$ 0.09 (10)	125 $\pm$ 20 (10)	—	—
25	0	885 $\pm$ 121 (8)	0.91 $\pm$ 0.09 (7)	522 $\pm$ 62 (7)	—	—
	0.1	657 $\pm$ 62 (6)	0.88 $\pm$ 0.07 (6)	319 $\pm$ 38 (6)	—	—
	1.0	417 $\pm$ 187 (3)	0.73 $\pm$ 0.21 (3)	55 $\pm$ 20 (3)	—	—

* Glucose equivalent was calculated from mean values for O_2 uptake and lactic acid production.

Incubation with pyruvate. In Ringer phosphate solution oxygen uptake was increased above the endogenous level (in agreement with the findings of Kornblueth, Yardeni-Yaron & Wertheimer, 1953) but not to the endogenous level found in bicarbonate solution (Table 3). In bicarbonate solution addition of pyruvate brought about no rise in oxygen uptake. Addition of iodoacetate did not inhibit oxygen uptake in the presence of pyruvate, in confirmation of the results of Meyerhof & Boyland (1931), nor was lactic acid formation inhibited. Pyruvic acid utilization was apparently diminished, but in view of the small number of experiments this last result is of doubtful significance.

DISCUSSION

The high rate of oxygen consumption observed for retina incubated in bicarbonate medium containing glucose (Q_{O_2} 30.5) is similar to that found by previous workers (Warburg, 1924b; Laser, 1937; Craig & Beecher, 1943a), but the rate in phosphate buffer (Q_{O_2} 8.5) is lower than that reported by Laser and by Craig & Beecher for rat and by Auricchio (1950) for rabbit retina. Kornblueth *et al.* (1953) have suggested that bicarbonate (or carbon dioxide) is necessary for continuous synthesis of tricarboxylic acid-cycle intermediaries and they found that oxygen uptake in phosphate buffer was increased by addition of glutamate and fumarate. If the effect of carbon dioxide is only to stimulate formation of intermediary metabolites addition of other compounds forming part of the

oxidative cycle should also increase oxygen uptake, but Craig & Beecher (1943b) did not find any increase on addition of succinate, and in our experiments pyruvate (which may be considered to be a source of cycle intermediaries) had a smaller effect than addition of bicarbonate (Table 3). Crane & Ball (1951a) found assimilation of carbon dioxide to be especially high in retina and suggest that malate is formed by assimilation of carbon dioxide to pyruvate with concurrent oxidation of reduced triphosphopyridine nucleotide. If this is so, the effect of bicarbonate (or carbon dioxide) on respiration may be due as much to the maintenance of a high concentration of oxidized triphosphopyridine nucleotide as to the formation of a necessary intermediary. Bicarbonate also increases lactic acid formation although the ratio of lactic acid produced to oxygen consumed is less in bicarbonate than in phosphate buffer (Table 4). If assimilation of carbon dioxide results in an increase in metabolism of pyruvate then reactions which produce pyruvate may also be stimulated and thus more pyruvate be available for reduction to lactate. It is possible that such a shift in reaction velocities could result in an increase in lactate formation although, at the same time, the ratio of lactate formed to oxygen utilized might fall.

Addition of iodoacetate had little effect on oxygen uptake during incubation in phosphate buffer. A similar lack of effect in other tissues has been interpreted by some workers (e.g. Barker *et al.* 1939) as evidence for oxidation by a non-glycolytic pathway, but the lack of inhibition may well be a

Table 3. *Effect of addition of pyruvate and monoiodoacetate on the oxygen consumption, lactic acid production and pyruvate utilization of ox retina*

Retina was incubated for 40 or 60 min. in Krebs-Ringer phosphate or bicarbonate solution at 37°. Results are given as means $\pm$ s.e.m., with the numbers of experiments in each group shown in parentheses.

Concn. of pyruvate (mM)	Concn. of monoiodoacetate (mM)	O ₂ uptake (μ moles/g. dry wt.)		Lactic acid production (μ moles/g. dry wt.)		Pyruvate utilization (μ moles/g. dry wt.)		Pyruvate equivalent* of O ₂ uptake and lactic acid production (μ moles/g. dry wt.)	
		40 min.	60 min.	40 min.	60 min.	40 min.	60 min.	40 min.	60 min.
0	0	280 $\pm$ 17 (12)	307 $\pm$ 21 (5)	(a) Krebs-Ringer phosphate		—	—	—	—
	0	387 $\pm$ 42 (7)	557 $\pm$ 25 (7)	39 $\pm$ 6 (8)	32 $\pm$ 10 (5)	—	—	—	—
	0.1	351 $\pm$ 9 (4)	506 $\pm$ 19 (4)	—	128 $\pm$ 13 (7)	—	304 $\pm$ 25 (7)	—	351
	1.0	305 $\pm$ 11 (2)	415 $\pm$ 9 (2)	—	172 $\pm$ 41 (4)	—	327 $\pm$ 23 (7)	—	375
0	0	494 $\pm$ 51 (12)	—	(b) Krebs-Ringer bicarbonate		—	—	—	—
	0	461 $\pm$ 62 (8)	—	40 $\pm$ 4 (8)	—	—	—	—	—
	0	524 $\pm$ 26 (4)	—	97 $\pm$ 16 (8)	—	347 $\pm$ 17 (2)	282	282	—
	1.0	—	—	167 $\pm$ 19 (4)	—	251 $\pm$ 21 (4)	376	376	—

* Pyruvate equivalent was calculated from mean values for O₂ uptake and lactic acid production.

Table 4. *Comparison of lactic acid produced and oxygen consumed during incubation of retina in Ringer phosphate and bicarbonate solutions containing glucose*

See Tables 1 and 2.

Concn. of glucose (mM)	Lactic acid produced (μ moles/g. dry wt.)	Oxygen used (μ moles/g. dry wt.)	Ratio (lactic acid produced: oxygen consumed)
Incubation in Ringer phosphate solution for 40 min.			
5	272	260	1:0.96
15	245	245	1:1.00
25	362	192	1:0.53
Incubation in Ringer phosphate solution for 60 min.			
5	403	351	1:0.87
15	416	364	1:0.88
25	495	277	1:0.56
Incubation in Ringer bicarbonate solution for 40 min.			
5	515	955	1:1.85
15	424	872	1:2.06
25	522	885	1:1.68

reflexion of a submaximal rate of oxidation, an explanation suggested by Heald (1953) to account for his observation that iodoacetate inhibited oxygen uptake of stimulated but not of resting brain tissue. Oxygen uptake of retina incubated in bicarbonate buffer was strongly inhibited by addition of iodoacetate but only when glucose was also present. This suggests that the effect was due to inhibition of glucose utilization rather than of some step in the oxidation process. Hexokinase in retina, as in other tissues, is partially inhibited by low concentrations of iodoacetate (D. S. Hoare, personal communication), so that in conditions where glucose utilization is high iodoacetate would be expected to reduce the amount used, whereas when utilization is low, as during incubation in phosphate buffer, sufficient may still be phosphorylated to allow oxidation, but not glycolysis, to continue uninhibited. The inhibition of lactic acid production would in these conditions be due to inhibition of triose phosphate dehydrogenase activity. The difference in effect of iodoacetate on oxygen uptake of retina in phosphate and bicarbonate buffers cannot therefore be used as evidence for the occurrence of different oxidative pathways in the two media, but this difference in effect in different metabolic conditions may perhaps explain the conflicting reports of previous workers who have studied the effect of iodoacetate on oxidative reactions.

SUMMARY

1. The rate of oxygen consumption of ox retina incubated in Ringer bicarbonate solution is increased by the addition of glucose but not by that

of pyruvate. When retina is incubated in Ringer phosphate solution the reverse is the case, for in the presence of glucose the rate of oxygen consumption is slightly depressed, although the initial rate is maintained for a longer period; addition of pyruvate causes a small rise in oxygen consumption.

2. Addition of monoiodoacetate at a concentration of mM causes only a small reduction in the amount of oxygen used during incubation in Ringer phosphate solution, although lactic acid production is strongly inhibited.

3. During incubation in Ringer bicarbonate solution containing glucose oxygen consumption is inhibited by 40% in a concentration of 0.1 mM-iodoacetate; an increase in concentration of iodoacetate to 1.0 mM does not bring about a significant further reduction in oxygen uptake.

We should like to thank The Royal Society for a grant to one of us (M.K.), which partially defrayed the expenses of this work.

REFERENCES

- Auricchio, G. (1950). *Ann. Ottalm.* **76**, 52.
 Barker, S. B., Shorr, E. & Malam, M. (1939). *J. biol. Chem.* **129**, 33.
 Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.* **138**, 535.
 Crabtree, H. G. (1929). *Biochem. J.* **23**, 536.
 Craig, F. N. & Beecher, H. K. (1943a). *J. gen. Physiol.* **26**, 467.
 Craig, F. N. & Beecher, H. K. (1943b). *J. gen. Physiol.* **26**, 473.
 Crane, R. K. & Ball, E. G. (1951a). *J. biol. Chem.* **188**, 819.
 Crane, R. K. & Ball, E. G. (1951b). *J. biol. Chem.* **189**, 289.
 Good, C. A., Kramer, H. & Somogyi, M. (1933). *J. biol. Chem.* **100**, 485.
 Graymore, C. N. & Tansley, K. (1959). *Brit. J. Ophthalm.* (in the Press).
 Heald, P. J. (1953). *Biochem. J.* **55**, 625.
 Kornblueth, W., Yardeni-Yaron, E. & Wertheimer, E. (1953). *Arch. Ophthalm., Chicago*, **50**, 45.
 Krebs, H. A. (1931). *Biochem. Z.* **234**, 278.
 Krebs, H. A. (1951). *Biochem. J.* **48**, 349.
 Laser, H. (1937). *Biochem. J.* **31**, 1671.
 Lundsgaard, E. (1930). *Biochem. Z.* **220**, 8.
 Meyerhof, O. & Boyland, E. (1931). *Biochem. Z.* **237**, 406.
 Noell, W. K. (1951). *J. cell. comp. Physiol.* **37**, 283.
 Noell, W. K. (1958). *Arch. Ophthalm., Chicago*, **60**, 702.
 Oguchi, T. (1938). *Acta Soc. Ophthalm. jap.* **42**, 583.
 Pardee, A. B. (1949). *J. biol. Chem.* **179**, 1085.
 Quastel, J. H. & Wheatley, A. H. M. (1932). *Biochem. J.* **26**, 725.
 Racker, E. (1956). *Ann. N.Y. Acad. Sci.* **63**, 1017.
 Saalow, G. (1937). *J. cell. comp. Physiol.* **10**, 385.
 Schubert, G. & Bornschein, H. (1951). *Experientia*, **7**, 461.
 Somogyi, M. (1945). *J. biol. Chem.* **160**, 61.
 Stannard, J. N. (1937). *Amer. J. Physiol.* **119**, 408.
 Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1945). *Manometric Techniques and Tissue Metabolism*, 1st ed. Minneapolis: Burgess Publishing Co.
 Warburg, O. (1924a). *Biochem. Z.* **152**, 51.
 Warburg, O. (1924b). *Biochem. Z.* **152**, 309.

The Fatty Acids of Human Milk from Mothers on Diets Taken *ad libitum**

BY W. INSULL, JUN. AND E. H. AHRENS, JUN.
The Rockefeller Institute, New York, N.Y.

(Received 29 September 1958)

The unique position of milk as a primary food and as a readily sampled product of active lipid metabolism emphasizes the desirability of obtaining detailed information on human-milk fatty acids. Fatty acids as glycerides comprise the bulk of milk lipids, less than 2% of which are cholesterol and phospholipid (Macy, Kelly & Sloan, 1953). The non-esterified fatty acids of milk, constituting about 2% of total milk lipids, have received slight attention. If analogous to those in serum (Dole, 1956; Gordon & Cherkas, 1956) they might have considerable physiological importance.

The major fatty acids of human milk lipids have been studied in a number of Laboratories (Bosworth, 1934; Hilditch & Meara, 1944; Baldwin & Longenecker, 1944; Brown & Orians, 1946).

* Supported in part by grants from the Nutrition Foundation and the U.S. Public Health Service (H-2539).

Analyses made by fractional distillation of the methyl esters, followed by crystallization and in some instances by spectroscopy, required sizeable quantities of starting materials; pooled milk specimens were used in all cases. Although studies on individual milks have not been feasible in such detail, Söderhjelm (1953) has defined the di- to hexa-ene contents of individual samples by ultraviolet spectrophotometry after isomerization with alkali. With gas-liquid chromatography of the methyl esters of fatty acids, introduced by James & Martin (1956) and improved by Lovelock & James (1959), precise all-inclusive analyses now can be made on multi-component mixtures totalling 50 µg. or less. Thus it becomes timely to refine the study of lactation by examining individual milks, in order to avoid any imprecisions due to pooling of samples.