2.5, and DNP caused a smaller or no further increase of respiration.

5. When sixty sperm samples of good motility from sixteen bulls were tested in the presence of fluoride, it was found that they could be grouped according to the type of their respiratory response to pyruvate and pyruvate + DNP. A correlation appeared to exist between the metabolic behaviour of sperm samples under the experimental conditions

described and the fertility of their donors as computed from their breeding record in artificial insemination.

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REFERENCES

- Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.* **138**, 535.
- Clifton, C. E. (1946). *Advanc. Enzymol.* **6**, 269.
- Clifton, C. E. (1952). In *The Enzymes*, ed. by Sumner, J. B. & Myrback, K., 2, pt. 2. New York: Academic Press.
- Friedemann, T. E. & Haugen, G. E. (1943). *J. biol. Chem.* **147**, 415.
- Gosh, D., Casida, L. E. & Lardy, H. A. (1949). *J. Anim. Sci.* **8**, 265.
- Jowett, M. & Quastel, J. H. (1935). *Biochem. J.* **29**, 2143.
- Krebs, H. A. (1937). *Biochem. J.* **31**, 661.
- Krebs, H. A. & Eggleston, L. V. (1940). *Biochem. J.* **34**, 442.
- Krebs, H. A. & Johnson, W. A. (1937). *Biochem. J.* **31**, 645.
- Lardy, H. A., Hansen, R. G. & Phillips, P. H. (1945). *Arch. Biochem.* **6**, 41.
- Lardy, H. A. & Phillips, P. H. (1941a). *Amer. J. Physiol.* **133**, 602.
- Lardy, H. A. & Phillips, P. H. (1941b). *Amer. J. Physiol.* **134**, 542.
- Lardy, H. A. & Phillips, P. H. (1943a). *Amer. J. Physiol.* **138**, 741.
- Lardy, H. A. & Phillips, P. H. (1943b). *J. biol. Chem.* **148**, 333, 343.
- Lardy, H. A. & Phillips, P. H. (1943c). *J. biol. Chem.* **149**, 177.
- Lardy, H. A. & Phillips, P. H. (1945). *Arch. Biochem.* **6**, 53.
- Lardy, H. A., Winchester, B. & Phillips, P. H. (1945). *Arch. Biochem.* **6**, 33.
- Loomis, W. F. & Lipmann, F. (1948). *J. biol. Chem.* **173**, 807.
- Mann, T. (1946). *Biochem. J.* **40**, 481.
- Mann, T. (1949). *Advanc. Enzymol.* **9**, 329.
- Melrose, D. R. & Turner, C. (1951). *Biochem. J.* **49**, i.
- Melrose, D. R. & Turner, C. (1952). *Proc. Soc. exp. Biol., N.Y.*, **80**, 298.
- Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
- Olson, R. E., Pearson, O. H., Miller, O. N. & Stare, F. J. (1948). *J. biol. Chem.* **175**, 489.
- Potter, V. R. (1947). *J. biol. Chem.* **169**, 17.
- Redenz, E. (1933). *Biochem. Z.* **257**, 234.
- Roe, J. H. (1934). *J. biol. Chem.* **107**, 15.
- Turner, C. (1951). *Biochem. J.* **50**, 145.
- Tosic, J. & Walton, A. (1950). *Biochem. J.* **47**, 199.
- Walton, A. (1945). *The Technique of Artificial Insemination*, 2nd ed. London: Holborn Surgical Instrument Co.
- Walton, A. & Edwards, J. (1938). *Proc. Amer. Soc. Anim. Prod.* p. 254.

An Effect of Bicarbonate on the Oxidation of Pyruvate by Kidney Homogenates

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Mitochondrial preparations from mammalian kidney, as Green, Loomis & Auerbach (1948) have shown, are capable of oxidizing pyruvate to completion and this reaction can be coupled with the phosphorylation of adenosinemonophosphate (AMP) or adenosinediphosphate (ADP). Experiments reported in this paper show that the oxidation of pyruvate stops under some conditions at the level of acetate. The factors that control complete and partial oxidation were investigated in detail because an experimental separation of the various steps of

pyruvate oxidation is necessary in studies of the quantitative aspects of oxidative phosphorylation, a problem to be dealt with in a subsequent paper.

EXPERIMENTAL

Collection of material. In the first experiments abattoir material was collected under ice and water. Later it was found that placing the tissue in a mixture of liquid and frozen 0.9% (w/v) aqueous KCl prevented the oedema which occurred in ice and water. Under 3 min. elapsed between the killing of the animal and immersing the kidney in the

cooling mixture in the case of sheep, and 20 min. in the case of cattle. Active preparations were obtained from both species.

Homogenates from kidneys kept in iced KCl for 24 hr. before homogenizing were as active as those prepared from fresh kidney. As about 50 g. of tissue are required for an experiment, abattoir kidney is more convenient and cheaper than rabbit kidney.

Preparation of the homogenates. Three methods were used. The first (preparation I) was similar to the preparation of 'cyclophorase' according to Green *et al.* (1948) and their method of naming the homogenate R_1 , R_2 , etc., according to the stage of washing, was also adopted. The period of storage of the kidney before homogenizing in cold KCl is indicated by a superscript giving the times in hours, e.g. $R_3^{24\text{hr}}$. The residue at the stage chosen was suspended in the required amount of ice-cold 0.9% KCl and if necessary stored up to 3 hr. at 0° until it was used.

The second method (preparation II) aimed at reducing the blank respiration and consisted in centrifuging the material obtained by homogenizing in a Waring Blender, for 2 min. at 700 g and discarding the precipitate. The supernatant was then treated as in the first method.

The third method (preparation III) was used when a preparation consisting almost entirely of mitochondria was required. Kidney cortex (25 g.) was minced and homogenized at 0° with a mixture of 70 ml. ice-cold 0.25 M-sucrose and 1 ml. N-NaOH in a stainless-steel homogenizer of the Potter-Elvehjem type. The resultant mixture was centrifuged at 0° and 700 g for 10 min. The residue was discarded and 8 ml. 1.5 N-KCl were added to the supernatant. The mixture was thoroughly stirred and then treated as in the first method.

General properties of the homogenates. The material spun down at the R_2 stage was a yellowish brown gel which became progressively lighter in colour with successive washings, and showed in general the properties of the rabbit 'cyclophorase' described by Green *et al.* (1948).

All preparations were reinforced with adenosinetriphosphate (ATP) and Mg ions, each to a final concentration of 0.001 M, and with phosphate buffer to a concentration of

To increase the accuracy of manometric measurement incubations were usually carried out at 20° to reduce the rate of metabolism. The washed homogenate was diluted in

Fig. 1. Distillation-diffusion unit used in the estimation of acetate. The unit was made by blowing a bulb on a Thunberg tube and fixing a ground-glass joint and stopper.

the proportion of 1 vol. of suspension to 3 vol. of 0.9% KCl. 2 ml. of the diluted suspension (60–70 mg. dry wt.) gave a steady O_2 uptake, on incubation with 0.0025 M-pyruvate and 0.004 M- $NaHCO_3$, of 300–400 μ l./hr. for about 2 hr. (Q_{O_2} ,

Fig. 2. Apparatus for use with distillation-diffusion units. The apparatus consists of two copper trays, the upper being heated and the lower cooled.

0.04 M. Unless otherwise indicated, the pH was 7.4. The O_2 uptake of some preparations was slightly increased by the addition of 0.2 ml. 1.86×10^{-4} M-cytochrome c and this was added as a routine measure.

μ l. O_2 uptake/mg. dry wt./hr., 5–6). In the absence of substrate, Q_{O_2} was usually less than 0.5.

Chemical estimations. O_2 uptake was measured in Warburg manometers. Pyruvic acid was estimated mano-

Table 1. *Recovery of acetate and acetic acid distilled from anhydrous sodium sulphate*

Indicator	Added material		Recovery	
	Substance	Quantity (μ moles)	μ moles	%
Bromothymol blue	Sodium acetate	0.890	0.868	97.5
		0.707	0.678	96.0
		0.528	0.550	104.0
		0.352	0.338	96.0
		0.176	0.183	104.0
Phenol red	Acetic acid	1.0	1.01	101.0
		1.5	1.51	100.5
		2.0	2.05	100.7
	Sodium acetate	1.0	0.992	99.2
		1.5	1.480	99.0
		2.0	1.965	98.4
	Sodium acetate in tissue extract	2.0	2.0	100.0

metrically by the yeast carboxylase method (Krebs & Johnson, 1937). Acetic acid was estimated by a distillation-diffusion method, utilizing principles introduced by Conway & Downey (1950) and Kirk (1950). It is based on the fact that the addition of anhyd. Na_2SO_4 increases the tension of acetic acid in solution (Conway & Downey, 1950) and thereby facilitates its distillation in a distillation-diffusion unit (Kirk, 1950). The units were adapted from Thunberg tubes and are of the shape shown in Fig. 1. The distillation bulb was half filled with anhyd. Na_2SO_4 ; 0.2–0.5 ml. of the acetate solution acidified with 2N- H_2SO_4 was then pipetted on top of the layer of Na_2SO_4 . Distilled water (1 ml.) and one drop of indicator (phenol red) were pipetted into the receiver and the assembled unit evacuated.

The units were then placed in the apparatus as shown in Fig. 2. The temperature of the distillation bulb was maintained at 50° and the receiver was cooled in ice. Under these conditions acetic acid was quantitatively transferred from the distillation bulb to the receiving tube in 20 min. At the end of this time the vacuum was released, the receiving tube removed, the sides rinsed with a little distilled water and its contents titrated with 0.01N- $\text{Ba}(\text{OH})_2$ free from carbonate. Bromothymol blue (Conway & Downey, 1950) was first used as indicator but was later replaced by phenol red which gave sharper end points. The method measures all volatile acids and under the conditions used pyruvic and hydrochloric acids gave positive results. These two acids were therefore rendered non-volatile by the addition of 0.2 ml. 0.1M-2,4-dinitrophenylhydrazine in 2N- H_2SO_4 and 0.1 ml. M- AgClO_4 , respectively, to 1 ml. of the solution to be analysed. A measured sample of the mixture, usually 0.5 ml., was then used for distillation. The precipitation of pyruvate and chloride may be carried out in the distillation bulb, Na_2SO_4 being added later, provided the total volume of liquid is not too large. Up to 0.5 ml. of liquid in the distillation bulb was found to be satisfactory for this procedure. A blank distillation of all reagents gave values of about 0.001–0.002 ml. 0.01N- $\text{Ba}(\text{OH})_2$ depending mainly on the contamination of the laboratory atmosphere. Recovery data are shown in Table 1.

RESULTS

The ratio of oxygen consumption to pyruvate utilization and acetate formation

The effect of substrate concentration. As shown by Green *et al.* (1948), rabbit-kidney 'cyclophorase'

oxidizes pyruvate to carbon dioxide and water. Under their conditions the ratio oxygen uptake/pyruvate consumed, hereafter referred to as O_2 /pyruvate ratio, was 2.5, which indicates complete oxidation. Under the same conditions this ratio was as low as 1.5 in sheep- or cattle-kidney preparations (Table 2).

Table 2. *The effect of substrate concentration on the oxidation of pyruvate by cattle-kidney homogenate*

(Samples (2 ml.) of a preparation I, $R_3^{34\text{hr}}$, suspended in 3 vol. 0.9% KCl. Standard amounts of ATP, cytochrome *c*, Mg and buffer; 0.5 ml. 0.1M- NaHCO_3 ; total vol. 4.0 ml. Temp. 40°, gas, O_2 .)

Pyruvate added (μ l.)	Pyruvate used (μ l.)	O_2 uptake (μ l.)	$\frac{\text{O}_2}{\text{Pyruvate}}$
1120	600	827	1.38
825	410	833	2.00
448	440	1034	2.35

Time curves of O_2 /pyruvate ratio with different substrate concentrations were obtained as follows:

A series of identical Warburg vessels was set up containing 0.2 ml. 0.1M- NaHCO_3 and pyruvate besides the standard additions. The side bulb contained 0.2 ml. 3M-acetate buffer, pH 5.0. The vessels were kept at 0° until they were assembled and put in the bath. The O_2 uptake was measured and the reaction was stopped in successive vessels at intervals by adding the acetate buffer from the side bulb. The vessels were removed from the bath, the soda swabbed out from the centre wells, and after rinsing the centre wells with 2N- H_2SO_4 and drying, 1 ml. portions of yeast carboxylase were put in the side bulbs and sticks of P in the centre wells. The manometers were reassembled, gassed with N_2 and the CO_2 output on mixing was measured.

The results (Table 3) indicate that for a given pyruvate concentration the O_2 /pyruvate ratio showed no major change during the period of incubation. It changed, however, with the pyruvate concentration. It was 2.5 with 240 μ l. or less pyruvate but was smaller at higher pyruvate con-

Table 3. *Ratio O₂/pyruvate at different pyruvate concentrations*

(Sheep-kidney preparation I.)

Exp. no.	Dry wt. of tissue (mg.)	Pyruvate added to 4.0 ml. (μl.)	Time of incubation (min.)	Pyruvate used (μl.)	O ₂ used (μl.)	$\frac{O_2}{\text{Pyruvate}}$
1	60.9	120	5	20	48	2.40
			10	39	96	2.46
			20	76	188	2.48
			30	114	284	2.49
2	60.9	240	5	18	45	2.50
			10	36	90	2.50
			20	72	180	2.50
			30	108	272	2.52
3	59.4	487	5	12	22	1.83
			10	24	44	1.83
			20	48	87	1.83
			30	70	124	1.77
4	71.0	496	10	55	78	1.42
			20	110	155	1.36
			30	165	235	1.42
			40	222	315	1.42
5	71.8	557	10	30	40	1.33
			20	60	80	1.33
			30	90	120	1.33

centrations. In all cases preparations with low blanks were used (see Tables 7 and 9), and it has been assumed that the observed oxygen consumptions in the presence of pyruvate are due solely to substrate oxidation, the endogenous respiration being completely inhibited.

Formation of acetate during the oxidation of pyruvate. If the O₂/pyruvate ratio falls below 2.5 some intermediate product must be accumulating. Distillation as described above for the acetic acid determination yielded a volatile acid behaving like acetic acid. No acetoacetate, acetoin, L-malate, fumarate, succinate or α-ketoglutarate were found, and in one case where a test was made a small amount of citrate amounting to 30 μl. was found, when 839 μl. of the substrate were used. To identify the volatile acid, pooled samples of the distillate from four manometer vessels were neutralized with 0.1N-sodium hydroxide and concentrated to approx. 0.2 ml. A portion of this material (5 μl.) was chromatographed on paper, using butanol-ethylamine according to Hiskox & Berridge (1950) as solvent. Standard spots of acetate, propionate and butyrate were chromatographed on the same paper. The unknown material ran as a single component in the same position as acetate. A further sample was tested with lanthanum nitrate according to the method of Krüger & Tschirsch (1929, 1930) and gave a positive result. These results indicated that the accumulating metabolite was acetate. Moreover, the amount of acetate accounted quantitatively for the low O₂/pyruvate ratios.

If the two overall reactions:

accounted completely for the removal of pyruvate, the amount of acetate formed can be calculated from the O₂/pyruvate ratio. Let the fraction of pyruvate disappearing that is oxidized to acetate be *a*.

$$\begin{aligned}\text{Then } O_2/\text{pyruvate} &= 0.5a + (1-a) 2.5, \\ a &= 1.25 - 0.5 O_2/\text{pyruvate}.\end{aligned}$$

Experiments (Table 4) show that the amounts of acetate produced and calculated from the O₂/pyruvate ratio approximately agree. Thus acetate is the only product of the incomplete oxidation of pyruvate which appears in appreciable quantities.

The effect of varying concentrations of bicarbonate on the Q_{o2} of kidney homogenates

In earlier experiments bicarbonate was added according to Green *et al.* (1948) to give a final concentration of 0.0125 M (1120 μl./4 ml.). Lowering the concentration to 0.0025 M (224 μl./4 ml.) resulted in a marked decrease of the rate of oxygen uptake in the presence of pyruvate, and in the O₂/pyruvate ratio (Table 5). The following experiments were carried out to decide whether the effect of bicarbonate was due to a change in the pH of the medium or to a direct action, such as participation in the carboxylation of pyruvate to oxaloacetate.

Effect of pH. To control the pH of the media containing bicarbonate, the experiments were carried out at a constant carbon dioxide pressure, maintained by diethanolamine-carbon dioxide buffers (Pardee, 1949; Krebs, 1951). Dickens & Šimer (1931) vessels containing in the outer ring the medium without tissue and in the centre compartment 3.0 ml. of diethanolamine-carbon dioxide

Table 4. *Formation of acetate by sheep-kidney homogenates when incubated with pyruvate*

(Incubation in Warburg vessels containing the standard additions +0.2 ml. 0.1N-NaHCO₃. Homogenate (2.0 ml.) suspended in 3 vol. of 0.9% KCl. Vessels in ice until put in bath; O₂ uptake measured; reaction stopped with 1.0 ml. 0.5N-HCl. 1.0 ml. sample analysed for residual pyruvate. 1.0 ml. sample analysed for acetate.)

Exp. no.	Preparation I	Pyruvate added (μl.)	Pyruvate used (μl.)	O ₂ uptake (μl.)	Fraction of pyruvate oxidized to acetate			Volatile acid formed (μl.)
					O ₂ Pyruvate	Acetate calculated (μl.)	(a)	
1	R ₃ ^{24hr.}	957	839	724	0.87	0.815	685	595
2	R ₃ ^{72hr.}	261	261	330	1.27	0.615	160	164
		1305	390	218	0.56	0.97	379	346
3	R ₃ ^{24hr.}	448	220	362	1.64	0.43	95	107
		672	296	320	1.08	0.71	210	209

Table 5. *The effect of bicarbonate concentration on the Q_{O₂} of kidney homogenates oxidizing pyruvate*

(Kidney homogenate R₃^{24hr.} (2.0 ml.) suspended in 3 vol. of 0.9% KCl. Volume 4 ml., temp. 20°, gas O₂. Vessels in ice until put in bath. Reaction stopped with 1.0 ml. 0.5N-HCl. 1.0 ml. sample analysed for residual pyruvate.)

Exp. no.	Preparation I	Pyruvate added (μl.)	Bicarbonate added (μl.)	Q _{O₂}	O ₂
					Pyruvate
1	Ox kidney	825	1120	6.9	2.50
			224	2.2	1.50
2	Sheep kidney	898	896	6.3	1.43
			672	6.0	1.31
			448	4.9	1.07
			224	3.0	0.80

buffer, were gassed in the bath with the appropriate carbon dioxide-oxygen mixture until equilibrated. The manometers were removed from the bath and the side-arm stopper leading to the outer ring was removed. The mitochondrial suspension was quickly pipetted into the side arm and the stopper replaced. The contents of the side arm were then

Table 6. *The effect of pH on the oxygen uptake of sheep-kidney mitochondrial preparations*

(Sheep kidney, R₃^{48hr.} Dickens & Šimer vessels were used as described in the text. 2.0 ml. of a suspension of 1 vol. of mitochondria in 3 vol. of 0.9% KCl. 224 μl. of pyruvate and 222 μl. NaHCO₃ in 4 ml. Temp. 20°.)

Type of preparation	pH	CO ₂ in gas phase (%)	Q _{O₂}	
			1st 30 min.	2nd 30 min.
II	7.0	1.18	1.56	0.78
	7.6	0.23	3.05	1.57
	8.0	0.09	3.61	2.36
III	7.0	1.18	0.98	0.88
	7.4	0.50	3.43	1.73
	8.0	0.09	6.46	5.88

tipped into the medium and the manometers were replaced in the bath and quickly regassed for a few seconds. The measurements (Table 6) show that respiration increased with pH within the range of pH tested; moreover the respiration was better maintained at the higher pH.

Effect of bicarbonate concentration at constant pH. Diethanolamine-carbon dioxide buffers were again used. In the range tested (0–0.01354M), increasing concentrations of bicarbonate raised Q_{O₂} in all experiments; the magnitude of the effect varied and depended on the level of the blank respiration. When the blank of preparation I was relatively high the increase on addition of bicarbonate at pH 7.4 was relatively small. Preparation II gave lower blanks, and generally a greater increase in respiration on addition of bicarbonate than preparation I. Illustrative data are shown in Table 7. The maximum increase caused by bicarbonate was over 100%. The effect of bicarbonate can be demonstrated at different pH values (Table 8), but the magnitude of the effect varies with the pH. The same concentration of bicarbonate (0.00752M) increased the rate by 258% at pH 7.0, 119% at pH 7.6 and 30% at pH 8.0.

The replacement of bicarbonate by fumarate in the oxidation of pyruvate. Green *et al.* (1948) stated that either bicarbonate or a member of the citric acid cycle was necessary for the oxidation of pyruvate by rabbit-kidney cyclophorase. The relative efficiencies of fumarate and bicarbonate in promoting pyruvate oxidation by preparations I and III were tested at constant pH. At pH 7.0 and 7.6 additions of either 0.0075M-bicarbonate or 0.00025M-fumarate increased respiration of preparations I and III, the effects being about equal during the first half hour

Table 7. *The effect of bicarbonate at pH 7.4 on the oxygen uptake of sheep-kidney homogenates*

(Conditions as in Table 6. High blank means that the Q_{O_2} was 40–60% of the value with pyruvate alone as substrate; low blank means that Q_{O_2} was less than 10%.)

Exp. no.	Type of preparation	NaHCO ₃ concn. (M)	CO ₂ in gas phase (%)	Q _{O₂}	
				1st 30 min.	2nd 30 min.
1	I (high blank)	0.00275	0.43	10.69	—
		0.00752	1.18	11.50	—
		0.0135	2.13	11.65	—
2	I (high blank)	0.00147	0.23	11.17	—
		0.00752	1.18	13.10	—
3	I (high blank)	0.000572	0.09	8.35	—
		0.00752	1.18	8.70	—
4	I (low blank)	0.00147	0.23	5.77	4.87
		0.00275	0.43	6.40	6.33
		0.00752	1.18	10.22	8.36
5	I (low blank)	0	0	6.57	5.55
		0.00752	1.18	9.70	8.13
6	I (low blank)	0	0	6.57	—
		0.00752	1.18	9.72	—
7	I (low blank)	0.000572	0.09	6.84	6.94
		0.00147	0.23	7.91	7.32
		0.00275	0.43	9.38	7.61
		0.00752	1.18	9.25	8.77
8	II	0.000572	0.09	3.73	2.86
		0.00275	0.43	5.70	3.79
		0.00752	1.18	8.60	8.12
9	II	0	0	6.56	—
		0.00147	0.23	10.05	—
		0.00752	1.18	11.13	—
		0.01354	2.13	11.40	—

Table 8. *The effect of bicarbonate on the oxygen uptake of sheep-kidney mitochondrial preparations at different pH values with pyruvate as substrate*

(Conditions as in Table 6 except that 1.0 ml. 0.1 M-phosphate buffer was used in each vessel.)

Type of preparation	pH	Concn. of added bicarbonate (M)	CO ₂ in gas phase (%)	Q _{O₂}		
				1st 30 min.	2nd 30 min.	3rd 30 min.
I R_3^{24} hr.	7.0	0	0	2.49	1.89	0.96
		0.00752	3.03	3.29	3.28	2.56
I R_2^{24} hr.		0	0	2.43	1.53	0.90
		0.00752	3.03	4.29	4.38	3.93
III R_4^{24} hr.	7.0	0	0	3.33	1.71	0.88
		0.00752	3.03	13.99	12.82	2.33
I R_3^{24} hr.	7.6	0	0	3.91	4.64	—
		0.00752	0.75	5.10	5.93	—
I R_3^{24} hr.	7.6	0	0	3.35	3.57	—
		0.00752	0.75	5.12	5.82	—
III R_4^{24} hr. aged 4 hr. before using	7.6	0	0	1.72	0.51	—
		0.00752	0.75	3.78	1.55	—
I R_3^{24} hr.	8.0	0	0	6.93	5.71	—
		0.00752	0.23	7.40	6.63	—
I R_3^{24} hr.	8.0	0	0	7.33	5.56	—
		0.00752	0.23	7.13	6.62	—
III R_4^{24} hr.	8.0	0	0	7.21	3.97	1.25
		0.00752	0.23	9.44	7.23	4.40

Table 9. *The effect of bicarbonate and fumarate on the oxygen uptake of sheep-kidney mitochondrial preparations at different pH levels*

(Conditions as in Table 6. Pyruvate added, 224 μ l.; bicarbonate and fumarate as shown. Appropriate diethanolamine- CO_2 buffers and O_2 - CO_2 gas mixture. Low blank means that the Q_{O_2} is less than 10% of the value with pyruvate alone as substrate.)

Type of preparation	pH	Concn. of additions		Q_{O_2}		
		Fumarate (M)	Bicarbonate (M)	1st 30 min.	2nd 30 min.	3rd 30 min.
I $R_3^{24\text{hr.}}$ (low blank)	7.0	0	0	2.49	1.89	0.96
		0.00025	0	6.03	4.02	2.59
		0	0.00752	3.29	3.28	2.56
		0.00025	0.00752	5.49	5.49	4.60
I $R_3^{24\text{hr.}}$ (low blank)	7.0	0	0	2.43	1.53	0.90
		0.00025	0	5.54	3.76	2.83
		0	0.00752	4.29	4.38	3.93
		0.00025	0.00752	5.18	5.62	4.73
III $R_4^{24\text{hr.}}$	7.0	0	0	3.33	1.71	0.88
		0.00025	0	13.10	6.43	3.30
		0	0.00752	13.99	12.82	2.33
		0.00025	0.00752	16.50	9.45	5.20
I $R_3^{24\text{hr.}}$ (low blank)	7.6	0	0	3.91	4.64	—
		0.00025	0	5.18	6.16	—
		0	0.00752	5.10	5.93	—
		0.00025	0.00752	6.01	6.26	—
III $R_4^{24\text{hr.}}$ aged 4 hr. before use	7.6	0	0	1.72	0.51	—
		0.00025	0	4.32	1.34	—
		0	0.00752	3.78	1.55	—
		0.00025	0.00752	9.37	6.26	—
I $R_3^{24\text{hr.}}$ (low blank)	8.0	0	0	6.93	5.71	—
		0.00025	0	8.36	7.25	—
		0	0.00752	7.4	6.63	—
		0.00025	0.00752	7.9	7.35	—
I $R_3^{24\text{hr.}}$ (low blank)	8.0	0	0	7.33	5.56	—
		0.00025	0	8.73	7.34	—
		0	0.00752	7.13	6.62	—
		0.00025	0.00752	8.74	7.66	—
III $R_3^{24\text{hr.}}$	8.0	0	0	7.36	7.36	3.44
		0.00025	0	6.56	7.74	4.53
		0	0.00752	7.52	6.36	3.63
		0.00025	0.00752	8.47	6.36	4.57
III $R_3^{24\text{hr.}}$	8.0	0	0	7.21	3.97	1.25
		0.00025	0	10.21	6.46	2.13
		0	0.00752	9.44	7.23	4.40
		0.00025	0.00752	9.15	7.18	4.65

Table 10. *The utilization of pyruvate by sheep-kidney mitochondria without bicarbonate at pH 7.0, 7.4, 7.6 and 8.0*

(Technique as previously described but vessels contained 1.0 ml. 0.1 M buffer in the main compartment of the vessel and 0.2 ml. 3 M-acetate buffer, pH 4.4, in the side bulb. The homogenate was prepared by method III. Incubation time, 50 min.)

Exp.	Pyruvate added (μ l.)	pH	Q_{O_2}	Pyruvate used (μ l.)	O_2 used (μ l.)	$\frac{\text{O}_2}{\text{Pyruvate}}$	Calculated formation of acetate from pyruvate (%)
1	405	7.0	2.02	171	118	0.69	90.5
		7.4	2.43	168	142	0.84	83.0
2	352	7.6	2.35	143	137	0.96	77.0
		8.0	2.95	158	172	1.09	70.5

of incubation. On incubation beyond 30 min. bicarbonate was more effective at pH 7.0 or 7.6 than fumarate. At pH 8.0 increases were not always observed, but when they occurred both bicarbonate and fumarate had again about the same effects (Table 9).

Oxidation of pyruvate without added bicarbonate or fumarate

Washed sheep-kidney homogenates were found to be capable of oxidizing pyruvate without added bicarbonate or fumarate, especially at a high pH (Tables 7-9). In some experiments the O_2 /pyruvate ratio was measured and values of 0.69-1.09 were obtained (Table 10). This means that 70.5-90.5% of the pyruvate utilized was oxidized to acetate, while the remainder was completely oxidized.

DISCUSSION

According to the experimental conditions the oxidation of pyruvate in kidney homogenate may be complete, or lead to the formation of acetate. An aerobic formation of acetate from pyruvate has been demonstrated before in brain homogenates, but not in the homogenates of other animal tissues (Ochoa, 1951). Whether the oxidation is complete or incomplete depends on the pyruvate concentration and on the concentration of bicarbonate or a precursor of oxaloacetate. Complete oxidation of pyruvate is obtained in the presence of 0.0075M-bicarbonate or 0.00025M-fumarate and a low concentration (0.0025M) of pyruvate. High pyruvate concentrations, above 0.0025M, and low bicarbonate concentrations result in incomplete oxidation and formation of acetate. Increase of pH has an effect similar to, but smaller than, the effect of bicarbonate. Under the conditions used by

Green *et al.* (1948) it is not possible to say how much of the stimulation of respiration obtained was due to bicarbonate and how much due to changed pH.

The relative ineffectiveness of bicarbonate at pH 8.0 is probably due to the saturation of the tissue with endogenous bicarbonate and carbon dioxide. This would also account for the comparatively high O_2 /pyruvate ratio at pH 8.0 without added bicarbonate. It appears probable that bicarbonate has no special role in the oxidation of pyruvate by mitochondria other than as supplier of carbon dioxide for carboxylation.

SUMMARY

1. The preparation and properties of respiring particulate preparations from homogenized sheep and ox kidney are described. Kidneys yielded fully active preparations after storage in frozen 0.9% potassium chloride for 24 hr.
2. The estimation of volatile acid (acetate) by the use of a distillation-diffusion apparatus is described.
3. Complete oxidation of pyruvate occurred only when bicarbonate (0.0075M) or fumarate (0.00025M) was present and the concentration of pyruvate was below about 0.0025M.
4. Under other conditions the oxidation of pyruvate was incomplete and acetic acid accumulated.
5. Bicarbonate increased the rate of oxygen uptake at pH 7.0, 7.4 and 7.6 but not consistently at pH 8.0. The increased oxygen uptake was accompanied by a reduction in the accumulation of acetate. Increase of pH from 7.0 to 8.0 had an effect similar to, but smaller than, that of bicarbonate.

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REFERENCES

- Conway, E. J. & Downey, M. (1950). *Biochem. J.* **47**, iv.
Dickens, F. & Šimer, F. (1931). *Biochem. J.* **25**, 973.
Green, D. E., Loomis, W. F. & Auerbach, V. H. (1948). *J. biol. Chem.* **172**, 389.
Hiskox, E. R. & Berridge, N. J. (1950). *Nature, Lond.*, **166**, 522.
Kirk, P. L. (1950). *Analyt. Chem.* **22**, 611.
Krebs, H. A. (1951). *Biochem. J.* **48**, 349.
Krebs, H. A. & Johnson, W. A. (1937). *Biochem. J.* **31**, 645.
Krüger, D. & Tschirsch, E. (1929). *Ber. dtsch. chem. Ges.* **62**, 2776.
Krüger, D. & Tschirsch, E. (1930). *Ber. dtsch. chem. Ges.* **63**, 826.
Ochoa, S. A. (1951). *Phys. Rev.* **31**, 71.
Pardee, A. B. (1949). *J. biol. Chem.* **179**, 349.