The *in vitro* Oxidation of Pyruvic and α -Ketobutyric Acids by Ground Preparations of Pigeon Brain. The Effect of Inorganic Phosphate and Adenine Nucleotide

By C. LONG, *From the Department of Biochemistry, The Queen's University, Belfast*

(Received 10 December 1942)

Quantitatively, pyruvate oxidation in pigeon brain has mainly been investigated with minced tissue [Peters & Thompson, 1934; McGowan, 1937; Long, 1938]. While most of the pyruvate is oxidized completely to CO_2 and water, small amounts undergo either oxidative decarboxylation (reaction 1; $R = \text{H}$) or anaerobic dismutation (reaction 2; $R = \text{H}$).

α -Ketobutyric acid is not a normal metabolite in biological systems, nor does the corresponding *l*- α -amino-butyric acid seem to occur naturally [Dunn, 1938], so that the activation of this keto-acid by enzymes must be regarded as an artefact. Nevertheless, its behaviour towards minced pigeon brain has led to valuable conclusions concerning the mechanism of pyruvic acid utilization [McGowan & Peters, 1937; Long & Peters, 1939; Long, 1942].

Minced pigeon brain could not completely oxidize α -ketobutyric acid, although oxidative decarboxylation (reaction 1; $R = \text{CH}_3$) and anaerobic dismutation (reaction 2; $R = \text{CH}_3$) occurred readily [Long & Peters, 1939]. Nevertheless, pyruvic and α -ketobutyric acids were equally rapidly utilized [Long, 1942], that fraction which was completely oxidized in the case of pyruvic acid merely undergoing oxidative decarboxylation in the case of α -ketobutyric acid.

Banga, Ochoa & Peters [1939 *a, b*], using dialysed pigeon brain suspensions in which the cell structure of the tissue was destroyed by grinding, found that pyruvate oxidation was stimulated by the addition of inorganic phosphate, adenine nucleotide and fumarate. Ochoa [1939] showed that Mg^{++} was also required. This enzyme system seems to have little in common with the pyruvate oxidation system as it occurs in the minced tissue, the respiration of which is not affected by the addition of these dialysable components; but evidence will be presented to show that this is not due to the presence in the minced brain of these activators.

In the present work enzyme studies with ground preparations of pigeon brain have been used to assess the effects of added inorganic phosphate and adenine nucleotide on the oxidation of both pyruvic

and α -ketobutyric acids: a further communication will deal with fumarate catalysis. The oxidation of both pyruvate and α -ketobutyrate has been found to be stimulated by inorganic phosphate. That of pyruvate was also markedly increased by the addition of adenine nucleotide, but the latter's effect on α -ketobutyrate oxidation was only very slight. The increased O_2 uptake, pyruvate utilization and O_2 /pyruvate ratio, together with the lowered R.Q., which were observed in presence of adenine nucleotide, showed that pyruvate was much more completely oxidized in the presence of the nucleotide than in its absence.

EXPERIMENTAL

Materials

Pyruvic and α -ketobutyric acids [Peters, 1938; Long & Peters, 1939]. The pure crystalline Na salts were used. Final concentration $M/100$, unless otherwise stated.

Adenine nucleotide. Three preparations were employed: (1) muscle adenylic acid (a gift from Prof. R. A. Peters), (2) adenylypyrophosphoric acid (Henning), and (3) Ca acid adenylypyrophosphate (a gift from Prof. T. H. Milroy). This last specimen was freed from Ca by Na oxalate. All three preparations, after neutralization, had the same effect on the oxidation of pyruvate by dialysed suspensions of pigeon brain containing $M/20$ phosphate buffer, $\text{pH } 7.3$. Adenine nucleotide was used throughout in concentration producing the maximum effect ($0.1 \text{ mg./ml.} = 3 \times 10^{-4} M$).

Methods

(1) The estimation of pyruvic and α -ketobutyric acids

This was by the method of Lu [1939] as modified by Long [1942].

(2) The estimation of inorganic phosphate

The method of Berenblum & Chain [1938] was applied in the following way to small volumes (2 ml.) of protein-free filtrates containing very low concentrations ($0.5\text{--}2.5 \times 10^{-4} M$) of inorganic phosphate.

Into three test tubes (a), (b) and (c) were measured: (a) 2 ml. of the 5% trichloroacetic acid, protein-free filtrate,

(b) 2 ml. of the standard phosphate ($2 \times 10^{-4} M$) in 5% $CCl_3.COOH$, and (c) 2 ml. 5% $CCl_3.COOH$ (control). 10N H_2SO_4 (0.1 ml.), 5% ammonium molybdate (1 ml.) and isobutanol (4 ml.) were added to each. The contents were rapidly drawn into and expelled from a teat-pipette about 30 times. The lower aqueous layers were removed, by means of the corresponding pipettes, and discarded. The isobutanol extracts were then washed by extracting twice with 2 ml. N H_2SO_4 . 0.4% $SnCl_2$ in N H_2SO_4 (3 ml.), freshly prepared from 40% $SnCl_2$ in conc. HCl, was then added to each. After thorough mixing and removal of the aqueous layer, each extract was made up to 10 ml. with ethanol. The absorption intensities were compared in a Hilger Spekker absorptiometer, using an orange filter (Hilger no. 3). The concentration of inorganic phosphate is linearly related to the absorption.

(3) The preparation of the enzyme suspensions

(a) *For anaerobic experiments.* Exhaustive washing of the tissue was necessary in order to produce a very low residual activity. The method of preparation [cf. Long & Peters, 1939] was as follows:

The cerebrum and optic lobes (about 1.5 g.) were removed as rapidly as possible from a freshly killed pigeon, and ground, ice-cold, in a mortar for 10 min. 0.9% KCl (6 ml.), ice-cold, was then added in portions and the grinding continued for a further 5 min. After passing through muslin, the brain suspension was centrifuged and the supernatant fluid discarded. The deposit was washed twice with 0.9% KCl and then three times with 0.2% KCl (6 ml. each time), keeping throughout as cold as possible and allowing the salt solutions to remain in contact with the enzyme for about 15 min. before each centrifugation. The washed, almost colourless, suspension, now free from cytochromes, was made up to 10 ml. with 0.2% KCl. 1 ml. suspension was equivalent to 150 mg. fresh brain tissue.

(b) *For aerobic experiments.* The preparation of dialysed brain suspensions, except in the temperature during extraction, resembled that of the 'dispersions' described by Banga *et al.* [1939b].

The cerebrum and optic lobes (about 4.5 g.) from three pigeons were placed in an ice-cold mortar and ground for 10 min. The contents warmed to room temperature during grinding. 0.9% KCl (9 ml.), ice-cold, was then added in portions and the grinding continued for 5 min. The brain suspension was passed through muslin, pipetted into two freshly prepared collodion sacs and dialysed for 3 hr. (unless otherwise stated) against 0.4% KCl (3 l.) at below 2° . During dialysis, the sacs were mechanically agitated. 1 ml. suspension was equivalent to 333 mg. fresh brain tissue.

(4) Thunberg technique

The Thunberg tubes [Keilin, 1929] contained 1 ml. of the enzyme suspension (added last), and substrates and other constituents to make the total volume 1.8 ml., the pH of the mixture being 7.3. The hollow stopper contained 0.2 ml. methylene blue (1:5000). The tubes were twice exhausted and filled with N_2 and again exhausted. At zero time the methylene blue was added to the other constituents; temperature 38° . Duplicate determinations were always made. $100 \times$ the reciprocal of the reduction time (t min.) was taken as a measure of the activity of the system.

(5) Respiration technique

1 ml. of the enzyme suspension, prepared as under (3) (b), was shaken at 28° in air in a total volume of 2 ml. at pH 7.3, usually for 40 min. O_2 uptakes were determined in Barcroft flasks, with filter paper soaked in 2N KOH (0.2 ml.) in the centre well. O_2 uptake was determined in duplicate or triplicate, and the agreement was $\pm 1.5\%$. If O_2 uptakes were not required, the suspension was shaken in 50 ml. Erlenmeyer flasks in the thermostat at 28° . For α -keto-acid or phosphate estimations, 25% $CCl_3.COOH$ (1 ml.) was added to the medium at the beginning and end of the respiration period. It was allowed to stand 15 min., the protein precipitate was filtered off, and the flasks and filters rinsed with 5% $CCl_3.COOH$ (3×2 ml.). After standing overnight, the volumes were made up to 10 ml. and aliquots taken for α -keto-acid or phosphate estimation.

RESULTS

(1) The effect of inorganic phosphate on the oxidation of pyruvic and α -ketobutyric acids by ground preparations of pigeon brain

Lipmann [1937] has shown that inorganic phosphate or arsenate is necessary for O_2 uptake with acetone-dried preparations of *B. delbrückii* in the presence of pyruvate and methylene blue. The relation of O_2 absorbed and CO_2 produced to pyruvate utilized, indicated oxidative decarboxylation to acetic acid. Banga *et al.* [1939b] observed that phosphate markedly increased the O_2 uptake of dialysed pigeon brain suspensions in presence of pyruvate, adenine nucleotide, fumarate and Mg^{++} . They inferred that the respiration would be zero in the complete absence of inorganic phosphate. To simplify the problem, the experiments described in this section were carried out in order to test the effect of inorganic phosphate, only, on the oxidation of pyruvate and α -ketobutyrate by pigeon brain suspensions, in the absence of other factors.

Anaerobic experiments. The time taken by the system, dehydrogenase preparation and α -keto-acid, to reduce 0.2 ml. methylene blue (1:5000) was determined by the Thunberg technique in the presence and absence of phosphate buffer, $M/20$, pH 7.3. The osmotic pressure and Na^+/K^+ ratio of the media were kept constant throughout, isotonic phosphate and chloride being interchanged.

There is no doubt that inorganic phosphate exerts a powerful catalytic effect on the anaerobic oxidation of both pyruvate and α -ketobutyrate (Tables 1 and 2). The increased activity due to phosphate addition is quantitatively the same for the two α -keto-acids.

Two typical curves connecting phosphate concentration and activity are shown in Fig. 1, in which the effect of phosphate in the absence of α -keto-acid is also included in one case. The residual concentration of phosphate in the tissue was found to be $0.75 \times 10^{-4} M$. Very small concentrations of inorganic phosphate exert a pronounced catalytic

Table 1. *The anaerobic oxidation of pyruvate and α -ketobutyrate in the presence and absence of inorganic phosphate*

Substrate	Activity (100/t)					
	Exp. 1			Exp. 2		
	Control	Phosphate	Diff.	Control	Phosphate	Diff.
Nil	0.5	0.5	0.0	1.1	2.5	1.4
Pyruvate	0.5	15.4	14.9	5.3	14.8	9.5
α -Ketobutyrate	0.5	14.8	14.3	4.0	13.8	9.8

t = reduction time (min.).

effect on the oxidation, and extrapolation to zero phosphate concentration shows that some α -keto-acid oxidation still occurs.

Fig. 1. The effect of inorganic phosphate concentration on the activity of the α -keto-acid dehydrogenase system from pigeon brain. Curve A, pyruvate; curve B, α -ketobutyrate; curve C, no substrate. Curves B and C refer to the same experiment, curve A to a separate experiment.

Arsenate also exerts a definite effect on the anaerobic oxidation of both pyruvate and α -ketobutyrate, and this effect is quantitatively the same

for the two α -keto-acids (Table 2). At equivalent concentration (*M*/20), arsenate appears to be about 70–75% as active as phosphate.

Table 2. *The replacement of phosphate by arsenate in the anaerobic oxidation of pyruvate and α -ketobutyrate*

Exp.	Substrate	Activity (100/t)				
		Control	Phosphate	Diff.	Arsenate	Diff.
1	Nil	0.5	0.7	0.2	0.7	0.2
	Pyruvate	0.9	6.9	6.0	5.7	4.8
	α -Ketobutyrate	0.5	6.5	6.0	5.3	4.8
2	Nil	0.5	0.9	0.4	0.9	0.4
	Pyruvate	2.1	10.0	7.9	7.5	5.4
	α -Ketobutyrate	0.9	9.3	8.4	7.1	6.2

t = reduction time (min.).

Aerobic experiments. Table 3 shows that the net O_2 uptake due to oxidation of pyruvate or α -ketobutyrate by brain suspensions is approximately doubled on addition of *M*/20 phosphate. The osmotic pressure and Na^+/K^+ ratio of the media were again kept constant throughout.

The catalytic effect of phosphate shown in Table 3 does not appear to be as great as that obtained under anaerobic conditions. The residual inorganic phosphate concentration of the tissue, however, is very different in the two cases. The dehydrogenase preparation contained only about $0.75 \times 10^{-4} M$, whereas the dialysed tissue suspension contained about $1.1 \times 10^{-3} M$ phosphate. The comparison between the aerobic and anaerobic oxidations should therefore be made at two definite phosphate concentrations, e.g. $1.1 \times 10^{-3} M$ and $5 \times 10^{-2} M$. Thus in one anaerobic experiment with α -ketobutyrate as substrate, the activity at $1.1 \times 10^{-3} M$ was 3.5, increasing to 7.4 at $5 \times 10^{-2} M$ phosphate; increase about 114%. This figure agrees quite closely with the percentage increase found for the catalytic effect in respiring pigeon brain suspensions.

In contrast to its stimulating action on the anaerobic oxidation of α -keto-acids, arsenate did not cause an increased respiratory rate when tested with the dialysed brain suspension in the presence of pyruvate or α -ketobutyrate (Table 4). Not only is the net O_2 uptake due to α -keto-acid oxidation

Table 3. *The effect of inorganic phosphate on the respiration of dialysed pigeon brain suspensions in the presence of pyruvate and α -ketobutyrate*

Substrate	Exp.	O ₂ uptake (μl./40 min.)						Net increase %
		No added phosphate			Added phosphate (M/20)			
		Control	With substrate	Net	Control	With substrate	Net	
Pyruvate	1	52.4	100.5	48.1	51.0	149.9	98.9	103
α-Ketobutyrate	2	51.8	79.5	17.7	60.7	117.0	56.3	103
	3	54.1	75.3	21.2	65.4	108.0	42.6	101
	4	52.4	62.5	10.1	51.0	75.0	24.0	138

decreased by arsenate addition, but so also is the residual respiration rate. The cause of this generalized inhibition has not been investigated.

Table 4. *The effect of arsenate on the respiration of dialysed pigeon brain suspensions in the presence of pyruvate and α -ketobutyrate*

Substrate	Exp.	O ₂ uptake (μ l./40 min.)					
		No arsenate added			Arsenate added (M/40)		
		Control	With substrate	Net	Control	With substrate	Net
Pyruvate	1	52.4	100.5	48.1	43.4	80.6	37.2
α -Ketobutyrate	2	49.4	76.1	26.7	38.4	50.5	12.1
	3	52.4	62.5	10.1	43.4	50.3	6.9

The experiments recorded in Table 5 show that although the addition of phosphate to brain suspensions brings about an increased utilization of α -keto-acid in all cases, this increase, which does not exceed 50 %, is perhaps less than might have been expected. The effect of various phosphate concentrations on the utilization of α -keto-acid is shown in Fig. 2 (two typical experiments, using

Fig. 2. The effect of inorganic phosphate concentration on the utilization of α -keto-acids by respiring dialysed pigeon brain suspensions. Curve A, pyruvate; curve B, α -ketobutyrate. The two curves refer to separate experiments.

pyruvate and α -ketobutyrate as substrates). Extrapolation of the curves to zero phosphate concentration shows that even in the complete absence of inorganic phosphate there may be an appreciable utilization of α -ketobutyrate. It is difficult to draw any definite conclusion regarding the utilization of pyruvate at zero phosphate concentration, since the curve is so steep at the point of extrapolation.

Table 5. *The utilization of pyruvate and α -ketobutyrate in the presence and absence of added inorganic phosphate*

Substrate	Exp.	Dialysis hr.	Added phosphate conc. $M \times 10^{-3}$	α -Keto-acid utilization μ mol.		Increase %
				Nil	Phosphate	
Pyruvate	1	3	50	1.99	3.08	55
	2	3	10	2.92	4.31	47
	3	3	10	2.80	4.01	50
	4	3	5	1.55	1.80	16
	5	24	1.2	0.69	0.83	22
α -Ketobutyrate	6	3	5	1.96	2.14	9
	7	7	5	1.51	1.89	25

(2) *The comparative utilization of pyruvic and α -ketobutyric acids by washed preparations of minced pigeon brain and dialysed pigeon brain suspensions in the presence of phosphate*

The quantitative comparison of keto-acid utilization by these two systems has not previously been made. The results have been expressed in terms of μ mol. of α -keto-acid utilized in 40 min. by an amount of enzyme preparation corresponding to 1 g. fresh tissue. The washed mince was found to contain an average of 87.8 % water, as compared with 79.8 % in the fresh brain tissue. Taking this factor into account, the average values found in a number of experiments for α -keto-acid utilization are quoted in Table 6. The figures indicate (1) that pyruvic and α -ketobutyric acids are equally rapidly utilized by washed pigeon brain mince [cf. Long, 1942], (2) that the utilization of α -ketobutyric acid is the same in washed mince and dialysed suspensions, and (3) that pyruvic acid is more rapidly utilized by dialysed suspensions than by washed mince. This last point will be referred to later.

Table 6. *The utilization of pyruvate and α -ketobutyrate by washed pigeon brain mince and dialysed pigeon brain suspensions in the presence of phosphate*

Phosphate concentration—M/20 in suspensions, M/10 in minced brain. Respiration at 28° in air, for 40 min.

	Pyruvic acid		α -Ketobutyric acid	
	Washed mince	Dialysed suspension	Washed mince	Dialysed suspension
No. of determinations	3	14	5	13
α -Keto-acid utilization, μ mol./g. tissue/40 min.	10.4	13.5	9.6	9.6
Standard deviation of mean (ϵ)	0.3	0.9	0.8	0.8

(3) *The effect of adenine nucleotide on the oxidation of pyruvic and α -ketobutyric acids by ground preparations of pigeon brain*

Banga *et al.* [1939b] observed that the omission of adenine nucleotide from the system (dialysed

pigeon brain suspension, pyruvate, inorganic phosphate, adenine nucleotide, fumarate and Mg^{++}) much reduced the rapid respiration rate. The following series of experiments was undertaken to discover what was the action of adenine nucleotide on the simplified system, dialysed brain suspension, pyruvate and inorganic phosphate only. In some experiments pyruvate was replaced by α -ketobutyrate.

The anaerobic oxidation of pyruvic and α -ketobutyric acids in the presence and absence of adenine nucleotide. Adenine nucleotide was found to be without effect on the anaerobic oxidation of either pyruvate or α -ketobutyrate (Thüning technique; Table 7).

Table 7. The anaerobic oxidation of pyruvate and α -ketobutyrate in the presence and absence of adenine nucleotide

M/20 phosphate present throughout						
Activity (100/t)						
Substrate	Exp. 1			Exp. 2		
	Adenine nucleotide			Adenine nucleotide		
	Nil	0.5	Diff.	Nil	1.0	Diff.
Nil	0.5	0.5	0.0	1.0	1.0	0.0
Pyruvate	15.4	12.5	-2.9	11.1	11.1	0.0
	Exp. 3			Exp. 4		
	Adenine nucleotide			Adenine nucleotide		
Nil	0.9	1.1	0.2	0.7	0.8	0.1
Pyruvate	11.8	12.9	1.1	—	—	—
α -Ketobutyrate	—	—	—	10.5	11.4	0.9

t = reduction time (min.).

Table 8. The effect of adenine nucleotide on the respiration of dialysed pigeon brain suspensions in the presence of pyruvate and α -ketobutyrate

Suspension dialysed for 3 hr. Respiration at 28° in air for 40 min. M/20 phosphate present throughout. 1 ml. suspension = 333 mg. fresh brain tissue.

		O ₂ uptake (μl.)					
		No addition			Adenine nucleotide		
		Nil	With sub- strate	Net	Nil	With sub- strate	Net
Pyruvate	1	56.2	135.2	79.0	64.7	291.0	226.3
	2	105.0	196.7	91.7	140.3	370.0	229.7
	3	71.0	119.7	48.7	95.3	272.0	176.7
	4	73.7	130.6	56.9	110.0	325.0	215.0
	5	64.1	151.2	87.1	76.3	271.0	194.7
	6	38.1	93.5	55.4	49.5	235.5	186.0
	7	88.6	159.2	70.6	118.1	305.0	186.9
	8	70.5	126.5	56.0	102.2	265.5	163.3
	9	61.0	132.2	71.2	84.2	319.0	234.8
	10	58.7	141.8	83.1	70.1	284.5	215.4
	Av.	68.7	138.7	70.0	91.1	293.9	202.8
α-Keto- butyrate	11	74.3	142.4	68.1	88.5	154.2	65.7
	12	71.0	126.0	55.0	74.2	133.8	59.6
	13	83.7	130.7	47.0	89.1	139.4	50.3
	14	71.0	113.3	42.3	95.3	151.8	56.5
	15	43.6	70.9	27.3	49.0	95.0	46.0
	Av.	68.7	116.7	48.0	79.2	134.8	55.6

The effect of adenine nucleotide on the O₂ uptake of dialysed pigeon brain suspensions in the presence of pyruvic and α -ketobutyric acids. The suspensions contained added inorganic phosphate. With pyruvate, the average net O₂ uptake due to oxidation of this substrate, in the absence of added adenine nucleotide, was about 70 μl./333 mg. tissue/40 min. (Table 8). The value was almost trebled when adenine nucleotide was added (cf. columns 4 and 7). With α -ketobutyrate as substrate, on the other hand, adenine nucleotide seemed to have little effect on the net O₂ uptake, although in two cases (Exps. 14 and 15) a definite increase was observed.

It should be noted that in order to observe any catalytic effect of adenine nucleotide on pyruvate oxidation by dialysed pigeon brain suspensions, inorganic phosphate must always be included in the respiration medium. In the experiment quoted below the effect of phosphate was studied on the respiration of dialysed pigeon brain suspension with 3×10^{-4} M adenine nucleotide present throughout.

O ₂ uptake (μl.)					
No phosphate added			M/20 phosphate added		
Nil	Pyruvate	Net	Nil	Pyruvate	Net
17.0	35.9	18.9	58.6	209.5	150.9

In the absence of phosphate, the net O₂ uptake due to pyruvate oxidation is of the same order as that obtained under comparable experimental conditions in the absence of both phosphate and adenine nucleotide (Tables 3 and 4). In the presence of phosphate, however, the net O₂ uptake is increased about eightfold.

The effect of adenine nucleotide on the utilization of pyruvic and α -ketobutyric acids by dialysed pigeon brain suspensions. The utilization of pyruvate is approximately doubled when adenine nucleotide is added (Table 9). Under similar conditions (Table 9,

Table 9. The effect of adenine nucleotide on the utilization of pyruvate and α -ketobutyrate by dialysed pigeon brain suspensions

					α -Keto-acid utilization μmol.	
Substrate	Exp.	Dialysis hr.	No addition		Adenine nucleotide	
			Nil	With substrate	Nil	With substrate
Pyruvate	1	3	4.74	10.05	—	—
	2	3	5.81	10.27	—	—
	3	3	4.53	9.90	—	—
	4	3	3.07	7.83	—	—
	5	3	5.32	9.89	—	—
	6	3	4.20	10.18	—	—
	7	3	4.27	9.24	—	—
	8	3	4.04	7.99	—	—
	Av.		4.50	9.43	—	—
	9	3	4.57	4.60	—	—
α -Ketobutyrate	10	3	3.17	4.27	—	—
	Av.		3.87	4.43	—	—
	11	8	1.75	2.43	—	—
	12	8	2.13	3.35	—	—
	Av.		1.94	2.89	—	—

Exps. 9 and 10), there exists no corresponding effect with α -ketobutyrate as substrate. When, however, the brain suspension is dialysed for longer periods, the effect of adenine nucleotide becomes more evident (Table 9, Exps. 11 and 12). This observation, taken in conjunction with the occasional effect of adenine nucleotide on the O_2 uptake of brain suspensions respiring in α -ketobutyrate (Table 8, Exps. 14 and 15), indicates that the nucleotide does play a definite part in α -ketobutyrate oxidation. It appears that the α -ketobutyrate oxidation system is saturated by minimal concentrations of adenine nucleotide, so that after a period of dialysis lasting only 3 hr., sufficient usually remains associated with the enzyme to produce maximal effects.

(4) *The molecular ratios of net O_2 absorbed to α -ketobutyrate utilized for pyruvic and α -ketobutyric acid oxidation in respiring pigeon brain suspensions*

McGowan [1937] found for washed preparations of minced pigeon brain that 1.77 μ mol. O_2 were absorbed per μ mol. pyruvic acid utilized. From R.Q. data [Long & Peters, 1939] the corresponding molecular ratio for α -ketobutyric acid may be calculated. It lies between 0.37 and 0.45. This ratio has now been determined experimentally by estimation of α -ketobutyric acid [Long, 1942], and for comparison with respiring suspensions of pigeon brain; the values obtained are included in Table 10.

Table 10. *The molecular ratio of net O_2 absorbed to α -ketobutyric acid utilized by washed preparations of minced pigeon brain*

Washed minced pigeon brain prepared according to Long & Peters [1939]. Initial concentration of α -ketobutyrate, M/50.

Exp.	Temp. of respiration ° C.	O_2 uptake (μ mol./g./hr.)			α -Keto-butyric acid utilization (μ mol./g./hr.)	O_2/α -ketobutyrate (molecular ratio)
		Control	α -Keto-butyrate	Net		
1	28	6.92	10.18	3.26	9.75	0.33
2	28	6.63	9.16	2.53	6.14	0.41
3	28	7.01	10.38	3.37	8.51	0.40
4	28	9.26	13.55	4.29	12.88	0.33
Av.		7.45	10.82	3.37	9.32	0.36
5	38	10.68	19.69	9.01	21.23	0.42
6	38	9.96	20.22	10.26	27.02	0.38
7	38	8.65	17.84	9.19	21.89	0.42
Av.		9.76	19.25	9.49	23.38	0.41

The O_2 /pyruvate ratio for dialysed pigeon brain suspensions in the presence and absence of added adenine nucleotide. Adenine nucleotide addition approximately doubles the pyruvate utilization and trebles the net O_2 uptake (cf. Tables 8 and 9). Thus it might be inferred that pyruvate oxidation is more complete in the presence of adenine nucleotide than in its absence. Table 11 fully confirms this expectation, since the O_2 /pyruvate ratio averages 0.70 in

the absence of added adenine nucleotide, and 0.96 in its presence. For that fraction of the pyruvate utilization which is specifically catalysed by adenine nucleotide, the molecular ratio, if it be considered separately, is 1.20 μ mol. O_2 / μ mol. pyruvic acid. It should be noted that this value is much smaller than the corresponding figure obtained by McGowan [1937] for pyruvate oxidation in the minced tissue (O_2 /pyruvate = 1.77).

Table 11. *The molecular ratio O_2 /pyruvate for dialysed pigeon brain suspensions in the presence and absence of added adenine nucleotide (A.N.)*

Exp.	Net O_2 uptake μ mol.			Pyruvic acid utilization μ mol.			O_2 /pyruvate		
	Control	A.N.	Diff.	Control	A.N.	Diff.	Control	A.N.	Diff.
1	3.48	9.94	6.46	4.74	10.05	5.31	9.73	0.99	1.22
2	4.02	9.82	5.80	5.81	10.27	4.46	0.69	0.96	1.30
3	2.50	9.45	6.95	4.53	9.90	5.37	0.55	0.96	1.29
4	2.43	8.17	5.74	3.07	7.83	4.76	0.79	1.04	1.21
5	3.10	8.20	5.10	5.32	9.89	4.57	0.58	0.83	1.12
6	3.13	9.73	6.60	4.20	10.18	5.98	0.75	0.96	1.10
7	3.65	9.42	5.77	4.37	9.24	4.97	0.84	1.02	1.18
Av.	3.19	9.25	6.06	4.56	9.62	5.06	0.70	0.96	1.20

In considering the effect of adenine nucleotide on α -ketobutyrate oxidation (Table 9), it was concluded that after 3 hr. dialysis some adenine nucleotide still remains associated with the enzyme. Thus the O_2 /pyruvate ratio would be much lower than 0.70 in the complete absence of adenine nucleotide, were it possible to realize this condition. Quite apart from this consideration, however, the mechanism of pyruvate oxidation is undoubtedly different in the presence, and in the absence, of adenine nucleotide, as will be shown later.

The O_2/α -ketobutyrate ratio for dialysed suspensions of pigeon brain. For washed preparations of minced pigeon brain, the O_2/α -ketobutyrate ratio (Table 10) averages about 0.39, a figure in agreement with previous work on the R.Q. for α -ketobutyrate oxidation under similar conditions. In Table 12 are recorded some corresponding values for this ratio, using dialysed pigeon brain suspensions. Series A (Table 12) refers to determinations carried out under the usual conditions. It will be seen that the O_2/α -ketobutyrate ratio is very similar to that obtained with the minced brain preparations.

It will also be noted, however, that if the brain is ground under rigorously ice-cold conditions, as recommended by Banga *et al.* [1939b], then the O_2/α -ketobutyrate ratio is consistently above 0.5 (series B, Table 12). This is the first definite indication that under certain conditions α -ketobutyrate can be oxidized to an extent greater than corresponds with oxidative decarboxylation. There seems to be little doubt that residual adenine nucleotide is responsible for this, since pyruvate oxidation

under the same conditions is very great in the absence of added adenine nucleotide, and addition of the latter does not produce the usual large catalytic effect (Table 13). A possible explanation is that adenine nucleotide exists in a combined form in the fresh brain, and that this combination, although stable under ice-cold conditions, breaks down at room temperature in the absence of an organized cell structure.

Table 12. *The O_2/α -ketobutyrate ratio for pigeon brain suspensions*

Exp.	O_2 uptake (μ mol.)			α -Keto-butyric acid utilization μ mol.	O_2/α -keto-butyrate
	Control	α -Keto-butyrate	Net		
Series A					
1	2.36	4.08	1.72	4.16	0.41
2	3.80	5.63	1.83	4.20	0.44
3	2.21	3.13	0.92	2.57	0.36
Av.	2.79	4.28	1.49	3.64	0.41
Series B					
4	2.77	4.81	2.04	2.35	0.87
5	3.30	5.73	2.43	4.26	0.57
6	2.33	4.91	2.58	4.11	0.63
7	2.67	5.57	2.90	5.09	0.57
Av.	2.77	5.26	2.49	3.95	0.64

Table 13. *The O_2 uptake of dialysed pigeon brain suspensions, prepared under rigorously ice-cold conditions, respiring in pyruvic acid*

Exp.	O_2 uptake (μ l./40 min.)					
	No adenine nucleotide added			Adenine nucleotide added		
	Control	Pyruvate	Net	Control	Pyruvate	Net
1	83.7	250.8	167.1	89.1	281.3	192.9
2	111.3	215.5	104.2	151.8	304.7	152.9
3	138.5	315.5	177.0	—	—	—
4	93.4	237.0	143.6	—	—	—
5	92.7	262.0	169.3	—	—	—
6	86.6	245.0	158.4	—	—	—
7	79.9	207.0	127.1	—	—	—
8	57.1	217.0	159.9	—	—	—
9	80.9	268.5	187.6	—	—	—
Av.	90.5	246.5	156.0	—	—	—
	(cf. 67.1)	134.2	67.1	for 23 expts. under normal conditions (Tables 8 and 14))		

(5) *The R.Q. of dialysed pigeon brain suspensions in the presence of pyruvic and α -ketobutyric acids*

For washed preparations of minced pigeon brain, McGowan [1937] obtained an average value for the R.Q. in pyruvate of 1.30, after allowing for residual gas exchange. For the R.Q. of α -ketobutyrate oxidation under similar conditions, the average value 2.37 was obtained [Long & Peters, 1939]. Both figures agree well with other experimental facts.

As might be expected, the more complete oxidation of pyruvate by dialysed suspensions of pigeon brain which is observed in the presence of adenine nucleotide is accompanied by a lowered R.Q. Furthermore, the similarity between the O_2/α -ketobutyrate ratios for washed pigeon brain mince and

dialysed suspensions under normal conditions, leads, as expected, to identical values for the R.Q. The R.Q. determinations were made by the indirect method of Long & Peters [1939].

The R.Q. for pyruvate oxidation by dialysed pigeon brain suspensions in the presence of inorganic phosphate and absence of added adenine nucleotide. Many factors contribute to the variation in the net O_2 uptakes observed with dialysed pigeon brain suspensions respiring in presence of pyruvate and inorganic phosphate only. The most important is the temperature of the grinding process, already mentioned: under ice-cold conditions high values were obtained. Other factors which tend to produce high values are (a) the use of less permeable dialysis sacs, (b) shorter duration of the dialysis, (c) shorter periods of grinding, and (d) lower temperature of dialysis. While (b) and (c) can easily be reproduced, it is more difficult to control (a) and (d); hence some scatter of the results is inevitable. The variable throughout appears to be the amount of residual adenine nucleotide still associated with the enzyme preparation.

Table 14. *The R.Q. for pyruvate oxidation by dialysed pigeon brain suspensions in the presence of inorganic phosphate only*

M/20 phosphate present throughout. 40 min. respiration in air at 28°.

Exp.	O_2 uptake (μ l.)			CO_2 production (μ l.)			R.Q.	
	Control	Pyruvate	Net	Control	Pyruvate	Net	Control	Net
Series A								
1	66.6	106.3	39.7	51.6	143.8	92.2	0.78	2.35
2	65.6	112.6	47.0	49.0	163.6	114.6	0.75	2.44
3	58.4	107.2	48.8	53.1	146.0	92.9	0.91	1.90
4	69.3	123.1	53.8	66.1	168.7	102.6	0.95	1.91
5	70.5	126.5	56.0	42.5	152.1	109.6	0.60	1.96
6	89.9	158.2	68.3	73.3	203.6	130.3	0.82	1.91
Av.	70.0	122.3	52.3	55.9	163.0	107.1	0.80	2.05
Series B								
7	50.1	118.7	68.6	31.1	155.6	124.5	0.62	1.82
8	80.3	149.7	69.4	77.3	202.0	124.7	0.96	1.80
9	63.2	134.2	71.0	47.6	177.8	130.2	0.75	1.84
10	74.7	149.5	74.8	46.7	206.9	160.2	0.63	2.14
11	54.1	129.8	75.7	47.1	179.0	131.9	0.87	1.74
12	43.7	125.8	82.1	36.3	182.5	146.2	0.83	1.78
13	95.8	194.0	98.2	72.8	263.8	191.0	0.76	1.94
Av.	66.0	143.1	77.1	51.3	195.4	144.1	0.78	1.87

In Table 14 are recorded determinations of the R.Q. for pyruvate oxidation by dialysed pigeon brain suspensions, in the presence of phosphate and absence of added adenine nucleotide. Series A and B, respectively, include those experiments in which smaller and larger net O_2 uptakes have been observed. In Series A, a low value for the net O_2 uptake is accompanied by a high value for the net R.Q. In Series B, the higher average net O_2 uptake corresponds with a lower net R.Q. Since there is reason to believe that appreciable amounts of re-

sidual adenine nucleotide are active in Series B, the conclusion may be drawn that this activator is responsible for the lowering of the net R.Q. Complete confirmation for this is provided in the next section. The average residual gas exchange is the same in both Series A and B.

The R.Q. for pyruvate oxidation by dialysed pigeon brain suspensions in the presence of inorganic phosphate and adenine nucleotide. When excess adenine nucleotide is added to the enzyme system, the extent of scatter among a series of R.Q. determinations for pyruvate oxidation should be much reduced, since the most important variable, namely adenine nucleotide present in suboptimal concentration, is no longer operative. Table 15 shows the individual figures for eight determinations of the R.Q. under these conditions. The net O₂ uptake due to pyruvate oxidation is fairly constant, and amounts to nearly three times the average figure (64.7 μ l.) observed in the absence of added adenine nucleotide (Table 14). The effect of adenine nucleotide is to lower the net R.Q. appreciably.

Table 15. *The R.Q. for pyruvate oxidation by dialysed pigeon brain suspensions in the presence of adenine nucleotide and inorganic phosphate*

$3 \times 10^{-4} M$ adenine nucleotide present throughout. Other conditions similar to those in Table 14.

Exp.	O ₂ uptake (μ l.)			CO ₂ production (μ l.)			R.Q.	
	Con- trol	Pyru- vate	Net	Con- trol	Pyru- vate	Net	Con- trol	Net
1	63.5	254.0	190.5	45.7	366.7	321.0	0.72	1.69
2	68.4	264.6	196.2	47.1	383.6	336.5	0.69	1.72
3	103.9	296.5	192.6	73.9	380.7	306.8	0.70	1.59
4	90.2	270.3	180.1	66.7	361.5	294.8	0.74	1.64
5	122.9	284.0	161.1	87.7	364.7	277.0	0.71	1.72
6	81.0	257.5	176.5	52.2	345.5	293.3	0.65	1.66
7	125.0	316.5	191.5	79.9	399.2	319.3	0.64	1.66
8	112.0	288.5	176.5	79.2	361.3	282.1	0.71	1.60
Av.	95.9	279.0	183.1	66.6	370.4	303.8	0.69	1.66

The R.Q. for that part of the pyruvate oxidation in dialysed pigeon brain suspensions which is specifically catalysed by adenine nucleotide. In order to obtain the R.Q. for the adenine nucleotide-catalysed oxidation of pyruvate, it is best to determine the individual R.Q.'s in the presence, and in the absence, of added adenine nucleotide and of pyruvate, simultaneously on the same sample of dialysed enzyme. In practice this is difficult, since with duplicate estimations 24 Barcroft manometers would be required. The general method adopted here has been to use those results shown in Tables 14 and 15, which give the R.Q. values for pyruvate oxidation in the presence, and in the absence, of added adenine nucleotide. Although the R.Q. in the absence of added adenine nucleotide varies widely, the scatter among the individual values, presumably due to variable suboptimal quantities of adenine nucleo-

tide in the enzyme preparation, should give the same net effect of adenine nucleotide for both series, A and B. This was the case (Table 16).

Table 16. *The R.Q. for that part of the pyruvate oxidation which is specifically catalysed by adenine nucleotide*

	Net O ₂ uptake (μ l.) due to pyruvate			Net CO ₂ production (μ l.) due to pyruvate			Net R.Q.
	No addi- tion	Added A.N.	Net	No addi- tion	Added A.N.	Net	
Series A	52.3	183.1	130.8	107.1	303.8	196.7	1.50
Series B	77.1		106.0	144.1		159.7	1.51
Av.	64.7	183.1	118.4	125.6	303.8	178.2	1.50

The R.Q. for α -ketobutyrate oxidation by dialysed pigeon brain suspensions. Under normal conditions, the net R.Q. for α -ketobutyrate oxidation by dialysed pigeon brain suspensions is identical with that previously found by Long & Peters [1939] for washed preparations of minced pigeon brain. The average value (2.38) for three determinations (Table 17, Series A) agrees closely with the figure 2.37 for minced brain. This is not unexpected, in view of the similarity of the ratio O₂/ α -ketobutyrate for the two enzyme preparations.

When the R.Q. for α -ketobutyrate oxidation was determined with dialysed suspensions prepared under rigorously ice-cold conditions, it was found to be considerably lower than the normal value (Table 17, Series B). This is in accordance with the earlier observation that the O₂/ α -ketobutyrate ratio is markedly increased under these conditions, as shown in Table 12.

Table 17. *The R.Q. for α -ketobutyrate oxidation by dialysed pigeon brain suspensions*

$M/20$ phosphate present throughout. 40 min. respiration at 28° in air.

Exp.	O ₂ uptake (μ l.)			CO ₂ production (μ l.)			R.Q.	
	Con- trol	α -Keto- buty- rate	Net	Con- trol	α -Keto- buty- rate	Net	Con- trol	Net
Series A								
1	85.0	125.7	40.7	59.1	156.0	96.9	0.70	2.38
2	69.7	104.1	34.4	60.5	135.2	74.7	0.87	2.17
3	76.5	115.3	38.8	52.0	151.4	99.4	0.68	2.56
Av.	77.0	115.0	38.0	57.2	147.5	90.3	0.74	2.38
Series B								
4	59.7	124.7	65.0	34.9	143.6	108.2	0.59	1.67
5	48.2	96.5	48.3	21.1	106.2	85.1	0.44	1.76
Av.	53.9	110.6	56.7	28.0	124.7	96.7	0.52	1.71

(6) *The amounts of free and combined CO₂ formed during the oxidation of pyruvic acid by dialysed pigeon brain suspensions*

In the experiments already described on the determination of the R.Q. values for pyruvate oxida-

tion, the total CO_2 produced was obtained by the summation of the amounts of free and bound CO_2 . The free CO_2 formed was estimated by the difference in gas absorption in the presence and absence of KOH-papers; the bound CO_2 was found by liberation of CO_2 by acid at the beginning and end of the respiration period. The usual controls in the absence of substrate were made throughout. It has been found that under any given set of experimental conditions both the total and relative amounts of free and bound CO_2 formed are remarkably constant, and that the omission of substrate, or the addition of adenine nucleotide, is accompanied by a new set of values (Table 18).

A pyruvate ion utilized by oxidative decarboxylation

must be replaced by an acetate ion and 1 mol. free CO_2 . A pyruvate ion completely oxidized,

however, is replaced by a bicarbonate ion and 2 mol. CO_2 . Thus in the first example all the CO_2 formed appears as gas, and in the second case two-thirds appear as gas and one-third as bicarbonate.

The experiments would best be performed in bicarbonate buffer saturated with O_2 containing

5% CO_2 , but in fact the medium used was initially free from bicarbonate and the respiration was conducted in air. It has been observed, however, that during the equilibration period a considerable amount of bicarbonate is formed (about 70 and 40 μl . in the presence and absence of pyruvate, respectively). During the respiration period bicarbonate is formed much less rapidly, whereas the gaseous CO_2 formed is very considerable. Apparently equilibrium between the medium and its atmosphere has been reached at the beginning of the respiration period. Measurement of the amounts of CO_2 formed as gas and bicarbonate under different conditions are therefore possible; for pyruvate oxidation in the presence and absence of added adenine nucleotide the results are given in Table 18. The figures for that part of the pyruvate oxidation which is specifically catalysed by adenine nucleotide are shown in Table 19, from which it will be seen that about two-thirds of the CO_2 produced appears as gas (CO_2 gas formation) and one-third remains in solution (bicarbonate formation). These results are discussed later.

Table 19. CO_2 production as gas and bicarbonate from that part of the pyruvate which is utilized specifically by adenine nucleotide (A.N.) catalysis

	Distribution of CO_2 production ($\mu\text{l}/40$ min.)					
	CO_2 gas formation			Bicarbonate formation		
	No addition	Added A.N.	Net	No addition	Added A.N.	Net
Series A	76.7	211.4	134.7	30.4	92.4	62.0
Series B	102.4		109.0	41.7		50.7
Av.	89.5	211.4	121.9	36.1	92.4	56.3

Table 18. CO_2 distribution as gas and bicarbonate during the oxidation of pyruvate by dialysed suspensions of pigeon brain

Exp.	Distribution of total CO_2 production ($\mu\text{l}/40$ min.)					
	CO_2 gas formation			Bicarbonate formation		
	Control	Pyruvate	Net	Control	Pyruvate	Net
No adenine nucleotide added, Series A						
1	47.0	108.1	61.1	4.6	35.7	31.1
2	42.6	123.7	81.1	6.4	39.9	33.5
3	40.8	110.1	69.3	12.3	35.9	23.6
4	49.0	125.3	76.5	17.1	43.2	26.1
5	35.6	113.3	75.7	6.9	40.8	33.9
6	59.7	156.0	96.3	13.6	47.6	34.0
Av.	45.8	122.5	76.7	10.1	40.5	30.4
No adenine nucleotide added, Series B						
7	26.7	115.7	89.0	4.4	39.9	35.5
8	58.3	147.2	88.9	19.0	54.8	35.8
9	41.2	132.7	91.5	6.4	45.1	38.7
10	45.6	156.3	110.7	1.1	50.6	49.5
11	42.6	138.3	95.7	4.5	40.7	36.2
12	33.8	144.5	110.7	2.5	38.0	35.5
13	56.1	186.1	130.0	16.7	77.7	61.0
Av.	43.5	145.9	102.4	7.8	49.5	41.7
Adenine nucleotide added						
1	42.1	262.1	220.0	3.6	104.6	101.0
2	40.0	269.1	229.1	7.1	114.5	107.4
3	63.1	275.6	212.5	10.8	105.1	94.3
4	57.1	259.9	202.8	9.6	101.6	92.0
5	69.6	269.0	199.4	18.1	95.7	77.6
6	45.8	251.3	205.5	6.4	94.2	87.8
7	74.8	295.1	220.3	5.1	104.1	99.0
8	69.4	271.3	201.9	9.8	90.0	80.2
Av.	57.8	269.2	211.4	8.8	101.2	92.4

DISCUSSION

The collective data presented in this paper, although still incomplete, allow a fairly detailed comparison to be made of the pyruvic acid oxidation processes occurring in minced and in ground pigeon brain. Knowledge of the behaviour of α -ketobutyric acid towards the same enzyme preparations has provided the initial clues to the problem. It has been shown (Table 6) that α -ketobutyric acid is equally rapidly utilized by minced and by ground pigeon brain under similar conditions. Furthermore, identical values have been obtained for the O_2/α -ketobutyrate ratio and the R.Q. for the two enzyme preparations. It therefore seems justifiable to draw the conclusion that those enzymes responsible for oxidative decarboxylation and anaerobic dismutation, reactions which are known to utilize α -ketobutyric acid in minced brain, are also operative in the ground tissue.

Pyruvic and α -ketobutyric acids have previously been shown [Long, 1942] to be equally rapidly utilized by respiring minced pigeon brain. In

ground preparations (suspensions), on the other hand, pyruvate has now been found to be more rapidly utilized than α -ketobutyrate. This observation can be explained in the following manner. It has been shown in the experimental section that under certain conditions, such as the rigorously ice-cold grinding of the brain, the tissue preparation contains appreciable amounts of adenine nucleotide, even after 3 hr. dialysis, and it is probable that even under the most favourable conditions obtainable, suboptimal amounts of adenine nucleotide are always associated with the tissue enzyme. This fact becomes important when it is borne in mind that pyruvate utilization by ground preparations of pigeon brain is very susceptible to catalytic amounts of adenine nucleotide (Table 9), whereas α -ketobutyrate utilization is not nearly so sensitive, a definite effect in this case being difficult to detect. It seems certain, therefore, that if adenine nucleotide could be excluded completely from the system, pyruvic acid utilization would be much lower than that normally observed, and pyruvic and α -ketobutyric acids would probably be equally rapidly utilized. When the ground tissue enzyme was thoroughly extracted with hypotonic saline, as in the anaerobic oxidation experiments, the reduction time of methylene blue was found to be the same with either pyruvate or α -ketobutyrate as substrate (Tables 1 and 2). This is evidence in favour of equal rates of oxidation of pyruvic and α -ketobutyric acids by ground preparations of pigeon brain in the presence of a phosphate buffer and in the complete absence of adenine nucleotide.

The reactions involved in the oxidation of pyruvic acid by dialysed pigeon brain suspensions, respiring in phosphate buffer, must now be considered. McGowan [1937], using minced pigeon brain, found the O_2 /pyruvate ratio to be 1.77 mol. O_2 absorbed/mol. pyruvic acid utilized. The corresponding ratio for dialysed suspensions, under comparable conditions, has been found to be 0.70 (Table 11). This indicates that pyruvate is much less completely oxidized by ground brain suspensions. Even this value, however, is too high, since some of the pyruvate is utilized by residual adenine nucleotide catalysis, which requires 1.20 mol. O_2 /mol. pyruvic acid. The true figure in the complete absence of adenine nucleotide is therefore probably much lower, and is no doubt very similar to the O_2 / α -ketobutyrate ratio (0.41). This is confirmed by the net R.Q. values. McGowan [1937] reported the R.Q. for pyruvate oxidation in minced brain to be 1.30. In this work the corresponding figure for dialysed suspensions was found to vary between 1.74 and 2.44. Furthermore, low values for the net O_2 uptake due to pyruvate oxidation are accompanied by high R.Q. values. Allowing for the pyruvate which is oxidized by residual adenine nucleotide catalysis,

the true R.Q. in the complete absence of adenine nucleotide would probably be similar to the R.Q. for α -ketobutyrate oxidation under the same conditions (2.38). Both lines of evidence strongly suggest that in the complete absence of adenine nucleotide, pyruvate and α -ketobutyrate oxidations would proceed according to the same reactions, i.e. oxidative decarboxylation and anaerobic dismutation. Presumably the process of grinding the brain tissue, and the consequent destruction of the cell structure, alters the conditions necessary for the complete oxidation of pyruvate to CO_2 and water. The latter occurs to the extent of about 70% of the pyruvate utilized by respiring minced brain [Long, 1938]. This fraction of the pyruvate which disappears is probably utilized by the alternative process of oxidative decarboxylation when the oxidation is carried out in the presence of ground preparations of pigeon brain.

If these deductions are correct, namely that dialysed pigeon brain suspensions, respiring in a phosphate-buffered inorganic medium, utilize pyruvic acid by oxidative decarboxylation and anaerobic dismutation exclusively, pyruvic acid certainly undergoes a totally different type of oxidation in the presence of adenine nucleotide. So far there is no evidence that adenine nucleotide exerts any influence on either of these reactions, and presumably adenine nucleotide opens up a new reaction path for pyruvic acid. This view is supported by the increased utilization of pyruvic acid when adenine nucleotide is added, and the completely different set of values obtained for the O_2 /pyruvate ratio and the R.Q. for that part of the pyruvate oxidation which is specifically catalysed by adenine nucleotide.

If that part of the pyruvate utilization which is specifically catalysed by adenine nucleotide only be considered, the O_2 /pyruvate ratio of 1.20 and R.Q. value of 1.50 indicate that 1.80 mol. CO_2 are produced by the oxidation of 1 mol. pyruvic acid, i.e. 60% of the carbon of pyruvic acid is converted into CO_2 . So far then the reaction can be expressed in the form

Further, if the CO_2 produced in the form of bicarbonate is one-third of the total CO_2 (Table 19), then 6 mol. CO_2 are formed as gas and 3 mol. as bicarbonate. In order to balance the total acidic groups taking part in the reaction, 2 mol. fixed acid must also be produced:

The constitution of the fixed acid or acids is not known. The three most likely possibilities are that

there are (1) two 3-carbon monobasic acids, (2) one 2-carbon and one 4-carbon monobasic acid, (3) one 6-carbon dibasic acid. Allowing for the 4 or 5 mol. water formed, there are various possibilities, which would fit the equation perfectly. Speculation is here of little value, and characterization and determination of the fixed acids must be awaited.

SUMMARY

1. Inorganic phosphate or arsenate is required for the anaerobic oxidation of both pyruvate and α -ketobutyrate by ground preparations of pigeon brain with methylene blue as H-acceptor.

2. Inorganic phosphate stimulates the O_2 uptake and the utilization of pyruvate and of α -ketobutyrate by respiring preparations of ground pigeon brain (suspensions). Arsenate is slightly inhibitory.

3. α -Ketobutyrate is utilized at the same rate under comparable conditions by minced and ground preparations of pigeon brain. Pyruvate is more rapidly utilized by ground than by minced brain. This apparent discrepancy is discussed.

4. Adenine nucleotide markedly increases the

net O_2 uptake and the pyruvate utilization of pigeon brain suspensions when inorganic phosphate is present. It stimulates α -ketobutyrate oxidation under certain conditions, but normally its influence is difficult to detect.

5. Normally the O_2/α -ketobutyrate ratio and the R.Q. for α -ketobutyrate oxidation is the same for brain suspensions as for minced brain.

6. The O_2 /pyruvate ratio, R.Q. and CO_2 distribution as gas and bicarbonate have been determined in the presence and absence of added adenine nucleotide. For that part of the pyruvate oxidation specifically catalysed by adenine nucleotide, the following values have been obtained: O_2 /pyruvate ratio, 1.20; R.Q. 1.50; gaseous CO_2 /bicarbonate CO_2 , 2.0.

7. The mechanisms of pyruvate oxidation in minced and ground pigeon brain preparations are compared. The mode of oxidation of pyruvate by pigeon brain suspensions in the presence of inorganic phosphate and adenine nucleotide is discussed.

The author thanks Prof. D. C. Harrison for his interest in the work.

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The Biological Value of Mixed Proteins in Food Served in Royal Air Force Airmen's Messes

By T. F. MACRAE (R.A.F.V.R.), KATHLEEN M. HENRY AND S. K. KON,
*From the R.A.F. Institute of Pathology and Tropical Medicine and the
 National Institute for Research in Dairying, University of Reading*

(Received 14 December 1942)

While much information is available on the biological value of isolated proteins and of mixed proteins of individual foodstuffs, relatively little is known about the biological value of the proteins (nitrogen) of mixed human dietaries. Supplementary relationships between proteins make it naturally impossible to calculate such values from those of separate constituents. Hence an assess-

ment of a whole diet is of greater practical importance than the evaluation of the contribution of its different components.

The experiments described below were carried out to determine the biological value of the combined proteins contained in the food served to airmen at four Royal Air Force Stations in this country during October–December, 1941.