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Effects of Ischaemia on Content of Metabolites in Rat Liver and Kidney *in vivo*

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1. The time-course of changes in content of intermediates of glycolysis in rat liver and kidney cortex after severance of blood supply was investigated. 2. The decline in content of ATP was more rapid in kidney (1.7–0.5 μmol/g in 30s) than in liver (2.7–1.6 μmol/g in 60s). In both tissues AMP and P_i accumulated. 3. Net formation of lactate was 1.7 μmol/g during the second minute of ischaemia in liver from well-fed rats, 1.1 μmol/g in liver from 48h-starved rats, and about 1.0 μmol/g during the first 30s of ischaemia in kidney. Net formation of α-glycerophosphate was rapid, especially in liver. 4. In kidney the concentration of β-hydroxybutyrate rose, but that of α-oxoglutarate and acetoacetate decreased. 5. In both organs the concentrations of fructose diphosphate and triose phosphates increased during ischaemia and those of other phosphorylated C_3 intermediates decreased. 6. The concentration of the hexose 6-phosphates rose rapidly during the first minute of ischaemia in liver, but decreased during renal ischaemia. 7. In kidney the content of glutamine fell after 2min of ischaemia, and that of ammonia and glutamate rose. 8. The redox states of the cytoplasmic and mitochondrial NAD couple in kidney cortex were similar to those in liver. 9. The regulatory role of glycogen phosphorylase, pyruvate kinase and phosphofructokinase is discussed in relation to the observed changes in the concentrations of the glycolytic intermediates.

Lowry, Passonneau, Hasselberger & Schulz (1964) were the first to investigate the time-course of the changes in content of intermediates of glycolysis and related metabolites that occurred in ischaemic tissue *in vivo*. They determined intermediates in mouse brain at various times after decapitation and obtained quantitative information on the increased glycolytic flux during ischaemia and on the enzymic steps controlling the rate of brain glycolysis. Analogous experiments have been carried out on rat heart by Kraupp *et al.* (1966) and on kidney by Needleman, Passonneau & Lowry (1968). Changes in non-glycolytic metabolites in rat liver after ischaemia have been investigated by Brosnan, Krebs & Williamson (1970).

In liver and kidney cortex, anaerobiosis causes an acceleration of glycolysis, which may be associated with inhibition of gluconeogenesis (see review by Newsholme & Gevers, 1967). In the present work the time-course of the changes in the contents of intermediates of glycolysis and related processes in ischaemic liver and kidney was investigated, to elucidate the enzymic sites at which glycolysis is accelerated by anoxia, and the concentrations of enzymic modifiers, such as adenine nucleotides. The measurements also clarify some of the immediate metabolic consequences of manipulations that potentially involve hypoxia, such as removal of organs, or operative procedures, e.g. before organ perfusion.

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EXPERIMENTAL

Freeze-clamping of liver or kidney. Albino Wistar rats (250–330g) were killed by cervical dislocation (with minimal stress to the animal). Immediately afterwards either the liver was exposed through a ventral incision, or the left kidney through a flank incision. In liver experiments, one lobe was cut off and immediately freeze-clamped at the temperature of liquid N_2 (Wollenberger, Ristau & Schoffa, 1960). Blood vessels to the rest of the liver were then cut, the organ was left *in situ*, and further lobes were removed and freeze-clamped after 1, 2 and 5 min. In the comparable kidney experiments, the left kidney was pulled free of vessels at the hilum and freeze-clamped. The right kidney was then separated from its

blood supply and left *in situ* for 30, 60 or 120s before being freeze-clamped. 'Initial' samples of liver or kidney were frozen within 8–12s of cervical dislocation, and within 5s of severance of blood vessels. The initial samples were collected as rapidly as possible; for this reason liver and kidney were never taken from the same rat.

When left renal venous blood was to be sampled, as well as the right kidney, rats were anaesthetized with a minimal intraperitoneal dose of commercial Nembutal (about 12mg/300g rat). The abdomen was then opened, and heparin (200i.u.) was injected into the inferior vena cava; after 1min blood (1ml) was taken from the left renal vein and immediately mixed with 1ml of 12% (w/v) $HClO_4$ acid at 0°C. The right kidney was then decapsulated *in situ*; after a further 1min (and only when there was a strong aortic pulse and good oxygenation of the arterial blood) the renal vessels were cut, and the kidney was rapidly freeze-clamped. The interval between freeze-clamping and cutting of the vessels was less than 2s, and a rush of blood from the renal artery provided further evidence that the organ had not been ischaemic.

A difficulty in interpretation of metabolite concentrations in whole organs arises from heterogeneity of tissues. To obtain information on concentrations of metabolites in hypoxic medulla, inner plus outer medulla were cut away from cortex during the 2min after the severance of blood supply, and then dropped into liquid N_2. In addition, to test the extent to which metabolite concentrations in the whole kidney reflect those of the cortex, cortex was largely freed of medulla as follows. The whole kidney was placed on the lower half of the cooled tongs, which were then closed with intermediate strength. About half the tissue, including the area of medulla, was broken off with a spatula while frozen, and discarded. The remaining half, which was extracted as usual, is referred to as 'cortex' in the text.

Preparation of tissue extracts. Frozen tissue, still in liquid N_2, was ground with a pestle and mortar. The powder was weighed into a centrifuge tube (cooled in liquid N_2) and homogenized in 4vol. of 6% (w/v) $HClO_4$. After 30min at 0°C extracts were centrifuged at 35000*g* for 15min at 0°C. Supernatants were decanted and neutralized with a measured volume of 30% KOH (see Gevers & Krebs, 1966). After a further 30min at 0°C, $KClO_4$ was removed by centrifugation, and the supernatant was used for determination of metabolites. Blood samples were similarly treated.

Analytical methods. Glucose, pyruvate, lactate, malate, aspartate, α-oxoglutarate, glutamate, triose phosphate, fructose 1,6-diphosphate, α-glycerophosphate, glucose 6-phosphate, fructose 6-phosphate and adenine nucleotides were determined as described by Gevers & Krebs (1966). Phosphoenolpyruvate and 2- and 3-phosphoglycerate were determined by the method of Czok & Eckert (1963), glucose 1-phosphate as described by Bergmeyer & Klotzsch (1965), citrate with citrate lyase (Gruber & Moellering, 1966), P_i as phosphomolybdate (Martin & Doty, 1949), ketone bodies and alanine as described by Williamson, Lopes-Vieira & Walker (1967*b*), ammonia with glutamate dehydrogenase (Kirsten, Gerez & Kirsten, 1963), and glutamine as glutamate after hydrolysis with *Escherichia coli* glutaminase (Lund, 1969). Glycogen was determined as described by Krebs, Bennett, de Gasquet, Gascoyne & Yoshida (1963).

Rejection of samples. The [lactate]/[pyruvate] ratio was

Table 1. *Contents of intermediates of glycolysis and of adenine nucleotides in liver of well-fed rats after severance of blood supply*

The vessels to the liver of the well-fed rats were cut and the organ was left *in situ* (at 37°C). After various times lobes were frozen and metabolites determined in the extracts. For experimental details see the text. Results are the means ±S.E.M. of three experiments. Abbreviations: PEP, phosphoenolpyruvate; 2PG, 2-phosphoglycerate; 3PG, 3-phosphoglycerate; Triose-P, sum of triose phosphates; αGP, α-glycerophosphate; FDP, fructose 1,6-diphosphate; F6P, fructose 6-phosphate; G6P, glucose 6-phosphate.

Time after cutting vessels (s)	Content of metabolites (μmol/g of fresh liver)														[ATP][AMP]/[ADP]²
	Lactate	Pyruvate	PEP	2PG	3PG	Triose-P	αGP	FDP	F6P	G6P	P_i	ATP	ADP	AMP	Total adenine nucleotides	
0	0.45 ±0.21	0.034 ±0.01	0.099 ±0.02	0.034 ±0.005	0.28 ±0.05	0.017 ±0.005	0.14 ±0.02	<0.010	0.05 ±0.01	0.16 ±0.02	3.82 ±0.36	2.74 ±0.23	1.34 ±0.16	0.26 ±0.04	4.34	0.40
60	1.76 ±0.50	0.022 ±0.01	0.058 ±0.005	0.022 ±0.005	0.21 ±0.03	0.030 ±0.005	0.73 ±0.11	0.013 ±0.005	0.16 ±0.03	0.59 ±0.07	5.48 ±0.38	1.61 ±0.19	1.81 ±0.09	0.85 ±0.07	4.27	0.42
120	3.44 ±0.27	0.023 ±0.01	0.035 ±0.005	<0.010	0.14 ±0.01	0.042 ±0.015	1.60 ±0.06	0.021 ±0.0	0.14 ±0.02	0.53 ±0.10	5.78 ±0.35	1.39 ±0.17	1.66 ±0.09	0.84 ±0.05	3.89	0.42
300	6.56 ±0.71	0.022 ±0.01	0.014 ±0.005	<0.010	0.05 ±0.01	0.042 ±0.02	2.38 ±0.19	0.026 ±0.01	0.20 ±0.07	0.80 ±0.28	7.48 ±0.33	0.63 ±0.03	1.43 ±0.07	1.38 ±0.16	3.44	0.43

used as an indicator of adequate oxygenation of kidneys rapidly removed and freeze-clamped. In the majority of samples this ratio was less than 20. About 20% of samples gave high values of 25–100, and this proportion was not dependent on the nutritional state of the rats. It is likely that these kidneys had been somewhat ischaemic; therefore all samples in which the above ratio was greater than 25 were rejected.

Calculation of results. Concentrations of metabolites in the tissue were calculated as follows: 1g of tissue was presumed to contain 0.75ml of water. On addition of 4ml of 6% $HClO_4$/g of frozen powder, the total fluid volume (i.e. volume of supernatant after centrifugation) became 4.75ml, which was assumed to contain all acid-soluble material from 1g of tissue. No corrections for content of blood or extracellular fluid were made.

RESULTS

Content of intermediates of glycolysis and of adenine nucleotides in liver after severance of blood supply. When the blood supply was cut there was a rapid rise in the concentrations of lactate and α-glycerophosphate and a progressive dephosphorylation of the adenine nucleotides in the livers of well-fed (Table 1) and of starved rats (Table 2). After a lag period of less than 1min the rate of lactate formation increased and after 2min it slowly fell. The highest rates of lactate formation (between 1 and 2min after cutting of the vessels) were 1.7μmol/min per g in the liver of well-fed rats and 1.1μmol/min per g in the liver of 48h-starved rats. The dephosphorylation of the adenine nucleotides was more rapid in the liver of starved than of well-fed rats. The sum of the three nucleotides remained constant during the first 1min but had fallen by about 25% after 5min. P_i increased rapidly, mainly as the result of the dephosphorylation of adenine nucleotides. Throughout 5min of ischaemia, a relatively constant quantity of phosphate was present as the sum of that in adenine nucleotides (counting ATP=3, ADP=2), P_i, and α-glycerophosphate: 15.1 or 16.0μmol/g initially (rats well-fed or starved respectively), and 16.0 or 17.2 after 5min of ischaemia (see Tables 1 and 2).

The concentration of glucose 6-phosphate increased, especially during the first 1min of ischaemia, presumably because of glycogen breakdown. This confirms the observations of Burch (1965) and Young (1966). Within 5min, glucose 1-phosphate content rose from 0.02 to 0.06μmol/g (averages of two measurements). The glucose content of the liver (not listed in the tables) rose from 1.33 to 8.0μmol/g during the first 5min in the liver of starved rats, after which time the glycogen content, expressed in glucose equivalents, was still 9μmol/g. The increase in glucose content in the ischaemic liver of fed rats was even greater, from 4.5 to 30μmol/g in 5min. The concentrations of triose phosphate and fructose diphosphate also rose

Table 2. *Contents of intermediates of glycolysis and of adenine nucleotides in livers of starved rats after severance of blood supply*

The experiment reported in Table 1 was repeated with 48h-starved rats. Details and abbreviations are as given in Table 1. Results are the average of measurements from two livers.

Time after cutting vessels (s)	Concentration of metabolites (μmol/g of fresh liver)															[ATP][AMP]/[ADP]²
	Lactate	Pyruvate	PEP	2PG	3PG	Triose-P	αGP	FDP	F6P	G6P	P_i	ATP	ADP	AMP	Total adenine nucleotides	
0	0.31	0.022	0.130	0.028	0.29	0.013	0.23	<0.010	0.04	0.19	6.2	1.66	1.97	0.64	4.27	0.27
60	0.93	0.015	0.039	0.016	0.14	0.027	0.77	0.030	0.06	0.29	9.1	0.87	1.68	1.65	4.20	0.51
120	2.05	0.020	0.023	0.016	0.10	0.034	1.40	0.025	0.07	0.29	8.9	0.85	1.43	1.65	3.93	0.68
300	3.46	0.015	0.013	<0.010	0.04	0.032	2.05	0.040	0.06	0.24	10.7	0.43	1.01	1.98	3.42	0.83

during ischaemia, whereas the concentrations of other C_3 phosphorylated intermediates and pyruvate fell.

Although the concentrations of ATP, ADP and AMP changed markedly during ischaemia the mass-action ratio of the adenylate kinase system in livers of well-fed rats remained virtually constant (Table 1) confirming the observation of Brosnan *et al.* (1970). The concentrations of P_i, ADP and AMP were somewhat higher in the livers of starved rats and those of ATP were lower (see also Start & Newsholme, 1968); in livers of starved rats the myokinase mass-action ratio rose during ischaemia (Table 2).

Content of intermediates of glycolysis and of adenine nucleotides in kidney after severance of blood supply. In general the changes in amounts of the renal intermediates were similar to those of the liver (Table 3). The concentration of lactate increased rapidly during the first 30s of ischaemia, at a rate of about 2 μmol/min per g. This rate compares with a rate of about 1.0, calculated for whole kidney from published results obtained with slices, presuming the kidney to be 15% medulla (see Wu, 1965; Underwood & Newsholme, 1967*b*; Gaja, Ragnotti, Cajone & Bernelli-Zazzera, 1967; György, Keller & Brehme, 1928). After 2min of ischaemia the glucose content fell by about 1.2 μmol/g, when the increase in lactate was 2.5 μmol/g. The concentration of ATP fell more rapidly than in liver and, as in liver, there was a slight loss of total adenine nucleotides during ischaemia. Unlike liver, there was no difference in the state of phosphorylation or the rate of dephosphorylation of adenine nucleotides between kidneys of well-fed and starved rats (not recorded in the tables).

The major difference between kidney and liver was the decrease in content of glucose 6-phosphate during renal ischaemia, which can be accounted for by the absence in kidney of major glycogen stores and glycogenolysis. The amount of α-glycerophosphate formed was less than in liver. The concentrations of the C_3 intermediates, except triose phosphate, decreased and those of fructose 1,6-diphosphate and triose phosphate increased.

Comparison of the concentrations of intermediates in 'cortex' and whole kidney revealed no major differences. In ischaemic medulla the content of lactate and pyruvate was somewhat higher than in 'cortex'.

Content of non-glycolytic intermediates in kidney after severance of blood supply. In ischaemic kidney there was a rise in content of glutamate and a parallel decrease in glutamine concentration (Table 4); these changes did not occur in ischaemic liver (Brosnan *et al.* 1970). The renal content of α-oxoglutarate, pyruvate and acetoacetate fell during ischaemia (Table 4). The associated increases in

Table 3. *Contents of intermediates of glycolysis and of adenine nucleotides in kidney after severance of blood supply*

Well-fed rats were either killed by cervical dislocation or anaesthetized with Nembutal as described in the text. Abbreviations are as given in Table 1. Results are means ± S.E.M., with number of observations in parentheses, except that the values for medulla are the average of two pooled samples, each comprising inner plus outer medulla from six kidneys.

Tissue extract	Treatment of rat	Time after severing vessels (s)	Content of metabolites (μmol/g of fresh kidney)																
			Lactate	Pyruvate	PEP	2PG	3PG	Triose-P	αGP	FDP	F6P	G6P	P_i	ATP	ADP	AMP	Glucose	Total adenine nucleotides	$\frac{[ATP][AMP]}{[ADP]^2}$
Whole kidney	Nembutal anaesthesia	0	0.35 ±0.11 (5)	0.048 ±0.01 (6)	0.047 ±0.01 (4)	<0.010	0.100 ±0.01 (4)	0.014 ±0.005 (4)	0.13 ±0.01 (6)	<0.010	0.017 ±0.005 (5)	0.069 ±0.014 (5)	2.81 ±0.15 (3)	1.71 ±0.02 (5)	0.96 ±0.08 (5)	0.25 ±0.06 (5)	—	2.92	0.46
Whole kidney	Cervical dislocation	0	0.47 ±0.06 (13)	0.027 ±0.005 (13)	0.035 ±0.005 (13)	0.014 ±0.005 (12)	0.085 ±0.01 (12)	0.016 ±0.005 (12)	0.12 ±0.01 (13)	0.011 ±0.005 (13)	0.013 ±0.005 (10)	0.039 ±0.005 (12)	4.05 ±0.17 (9)	1.43 ±0.08 (7)	1.34 ±0.05 (7)	0.42 ±0.03 (7)	2.72 ±0.15 (6)	3.19	0.33
Whole kidney	Cervical dislocation	30	2.07 ±0.07 (3)	0.021 ±0.005 (3)	0.014 ±0.005 (3)	<0.010	0.045 ±0.01 (3)	0.032 ±0.005 (3)	0.36 ±0.01 (3)	0.017 ±0.005 (3)	<0.010	0.021 ±0.005 (3)	6.08 ±0.23 (3)	0.44 ±0.02 (3)	1.30 ±0.04 (3)	1.35 ±0.05 (3)	—	3.09	0.35
Whole kidney	Cervical dislocation	60	2.10 ±0.03 (4)	0.012 ±0.005 (4)	0.012 ±0.005 (4)	<0.010	0.040 ±0.005 (4)	0.032 ±0.005 (4)	0.31 ±0.04 (4)	0.023 ±0.005 (4)	<0.010	0.019 ±0.005 (5)	7.18 ±0.40 (4)	0.45 ±0.03 (4)	1.17 ±0.04 (4)	1.56 ±0.03 (4)	—	3.18	0.51
Whole kidney	Cervical dislocation	120	3.04 ±0.19 (12)	0.010 ±0.005 (12)	0.013 ±0.005 (12)	<0.010	0.024 ±0.005 (12)	0.038 ±0.005 (11)	0.40 ±0.02 (12)	0.043 ±0.005 (11)	<0.010	<0.010	8.73 ±0.19 (12)	0.37 ±0.02 (4)	0.79 ±0.02 (4)	1.20 ±0.03 (4)	1.53 ±0.08 (4)	2.36	0.71
'Cortex'	Cervical dislocation	0	0.61 ±0.13 (5)	0.036 ±0.005 (5)	0.042 ±0.01 (5)	<0.010	0.087 ±0.01 (5)	0.032 ±0.01 (5)	0.09 ±0.01 (5)	<0.010	0.014 ±0.005 (5)	0.050 ±0.01 (5)	3.76 ±0.18 (3)	1.50 ±0.08 (4)	0.91 ±0.07 (4)	0.17 ±0.06 (4)	—	2.58	0.31
Medulla	Cervical dislocation	120	5.19	0.041	<0.010	<0.010	0.038	0.056	0.41	0.043	<0.010	0.016	5.50	0.54	0.54	1.03	—	2.11	0.19

lactate and β-hydroxybutyrate imply that these latter two changes at least are a consequence of reduction. Determination of these metabolites in 'cortex' gave values similar to those in whole kidney.

Measurement of the content of ammonia in kidney presents special problems because of its uneven distribution between tissue and tubular lumen. Denis, Preuss & Pitts (1964) report that the intracellular concentration may be calculated from that in renal venous blood, by presuming that barriers between blood and cells are permeable only to the free-base species (NH_3) and that this species equilibrates readily across the barrier. In this situation the gradient of the species NH_4^+ is proportional to that of H^+. Since the pK of ammonia is above 9.0, ammonia in biological fluids near neutral pH consists almost entirely of NH_4^+, so that the concentration gradient of total ammonia ($NH_3+NH_4^+$, i.e. the entity measured) would also approximately equal the H^+ gradient.

In the present work, an estimate of the intramitochondrial ammonia concentration of the kidney cortex was required to calculate the redox state of the mitochondrial NAD couple by the method of Williamson, Lund & Krebs (1967*a*). In this method, cytoplasmic and mitochondrial concentrations of certain metabolites (including ammonia, but not nicotinamide nucleotides) are presumed to be equal, and the pH of both compartments is presumed to be 7.0. Therefore, since the renal venous pH is about 7.4, the calculated intracellular (i.e. presumed intramitochondrial) ammonia content given in Table 4 is 2.4 times that in renal venous blood. The values of the redox states of the cytoplasmic and mitochondrial NAD couples in rapidly frozen (non-ischaemic) kidney of well-fed or starved rats (Table 5), calculated as described by Williamson *et al.* (1967*a*), are similar to those of liver. The total content of ammonia in kidney, which was greater than the calculated intracellular value (presumably due to the higher concentration in the tubular lumen), increased during ischaemia (Table 4).

DISCUSSION

Onset of anaerobic glycolysis in ischaemic liver or kidney. The oxygen content of the blood in liver or kidney at the moment of separation from blood supply may be calculated to be about 1.5 μmol/g fresh wt. of tissue (see Brosnan *et al.* 1970). This would provide less than a 1 min supply of oxygen for the liver, and much less for the kidney, which consumes about 5 μmol of O_2/min per g (Nishi-itsutsuji-Uwo, Ross & Krebs, 1967). Hence it is not surprising that the onset of the maximal rate of lactate formation occurred within 1 min of severance of blood supply to these organs. The alterations in content of intermediates during this initial phase of

Table 4. *Content of non-glycolytic intermediates in kidney of well-fed or starved rats after severance of blood supply*

Rats were anaesthetized with Nembutal. Calculation of intracellular NH_4^+ content and experimental details are described in the text. The values for citrate in kidney from well-fed rats are from animals that were killed by cervical dislocation.

Nutritional state	Tissue extract	Time after cutting vessels (s)	Content of intermediates (μmol/g of fresh kidney)													Renal venous $[NH_4^+]$ (μmol/ml of blood)	Calculated intracellular $[NH_4^+]$ (μmol/g of fresh kidney
			Glutamine	Glutamate	α-Oxoglutarate	Total tissue NH_4^+	Alanine	Aspartate	Citrate	Malate	Acetoacetate	β-Hydroxybutyrate	Lactate	Pyruvate	ATP	ADP	AMP		
Well-fed	Whole kidney	0	1.72 ±0.17 (6)	3.01 ±0.23 (9)	0.30 ±0.06 (6)	0.88 ±0.10 (6)	0.86 ±0.06 (6)	1.11 ±0.06 (6)	0.39 ±0.08 (7)	0.37 ±0.05 (6)	0.034 ±0.01 (6)	0.088 ±0.02 (6)	1.17 ±0.15 (6)	0.089 ±0.01 (6)	1.71 ±0.02 (5)	0.96 ±0.08 (5)	0.25 ±0.06 (5)	0.12 ±0.02 (6)	0.29
Well-fed	Whole kidney	120	1.03 ±0.15 (5)	5.17 ±0.19 (8)	0.04 ±0.01 (5)	1.54 ±0.12 (5)	—	0.94 ±0.03 (5)	0.45 ±0.06 (9)	0.47 ±0.03 (5)	< 0.02	0.135 ±0.03 (5)	4.05 ±0.32 (6)	0.029 ±0.06 (5)	—	—	—	—	
Well-fed	'Cortex'	0	1.28 ±0.08 (3)	2.38 ±0.17 (4)	0.34 ±0.03 (4)	—	—	0.87 ±0.09 (3)	0.43 ±0.02 (4)	0.27 ±0.04 (4)	—	—	0.94 ±0.16 (4)	0.074 ±0.01 (4)	1.88 ±0.10 (4)	0.58 ±0.04 (4)	0.13 ±0.04 (4)	—	
Starved for 48 h	Whole kidney	0	1.09 ±0.05 (8)	2.70 ±0.02 (12)	0.24 ±0.02 (11)	1.71 ±0.14 (8)	0.52 ±0.04 (3)	0.75 ±0.03 (6)	—	0.27 ±0.02 (8)	0.169 ±0.02 (11)	1.18 ±0.10 (11)	0.83 ±0.10 (11)	0.056 ±0.05 (11)	1.99 ±0.17 (3)	0.92 ±0.14 (3)	0.21 ±0.03 (3)	0.09 ±0.02 (7)	0.22
Starved for 48 h	Whole kidney	120	0.75 ±0.05 (5)	3.50 ±0.12 (5)	0.04 ±0.01 (7)	3.25 ±0.50 (7)	0.55 ±0.05 (4)	0.74 ±0.03 (5)	—	0.30 ±0.02 (7)	0.060 ±0.02 (7)	1.05 ±0.17 (7)	3.74 ±0.04 (7)	0.024 ±0.01 (7)	—	—	—	—	

Table 5. *Redox state of the NAD couple in kidney*

Metabolite concentration ratios are from the results in Table 4 (aerobic kidneys). The [free NAD^+]/[free NADH] ratio is calculated as described by Williamson *et al.* (1967*b*).

Nutritional state	Metabolite concentration ratio			Calculated [free NAD^+]/[free NADH] ratio		
				Cytoplasm	Mitochondria	
	[Lactate]/[pyruvate]	[β-Hydroxybutyrate]/[acetoacetate]	[Glutamate]/[α-oxoglutarate][NH_4^+]	From lactate dehydrogenase	From β-hydroxybutyrate dehydrogenase	From glutamate dehydrogenase
Well-fed	13.1	2.6	36	695	7.8	7.2
Starved for 48h	14.8	7.0	51	615	2.9	5.1

ischaemia suggest that glycogenolysis (in liver), phosphofructokinase and pyruvate kinase are the regulatory sites at which this acceleration of glycolysis is achieved. In the following discussion, the reasons for this conclusion are presented.

Activation of hepatic glycogen breakdown during ischaemia. The most striking changes induced by ischaemia are the rapid formation of glucose and glucose 6-phosphate, followed by the formation of lactate. These changes are presumably due to activation of glycogenolysis, which is probably connected with the rise of the concentrations of AMP and P_i (which is a substrate of glycogen phosphorylase) and the decrease in ATP, an inhibitor of this enzyme (see Maddaiah & Madsen, 1966).

Effects of ischaemia on the activity of phosphofructokinase. The gradual rise of the concentrations of triose phosphates and fructose diphosphate, and the fall of the concentrations of hexose 6-phosphates in kidney after cessation of blood flow confirms the findings of Wu (1965) and Underwood & Newsholme (1967*b*), obtained with kidney-cortex slices (incubated anaerobically) and may be interpreted as the result of an activation of phosphofructokinase by the increase in AMP and P_i, and by the decrease in ATP.

Although the properties of liver phosphofructokinase (Underwood & Newsholme, 1965*b*; Brock, 1969) are similar to those of the kidney enzyme (Underwood & Newsholme, 1967*a*), the effects of anaerobiosis on the content of hexose 6-phosphate differ in the two tissues, presumably because of the greater glycogen content of the liver. Since the hepatic fructose 6-phosphate concentration was well below 1mM throughout 5min of ischaemia, and since rat liver phosphofructokinase does not become saturated with fructose 6-phosphate until the concentration exceeds 1mM (Underwood & Newsholme, 1965*b*), the increased flux through phosphofructokinase in ischaemic liver was at least partly a response to increased provision of substrate.

In both kidney and liver, inhibition of fructose 1,6-diphosphatase activity, especially by an increase in AMP (Mendicino & Vasarhely, 1963; Underwood & Newsholme, 1965*a*), could also contribute to anaerobic acceleration of glycolysis.

Pyruvate kinase during ischaemia in liver and kidney. In liver and kidney, 2- and 3-phosphoglycerate and phosphoenolpyruvate gradually decreased as ischaemia progressed, i.e. as lactate concentration increased. These changes imply an acceleration of the pyruvate kinase reaction, which could be caused by de-inhibition through a decrease in ATP (Tanaka, Sue & Morimura, 1967; Rozengurt, de Asua & Carminatti, 1969) and by activation through an increase in fructose diphosphate (Taylor & Bailey, 1967).

Redox balance in ischaemic tissue. When lactate and malate are formed from glucose in ischaemic tissue reductions and oxidations balance. Since there was no possibility of addition or removal of metabolites from the ischaemic organs in the present experiments, other metabolic changes must also balance with respect to oxidoreductions as well as with respect to carbon. Thus the oxidations that accompany the formation of α-glycerophosphate and β-hydroxybutyrate, and of glutamate in kidney, require definition. This is complicated by the fact that some intermediates, e.g. α-oxoglutarate and pyruvate, can react either as oxidants or reductants. The present measurements do not provide answers to the questions of how much α-oxoglutarate was oxidized to succinate or reduced to citrate or glutamate, of how much pyruvate was reduced to lactate or oxidized to acetyl-CoA and acetoacetate or of how much oxaloacetate, citrate or glutamate were formed from pyruvate.

A key question is whether pyruvate is converted into acetyl-CoA or tricarboxylic acid-cycle inter-

mediates in anaerobic tissue. Net formation of oxaloacetate, α-oxoglutarate and succinate from pyruvate is known to occur in pigeon liver homogenates in aerobic conditions (Evans, 1940; Krebs & Eggleston, 1940); these products, and also citrate and β-hydroxybutyrate, can be formed from pyruvate in anaerobic mammalian systems, including liver and kidney (Krebs & Johnson, 1937; Krebs, Eggleston, Kleinzeller & Smyth, 1940). Citrate can form glutamate and tricarboxylic acid-cycle intermediates anaerobically in liver homogenates (Bartley, Sobrinho-Simoes, Notton & Montesi, 1959). The present experiments suggest that these reactions can occur in ischaemic (intact) liver and kidney. However, it is not clear which specific reactions generate the net quantity of NADH from NAD^+ under anaerobic conditions that is required to form α-glycerophosphate or glutamate.

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