Carbohydrate Metabolism in the Isolated Perfused Rat Kidney

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1. Anaerobic formation of lactate from glucose by isolated perfused rat kidney ($411 \mu\text{mol/h per g dry wt.}$) was three times as fast as in aerobic conditions ($138 \mu\text{mol/h per g.}$)
2. In aerobic or in anaerobic conditions, the ratio of lactate production to glucose utilization was about 2.
3. Starvation or acidosis caused a decline of about 30% in the rate of aerobic glycolysis.
4. The rate of formation of glucose from lactate by perfused kidney from a well-fed rat, in the presence of 5 mM-acetoacetate ($83 \mu\text{mol/h per g dry wt.}$), was of the same order as the rate of aerobic glycolysis.
5. During perfusion with physiological concentrations of glucose (5 mM) and lactate (2 mM) there were negligible changes in the concentration of either substrate.
6. Comparison of kidneys perfused with lactate, from well-fed or starved rats, showed no major differences in contents of intermediates of gluconeogenesis.
7. The tissue concentrations of hexose monophosphates and C_3 phosphorylated glycolytic intermediates (except triose phosphate) were decreased in anaerobic conditions.
8. Aerobic metabolism of fructose by perfused kidney was rapid: the rate of glucose formation was $726 \mu\text{mol/h per g dry wt.}$ and of lactate formation $168 \mu\text{mol/h per g (dry wt.)}$. Glycerol and D-glyceraldehyde were also released into the medium.
9. Aerobically, fructose generated high concentrations of glycolytic intermediates.
10. Anaerobic production of lactate from fructose ($74 \mu\text{mol/h per g dry wt.}$) was slower than the aerobic rate.
11. In both anaerobic and aerobic conditions the ratio [lactate]/[pyruvate] in kidney or medium was lower during perfusion with fructose than with glucose.
12. These results are discussed in terms of the regulation of renal carbohydrate metabolism.

From experiments on kidney-cortex slices it is known that lactate formation is negligible in aerobic conditions, but is appreciable in anaerobic conditions (for review, see Newsholme & Gevers, 1967). Similarly, lactate accumulates in ischaemic kidney cortex *in vivo* (Hems & Brosnan, 1970). Renal medulla, on the other hand, has a relatively high glycolytic capacity, which in slices is manifested aerobically as well as anaerobically though the aerobic rate is slower (Györgi *et al.*, 1928).

The rate of glycolysis has not been measured previously in the intact kidney. The perfused organ offers the opportunity of measuring metabolic processes while conditions such as pH or the oxygen concentration are varied. It is further possible to freeze-clamp the tissue at a time when the rate of glycolysis is constant. Thus by measurements of tissue intermediate concentrations, information may be gained on the regulatory steps of the glycolytic pathway.

Results of such experiments are reported in the

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present paper. In particular, a comparison has been made between aerobic and anaerobic glycolysis. Glucose was the substrate in most perfusions, but fructose was also investigated in view of its known rapid aerobic metabolism in kidney (Krebs & Lund, 1966) and liver (Woods *et al.*, 1970).

Experimental

Animals

Male albino Wistar rats (280–350 g) were fed on a standard diet of rat cubes. Starvation was for 48 h. Acidosis was induced by replacing the drinking water with 1.5% (w/v) NH_4Cl soln. for 7–10 days (see Hems & Brosnan, 1971). The mean plasma bicarbonate concentration (mM) in acidotic rats was 20 ± 2 (5) and in normal rats 25 ± 1 (11).

Perfusion of isolated kidney

The operative technique, bicarbonate–albumin–saline perfusion medium and apparatus described by Nishiitsutsuji-Uwo *et al.* (1967) were employed, except that a pulsatile renal arterial input was generated with a roller occlusion pump (see

Weidemann *et al.*, 1969). Albumin was dialysed against bicarbonate-buffered saline (Krebs & Henseleit, 1932) for 60h (five changes), but was not freed from bound fatty acids.

In standard medium the gas phase was $N_2 + CO_2$ (95:5) in anaerobic experiments and $O_2 + CO_2$ (95:5) in aerobic experiments. HCN (as neutralized KCN) was added to a concentration of 1mM, immediately before anaerobic perfusions. Bicarbonate was 25mM in the medium at pH 7.4 and about 10mM at pH 7.1.

Extracts of freeze-clamped (Wollenberger *et al.*, 1960) kidney and perfusion medium were prepared in $HClO_4$ as described by Weidemann *et al.* (1969). Kidneys were freeze-clamped 40min after the start of perfusion.

Analytical methods

These were as described by Weidemann *et al.* (1969). Fructose 1-phosphate was kindly determined by Mr. L. V. Eggleston, by the method of Eggleston (1970). Glycerol and D-glyceraldehyde were determined by the method of Pinter *et al.* (1967). Although extracts of perfusion medium sometimes contained D-glyceraldehyde, measurements with standard solutions showed that D-glyceraldehyde gave a slower reduction of NAD^+ during the assay. The glycerol-dependent reaction was complete within 5min. Reduction of triose to glycerol was carried out with $NaBH_4$ (Pinter *et al.*, 1967); total glycerol was then determined. Dihydroxyacetone was determined as described by Wieland (1963); none was detected in the perfusion medium.

Results

Aerobic formation of lactate from glucose by the perfused kidney

The time-course of aerobic lactate formation by the isolated perfused kidney was linear for 1–2h, and the

rate (about $130 \mu\text{mol/h}$ per g dry wt. of kidney; Table 1) was of the order that would be expected from published experiments on slices of rat kidney medulla (Györgi *et al.*, 1928) if medulla is presumed to be about 15% of the renal mass. Hence the contribution of the cortex to aerobic glycolysis in the perfused kidney was negligible, in agreement with the observation that rates of aerobic glycolysis are low in rat kidney-cortex slices (Györgi *et al.*, 1928; Underwood & Newsholme, 1967; Weidemann & Krebs, 1969).

In aerobic perfusions the glucose removed from the medium (Table 1) approximately corresponded to the lactate formed (since $1 \text{ mol of glucose} \equiv 2 \text{ mol of lactate}$). Pyruvate also appeared regularly in the medium and the ratio $[\text{lactate}]/[\text{pyruvate}]$ was about 12 at all glucose concentrations during perfusion of kidneys from fed rats.

In starved or acidotic rats the capacity for renal gluconeogenesis is increased (Krebs *et al.*, 1963; Goodman *et al.*, 1966). Hence it was decided to measure renal glycolysis in these conditions. When the pH of the perfusion medium was 7.1, or if the rat was acidotic or starved, the rate of aerobic lactate production tended to be somewhat lower than in control perfusions (Table 1).

Anaerobic formation of lactate from glucose in the perfused kidney

Lactate formation was linear with time, for at least 2h, in anaerobic perfusions with HCN. If cyanide was not present the initial rate was slower and increased during perfusion, presumably as anaerobiosis became complete. Hence cyanide was always added in anaerobic perfusions.

Anaerobic lactate production was dependent on glucose concentration over the range 0–10mM (Table 2). The maximal observed rate of lactate formation ($411 \mu\text{mol/h}$ per g dry wt. of kidney; Table 2) was

Table 1. Rates of production of lactate from glucose in isolated aerobic perfused kidney

Kidneys were perfused aerobically with standard medium. Other details are given in the text. Results are means $\pm$ S.E.M. (number of observations in parentheses).

Nutritional state of rats	Glucose in medium (mM)	pH of medium	Changes in the medium ($\mu\text{mol/h}$ per g dry wt.)	
			Lactate production	Glucose removal
Well-fed	1	7.4	71 ± 3 (6)	38 ± 3 (4)
	5	7.4	138 ± 3 (3)	65 ± 8 (3)
	10	7.4	121 ± 8 (5)	—
	10	7.1	75 ± 14 (4)	—
Starved (48h)	10	7.4	83 ± 9 (5)	—
Acidotic (7–10 days)	10	7.4	76 ± 6 (4)	—

Table 2. *Rates of production of lactate from glucose in isolated anaerobic perfused kidney*

Kidneys were perfused anaerobically with standard medium at pH 7.4 containing HCN (1 mM). Other details are given in the text. Results are means $\pm$ S.E.M. (number of observations in parentheses).

Nutritional state of rats	Glucose in medium (mM)	Changes in the medium ($\mu\text{mol/h per g dry wt.}$)	
		Lactate production	Glucose removal
Well-fed	0	<15	—
	1	233 $\pm$ 19 (6)	157 $\pm$ 15 (6)
	5	328 $\pm$ 16 (6)	170 $\pm$ 15 (4)
	10	411 $\pm$ 25 (10)	—
Starved (48 h)	10	341 $\pm$ 30 (4)	—

again of the order that would be expected from published experiments with slices of rat kidney cortex or medulla, on the assumption that medulla constitutes about 15% of the renal mass. This may be calculated from the observations that rates of anaerobic lactate formation in rat kidney slices (from glucose concentrations of at least 5 mM) are about 1200 $\mu\text{mol/h per g dry wt.}$ for the medulla (Györgi *et al.*, 1928; Gaja *et al.*, 1967) and 200–300 for cortex (Györgi *et al.*, 1928; Gaja *et al.*, 1967; Wu, 1965; Underwood & Newsholme, 1967).

In anaerobic perfusions, glucose removal from the medium was faster than in aerobic perfusions (Table 2) and again corresponded to the amount of lactate formed. Pyruvate formation was negligible in anaerobic perfusions, and the ratio [lactate]/[pyruvate] in the medium was greater than 50.

Utilization of lactate by the perfused kidney

As well as forming lactate from glucose, the kidney is able to form glucose from lactate by gluconeogenesis (Krebs *et al.*, 1963; Nishiitsutsuji-Uwo *et al.*, 1967). Hence it was decided to compare the rates of these processes. In the perfused kidney from fed rats, the rate of gluconeogenesis from lactate (10 mM) was 32 $\mu\text{mol/h per g dry wt.}$ (Table 3) and 83 $\mu\text{mol/h per g dry wt.}$ if stimulated by the addition of acetoacetate (Table 3; see Krebs *et al.*, 1965, for discussion of this effect). Thus the rate of gluconeogenesis in fed rats attained a magnitude similar to the maximal rate of aerobic glycolysis. In rats starved for 48 h the maximal aerobic rates of these processes were also of a similar order (compare Tables 1 and 3).

Perfusion of kidney with combinations of glucose and lactate

Under anaerobic conditions, in the presence of an initial lactate concentration of 2 or 4 mM, the rate of

formation of further lactate from 10 mM-glucose by the perfused kidney was the same as in the absence of added lactate. However, in aerobic conditions, the presence of initial added lactate did affect the rate of glycolysis. Thus if both lactate and glucose were initially 5 mM in perfusions of kidney from well-fed rats, lactate disappeared from the medium whereas glucose did not. These changes are in the opposite direction to those observed in the presence of glucose alone. This observation, and the fact that maximal rates of glycolysis and gluconeogenesis are of the same order, raised the possibility that, at intermediate lactate and glucose concentrations, net changes in the concentrations of both substrates would be minimal. This was confirmed experimentally: in two perfusions of kidney from well-fed rats, with glucose (5 or 10 mM) and lactate (2 mM), changes in the concentration of glucose or lactate were negligible, over 0–90 min.

Metabolism of fructose by the perfused kidney

In aerobic perfusions of kidney from well-fed rats with fructose (10 mM) the rate of glucose formation was rapid (726 $\mu\text{mol/h per g dry wt.}$ of kidney; Table 4) and lactate production was slightly faster than from 10 mM-glucose (compare Tables 1 and 4). In contrast to the anaerobic perfusions with glucose, anaerobic lactate formation from fructose was slower than that in aerobic conditions (Table 4).

During perfusion with fructose the ratio [lactate]/[pyruvate] in the medium, under aerobic or anaerobic conditions, was 6 (mean of four perfusions) or 26 (three perfusions) respectively (Table 4). These ratios are less than those in the presence of 10 mM-glucose in comparable conditions (12 and >50 respectively).

In aerobic perfusions with 10 mM-fructose, glycerol was released into the perfusion medium at an initial rate of 46 $\pm$ 10 (3) $\mu\text{mol/h per g dry wt.}$ of kidney. Anaerobically, the rate was 9 $\mu\text{mol/h per g}$ (average of two experiments). The rate of release of glycerol

Table 3. *Rates of production of glucose from lactate in isolated perfused kidney*

The conditions of perfusion were as indicated in Table 1. Other details are given in the text. Results are means $\pm$ S.E.M. (number of observations in parentheses).

Nutritional state	Lactate in medium (mM)	pH of medium	Changes in the medium	
			Glucose production (μ mol/h per g dry wt.)	Ratio [lactate]/[pyruvate]
Well-fed	5	7.4	23 $\pm$ 4 (7)	75 (3)
	5	7.1	25 $\pm$ 4 (6)	
	10	7.4	32 $\pm$ 3 (3)	
	10*	7.4	83 $\pm$ 12 (4)	
Starved	5	7.4	†72	61 (5)
	5	7.1	65 $\pm$ 8 (3)	
	10	7.4	98 $\pm$ 7 (11)	

* Acetoacetate (5mM) added to medium.

† From Nishiitsutsuji-Uwo *et al.* (1967).

Table 4. *Metabolism of fructose (10mM) by isolated perfused kidney*

Kidneys from well-fed rats were perfused with standard medium at pH 7.4. Other details are given in the text. Results are means $\pm$ S.E.M. (number of observations in parentheses).

Gas phase (containing 5% CO ₂)	Changes in the medium		
	Glucose production (μ mol/h per g dry wt.)	Lactate production (μ mol/h per g dry wt.)	Ratio [lactate]/[pyruvate]
O ₂	726 $\pm$ 17 (4)	168 $\pm$ 15 (4)	6 (4)
N ₂	<10	74 $\pm$ 7 (3)	26 (3)

plus D-glyceraldehyde was 111 μ mol/h per g. No dihydroxyacetone was detected in the medium in aerobic or anaerobic conditions. Since D-glyceraldehyde is a product of the fructose 1-phosphate aldolase reaction, this was presumably the route of its formation from fructose. Glycerol was probably formed by reduction of the free D-glyceraldehyde. Similar results have been reported for the perfused rat liver (Freedland, 1971).

Content of metabolites in perfused kidney

To elucidate the enzymic steps at which alteration in the rates of glycolysis were brought about by anaerobiosis, concentrations of glycolytic pathway intermediates were measured in the perfused kidney (Table 5). In a comparison of aerobic and anaerobic perfusions with glucose (10mM), the anaerobic kidney contained lower concentrations of hexose 6-phosphates and C₃ phosphorylated intermediates (other than triose phosphate) and increased concentrations of triose phosphate and fructose 1,6-diphosphate (Table 5). These changes, brought about by anaerobiosis, are similar to those that occur *in vivo* in kidney rendered ischaemic by severance of blood supply (Hems & Brosnan, 1970).

In aerobic perfusions with fructose (10mM) the renal concentrations of glycolytic intermediates were high, compared with those in aerobic perfusions with glucose or lactate or in anaerobic perfusions with fructose (Table 5). The renal concentration of fructose 1-phosphate was 4.7 μ mol/g during perfusion with fructose aerobically (average of two perfusions) and 0.53 anaerobically (one perfusion); the concentration of this intermediate was 0.48 μ mol/g during aerobic perfusion with 10mM-lactate (one perfusion). The glycerol concentration in the kidney perfused with fructose was 0.17 μ mol/g in aerobic conditions (one perfusion) and 0.06 μ mol/g anaerobically (average of two perfusions).

In view of their role as modifiers of metabolic processes, the concentrations of adenine nucleotides were measured in perfused kidney (Table 5). In aerobic perfusions, fructose caused a slight alteration in that there was a decrease in ATP and an increase in ADP, compared with values during perfusion with glucose. In anaerobic perfusions with either glucose or fructose, the total renal concentration of adenine nucleotides was markedly decreased compared with that in aerobic perfused kidney (Table 5). This may be explained by the properties of the enzymes that degrade AMP, such as the de-inhibition of 5'-nucleo-

Table 5. *Content of intermediate metabolites in the isolated perfused kidney on addition of glucose, fructose or lactate to the perfusion medium*

Kidneys from well-fed rats were freeze-clamped after 40min perfusion. For experimental details see the text. The contents are means $\pm$ S.E.M. of three perfusions except for the aerobic glucose experiments, where the number of observations was four. The values for perfused kidney from rats starved for 48h, which are included for comparison, are from Weidemann *et al.* (1969).

Metabolite	Substrate ... Gas phase (containing 5% CO ₂) ...	Content of metabolite (μ mol/g fresh wt.)					
		Glucose (5mm) O ₂	Glucose (5mm) N ₂	Fructose (10mm) O ₂	Fructose (10mm) N ₂	Lactate (10mm) O ₂	Lactate (10mm) (starved rat) O ₂
Lactate		1050 $\pm$ 145	1590 $\pm$ 330	2320 $\pm$ 230	465 $\pm$ 35	8080 $\pm$ 380	8380 $\pm$ 232
Pyruvate		35 $\pm$ 10	23 $\pm$ 10	330 $\pm$ 8	29 $\pm$ 10	103 $\pm$ 10	89 $\pm$ 11
Phosphoenolpyruvate		12 $\pm$ 5	<10	155 $\pm$ 9	30 $\pm$ 4	<10	13 $\pm$ 2
2-Phosphoglycerate		<10	<10	75 $\pm$ 3	<10	<10	<10
3-Phosphoglycerate		35 $\pm$ 5	17 $\pm$ 5	350 $\pm$ 17	38 $\pm$ 2	30 $\pm$ 5	26 $\pm$ 5
Triose phosphate		16 $\pm$ 5	48 $\pm$ 5	50 $\pm$ 7	55 $\pm$ 25	13 $\pm$ 5	21 $\pm$ 2
α -Glycerophosphate		160 $\pm$ 25	480 $\pm$ 55	980 $\pm$ 110	550 $\pm$ 45	440 $\pm$ 15	604 $\pm$ 80
Fructose diphosphate		<10	42 $\pm$ 5	14 $\pm$ 6	<10	<10	<10
Fructose 6-phosphate		<10	<10	100 $\pm$ 20	<10	<10	14 $\pm$ 3
Glucose 6-phosphate		55 $\pm$ 5	20 $\pm$ 5	320 $\pm$ 60	<10 $\pm$ 6	34 $\pm$ 10	54 $\pm$ 18
Malate		105 $\pm$ 10	60 $\pm$ 10	270 $\pm$ 20	65 $\pm$ 20	430 $\pm$ 70	330 $\pm$ 40
Glutamate		1920 $\pm$ 35	1485 $\pm$ 225	1940 $\pm$ 180	1390 $\pm$ 70	2570 $\pm$ 215	2750 $\pm$ 150
α -Oxoglutarate		1010 $\pm$ 105	225 $\pm$ 130	470 $\pm$ 90	<10	1970 $\pm$ 350	1070 $\pm$ 150
P _i		3600 $\pm$ 265	5960 $\pm$ 245	4110 $\pm$ 530	5780 $\pm$ 280	3840 $\pm$ 200	4790 $\pm$ 420
ATP		1130 $\pm$ 175	135 $\pm$ 30	960 $\pm$ 105	90 $\pm$ 50	1145 $\pm$ 15	960 $\pm$ 60
ADP		470 $\pm$ 45	145 $\pm$ 20	800 $\pm$ 210	210 $\pm$ 55	620 $\pm$ 110	640 $\pm$ 40
AMP		140 $\pm$ 10	240 $\pm$ 70	230 $\pm$ 55	200 $\pm$ 45	180 $\pm$ 35	250 $\pm$ 20
Sum of ATP, ADP, AMP		1740	520	1990	500	1945	1850

tidase if there is a decrease in tissue ATP concentration (see Weidemann *et al.*, 1969, for discussion).

The enhancement of renal gluconeogenesis by starvation (Krebs *et al.*, 1963) was investigated by measurement of the concentrations of intermediates in the perfused kidney (Table 5). An investigation of this kind in the intact rat has shown that enhancement of renal gluconeogenesis by starvation may be brought about at the enzymic steps catalysed by phosphoenolpyruvate carboxykinase and fructose diphosphatase (Hems & Brosnan, 1971). However, in the kidney perfused with lactate, comparison between the fed and the starved rat did not reveal a clear-cut pattern of change of concentration of gluconeogenic intermediates (Table 5), despite the threefold increase in the rate of gluconeogenesis from lactate after starvation (Table 3).

Discussion

Fuel of respiration of the whole kidney

The rate of aerobic glycolysis in the perfused kidney was of the order expected if the renal medulla

was making the major or sole contribution to lactate formation. This, and the observation that the ratio of lactate production to glucose uptake in the aerobic perfused kidney was about 2, suggest that in the conditions of the present experiments (i.e. glucose as sole exogenous carbohydrate, plus dialysed albumin-saline that did not contain fatty acids in excess of physiological amounts) oxidation of glucose by the kidney was negligible. This conclusion is in agreement with observations suggesting that the major fuels of respiration of the kidney cortex are fatty acids and ketone bodies (see Weidemann & Krebs, 1969).

Effect of anaerobiosis on glycolysis

The increase in fructose diphosphate and the decrease in C₃ phosphorylated intermediates (other than triose phosphate) in the anaerobic kidney perfused with glucose resemble the effects produced *in vivo* by severance of the blood supply of the kidney (Hems & Brosnan, 1970). These changes support the previous suggestion of Hems & Brosnan (1970) that during the acceleration of renal cortical glycolysis by

anaerobiosis the phosphofructokinase and pyruvate kinase steps are activated.

Control of renal fructose metabolism

In aerobic conditions, perfusion of the kidney with fructose generated high renal concentrations of the intermediates of carbohydrate metabolism, confirming and expanding the results of Bowman (1970) and Burch *et al.* (1970). These high intermediate concentrations were associated with a high rate of gluconeogenesis from fructose.

The formation of lactate during aerobic perfusion with fructose could largely reflect utilization by the medulla of glucose produced in the cortex. This would explain the decreased anaerobic formation of lactate from fructose (since cortical gluconeogenesis would be slower in anaerobic conditions), in conjunction with the fact that fructose is less rapidly taken up by the kidney in anaerobic than aerobic conditions (see below). These considerations do not exclude the possibilities of slow rates of aerobic or anaerobic formation of lactate from fructose by the cortex, or of medullary utilization of fructose. The decreased rate of formation of lactate and glucose from fructose in anaerobic perfused kidney is probably a consequence of a diminished rate of phosphorylation, as shown by the decreased concentration of fructose 1-phosphate and other glycolytic intermediates.

Inter-relationship between glycolysis and gluconeogenesis in the perfused kidney

In general, the rates of gluconeogenesis in the isolated perfused kidney are rather less than those in cortex slices from rats in the same metabolic state (e.g. compare Nishiitsutuji-Uwo *et al.*, 1967, and Krebs *et al.*, 1963). Thus, if one considers lactate as substrate, the rates of gluconeogenesis were 32 $\mu\text{mol/h}$ per g dry wt. of tissue in perfused kidney of fed rats, and 98 $\mu\text{mol/h}$ per g in starved rats. In slices the corresponding rates are 110 and 200 respectively (Krebs *et al.*, 1963). This disparity is partly due to reconsumption of glucose by the renal medulla during perfusion. Thus if one subtracts 10–40 $\mu\text{mol/h}$ per g dry wt. (the likely rate of medullary glucose consumption during gluconeogenesis) from the rates of gluconeogenesis by cortex slices, and corrects for the cortex being about 85% of the total kidney weight, the resulting rates more closely resemble the rates of gluconeogenesis observed in the isolated perfused kidney.

The perfusions with combinations of glucose and lactate showed that aerobically (in the conditions of the perfusions), if the glucose concentration was 5–10 mM and lactate about 2 mM, these concentrations

were approximately maintained by the kidney, presumably as a result of a balance between cortical synthesis of glucose from lactate, and medullary glycolysis. Since the above concentrations approximate to those in blood, such 'autoregulation' by the kidney of circulating glucose and lactate concentrations could occur *in vivo*, thus according a particular metabolic significance to the proportions and properties of the combination of cortex and medulla.

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References

- Bowman, R. H. (1970) *J. Biol. Chem.* **245**, 1604
- Burch, H. B., Lowry, O. H., Meinhardt, L., Max, P., Jr. & Chyu, K.-J. (1970) *J. Biol. Chem.* **245**, 2092
- Eggleson, L. V. (1970) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), vol. 2, p. 1276, Academic Press, New York
- Freedland, R. A. (1971) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **30**, 258 (Abstr.)
- Gaja, G., Ragnotti, G., Cajone, F. & Bernelli-Zazzera, A. (1967) *Biochem. J.* **105**, 647
- Goodman, A. D., Fuisz, R. E. & Cahill, G. F., Jr. (1966) *J. Clin. Invest.* **45**, 612
- Györgi, P., Keller, W. & Brehme, T. (1928) *Biochem. Z.* **200**, 356
- Hems, D. A. & Brosnan, J. T. (1970) *Biochem. J.* **120**, 105
- Hems, D. A. & Brosnan, J. T. (1971) *Biochem. J.* **123**, 391
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33
- Krebs, H. A. & Lund, P. (1966) *Biochem. J.* **98**, 210
- Krebs, H. A., Bennett, D. A. H., de Gasquet, P., Gascoyne, T. & Yoshida, T. (1963) *Biochem. J.* **86**, 22
- Krebs, H. A., Speake, R. N. & Hems, R. (1965) *Biochem. J.* **94**, 712
- Newsholme, E. A. & Gevers, W. (1967) *Vitam. Horm. (New York)* **25**, 1
- Nishiitsutuji-Uwo, J., Ross, B. D. & Krebs, H. A. (1967) *Biochem. J.* **103**, 852
- Pinter, J. K., Hayashi, J. A. & Watson, J. A. (1967) *Arch. Biochem. Biophys.* **121**, 404
- Underwood, A. H. & Newsholme, E. A. (1967) *Biochem. J.* **104**, 300
- Weidemann, M. J. & Krebs, H. A. (1969) *Biochem. J.* **112**, 149
- Weidemann, M. J., Hems, D. A. & Krebs, H. A. (1969) *Biochem. J.* **115**, 1
- Wieland, O. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), p. 244, Academic Press, New York
- Wollenberger, A., Ristau, O. & Schoffa, G. (1960) *Pflügers Arch. Gesamte Physiol.* **270**, 399
- Woods, H. F., Eggleson, L. V. & Krebs, H. A. (1970) *Biochem. J.* **119**, 501
- Wu, R. (1965) *J. Biol. Chem.* **240**, 2373