Effect of Ischaemic Limb Injury on the Rates of Metabolism of Ketone Bodies in Starved Rats

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1. Rats starved for 30h were injected with trace amounts of [3-¹⁴C]acetoacetate and β -hydroxy[3-¹⁴C]butyrate 1 h after ischaemic limb injury in a 20°C environment, and the concentrations and radioactivities of blood ketone bodies were determined at intervals. 2. Starvation alone raised the rates of production and utilization of β -hydroxybutyrate plus acetoacetate about 3.7-fold, but lowered their metabolic clearance rates by about 50%. In the starved rat ketone-body oxidation could account for up to 30% of whole body O₂ consumption. 3. Injury in starved rats lowered the rates of production and utilization of both β -hydroxybutyrate and acetoacetate, the combined fall of about 37% slightly exceeding the concomitant fall in whole-body O₂ consumption. The concentration of β -hydroxybutyrate decreased after injury, but its metabolic clearance rate was unaltered; the concentration of acetoacetate rose slightly and its metabolic clearance rate fell.

The first few hours after severe injury are characterized by striking metabolic changes (Stoner & Heath, 1973), which can often only be studied in detail in experimental animals (Stoner, 1969a). A useful experimental model is bilateral hind-limb ischaemia, which leads to reproducible tissue damage and fluid loss (Stoner, 1961). In the post-absorptive rat (see Heath & Threlfall, 1968) in a 20°C environment, ischaemic limb injury leads to a decrease in the rates of whole-body O₂ consumption (Stoner, 1969b), glucose disposal and oxidation (Ashby *et al.*, 1965; Heath & Corney, 1973) and free fatty acid oxidation (Heath & Stoner, 1968). In contrast, both the concentrations of ketone bodies in the blood and their rates of utilization (disposal) are increased (Barton, 1973). A rise in concentration has also been found in some, but not all, human patients after severe injury (Smith *et al.*, 1975).

In starvation the shift from carbohydrate to fat catabolism leads to higher basal values of both the turnover and concentration of ketone bodies (Bates *et al.*, 1968). Under these conditions limb ischaemia at 20°C again leads to a fall in O₂ consumption (R. A. Little & H. B. Stoner, unpublished work) and free fatty acid oxidation (Heath & Stoner, 1968). The concentrations of ketone bodies in the blood also fall (Barton, 1971), suggesting that in the starved rat, unlike in the post-absorptive rat, ketone-body utilization may be lower after injury.

In the present work the rates of utilization of β -hydroxybutyrate and acetoacetate in injured and control starved rats have been determined by single injections of ¹⁴C-labelled ketone bodies. In the steady state the rates of ketone-body utilization are

equal to the rates of production. Values for the metabolic clearance rates and rate coefficients for utilization of ketone bodies have also been calculated directly to investigate the effects of injury on the process of utilization.

Experimental

Theory and experimental design

The theory of the method used has been described in detail by Heath & Barton (1973) and Barton (1973). Rats given single intravenous injections of [3-¹⁴C]-acetoacetate or β -hydroxy[3-¹⁴C]butyrate were killed at intervals and the concentrations and radioactivities of both ketone bodies in the blood were determined. From the four areas to infinite time under the specific activity–time curves the sum of the rates of utilization of β -hydroxybutyrate and acetoacetate was calculated. To obtain the rates separately allowance had to be made for interconversion of β -hydroxybutyrate and acetoacetate in the extra-hepatic tissues, but experiments in hepatectomized control rats showed that the correction needed was very small (approx. 2%). The rate coefficients and metabolic clearance rates for utilization of β -hydroxybutyrate and acetoacetate were also calculated, by using quantity of label–time curves instead of specific activity–time curves.

This procedure differed in one important respect from that used for post-absorptive rats (Barton, 1973). In the latter the rates of utilization of β -hydroxybutyrate and acetoacetate could be shown to be proportional to their concentrations and were therefore calculated by multiplying rate coefficients

by blood pool sizes (concentration $\times$ blood volume). In the present study this was not the case and so the use of specific activity-time curves was appropriate (Heath & Barton, 1973; Cremer & Heath, 1974). The areas under these curves were calculated like those under radioactivity-time curves (Heath & Barton, 1973; Barton, 1973).

Animal techniques

Rats. Male Wistar rats of the Porton strain were housed and experiments were done in a room maintained at 18–22°C and lit from 07:00 to 19:00h daily. M.R.C. Diet 41B was available until the day before the experiment; when samples were taken, the rats had been starved for about 30h. The starved body weights, on which all results are based, were in the range 216–253g (mean 234.7g). Rats were subjected to bilateral hind-limb ischaemia for 4h with rubber tourniquets (Rosenthal, 1943) applied under diethyl ether anaesthesia at 09:00–10:30h, and control rats were anaesthetized for a similar period (about 5min). Experiments were designed so that the mean of the interval between injection of the isotope and sampling was always 1h after removing the tourniquets, and at the same time of day (14:00–15:30h) in controls.

Injection and blood sampling in intact rats. Rats were injected with 0.25–0.4 μ Ci of [3-¹⁴C]acetoacetate or β -hydroxy[3-¹⁴C]butyrate/100g body wt., specific activity 4.8 μ Ci/ μ mol, prepared from ethyl [3-¹⁴C]-acetoacetate (The Radiochemical Centre, Amersham, Bucks., U.K.) as described by Barton (1973). For the simultaneous estimation of blood volume (see below), the radiolabelled ketone body was injected together with ¹³¹I- or ¹²⁵I-labelled human serum albumin (The Radiochemical Centre) previously passaged for 3.5h through a starved rat (McFarlane, 1957). The radioactive dose (in 0.2ml serum) was generally injected via a tail vein cannula into unanaesthetized rats and washed in with 0.2ml of 0.9% NaCl, but for the earliest samples (10s after injection) this procedure was too slow and injections were given directly into a lateral tail vein in rats under diethyl ether anaesthesia. Rats were decapitated at intervals after injection and blood was collected from the severed neck. For the samples taken 10s after injection, accurate timing was achieved by terminating the collection 2s after decapitation; in injured rats this gave insufficient blood for determination of ketone-body concentrations, so a second sample was collected for this purpose. All data were normalized to a dose of 0.4 μ Ci of radiolabelled β -hydroxybutyrate or acetoacetate/100g body wt.

Infusion and blood sampling in hepatectomized rats. Under diethyl ether anaesthesia, control rats were hepatectomized and infused with [3-¹⁴C]acetoacetate or β -hydroxy[3-¹⁴C]butyrate together with unlabelled

ketone bodies and glucose to compensate for their lack of production by the liver (Barton, 1973). The rates of infusion were 2.7–3.6 μ mol/min per 100g body wt. for D-(–)- β -hydroxybutyrate, 1.8–2.4 μ mol/min per 100g body wt. for acetoacetate, 4.2–5.4 μ mol/min per 100g body wt. for glucose and 0.07–0.23 μ Ci/min per 100g body wt. for the radiolabelled ketone body. Blood was collected from the aorta at times up to 30min from the start of the infusion, each rat contributing one sample. Data from these experiments were normalized to an infusion rate of 0.15 μ Ci/min per 100g body wt.

Analytical methods

Blood volume. The blood volumes needed for calculating quantities of label in the blood were determined in two ways in each rat. The dilution of the injected radiolabelled albumin gave an 'estimated' blood volume (Gregersen & Rawson, 1959) and a 'calculated' value was derived from the haematocrit by assuming that erythrocytes constituted a constant proportion of body weight. Since results in the rabbit show that the erythrocyte volume and the F_{cells} ratio (ratio of whole body haematocrit to measured great vessel haematocrit) are unchanged after limb ischaemia (Little, 1974), the data of Heath (1973) were used for injured as well as control rats. At short times after injection the 'calculated' blood volume tended to exceed the 'estimated' value owing to incomplete mixing of the injected dose in the circulation, and at long times there were discrepancies due to disappearance of labelled albumin, for which only approximate allowance was made. At intermediate times (1.5–5min) the ratio between the 'estimated' and 'calculated' values was 0.986 ± 0.053 (mean $\pm$ s.d.; 24 samples) in control rats and 0.973 ± 0.059 (mean $\pm$ s.d.; 22 samples) in the injured. This confirms that after limb ischaemia in the rat there is no loss of erythrocytes, which would lead to overestimation of the 'calculated' volume. In general, except for the discrepant values at long times after injection, 'estimated' volumes were used for calculating quantities of label; this corrected for slow mixing into the circulation.

Determination of concentrations and radioactivities. Chemicals and enzymes were from the sources given by Barton (1973).

In blood samples from intact (non-hepatectomized) rats, concentrations of β -hydroxybutyrate and acetoacetate were determined enzymically as described previously (Barton, 1973). Label in ketone bodies was determined by decarboxylation of acetoacetate (Mayes & Felts, 1967), but for conversion of β -hydroxybutyrate into acetoacetate 1.0ml of blood extract was mixed with 0.1ml of 0.25M-Tris buffer (pH 8.5), 0.1ml of 10mM-NAD⁺, 0.2ml of 0.5M-sodium oxaloacetate (pH 8.5), 10 μ l of

β -hydroxybutyrate dehydrogenase (about 15 units/ml) and 10 μ l of malate dehydrogenase (about 3600 units/ml). The mixture (1 ml) was left overnight at room temperature in a sealed ampoule, which was placed in freezing mixture before being opened for the decarboxylation. When treated in this way [14 C]-acetoacetate standards gave a recovery about 15% lower than when decarboxylated directly (see Barton, 1973); recovery of β -hydroxy[14 C]butyrate was similar to that of [14 C]acetoacetate. In each experiment a portion of the solution for injection was serially diluted with blood to give a standard, which was analysed exactly like the samples. After correcting for dilution the label recovered was taken as the actual dose injected; this allowed both for any impurity in it and for losses at any stage. A further correction was made for the additional loss described above. Label in the C-1 position of ketone bodies was determined only in samples at or after 2.5 min after injection, and was subtracted from the label in the C-2 to C-4 moiety to correct for recycling, which is accompanied by randomization of label between C-1 and C-3 in the acetoacetyl-CoA thiolase (EC 2.3.1.9) reaction (Lynen, 1955). The contribution of recycled label to the total label in blood ketone bodies gradually increased after injection, reaching about 20% at the latest times of sampling.

The specific activities of β -hydroxybutyrate and acetoacetate in blood from hepatectomized rats were determined as before (Barton, 1973).

Results

A 4 h period of bilateral hind-limb ischaemia is followed by fluid loss into the hind limbs (Wilson & Roome, 1936; Rosenthal, 1943). In the present experiments the haematocrits, corrected for trapped plasma, rose from 47.1 ± 2.5 (mean $\pm$ s.d.; 65 samples) in control rats to 57.2 ± 2.2 (mean $\pm$ s.d.; 64 samples) in the injured rats and the blood volumes calculated from them (see the Experimental section) fell from 6.51 ± 0.38 ml/100 g body wt. (mean $\pm$ s.d.; 65 samples) to 5.34 ± 0.23 ml/100 g body wt. (mean $\pm$ s.d.; 64 samples).

Ketone-body concentrations did not differ systematically between samples at different times after injection either in injured rats (about 1 h after limb ischaemia) or in controls, suggesting that ketone-body metabolism was substantially in a steady state during this period (Barton, 1973). The mean concentrations are given in Table 1. In agreement with previous results (Barton, 1971), the concentration of β -hydroxybutyrate in the blood fell after injury, but that of acetoacetate marginally increased; hence the blood [β -hydroxybutyrate]/[acetoacetate] ratio decreased ($P < 0.001$) from 3.07 ± 0.59 (mean $\pm$ s.d.; 63 samples) to 1.77 ± 0.80 (mean $\pm$ s.d.; 64 samples). (These values are only for unanaesthetized

rats. When diethyl ether anaesthesia was used to obtain samples 10 s after injection the ratio in injured rats was significantly higher and often above the control range, suggesting that the anaesthetic may have exacerbated the effects of the injury and hastened the onset of hypoxia in the liver.)

The specific radioactivity-time curves for blood ketone bodies after injection of [14 C]acetoacetate and β -hydroxy[14 C]butyrate are given in Figs. 1 and 2 respectively. As in post-absorptive rats (Barton, 1973), but in contrast with rats starved for 48 h (Bates *et al.*, 1968; McGarry *et al.*, 1970), the specific activities of β -hydroxybutyrate and acetoacetate never became equal.

From the areas under the specific activity-time curves (Figs. 1 and 2) the combined rate of utilization of both ketone bodies (Table 1) was calculated. In order to calculate the separate rates of utilization

Fig. 1. Specific activity-time curves in blood after injection of [14 C]acetoacetate

Bars represent the standard errors of specific activity (four to seven samples per point). Values at approx. 10 s after injection (in anaesthetized rats) were calculated from quantity of label in these rats and mean blood pool size in unanaesthetized rats. Values at zero time were calculated from quantity of label injected and mean pool size. (a) Control rats; (b) injured rats. ●, β -Hydroxybutyrate; ○, acetoacetate.

of β -hydroxybutyrate and acetoacetate allowance had to be made for their extrahepatic interconversion, which was estimated, in control rats only, by infusion of [14 C]acetoacetate and β -hydroxy[14 C]butyrate after hepatectomy (see the Experimental section). About 6 min after starting the infusions the specific activities of the two ketone bodies reached roughly constant values (Table 2). The specific activity of β -hydroxybutyrate was much lower than that of acetoacetate during [14 C]acetoacetate infusion and vice versa, as in post-absorptive rats (Barton, 1973) and rats starved for 48 h (McGarry *et al.*, 1970), showing that the rate coefficients for extrahepatic interconversion were very small (Table 3). Neglecting extrahepatic interconversion altered the rates and rate coefficients for utilization of ketone bodies by about 2%, and control data (Table 2) were used to make this small correction in injured rats.

The rate coefficients for interconversion of blood β -hydroxybutyrate and acetoacetate in the whole body were determined from the blood radioactivity-time curves (not shown), and those for interconversion via the liver were calculated by difference between the whole-body and extrahepatic values. All the interconversion coefficients are given in Table 3. After injury the overall rate coefficient for conversion of β -hydroxybutyrate into acetoacetate was unaltered, but that for conversion of acetoacetate into β -hydroxybutyrate fell.

The rates, rate coefficients and metabolic clearance rates for utilization of β -hydroxybutyrate and acetoacetate are given in Table 1. The fall in the total rate of utilization after injury is due to decreases in the rates for both β -hydroxybutyrate and acetoacetate. The metabolic clearance rate for acetoacetate also decreased, whereas that for β -hydroxybutyrate was unchanged; these findings are consistent with the concentration changes noted above, i.e. a slight rise for acetoacetate and a fall for β -hydroxybutyrate.

Fig. 2. Specific activity-time curves in blood after injection of β -hydroxy[14 C]butyrate

Further details are as in the legend to Fig. 1. (a) Control rats; (b) injured rats. ●, β -Hydroxybutyrate; ○, acetoacetate.

Table 1. Concentrations of ketone bodies in blood of injured and control starved rats, and rates, rate coefficients and metabolic clearance rates for their utilization

Data are given as means $\pm$ S.E. Rates, rate coefficients and metabolic clearance rates were calculated as described in the Experimental section, and their errors as described by Barton (1973). Differences from control values were significant at: $P < 0.001$ (*); $P < 0.02$ (†); $P < 0.05$ (‡).

	Control rats			Injured rats		
	Acetoacetate	β -Hydroxybutyrate	Acetoacetate + β -hydroxybutyrate	Acetoacetate	β -Hydroxybutyrate	Acetoacetate + β -hydroxybutyrate
Concentration (μ mol/ml) (63 samples per group)	0.47	1.41	1.90	0.53†	0.87*	1.40*
Rate of utilization (μ mol/min per 100 g body wt.)	± 0.01	± 0.04	± 0.04	± 0.02	± 0.04	± 0.05
Rate coefficient (min^{-1})	5.67	3.78	9.45	3.91*	2.08*	5.99*
	± 0.21	± 0.23	± 0.17	± 0.28	± 0.18	± 0.21
Metabolic clearance rate (ml/min per 100 g body wt.)	1.79	0.422	—	1.57‡	0.439	—
	± 0.08	± 0.024	—	± 0.07	± 0.044	—
	11.65	2.75	—	8.38*	2.34	—
	± 0.53	± 0.16	—	± 0.38	± 0.24	—

Table 2. *Specific activities in blood during infusion of labelled ketone bodies into hepatectomized control rats*

Control starved rats were hepatectomized and infused with glucose, unlabelled ketone bodies and [3-¹⁴C]acetoacetate or β -hydroxy[3-¹⁴C]butyrate. Each rat contributed one blood sample.

	Time (min)	10 ⁻³ × Specific activity (d.p.m./ μ mol)	
		Acetoacetate	β -Hydroxybutyrate
[¹⁴ C]Acetoacetate infusion (mean $\pm$ S.E.; 6 samples)	10–30	124.4 $\pm$ 7.6	6.69 $\pm$ 0.40
β -Hydroxy[¹⁴ C]butyrate infusion (2 samples)	7, 10	9.04, 9.80	95.2, 98.3

Table 3. *Rate coefficients for interconversion of blood ketone bodies*

All rate coefficients are given as means $\pm$ S.E. Values for whole body were calculated from areas under radioactivity-time curves, and those for extrahepatic tissues from data in Table 2 and from disposal rate coefficients in Table 1. Rate coefficients for interconversion via liver were calculated by difference.

	Rate coefficients (min ⁻¹)	
	Acetoacetate $\rightarrow$ β -Hydroxybutyrate	β -Hydroxybutyrate $\rightarrow$ Acetoacetate
Injured rats:		
Whole body	0.427 $\pm$ 0.037*	0.468 $\pm$ 0.049
Control rats:		
Whole body	0.630 $\pm$ 0.060	0.380 $\pm$ 0.026
Extrahepatic tissues	0.131 $\pm$ 0.010	0.035 $\pm$ 0.003
Liver	0.499 $\pm$ 0.061	0.345 $\pm$ 0.027

* Difference from control value significant at $P < 0.01$ (Student's *t* test).

Discussion

In the starved rat in a 20°C environment ischaemic limb injury nearly always results in death within a few hours (Threlfall & Stoner, 1954). However, after 1 h, when the present experiments were done, there is no evidence of hypoxia as judged by the [β -hydroxybutyrate]/[acetoacetate] and [lactate]/[pyruvate] ratios in the liver and blood (Threlfall, 1970; Barton, 1971).

Rates of production and utilization

Since the concentrations of ketone bodies in the blood did not change during the experimental period, the rates of ketone-body utilization must

have been very close to the rates of production by the liver. Thus in rats starved for about 30 h limb ischaemia led to a decrease of about 37% in the rate of ketone-body production (Table 1). This may have been due to a lower net rate of uptake of free fatty acids by the liver, since in the injured starved rat the plasma concentration of free fatty acids is decreased, but their fractional uptake by the liver is probably unchanged (Heath & Stoner, 1968). The fall in plasma free fatty acid concentration occurs in spite of sympathetic stimulation of lipolysis and is probably due to poor adipose-tissue perfusion (Stoner & Matthews, 1967).

Ketone bodies are mainly oxidized *in vivo* to CO₂; net conversion into fatty acids is also possible, but in practice is not extensive (Balasse & Havel, 1971). If complete oxidation is assumed the mean rates of utilization in Table 1 would require an O₂ consumption of 39 μ mol/min per 100 g body wt. in control rats and 25 μ mol/min per 100 g body wt. in injured rats. These values are equivalent to about 29% and 25% respectively of the whole-body O₂ consumption, which falls from 138 $\pm$ 5 μ mol/min per 100 g body wt. to 98 $\pm$ 5 μ mol/min per 100 g body wt. (mean $\pm$ S.E.; 11 samples per group) 1 h after limb ischaemia in starved rats (R. A. Little & H. B. Stoner, unpublished work). Thus in the starved rat the decrease in the rate of ketone-body utilization after injury paralleled the decrease in O₂ consumption and the importance of ketone bodies as a metabolic fuel was apparently retained.

The O₂ consumption required for ketone-body production from fatty acids could also be calculated and added to that required for ketone-body oxidation to give the total O₂ requirement for fatty acid oxidation via ketone bodies. This was equivalent to approx. 40% of the whole-body O₂ consumption in control starved rats and approx. 35% in injured rats, suggesting that in both groups about one-half of the overall oxidation of fatty acids took place via ketone-body synthesis and oxidation. The decrease in ketone-body turnover after injury was therefore consistent with the fall in the overall rate of oxidation of free fatty acids, as shown by the slower incorporation of injected [¹⁴C]palmitate into ¹⁴CO₂ (Heath & Stoner, 1968).

The present results can be compared with those in injured and control post-absorptive rats (Barton, 1973). The comparison between controls shows that the rate of ketone-body production rose about 3.7-fold during starvation to reach a value close to the maximum attainable in the perfused liver (Krebs & Hems, 1970). The increase was proportionally greater for β -hydroxybutyrate than for acetoacetate. After injury the fall in the rate of ketogenesis in the starved rat contrasted with the rise in the post-absorptive rat. This difference has not yet been explained.

Rate coefficients

Rate coefficients for ketone-body utilization have been calculated as metabolic clearance rates (the volume of blood which, if cleared of substrate in unit time, would give a rate of utilization equal to that in the whole animal). In control starved rats the values of the metabolic clearance rates (Table 1) were about one-half those in control post-absorptive rats (Barton, 1973). This was probably due to partial saturation of the enzymes involved in ketone-body utilization since in the post-absorptive state metabolic clearance rates can be lowered simply by raising the ketone-body concentrations by infusion (Bässler *et al.*, 1970; Balasse & Havel, 1971). One might therefore have expected that the lower concentration of β -hydroxybutyrate after injury would be associated with a higher metabolic clearance rate; however, this was not the case (Table 1). The metabolic clearance rate of acetoacetate was also lower than expected, since it fell substantially, whereas the blood concentration rose only slightly (Table 1). As in post-absorptive rats (Barton, 1973), the likely explanation is a fall in cardiac output (Takács *et al.*, 1962) leading to a decrease in tissue perfusion, and there was probably no impairment of ketone-body utilization within the cells.

Values could also be calculated for the rate coefficients for interconversion of blood β -hydroxybutyrate and blood acetoacetate and, in control rats only, the relative contributions of the liver and extra-hepatic tissues to this interconversion (Table 3). In control starved rats the rate coefficient for conversion of acetoacetate into β -hydroxybutyrate by the liver was much higher than in control post-absorptive rats (Barton, 1973); the liver thus became the major route for interconversion in both directions, and there was a rise in the overall rate coefficient for conversion of acetoacetate into β -hydroxybutyrate. After injury in the starved rat the value for the latter fell, but there was no decrease in the rate coefficient for conversion of β -hydroxybutyrate into acetoacetate, as seen in the injured post-absorptive rat (Barton, 1973). The significance of these findings is so far unknown.

Injury in man

Many of the metabolic changes after limb ischaemia in the rat resemble those typically seen after injury in man (Stoner, 1969a). One might therefore expect that, in subjects with moderate ketosis, severe injury would be followed by decreased rates of ketone-body production and utilization which, despite decreased metabolic clearance, would to some extent be reflected in lower concentrations in the blood. There are insufficient published data to substantiate these predictions; however, it is of interest that a series of patients during the first 20h after major

abdominal surgery failed to show the rise in blood ketone body concentrations expected during a comparable fast (Naftalin, 1962).

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References

- Ashby, M. M., Heath, D. F. & Stoner, H. B. (1965) *J. Physiol. (London)* **179**, 193–237
- Balasse, E. O. & Havel, R. J. (1971) *J. Clin. Invest.* **50**, 801–813
- Barton, R. N. (1971) *Clin. Sci.* **40**, 463–477
- Barton, R. N. (1973) *Biochem. J.* **136**, 531–543
- Bässler, K. H., Wagner, G. & Heicke, B. (1970) *Z. Gesamte Exp. Med.* **153**, 131–135
- Bates, M. W., Krebs, H. A. & Williamson, D. H. (1968) *Biochem. J.* **110**, 655–661
- Cremer, J. E. & Heath, D. F. (1974) *Biochem. J.* **142**, 527–544
- Gregersen, M. I. & Rawson, R. A. (1959) *Physiol. Rev.* **39**, 307–342
- Heath, D. F. (1973) *Br. J. Exp. Pathol.* **54**, 359–367
- Heath, D. F. & Barton, R. N. (1973) *Biochem. J.* **136**, 503–518
- Heath, D. F. & Corney, P. L. (1973) *Biochem. J.* **136**, 519–530
- Heath, D. F. & Stoner, H. B. (1968) *Br. J. Exp. Pathol.* **49**, 160–169
- Heath, D. F. & Threlfall, C. J. (1968) *Biochem. J.* **110**, 337–362
- Krebs, H. A. & Hems, R. (1970) *Biochem. J.* **119**, 525–533
- Little, R. A. (1974) *J. Physiol. (London)* **238**, 207–221
- Lynen, F. (1955) *Annu. Rev. Biochem.* **24**, 653–688
- Mayes, P. A. & Felts, J. M. (1967) *Biochem. J.* **102**, 230–235
- McFarlane, A. S. (1957) *Prog. Biophys. Biophys. Chem.* **7**, 115–163
- McGarry, J. D., Guest, M. J. & Foster, D. W. (1970) *J. Biol. Chem.* **245**, 4382–4390
- Naftalin, L. (1962) *Clin. Chim. Acta* **7**, 614–622
- Rosenthal, S. M. (1943) *Publ. Health Rep.* **58**, 1429–1436
- Smith, R., Fuller, D. J., Wedge, J. H., Williamson, D. H. & Alberti, K. G. M. M. (1975) *Lancet* **i**, 1–3
- Stoner, H. B. (1961) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **20**, Suppl. 9, 38–48
- Stoner, H. B. (1969a) *Ann. R. Coll. Surg. Engl.* **44**, 308–323
- Stoner, H. B. (1969b) *Br. J. Exp. Pathol.* **50**, 125–138
- Stoner, H. B. & Heath, D. F. (1973) *Br. J. Anaesth.* **45**, 244–251
- Stoner, H. B. & Matthews, J. (1967) *Br. J. Exp. Pathol.* **48**, 58–65
- Takács, L., Kállay, K. & Skolnik, J. H. (1962) *Circulation Res.* **10**, 753–757
- Threlfall, C. J. (1970) *Ciba Found. Symp.: Energy Metabolism in Trauma*, 127–138
- Threlfall, C. J. & Stoner, H. B. (1954) *Q. J. Exp. Physiol.* **39**, 1–9
- Wilson, H. & Roome, N. W. (1936) *Arch. Surg.* **32**, 334–345