The Appearance of Gluconeogenesis at Birth in Sheep

ACTIVATION OF THE PATHWAY ASSOCIATED WITH BLOOD OXYGENATION

By DEIRDRE M. WARNES and ROBERT F. SEAMARK
*Department of Obstetrics and Gynaecology, University of Adelaide, Adelaide,
South Australia 5001, Australia*

and F. JOHN BALLARD
*CSIRO Division of Human Nutrition, Kintore Avenue, Adelaide, South Australia 5000,
Australia*

(Received 27 September 1976)

1. Measurements of pyruvate carboxylase, mitochondrial phosphoenolpyruvate carboxykinase (GTP), hexose bisphosphatase and glucose 6-phosphatase in developing sheep liver showed substantial activities of all enzymes in the foetus, especially towards the end of gestation. Cytosol phosphoenolpyruvate carboxykinase (GTP) in livers of mid-term foetuses was only 10% of the activity at birth. 2. All enzymes except pyruvate carboxylase showed 1.5-2-fold increases after birth. 3. Gluconeogenesis from [^{14}C]lactate could not be detected in chronically cannulated sheep foetuses at any developmental stage and was not initiated by the infusion of adrenaline or glucagon. 4. An active pathway of gluconeogenesis was evident *in vivo* within 2 min after natural birth or within 4 min after Caesarian delivery of term lambs, and was delayed in prematurely delivered lambs until breathing was established and the blood fully oxygenated. 5. It is proposed that oxygen availability initiates gluconeogenesis in the newborn lamb.

Gluconeogenesis in foetal rat liver is prevented by the negligible activity of phosphoenolpyruvate carboxykinase (GTP) (Ballard & Hanson, 1967). However, even when this enzyme is induced by the injection of glucagon to foetuses *in utero*, the overall pathway cannot be detected (Philippidis & Ballard, 1969, 1970). Although we have interpreted this finding as being caused by a relatively hypoxic state in the rat foetus, we could not obtain direct evidence for this hypothesis. However, appropriate experiments are possible with chronically unstressed cannulated sheep foetuses, especially since we have shown in the preceding paper (Warnes *et al.*, 1977) the absence of gluconeogenesis from lactate in these animals.

In the present study we have followed the appearance of gluconeogenesis in chronically cannulated sheep foetuses by injecting pulses of [^{14}C]lactate before, during and after birth. Attempts have also been made to induce the pathway prematurely, and to relate the appearance of gluconeogenesis to the activity of phosphoenolpyruvate carboxykinase (GTP) and other key enzymes of gluconeogenesis.

Methods

Chemicals

Phosphoenolpyruvate, fructose 1,6-bisphosphate, IDP, malate dehydrogenase (NAD^+), phosphoglucose isomerase and glucose 6-phosphate dehydro-

genase were obtained from Boehringer, Mannheim, Germany; acetyl-CoA, NADH, NADP^+ , ATP and glucose 6-phosphate were from Sigma Chemical Co., St. Louis, MO, U.S.A.; $\text{NaH}^{14}\text{CO}_3$ and [$\text{U-}^{14}\text{C}$]lactate were purchased from The Radiochemical Centre, Amersham, Bucks., U.K.; adrenaline tartrate was from David G. Bull Lab. Pty. Ltd., Canterbury, N.S.W., Australia, and glucagon was from Eli Lilly, Indianapolis, IN, U.S.A.

Animals

Foetal lamb livers and liver samples from adult sheep were obtained from the SAMCOR abattoir, Gepps Cross, South Australia, Australia. Livers were placed in ice-cold 0.15M-NaCl for transport to the laboratory. The crown-rump length and weight of each foetus was measured for estimation of foetal age by reference to a nomogram constructed from data on sheep of a similar breed. Term has been taken as 150 days gestation. Livers from postnatal lambs were obtained from the CSIRO Glenelg Field Station, O'Halloran Hill, South Australia, Australia.

Cannulation of foetal and postnatal lambs was carried out as described in the preceding paper (Warnes *et al.*, 1977). The foetuses used were of known gestational age. For those animals in which gluconeogenesis was being measured during natural birth, the approach to parturition was shown by the increase in foetal plasma cortisol that occurred during the last days of gestation (Liggins *et al.*, 1974),

as well as by behavioural changes in the ewe. Gluconeogenesis was assessed in these animals by [^{14}C]lactate injections before birth. After birth of the lamb with cannulae intact, three-way stopcocks were attached to each cannula, the umbilical cord was ligated and further injections were carried out. The mother was allowed free access to the newborn lamb, but the lambs made no attempt to suckle during the experimental period.

Four lambs with cannulae implanted in the femoral artery and vein were delivered by midline laparotomy. The umbilical cords were ligated after the head was delivered and breathing established. The lambs were dried vigorously and kept warm. Injections of [^{14}C]lactate and blood sampling were carried out both before and after delivery.

Measurements of enzyme activities

Liver samples were homogenized in 4 vol. of 0.25 M-sucrose by using a glass homogenizer with Teflon pestle. A portion of this homogenate was used for the measurement of glucose 6-phosphatase activity.

A further 0.5 ml of homogenate was freeze-dried and then suspended in water for immediate assay of pyruvate carboxylase. The remaining homogenate was centrifuged at 100000g for 30 min and the supernatant used for the assay of hexose bisphosphatase and cytosol phosphoenolpyruvate carboxykinase (GTP). The pellet from this centrifugation was dispersed in water, freeze-dried and resuspended in water for the measurement of particulate phosphoenolpyruvate carboxykinase (GTP).

Glucose 6-phosphatase (EC 3.1.3.9) activity was measured as the amount of P_i released when glucose 6-phosphate was incubated with liver homogenate (Shull *et al.*, 1956). P_i was determined as described by Tausky & Shorr (1953).

Hexose bisphosphatase (EC 3.1.3.11) was assayed spectrophotometrically by the method described by Taketa & Pogell (1965).

Bicarbonate-fixation assays were used for the measurement of phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32) (Ballard & Hanson, 1967) and pyruvate carboxylase (EC 6.4.1.1) (Utter & Keech, 1963) activities.

Enzyme activities are expressed as μmol of product formed/min (units) per g of tissue for incubations carried out at 37°C.

Hormone infusions

Concentrations of glucose, fructose and lactate in blood, as well as $p\text{O}_2$, $p\text{CO}_2$ and pH, were measured post-operatively in foetuses with cannulae implanted in their femoral blood vessels [see the preceding paper (Warnes *et al.*, 1977)]. A minimum of 5 days elapsed between surgery and the infusion studies.

Infusion of adrenaline in 0.15 M-NaCl, of glucagon in 0.15 M-NaCl or of 0.15 M-NaCl alone was maintained at a rate of 0.1 ml/min with a Handley intra-venous-injection apparatus obtained from South-down Medical Developments, Harpenden, Herts., U.K. The foetuses treated with adrenaline received a priming dose of 10 μg , followed by a constant infusion at the rate of 1.75 $\mu\text{g}/\text{min}$. Foetuses infused with glucagon received an initial priming dose of 50 μg , followed by an infusion of 5 μg of glucagon/min.

Assessment of gluconeogenesis

Gluconeogenesis rates *in vivo* were measured as the extent of conversion of [^{14}C]lactate into glucose by using the separation methods described in the preceding paper (Warnes *et al.*, 1977). Foetuses or lambs received 10 μCi of [^{14}C]lactate via the femoral-arterial cannula and blood samples were taken from the femoral vein 2 min afterwards. Glucose radioactivity was measured as a fraction of glucose-plus-lactate radioactivity in these blood samples.

Results

Activities of key enzymes in gluconeogenesis

Glucose 6-phosphatase activity increases from 1.2 units/g of liver in foetuses at approx. 55 days gestation to 9.7 units/g of liver in term foetuses (Fig. 1a). There is a further increase after birth before the activity declines to the adult value of 8.8 units/g of liver.

Hexose bisphosphatase activity is present in foetal sheep liver at a considerable activity throughout the period of gestation studied (Fig. 1b). There is an increase in activity just before birth, but no further changes occur in suckling or adult animals.

The activity of hepatic pyruvate carboxylase increases more than twofold between mid-gestation and term, with a further increase in activity in the newborn (Fig. 1c). Livers of suckling animals have a slightly higher pyruvate carboxylase activity than that found in adult sheep.

Two distinct phosphoenolpyruvate carboxykinase (GTP) enzymes occur in liver, one of which is mitochondrial and the other is present in the cytosol (Ballard & Hanson, 1969). These enzymes in sheep liver can be separated by electrophoretic or chromatographic techniques (Ballard, 1971b) and presumably correspond to the rat liver enzymes that have been distinguished by immunochemical methods (Ballard & Hanson, 1969). The activity of the particulate enzyme is relatively constant in sheep liver during gestation and is present at an activity of 1.25–1.4 units/g of tissue (Fig. 2b). This activity increases twofold after birth before decreasing to an

Fig. 1. Changes in the activities of (a) glucose 6-phosphatase, (b) hexose biphosphatase and (c) pyruvate carboxylase, in developing sheep liver

Values are expressed as means $\pm$ S.E.M. for measurements on six to twelve animals at each developmental stage. T, term; A, adult.

adult activity of 1.45 units/g of liver. The cytosol phosphoenolpyruvate carboxykinase (GTP), on the other hand, is present at a very low activity in livers of mid-term foetuses (Fig. 2a) and increases tenfold between this stage and birth. The activity in adult sheep liver is similar to that found in livers of suckling animals. The percentage of total enzyme present in the cytosol fraction shows marked changes with development, being 11% in mid-gestation, 27% at 135 days gestation, 39% in term foetuses and suckling animals, and 52% in the adult.

The changes in activity of glucose 6-phosphatase and hexose biphosphatase agree with those published by Ballard & Oliver (1965), although the coupled enzymic assay used for the latter enzyme in present study gives somewhat higher activities. Edwards *et al.* (1975) report a postnatal increase

Fig. 2. Changes in the activities of (a) cytosol and (b) particulate phosphoenolpyruvate carboxykinase (GTP) in developing sheep liver

Values are expressed as means $\pm$ S.E.M. for measurements on six to twelve animals at each development stage. T, term; A, adult.

in the pyruvate carboxylase activity of sheep liver, but low total phosphoenolpyruvate carboxykinase (GTP) activity throughout gestation. Edwards *et al.* (1975) did not distinguish between cytosol and particulate activities of phosphoenolpyruvate carboxykinase (GTP).

Development of an active gluconeogenic pathway

In the preceding paper (Warnes *et al.*, 1977) we showed that gluconeogenesis from lactate does not occur in unanaesthetized unstressed foetal sheep. Since all key enzymes involved in gluconeogenesis are present in term foetuses at activities similar to those found in livers of adult sheep, the near-term foetus certainly has the capacity for gluconeogenesis. In an attempt to follow the appearance of actual gluconeogenic flux from lactate we inserted cannulae in the femoral blood vessels of sheep foetuses and injected sequential pulses of [14 C]lactate before birth and during the first few minutes after birth. Blood samples were withdrawn 2 min after each

radioactive pulse and the ratio of glucose radioactivity to glucose-plus-lactate radioactivity was measured (Fig. 3). No glucose was formed when [^{14}C]lactate was injected into foetuses. However, there was a gradual increase in the above ratio at increasing times after a pulse was given to postnatal animals.

We have chosen the earliest sampling point, 2 min, as an index of gluconeogenic flux. Although only 10% of the radioactivity is present in blood glucose at this time after injection of [^{14}C]lactate into suckling lambs compared with 75% 1 h after the injection (Fig. 3), the total glucose radioactivity at 2 min is substantial and changes in labelling rate are readily detected. Gluconeogenesis cannot be detected in foetal sheep even 10 min before natural delivery (Table 1), but when [^{14}C]lactate is injected immediately after birth and blood withdrawn 2 min later, conversion of lactate into glucose is found (Table 1, foetus no. 1). The second foetus tested in this way (foetus no. 2) showed an active gluconeogenic pathway at 6 min, the first sampling period after birth. With both animals the rate of gluconeogenesis increased further during the first hour after birth. The birth of animal no. 1 was accompanied by a rapid increase in blood oxygenation, and although blood gases were not measured in foetus no. 2, it was apparent from the change in blood colour that oxygenation had occurred by the first sampling period.

An additional four animals were delivered by Caesarian section, and 10 μCi pulses of [^{14}C]lactate given at the times indicated in Table 2. For each animal, measurements of glucose, lactate, $p\text{O}_2$, $p\text{CO}_2$ and pH were made in blood samples and the conversion of [^{14}C]lactate into glucose was followed. In the two animals where blood oxygenation was

rapid, lambs nos. 3 and 6, gluconeogenesis was detectable in the first postnatal sample. Blood oxygenation was delayed in the other two animals, possibly because they were at an earlier stage in

Fig. 3. Labelling of blood glucose after injection of [^{14}C]lactate into suckling (●) or chronically cannulated foetal (○) sheep

Blood samples were withdrawn at different times after the injection of 50 μCi of [^{14}C]lactate into each animal. After separation of glucose, fructose and lactate from deproteinized blood [see the preceding paper (Warnes *et al.*, 1977)], the ratio between radioactivity in glucose and in lactate-plus-glucose was determined. Values are means from experiments on five suckling animals between 5 and 28 days after birth and four foetuses of gestational ages 125 to 135 days.

Table 1. Development of gluconeogenic flux in naturally born lambs

Details of the measurements on each animal are given in the text. [^{14}C]Lactate was injected at the times indicated and blood withdrawn 2 min later. Glucose radioactivity in these samples is the percentage of radioactivity in blood glucose relative to radioactivity in lactate plus glucose (see Fig. 3).

	Time of [^{14}C]lactate injection (min after birth)	Blood					
		Glucose radioactivity	[Glucose] (mm)	[Lactate] (mm)	$p\text{O}_2$ (mmHg)	$p\text{CO}_2$ (mmHg)	pH
Foetus no. 1, term	-208	0	0.38	3.11	13	49	7.42
	-72	0	1.00	9.80	20	48	7.34
	0	0.7	1.53	11.83	44	51	7.25
	12	2.0	1.80	13.23	44	35	7.28
	46	4.7	1.34	12.15	44	35	7.36
Foetus no. 2, term	-30	0	0.64	5.33	—	—	—
	-19	0	1.16	6.04	—	—	—
	-10	0	1.83	5.94	—	—	—
	6	1.1	1.07	8.59	—	—	—
	19	1.5	2.62	7.80	—	—	—
	76	3.6	2.86	8.71	—	—	—

Table 2. *Development of gluconeogenic flux in Caesarian-born lambs*

Measurements are described in the text and in the legend to Fig. 3. Blood was sampled 2min after each injection of [^{14}C]lactate.

	Time of [^{14}C]lactate injection (min after delivery)	Blood					
		Glucose radioactivity	[Glucose] (mM)	[Lactate] (mM)	$p\text{O}_2$ (mmHg)	$p\text{CO}_2$ (mmHg)	pH
Foetus 3, 148 days	-2	0	1.34	5.37	16	40	7.39
	2	1.6	1.36	5.17	54	41	7.36
	17	2.8	1.28	3.94	44	40	7.39
	38	7.3	1.19	3.92	51	41	7.39
	65	7.1	1.15	4.46	54	35	7.44
Foetus 4, 139 days	-2	0	3.22	5.73	12	49	7.34
	9	0	3.94	6.96	24	57	7.19
	31	1.3	4.50	5.44	29	53	7.17
	54	2.1	4.94	5.22	37	55	7.15
	123	2.8	5.83	6.91	49	50	7.25
Foetus 5, 144 days	-10	0	0.87	2.89	19	40	7.43
	13	0	1.30	3.62	24	50	7.23
	32	2.1	1.52	2.54	39	54	7.25
	93	2.4	1.17	2.11	50	42	7.35
	118	2.2	0.77	2.38	54	42	7.38
Foetus 6, term	-2	0	1.91	9.28	19	54	7.31
	4	0.2	2.08	9.38	51	45	7.34
	68	1.5	3.96	7.62	—	—	—
	140	1.9	4.68	6.96	—	—	—

gestation. In these latter animals, glucose synthesis from lactate could not be detected after the first postnatal [^{14}C]lactate injection, but was found at 30min.

With the exception of the relation between blood oxygenation and gluconeogenesis noted above, no correlations were found between other blood measurements and gluconeogenesis. The concentration of blood glucose increased after birth in the two animals born naturally and in two of the lambs delivered by Caesarian section. However, the glucose was probably derived from glycogen rather than by gluconeogenesis, since in animal no. 4 the blood-glucose concentration had increased to 4mM before any gluconeogenesis could be detected. The post-natal glucose concentration is very much greater than the concentration of about 0.5mM found in unstressed foetuses (Tables 1, 3 and 4). It is also likely that the increase in blood lactate after natural birth (Table 1) occurs via breakdown of tissue glycogen, possibly caused by adrenaline release.

Attempts to induce gluconeogenesis prematurely in foetal sheep

Since gluconeogenesis in rat liver is stimulated by adrenaline and by glucagon (Exton & Park, 1966; Exton, 1972; Taunton *et al.*, 1972; Eisenstein *et al.*, 1974; Tilghman *et al.*, 1976), we have attempted to

produce an active pathway by the prolonged infusion of these hormones into foetal sheep.

Each hormone produced increases in foetal-blood glucose and lactate (Tables 3 and 4), which persisted throughout the infusion period. Blood fructose was not affected. However, pulse injections of [^{14}C]lactate followed by sampling after 2min gave no indication of glucose synthesis at any stage during the glucagon- or adrenaline-infusion experiments. A control infusion of 0.15M-NaCl into foetus no. 8 at 134 days of gestation produced no changes in blood glucose, lactate or fructose concentrations.

The three foetuses used in these experiments showed no undesirable effects of cannulation or from the hormonal treatments. As mentioned above, foetus no. 8 was infused on two occasions and was eventually born alive. Foetus no. 1 was first infused with adrenaline at age 119 days, then with glucagon at 131 days and was born naturally at 150 days with cannulae still functioning (see Table 1). Foetus no. 7 was also born alive.

Discussion

The key regulatory enzymes of gluconeogenesis [glucose 6-phosphatase, hexose biphosphatase, pyruvate carboxylase and phosphoenolpyruvate carboxykinase (GTP)] are all present at substantial activities in foetal sheep liver (Figs. 1 and 2). Although

Table 3. *Attempts to induce gluconeogenic flux by adrenaline infusion into foetal sheep*

Adrenaline was infused for 35 min in foetus no. 7 and for 22 min in foetus no. 1 at a rate of 1.75 µg/min after priming doses of 10 µg. Measurements were made on blood samples taken 2 min after each injection of [¹⁴C]lactate. Details of each measurement are given in the text and in the legend to Fig. 3.

	Time of [¹⁴ C]lactate injection (min after adrenaline)	Blood			
		Glucose radioactivity	[Glucose] (mm)	[Lactate] (mm)	[Fructose] (mm)
Foetus 7, 121 days	0	0	0.71	1.60	3.56
	2	0	0.86	1.93	3.64
	13	0	0.91	3.34	3.32
	24	0	1.21	4.01	3.40
	42	0	1.37	4.62	3.74
	68	0	1.30	4.11	3.82
Foetus 1, 119 days	0	0	0.43	1.61	3.53
	4	0	0.68	2.14	3.49
	9	0	1.18	3.86	3.46
	20	0	1.22	3.96	3.40
	28	0	0.80	4.27	3.60
	58	0	0.70	3.51	3.63

Table 4. *Attempts to induce gluconeogenic flux by glucagon infusion to foetal sheep*

Glucagon was infused for 75 min in foetus no. 8 and for 50 min in foetus no. 1 at a rate of 5 µg/min after initial priming doses of 50 µg. Measurements were made on blood samples taken 2 min after each injection of [¹⁴C]lactate. Details are given in the text and in the legend to Fig. 3.

	Time of [¹⁴ C]lactate injection (min after glucagon)	Blood		
		Glucose radioactivity	[Glucose] (mm)	[Lactate] (mm)
Foetus 8, 144 days	0	0	0.48	1.58
	8	0	0.63	1.65
	18	0	1.45	2.63
	28	0	1.73	3.22
	58	0	1.63	3.56
	73	0	1.70	3.40
Foetus 1, 131 days	0	0	0.52	1.71
	3	0	0.68	2.16
	10	0	1.04	2.66
	18	0	1.03	3.26
	28	0	1.15	3.19
	52	0	1.02	2.92
	66	0	1.09	2.21

cytosol phosphoenolpyruvate carboxykinase (GTP) is present in mid-term foetuses at only 10% of the activity at term, it is clear from studies on species in which the mitochondrial enzyme is the only form (Söling & Kleineke, 1976), that the cytosol enzyme has no unique role in gluconeogenesis. Clearly, the absence of gluconeogenesis from foetal sheep liver is not due to restrictive activities of these key enzymes.

If gluconeogenesis *in utero* is not limited by the activities of these enzymes, what can be limiting? Certainly substrate in the form of lactate is available.

In studies with developing rats, Philippidis & Ballard (1969, 1970) showed an absence of gluconeogenesis *in vivo* that was still apparent after phosphoenolpyruvate carboxykinase (GTP) had been induced with glucagon. A pertinent finding was an active gluconeogenic pathway in liver slices prepared from these glucagon-treated foetuses, a result which implied an inhibitory control *in vivo* that was not operating in liver slices. Further experiments with young rats demonstrated a decrease in gluconeogenesis *in vivo* in animals exposed to hypoxic conditions that were not sufficiently severe to decrease

the phosphorylation state of hepatic adenine nucleotides (Ballard 1971a,b). A tentative conclusion from these results was that gluconeogenesis could be limited by hypoxic conditions that were not otherwise deleterious to the young rat. Although direct evidence of foetal hypoxia in rats was not available, the phosphorylation state of liver adenine nucleotides was much lower in the foetus and in the immediate newborn than in animals 30 min after delivery (Ballard, 1971c). A further change was an oxidation of the cytosol NAD pool within 1 h after birth and the concomitant facilitation of lactate conversion into pyruvate (Ballard, 1971c). However, a major problem with all but the postnatal measurements in rats is the obligatory use of anaesthesia, and we were especially concerned that the ether used could have caused or could have contributed to the foetal hypoxia. Although chronic cannulation of foetal blood vessels obviates any problem of anaesthesia, foetal sheep do not have active gluconeogenesis. Yet within minutes of birth the young lamb is able to convert lactate into glucose, a facility that correlates with increased oxygenation of the blood.

An alternative explanation for the postnatal appearance of gluconeogenesis is that the hormone changes accompanying birth would either activate the pathway or induce limiting enzymes. Certainly changes in glucagon, insulin (Girard *et al.*, 1973) and probably adrenaline (Girard & Zeghal, 1975) occur at birth in the rat, and the direction of these changes is consistent with increased gluconeogenesis. However, the foetal sheep at term already has substantial activities of those hepatic enzymes that can be induced by glucagon or adrenaline in other species, and we were unable to produce an active pathway of gluconeogenesis even after prolonged infusion of the glucagon or adrenaline.

Two major problems remain to be resolved: the extent to which gluconeogenesis is absent from foetuses of other mammalian species, and an understanding of the cellular mechanisms involved in the initiation of gluconeogenesis. The first point cannot be answered until studies comparable with these are performed on other species. Nevertheless, gluconeogenesis is an energetically extravagant process for a foetus, since adequate amounts of glucose are available from the maternal circulation. Yet there is a useful advantage for the developing young to have all enzymes present to carry out gluconeogenesis as soon as the maternal transport of glucose is stopped, since hypoglycaemia will otherwise result. Such hypoglycaemia does occur in the neonatal rat (Dawkins, 1963; Yeung & Oliver, 1968), a species in which gluconeogenesis cannot occur during the hours required for phosphoenolpyruvate carboxykinase (GTP) induction. Hypoglycaemia has not to our knowledge been observed

in newborn sheep; rather hyperglycaemia is usually found in the first hour after birth (see Tables 1 and 2). Foetuses of most mammalian species have substantial activities of hepatic phosphoenolpyruvate carboxykinase (GTP) and other gluconeogenic enzymes (Ballard & Oliver, 1965; Mersman, 1971; Arinze, 1975; Tilghman *et al.*, 1976), and it is not unreasonable to infer that the situation described for the sheep occurs in other species.

We have no information on the cellular control of gluconeogenesis which would relate to the increased oxygenation after birth. We can only speculate that slight changes in the phosphorylation of adenine nucleotides and a relative oxidation of cellular redox states may be involved.

References

- Arinze, I. J. (1975) *Biochem. Biophys. Res. Commun.* **65**, 184-189
- Ballard, F. J. (1971a) *Biochem. J.* **121**, 169-178
- Ballard, F. J. (1971b) *Biochim. Biophys. Acta* **242**, 470-472
- Ballard, F. J. (1971c) *Biochem. J.* **124**, 265-274
- Ballard, F. J. & Hanson, R. W. (1967) *Biochem. J.* **104**, 866-871
- Ballard, F. J. & Hanson, R. W. (1969) *J. Biol. Chem.* **244**, 5625-5630
- Ballard, F. J. & Oliver, I. T. (1965) *Biochem. J.* **95**, 191-200
- Dawkins, M. J. R. (1963) *Ann. N.Y. Acad. Sci.* **111**, 203-211
- Edwards, E. M., Dhand, U. K., Jeacock, M. K. & Shepherd, D. A. L. (1975) *Biochim. Biophys. Acta* **399**, 217-227
- Eisenstein, A. B., Strack, I. & Steiner, A. (1974) *Metabolism* **23**, 15-23
- Exton, J. H. (1972) *Metabolism* **21**, 945-990
- Exton, J. H. & Park, C. R. (1966) *Pharmacol. Rev.* **18**, 181-188
- Girard, J. R. & Zeghal, N. (1975) *Biol. Neonat.* **26**, 205-213
- Girard, J. R., Cuendet, G. S., Marliss, E. B., Kervran, A., Rientort, M. E. & Assan, R. (1973) *J. Clin. Invest.* **52**, 3190-3200
- Liggins, G. C., Fairclough, R. J., Grieves, S. A., Kendall, J. Z. & Knox, B. S. (1974) *Recent Prog. Horm. Res.* **29**, 111-159
- Mersman, H. J. (1971) *Am. J. Physiol.* **220**, 1297-1302
- Phillipidis, H. & Ballard, F. J. (1969) *Biochem. J.* **113**, 651-657
- Phillipidis, H. & Ballard, F. J. (1970) *Biochem. J.* **120**, 385-392
- Shull, K. H., Ashmore, J. & Mayer, J. (1956) *Arch. Biochem. Biophys.* **62**, 210-216
- Söling, H.-D. & Kleineke, J. (1976) in *Gluconeogenesis: Its Regulation in Mammalian Species* (Hanson, R. W. & Mehlman, M. A., eds.), pp. 369-462, John Wiley, New York
- Taketa, K. & Pogell, B. M. (1965) *J. Biol. Chem.* **240**, 651-662
- Taunton, O. D., Stifel, F. B., Greene, H. L. & Herman, R. H. (1972) *Biochem. Biophys. Res. Commun.* **48**, 1663-1670

- Taussky, H. H. & Shorr, E. (1953) *J. Biol. Chem.* **202**, 675-685
- Tilghman, S. M., Hanson, R. W. & Ballard, F. J. (1976) in *Gluconeogenesis: Its Regulation in Mammalian Species* (Hanson, R. W. & Mehlman, M. A., eds.), pp. 47-91, John Wiley, New York
- Utter, M. F. & Keech, D. B. (1963) *J. Biol. Chem.* **238**, 2603-2608
- Warnes, D. M., Seamark, R. F. & Ballard, F. J. (1977) *Biochem. J.* **162**, 617-626
- Yeung, D. & Oliver, I. T. (1968) *Biochem. J.* **108**, 325-331