The activity of glycogen synthase phosphatase limits hepatic glycogen deposition in the adrenalectomized starved rat

Mathieu BOLLEN, Greet GEVERS and Willy STALMANS

Afdeling Biochemie, Faculteit Geneeskunde, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

(Received 28 March 1983/Accepted 12 May 1983)

1. Hepatocytes from adrenalectomized 48 h-starved rats responded to increasing glucose concentrations with a progressively more complete inactivation of phosphorylase. Yet no activation of glycogen synthase occurred, even in a K⁺-rich medium. 2. Protein phosphatase activities in crude liver preparations were assayed with purified substrates. Adrenalectomy plus starvation decreased synthase phosphatase activity by about 90%, but hardly affected phosphorylase phosphatase activity. 3. Synthase *b* present in liver extracts from adrenalectomized starved rats was rapidly and completely converted into the *a* form on addition of liver extract from a normal fed rat. 4. Glycogen synthesis can be slowly re-induced by administration of either glucose or cortisol to the deficient rats. In these conditions there was a close correspondence between the initial recovery of synthase phosphatase activity and the amount of synthase *a* present in the liver. The latter parameter was strictly correlated with the measured rate of glycogen synthesis *in vivo*. 5. The decreased activity of synthase phosphatase emerges thus as the single factor that limits hepatic glycogen deposition in the adrenalectomized starved rat.

During starvation for 2 days, the adrenalectomized rat loses the ability for prompt glycogen storage in the liver. There was no response to injected glucose (Dorsey & Munck, 1962), and after intra-gastric glucose administration the deposition of glycogen in the liver showed a lag of about 1.5 h before proceeding at a normal rate (Friedmann *et al.*, 1965, 1967). The lag *in vivo* is well explained by the failure of glycogen synthase to be activated (Mersmann & Segal, 1969). A similar lack of glycogen deposition and of synthase activation was noted when isolated livers from adrenalectomized starved rats were perfused with 30 mM-glucose (Glinsmann *et al.*, 1970; Whitton & Hems, 1976). Several groups have observed that the conversion of synthase *b* into *a* in liver homogenates from such animals was virtually non-existent (Mersmann & Segal, 1969; Glinsmann *et al.*, 1970; Tan & Nuttall, 1976).

In the normal liver phosphorylase *a* appears as the primary target for glucose, which facilitates the inactivation of the enzyme by phosphorylase phosphatase. The activation of glycogen synthase occurs in a second step, owing to the removal of phosphorylase *a*, which is a potent inhibitor of glycogen synthase phosphatase. The same phenomenon is evident in broken-cell preparations, where the activation of synthase is preceded by a lag that

corresponds to the inactivation of phosphorylase (Stalmans, 1976; Hers, 1981; Mvumbi *et al.*, 1983). Therefore the loss of the hepatic glycogenic response to glucose in the adrenalectomized starved animal might be explained at various levels: (i) an impaired inactivation of phosphorylase (Schwartz Sørensen, 1957; Miller *et al.*, 1973; Tan & Nuttall, 1976; Whitton & Hems, 1976) could be responsible for a lasting inhibition of synthase phosphatase; alternative proposals are (ii) a loss of synthase phosphatase activity (Tan & Nuttall, 1976) and (iii) the presence of synthase *b* in a 'non-substrate' form, resistant to the action of synthase phosphatase (Tan & Nuttall, 1976; Tan *et al.*, 1980).

The present work was intended to delineate the relative importance of these putative causes by experiments *in vivo* and on isolated hepatocytes. We find that glycogen synthase phosphatase emerges as the single limiting factor for hepatic glycogen deposition. Parts of this work have been published in a preliminary form (Bollen *et al.*, 1981; Doperé *et al.*, 1981).

Experimental

Materials, buffers and statistics

The sources of most relevant chemicals have been reported (Doperé *et al.*, 1980). ³H₂O was from The

Radiochemical Centre. Particulate glycogen was purified from dog liver by the phenol method (Laskov & Margoliash, 1963). Cortisol sodium succinate was a commercial pharmaceutical preparation (Roussel). Buffer A contained 0.25 M-sucrose, 0.5 mM-dithiothreitol and 50 mM-imidazole, adjusted at room temperature to pH 7.4.

Results are expressed as means $\pm$ S.E.M. for the numbers of observations indicated in parentheses. Vertical bars in graphs indicate $\pm$ S.E.M. Statistical treatment of the data was by Student's *t* test or by linear regression analysis.

Handling of animals and livers

Male Wistar rats weighing about 250 g were used either intact or after bilateral adrenalectomy. The adrenalectomized rats were given 0.15 M-NaCl as drinking water. They were either fed *ad libitum* or starved, and killed 7 days after surgery. Unless otherwise specified, 'starved' refers to food withdrawal in the morning, 48 h before death. Glucose (1 g in 3 ml of water) was administered by a stomach tube. Cortisol (10 mg in 0.5 ml) was injected intraperitoneally. $^3\text{H}_2\text{O}$ (20 mCi in 2 ml of 0.15 M-NaCl/kg body wt.) was injected intraperitoneally 30 min before death. All rats were killed by decapitation.

The liver was rinsed in ice-cold 0.15 M-NaCl and homogenized in a Potter-Elvehjem tube in 4 vol. of cold buffer A (except for the experiments related to Fig. 2). When indicated, the homogenate was centrifuged in the cold (10 min at 8000 g) and 1 ml of supernatant (extract) was filtered in the cold through a column of Sephadex G-25 (1.5 cm $\times$ 5 cm) equilibrated in buffer A. When necessary, results were corrected for the dilution caused by gel filtration (about 1.5-fold), as determined by assay of the protein concentration (Lowry *et al.*, 1951).

Rate of glycogen synthesis in vivo

This was measured by the incorporation of ^3H from $^3\text{H}_2\text{O}$ into glycogen, after the procedure of Postle & Bloxham (1980). Modifications concerned mainly the isolation of the glycogen. To 1.5 ml of homogenate (see above) was added 0.5 ml of 7 M-KOH and the mixture was heated in a stoppered tube for 20 min at 90°C. After cooling, 4 ml of ethanol was added and the mixture was kept overnight at -20°C to ensure complete precipitation of glycogen. The precipitate was packed by centrifugation (5 min at 2000 g), drained and dissolved in 0.3 ml of water. The solution was quantitatively transferred on to a square (6 cm $\times$ 6 cm) of filter paper (Whatman ET31), which was washed for 4 $\times$ 20 min in 66% (v/v) ethanol. The glycogen was then hydrolysed by incubation of the papers with a solution containing amyloglucosidase (Boehringer) as described by Chan & Exton (1976). Samples of the digest were used for the determination of ^3H

incorporation by liquid-scintillation counting and for determination of total glycogen as glucose (Dahlqvist, 1961).

Hepatocytes

The preparation and incubation were described by Bollen *et al.* (1983). In the regular incubations, the extracellular medium contained 144 mM- Na^+ and 6 mM- K^+ (Krebs & Henseleit, 1932); the 'K $^+$ -rich medium' contained 89 mM- Na^+ and 60 mM- K^+ . Samples of cell suspensions for enzyme assays were treated as described by Bollen *et al.* (1983).

Enzyme assays

All incubations were done at 25°C. Glycogen synthase *a* and *a* + *b* activities were measured as described by Doperé *et al.* (1980), on samples of liver homogenates diluted to contain 15 mM-EDTA and 75 mM-NaF. Hepatocyte preparations were assayed for glycogen synthase and phosphorylase as described by Bollen *et al.* (1983). One unit of these enzymes converts 1 μmol of substrate/min in the appropriate assay conditions.

Glycogen synthase phosphatase was measured in liver homogenates (final concn. 2%, w/v) by their ability to convert purified liver synthase *b* into *a* in the presence of 3 mM-AMP and 5 mM-magnesium acetate (Doperé *et al.*, 1980). The substrate was a pool of the forms termed *b*₂ and *b*₃ by Doperé *et al.* (1980). One unit of synthase phosphatase converts 1 unit of synthase *b* into *a*/min.

Phosphorylase phosphatase was determined in gel-filtered liver extracts (see above), with or without subsequent incubation with trypsin, by the rate of dephosphorylation of ^{32}P -labelled muscle phosphorylase (Doperé & Stalmans, 1982). One unit of phosphorylase phosphatase dephosphorylates 1 unit of phosphorylase *a*/min.

Extracellular and total lactate dehydrogenase activities in cell suspensions were measured as described by Krack *et al.* (1980).

Results

Effects of glucose on isolated hepatocytes

Hepatocytes from normal rats and from adrenalectomized starved rats were incubated in the presence of various concentrations of added glucose (5, 12, 32 or 62 mM). Only the prominent features are illustrated in Fig. 1. The initial concentration of phosphorylase *a* was distinctly higher in the control preparation, as expected from the similar difference in total phosphorylase (Chan *et al.*, 1979). However, in both types of hepatocytes there was a progressive glucose-promoted inactivation of phosphorylase, until similar minimal activities were reached at the highest glucose concentration. Although it is difficult to quantify the sensitivity of the

Fig. 1. Effects of two glucose concentrations on enzyme activities in hepatocytes from normal fed and from adrenalectomized starved rats

Hepatocytes from four normal fed rats and from three adrenalectomized starved rats were preincubated for 15 min in the presence of 2 mM-glucose before addition (arrow) of 10 mM-glucose (a) or 60 mM-glucose (b). Δ , Phosphorylase a; O, synthase a; $\bullet$, synthase a + b. Control cell preparations ($n = 4$) incubated for 10 min with 50 nM-glucagon contained 103 ± 12 units of phosphorylase a/g of protein.

phosphorylase inactivation to glucose, no obvious difference was apparent between the two types of hepatocytes.

A distinctive feature of the hepatocytes from adrenalectomized starved rats was the complete lack of activation of glycogen synthase at any glucose concentration (Fig. 1). This negative response requires both adrenalectomy and starvation (results not shown). When the concentration of extracellular K^+ is increased at the expense of Na^+ , the inactivation of phosphorylase, and accordingly the activation of synthase, are facilitated in normal hepatocytes (Hue *et al.*, 1975; results not shown). However, when hepatocytes from adrenalectomized starved rats were incubated in a K^+ -rich medium plus 62 mM-glucose, the activation of synthase was not improved.

The total concentration of glycogen synthase (a + b) was similar in the two types of hepatocytes and remained fairly constant throughout (Fig. 1). We found that this parameter can be used conveniently to monitor cell integrity. During long-term incubations of hepatocytes (up to 4 h) there was a good correlation between loss of total synthase and release of lactate dehydrogenase into the medium (eight observations on three cell preparations; correlation coefficient $r = 0.88$; $P < 0.01$). Leakage of lactate dehydrogenase and uptake of Trypan Blue are equivalent indices of cell integrity (Seglen, 1976).

Activation of glycogen synthase in gel-filtered liver extracts

Addition of AMP, especially in the presence of Mg^{2+} , relieves hepatic synthase phosphatase from

the inhibitory effect of phosphorylase *a* (Stalmans *et al.*, 1971; Mvumbi *et al.*, 1983), and thus ensures a rapid conversion of synthase *b* into *a* in incubated gel-filtered liver extracts (Fig. 2*a*). Complete conversion occurred in 20 min in liver extracts from normal and adrenalectomized fed rats; the rate of activation was considerably and progressively decreased when the adrenalectomized animals were starved for 1–3 days before the experiment. It was not evident whether, under the latter conditions, part of the synthase *b* might furthermore be resistant to the action of synthase phosphatase. Therefore, each preparation used in Fig. 2*a*) was supplemented with native synthase phosphatase by mixing with a gel-filtered liver extract from the normal rat (Fig. 2*b*). Full conversion of synthase *b* into *a* then occurred rapidly in all conditions. Similar patterns were obtained when unfiltered liver extracts were incubated without further ionic supplement, but all the conversions then occurred more slowly (results not shown).

Protein phosphatase activities on purified substrates

The activity of synthase phosphatase was quantitatively measured in dilute liver homogenates with purified hepatic synthase *b* as substrate, in the presence of AMP and Mg²⁺ (Table 1). With respect to normal fed rats, either adrenalectomy or starvation for 48 h decreased the activity by about one-third. A progressive, much more important, decrease occurred during starvation of the adrenalectomized rats.

Phosphorylase phosphatase activity was measured in gel-filtered liver extracts, prepared and assayed in conditions of minimal organelle damage; adrenalectomy plus prolonged (48 h) starvation caused a slight decrease in that activity (Table 1). When liver extracts are treated with trypsin before assay, a huge previously latent phosphorylase phosphatase activity is revealed (Laloux *et al.*, 1978;

Table 1. *Hepatic protein phosphatase activities during starvation of normal and adrenalectomized rats*

The activities of synthase phosphatase and of phosphorylase phosphatase were measured in crude liver preparations on purified substrates as described in the Experimental section. The mean values for the control group are indicated in square brackets in units/g of liver. Significance of difference from the normal fed group: **P* < 0.05; †*P* < 0.01; ‡*P* < 0.001.

Condition	Synthase phosphatase (% of control)	Phosphorylase phosphatase (% of control)	
		Basal	+ trypsin
Normal rats	[0.45]	[16]	[558]
Fed	100 ± 6 (4)	100 ± 4 (5)	100 ± 6 (5)
24 h starved	109 ± 5 (6)	—	—
48 h starved	67 ± 4 (4)†	—	—
Adrenalectomized rats			
Fed	70 ± 4 (5)†	—	—
24 h starved	23 ± 1 (3)‡	93 ± 9 (5)	122 ± 4 (5)*
48 h starved	9 ± 1 (9)‡	86 ± 3 (5)*	118 ± 3 (5)*

Fig. 2. *Conversion of endogenous synthase b into a in gel-filtered liver extracts*

The livers from a normal fed rat (■) and from adrenalectomized rats, fed (▲) or starved for 24 h (○), 48 h (◐) or 72 h (●), were homogenized in a Potter–Elvehjem tube in 2 vol. of a cold solution containing 0.25 M-sucrose, 10 mg of particulate glycogen/ml and 50 mM-glycylglycine, pH 7.4. A low-speed extract from each homogenate was gel-filtered as described in the Experimental section. The filtrates were incubated at 25°C in the presence of 3 mM-AMP and 5 mM-magnesium acetate, after the addition of an equal volume of either buffer A (a) or filtrate from the normal fed rat (b). At each time point samples were assayed for synthase *a* and for total synthase (the latter remaining constant).

Doperé & Stalmans, 1982; Table 1). The latter activity was somewhat higher in adrenalectomized rats starved for 24 h or 48 h.

Initial recovery after administration of glucose or cortisol

Hepatic glycogen synthesis can be slowly re-induced in adrenalectomized starved rats either by re-feeding (see the introduction) or by administration of a glucocorticoid (Glenn *et al.*, 1961; Munck & Koritz, 1962; Mersmann & Segal, 1969). Table 2 summarizes data for some parameters obtained after

forced glucose feeding or cortisol injection. There was a partial activation of glycogen synthase, especially after cortisol, without change in total synthase. Both the degree of synthase activation and the rate of glycogen accumulation were modest in comparison with observations on non-adrenalectomized starved or fed animals (see the Discussion section). However, the purpose of these experiments was to check simultaneously the activity of synthase phosphatase during the early stages of recovery. Such a comparison between three parameters in the individual livers is shown in Fig. 3.

Table 2. Recovery of hepatic glycogen synthesis and related parameters in adrenalectomized starved rats after treatment with cortisol or glucose

Adrenalectomized starved rats were injected with cortisol or received glucose by a stomach tube at the indicated time before death. The rate of hepatic glycogen synthesis *in vivo* was measured by the incorporation of ^3H from $^3\text{H}_2\text{O}$. Samples of the liver homogenate were also used for the assay of glycogen synthase, synthase phosphatase and glycogen as described in the Experimental section. Only values marked (*) are not significantly different from those for the adrenalectomized starved group ($P > 0.05$).

Condition (no. of animals)	Glycogen synthase (munits/g)		Synthase phosphatase (munits/g)	$10^{-3} \times$ Glycogen synthesis (c.p.m./g)	Glycogen (mg/g)
	$a + b$	a			
Normal fed (9)	$1464 \pm 80^*$	157 ± 17	501 ± 7	2.73 ± 0.22	43 ± 4
Adrenalectomized starved (6)	1457 ± 61	69 ± 7	40 ± 5	1.74 ± 0.15	0.5 ± 0.2
2 h after cortisol (4)	$1254 \pm 63^*$	143 ± 18	61 ± 4	$2.63 \pm 0.57^*$	$0.8 \pm 0.1^*$
3 h after cortisol (4)	$1310 \pm 148^*$	385 ± 62	121 ± 13	12.64 ± 2.73	3.9 ± 0.8
4 h after cortisol (3)	$1471 \pm 241^*$	426 ± 49	137 ± 23	15.00 ± 2.13	6.9 ± 1.0
45 min after glucose (3)	$1334 \pm 29^*$	134 ± 21	$45 \pm 5^*$	4.00 ± 0.79	$0.7 \pm 0.1^*$
90 min after glucose (5)	$1563 \pm 46^*$	232 ± 40	118 ± 17	9.91 ± 1.40	4.6 ± 0.8

Fig. 3. Correlation between synthase phosphatase and synthase a activities and the rate of glycogen synthesis in the liver during treatment of adrenalectomized starved rats with cortisol or glucose

The individual data from the adrenalectomized rats in Table 2 are graphically compared. The activity of synthase phosphatase is expressed as a percentage of the mean in normal fed controls. Adrenalectomized starved rats: ■, untreated; (○) cortisol-treated for 2 h, (●) 3 h, (●) 4 h; glucose-treated for (△) 45 min, (▲) 90 min. The cross (in *b*) indicates the mean $\pm$ s.e.m. for the normal fed group; the latter data were not included in the statistical analysis. (a) Correlation coefficient $r = 0.85$; $P < 0.001$. (b) $r = 0.95$; $P < 0.001$.

Irrespective of the treatment, there is a clear-cut linear relationship between (i) activity of synthase phosphatase and amount of synthase *a*, and (ii) amount of synthase *a* and the rate of glycogen synthesis *in vivo*; the latter relationship is identical for normal untreated rats.

Discussion

In normal animals glucose feeding and glucocorticoid administration cause a considerable deposition of glycogen in the liver. The mechanism of glucose action has been reviewed (see the introduction). Glucocorticoids appear to act via an increased activity of phosphorylase phosphatase, and via the synthesis of a protein that cancels the inhibitory effect of phosphorylase *a* on synthase phosphatase (Stalmans & Laloux, 1979). There is no reason to believe that these glycogenic agents would act differently in the adrenalectomized starved animal. However, the initial rates of glycogen accumulation (Table 2) are much smaller than those observed in similarly treated normal animals (Friedmann *et al.*, 1965; De Wulf & Hers, 1967). This, in turn, is explained by the limited activation of glycogen synthase after adrenalectomy plus starvation; we show that in the latter conditions, as in normal animals (De Wulf & Hers, 1968), the hepatic content of synthase *a* determines the rate of glycogen synthesis.

The question then arises as to what limits the activation of glycogen synthase in the liver of the adrenalectomized starved rat. The only important defect that we could observe was in the activity of glycogen synthase phosphatase. Furthermore, the initial extent of synthase activation *in vivo* by glucose or cortisol corresponded closely to the degree of recovery of synthase phosphatase. The nature of the decreased synthase phosphatase activity remains to be established.

A decrease of the apparent synthase phosphatase activity by about one-third, as occurs when fed rats are only adrenalectomized or only starved, does not at all impair the ability for prompt glycogen synthesis. A greater decrease in synthase phosphatase activity is observed when adrenalectomized rats are starved for 24 h. The activity loss becomes virtually complete (90% decrease) after starvation for 48 h (Tan & Nuttall, 1976; the present work). It should be noted that even small increments in this very low activity of synthase phosphatase correspond to a real increase in the ability for synthase activation *in vivo* (Fig. 3a). These observations may explain why Miller *et al.* (1973), who used 18-h starved adrenalectomized rats, still obtained a significant glucose-induced activation of glycogen synthase in liver perfusion studies. It should be recalled that the classical observations on the

deficient glycogen synthesis (see the introduction) stem from work with adrenalectomized rats starved for 48 h. Reports on adrenalectomized rats starved for 12–16 h are difficult to interpret (Kreutner & Goldberg, 1967; Nichols & Goldberg, 1972).

We have been unable to obtain clear evidence for the presence of synthase *b* in a non-substrate form in the liver of the adrenalectomized starved rats, as proposed by Tan & Nuttall (1976). Tan *et al.* (1980) reported that the refractoriness to synthase phosphatase disappeared on purification of the synthase *b*. The mechanism was not clarified.

The conversion of phosphorylase *a* into *b* occurred somewhat more slowly in liver homogenates or extracts obtained from adrenalectomized starved rats (Schwartz Sørensen, 1957; Tan & Nuttall, 1976). A drastic decrease (by 75%) of phosphorylase phosphatase activity, as measured on purified substrate, was reported by Tan & Nuttall (1976). Our results indicate a slight decrease in the native phosphorylase phosphatase activity, and a similar increase in the latent enzyme activity. These changes were statistically significant, but their biological significance is presumably minimal, since they do not cause an obvious defect in the hepatocyte (Fig. 1). Some earlier work may have suffered from imperfect assay methods for phosphorylase *a*. This problem has been discussed elsewhere (Bollen *et al.*, 1983).

We are grateful to Dr. F. Doperé for his continued interest and advice, and to Ms. N. Sente for expert technical assistance. This work was supported by the Belgian 'Fonds voor Geneeskundig Wetenschappelijk Onderzoek' (Grant 3.0051.82).

References

- Bollen, M., Doperé, F. & Stalmans, W. (1981) *Arch. Int. Physiol. Biochim.* **89**, B149–B150
- Bollen, M., Hue, L. & Stalmans, W. (1983) *Biochem. J.* **210**, 783–787
- Chan, T. M. & Exton, J. H. (1976) *Anal. Biochem.* **71**, 96–105
- Chan, T. M., Blackmore, P. F., Steiner, K. E. & Exton, J. H. (1979) *J. Biol. Chem.* **254**, 2428–2433
- Dahlqvist, A. (1961) *Biochem. J.* **80**, 547–551
- De Wulf, H. & Hers, H. G. (1967) *Eur. J. Biochem.* **2**, 57–60
- De Wulf, H. & Hers, H. G. (1968) *Eur. J. Biochem.* **6**, 558–564
- Doperé, F. & Stalmans, W. (1982) *Biochem. Biophys. Res. Commun.* **104**, 443–450
- Doperé, F., Vanstapel, F. & Stalmans, W. (1980) *Eur. J. Biochem.* **104**, 137–146
- Doperé, F., Bollen, M., Gevers, G. & Stalmans, W. (1981) *Arch. Int. Physiol. Biochim.* **89**, B165–B166
- Dorsey, J. L. & Munck, A. (1962) *Endocrinology* **71**, 605–608
- Friedmann, B., Goodman, E. H., Jr. & Weinhouse, S. (1965) *J. Biol. Chem.* **240**, 3729–3735

- Friedmann, B., Goodman, E. H., Jr. & Weinhouse, S. (1967) *Endocrinology* **81**, 486–496
- Glenn, E. M., Bowman, B. J., Bayer, R. B. & Meyer, C. E. (1961) *Endocrinology* **68**, 386–410
- Glinsmann, W., Pauk, G. & Hern, E. (1970) *Biochem. Biophys. Res. Commun.* **39**, 774–782
- Hers, H. G. (1981) in *Short-Term Regulation of Liver Metabolism* (Hue, L. & van de Werve, G., eds.), pp. 105–117, Elsevier/North-Holland Biomedical Press, Amsterdam
- Hue, L., Bontemps, F. & Hers, H. G. (1975) *Biochem. J.* **152**, 105–114
- Krack, G., Goethals, F., Deboyser, D. & Roberfroid, M. (1980) *Toxicology* **18**, 213–223
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
- Kreutner, W. & Goldberg, N. D. (1967) *Proc. Natl. Acad. Sci. U.S.A.* **58**, 1515–1519
- Laloux, M., Stalmans, W. & Hers, H. G. (1978) *Eur. J. Biochem.* **92**, 15–24
- Laskov, R. & Margoliash, E. (1963) *Bull. Res. Counc. Isr. Sect. A* **11**, 351–362
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mersmann, H. J. & Segal, H. L. (1969) *J. Biol. Chem.* **244**, 1701–1704
- Miller, T. B., Jr., Hazen, R. & Lerner, J. (1973) *Biochem. Biophys. Res. Commun.* **53**, 466–474
- Munck, A. & Koritz, S. B. (1962) *Biochim. Biophys. Acta* **57**, 310–317
- Mvumbi, L., Doperé, F. & Stalmans, W. (1983) *Biochem. J.* **212**, 407–416
- Nichols, W. K. & Goldberg, N. D. (1972) *Biochim. Biophys. Acta* **279**, 245–259
- Postle, A. D. & Bloxham, D. P. (1980) *Biochem. J.* **192**, 65–73
- Schwartz Sørensen, N. (1957) *Nature (London)* **180**, 857–858
- Seglen, P. O. (1976) *Methods Cell Biol.* **13**, 29–83
- Stalmans, W. (1976) *Curr. Top. Cell. Regul.* **11**, 51–97
- Stalmans, W. & Laloux, M. (1979) in *Glucocorticoid Hormone Action* (Baxter, J. D. & Rousseau, G. G., eds.), pp. 517–533, Springer-Verlag, Berlin
- Stalmans, W., De Wulf, H. & Hers, H. G. (1971) *Eur. J. Biochem.* **18**, 582–587
- Tan, A. W. H. & Nuttall, F. Q. (1976) *Biochim. Biophys. Acta* **445**, 118–130
- Tan, A. W. H., Tan, A. H. S. & Nuttall, F. Q. (1980) *Biochim. Biophys. Acta* **614**, 328–338
- Whitton, P. D. & Hems, D. A. (1976) *Biochem. J.* **156**, 585–592