Biochem. J. (1992) 284, 777–780 (Printed in Great Britain)

Mechanism linking glycogen concentration and glycogenolytic rate in perfused contracting rat skeletal muscle

Peter HESPEL*‡ and Erik A. RICHTER†

*Institute of Physical Education, Catholic University of Leuven, Tervuursevest 101, B-3001 Leuven, Belgium, and †August Krogh Institute, University of Copenhagen, Universitetsparken 13, DK-2100 Copenhagen Ø, Denmark

The influence of differences in glycogen concentration on glycogen breakdown and on phosphorylase activity was investigated in perfused contracting rat skeletal muscle. The rats were preconditioned by a combination of swimming exercise and diet (carbohydrate-free or carbohydrate-rich) in order to obtain four sub-groups of rats with varying resting muscle glycogen concentrations (range 10–60 μmol/g wet wt.). Pre-contraction muscle glycogen concentration was closely positively correlated with glycogen breakdown over 15 min of intermittent short tetanic contractions ($r = 0.75$; $P < 0.001$; $n = 56$) at the same tension development and oxygen uptake. Additional studies in supercompensated and glycogen-depleted hindquarters during electrical stimulation for 20 s or 2 min revealed that the difference in glycogenolytic rate was found at the beginning rather than at the end of the contraction period. Phosphorylase *a* activity was approximately twice as high ($P < 0.001$) in supercompensated muscles as in glycogen-depleted muscles after 20 s as well as after 2 min of contractions. It is concluded that glycogen concentration is an important determinant of phosphorylase activity in contracting skeletal muscle, and probably via this mechanism a regulator of glycogenolytic rate during muscle contraction.

INTRODUCTION

Muscle glycogen is the primary energy source in the initial stage of exercise and during continued muscular activity of high intensity [1–4]. Thus the magnitude of pre-exercise muscle glycogen stores is an important determinant of exercise performance [1,5]. In the muscle cell glycogen exists in a complex with the enzymes regulating its metabolism and with parts of the sarcoplasmic reticulum [6,7]. This glycogen–protein–sarcoplasmic-reticulum complex is a structural entity of the muscle cell, behaving as a functional enzymic unit in which glycogen synthesis and degradation are regulated [6–8]. The rate-limiting step in glycogen breakdown is catalysed by phosphorylase [9]. Contraction-induced activation of glycogenolysis probably primarily involves conversion of phosphorylase from its less active *b* form into its more active *a* form [10–12], in a reaction catalysed by phosphorylase kinase [9,13], as well as allosteric activation of phosphorylase *b* by AMP and IMP [14]. Furthermore, the increase in P_i during contractions may also be important in increasing glycogenolysis [10,15]. *In vivo*, adrenaline secreted from the adrenal medulla is required for maintaining glycogenolysis during prolonged exercise [12,16,17] by slowing the reversal of phosphorylase *a* activity [12]. In addition, several findings indicate also that glycogen itself is possibly an important regulator of its own rate of catabolism. Thus most [18–24], but not all [25–27], studies in humans as well as in rats show, for a given amount of exercise or muscle contraction, a positive relationship between pre-contraction muscle glycogen concentration and rate of glycogen breakdown. However, a study in rat plantaris muscle *in situ* failed to demonstrate an effect of glycogen itself on the rate of phosphorylase activation during electrical stimulation [28]. In that study, however, a possible direct effect of glycogen on phosphorylase activity might have been masked by short-term effects of exercise. In addition, extramuscular factors that may influence muscle glycogenolysis, such as plasma non-esterified fatty acids [29,30], insulin [31] and adrenaline [16,17,24] were not controlled.

Therefore, in the present study we investigated the influence of differences in pre-contraction muscle glycogen concentration on glycogenolytic rate and on phosphorylase activity in stimulated muscle of the isolated perfused rat hindlimb. Hindquarters were perfused with media of identical composition, in this way excluding the influence of differences in extramuscular factors. To avoid acute effects of exercise, the rats were rested in their cages for at least 19 h before the perfusion experiments. The data demonstrate that glycogen concentration is an important determinant of phosphorylase activity in contracting skeletal muscle.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 200–250 g were preconditioned in order to obtain four subgroups with varying muscle glycogen concentrations. A first group of rats (control rats) rested in their cages and had free access to regular rat chow until their hindquarters were perfused. A second group of rats (glucose-fed rats) also rested in their cages, but in addition to their normal rat chow they received a 20% (w/v) glucose drinking solution starting 48 h before perfusion. Rats from the third group (glycogen-depleted rats) were subjected to 2 h of swimming in water maintained at 32–35 °C, with weights (2.5% of body weight) attached to their tails. In the 24 h preceding the swim their food intake was restricted to 9 g, and after the swim they were fed on lard until perfusion between 19 and 25 h later. The last group of rats (supercompensated rats) swam in accordance with the same protocol as for the glycogen-depleted rats. However, after the swim they received *ad libitum* normal rat chow together with a 10% glucose drinking solution, until perfusion between 19 and 25 h later.

Experimental procedures

After the procedures of preconditioning, the rats were anaesthetized by an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt.) and prepared surgically for hindquarter perfusion as previously described [12,32]. Before insertion of the

‡ To whom correspondence should be addressed.

perfusion catheters, the rat was heparinized with 500 i.u. of heparin in the inferior vena ceva. The rat was killed with an intracardial injection of pentobarbital sodium immediately before being placed in the perfusion cabinet. The initial perfusate (200 ml) consisted of Krebs–Henseleit solution, 1–3-day-old washed bovine erythrocytes at a haematocrit of 30%, 5% (w/v) BSA (Cohn fraction V; Sigma, St. Louis, MO, U.S.A.), 6 mM-glucose, 0.15 mM-pyruvate and 0.5–1.0 mM-lactate originating from the erythrocytes. No insulin was added. The perfusate was continuously gassed with O_2/CO_2 (49:1), which yielded a pH of 7.3–7.4 and partial pressures of CO_2 and O_2 in arterial perfusate of typically 35–40 mmHg and 400 mmHg respectively.

The first 25 ml of perfusate that passed through the hindquarter was discarded, whereupon the perfusate was recirculated at a flow rate of 12.5 ml/min. After a 20 min equilibration period, the left gastrocnemius–plantaris muscles were freeze-clamped *in situ* with aluminium clamps cooled in liquid N_2, and resected. Subsequently, the common iliac vessels supplying the biopsied leg were tied off and a clamp was fixed tightly around the proximal part of the leg. Perfusion flow rate was decreased to 9 ml/min, resulting in similar perfusion pressures (50–70 mmHg) as for bilateral perfusion at a flow rate of 12.5 ml/min. The hindquarter was allowed to rest for 5 min before the right leg was immobilized and a hook-electrode was placed around the sciatic nerve and connected to a DISA stimulator (Disa Electronic, Herlev, Denmark). The resting length of the gastrocnemius–soleus–plantaris muscle group was then adjusted to obtain maximum active tension upon stimulation, whereupon the experimental period was begun. The muscles were made to contract isometrically by stimulating the sciatic nerve electrically with supramaximum (6–10 V) trains of 50 ms and 67 Hz, each impulse in the train lasting for 1 ms. The trains were delivered at a rate of 45 per min. During stimulation, tension developed by the gastrocnemius–soleus–plantaris muscle group was recorded with a locally constructed isometric muscle tension transducer. At the onset of electrical stimulation, perfusate flow was increased to 20 ml/min, which resulted in a perfusion pressure of 100–130 mmHg. At the end of the 15 min stimulation period the gastrocnemius–plantaris muscles of the stimulated leg were freeze-clamped *in situ* without interrupting the electrical stimulation or the flow of perfusate. Muscle samples were powdered under liquid N_2 and stored at −80 °C until analysed.

In additional perfusions in glycogen-depleted and supercompensated rats, the gastrocnemius–plantaris muscles of the stimulated leg were freeze-clamped *in situ* after either 20 s or 2 min of stimulation. The muscle samples were freeze-dried, dissected free of connective tissue and blood, and stored at −80 °C until analysed.

Analyses, calculations, statistics

Muscle glycogen was measured by a hexokinase method after acid hydrolysis [16]. Glycogen phosphorylase was assayed essentially as described by Gilboe and co-workers [33]. Total phosphorylase activity ($a+b$) was measured at 37 °C in the presence of AMP (3 mM) and phosphorylase a activity in the absence of AMP in the assay mixture.

Statistical evaluation of the data was done by unpaired t test. All data are presented as means ± S.E.M.

RESULTS

Muscle glycogen breakdown

Compared with control rats, muscle glycogen concentrations before contractions were on average 36% lower in rats that had undergone procedures for glycogen depletion, whereas they were 30% and 55% higher in glucose-fed and supercompensated rats respectively (Table 1). During electrical stimulation, muscle glycogen markedly decreased in all groups of rats, the decrease, however, being greater in muscle from rats with high pre-contraction glycogen levels (glucose-fed and supercompensated rats) than in rats with low (glycogen-depleted rats) or normal (control rats) glycogen levels (Table 1). Over the 15 min of muscle contraction, muscle glycogen breakdown was approximately twice as high in supercompensated rats as in glycogen-depleted rats, the latter in turn breaking down 31% less glycogen than control rats. In the total group of rats, muscle glycogen breakdown over 15 min of electrical stimulation was closely and positively correlated with the pre-contraction glycogen concentration ($r = 0.75$; $y = 2.95+0.43x$; $P < 0.001$) (Fig. 1).

To elucidate further the mechanism linking glycogen concentration and glycogen breakdown, additional studies were performed in glycogen-depleted and supercompensated hindquarters, perfused during 20 s or 2 min of electrical stimulation.

Table 1. Muscle glycogen concentration in perfused hindquarters before and after 15 min of contractions

Values are means ± S.E.M. Muscle glycogen concentrations were measured in one leg before and in the other leg after 15 min of intermittent short tetanic contractions. See the Materials and methods section for further details. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with corresponding value in control hindquarters.

Rat group	Glycogen concn. (μmol/g wet wt.)		
	Before contractions	After contractions	Breakdown
Glycogen-depleted ($n = 16$)	18.9±1.1***	7.4±1.0**	11.6±0.8***
Control ($n = 11$)	29.5±1.8	12.6±1.9	16.8±0.6
Glucose-fed ($n = 11$)	38.5±2.3**	20.3±1.9**	18.3±2.9
Supercompensated ($n = 18$)	45.8±2.0***	23.2±1.6***	22.6±1.8**

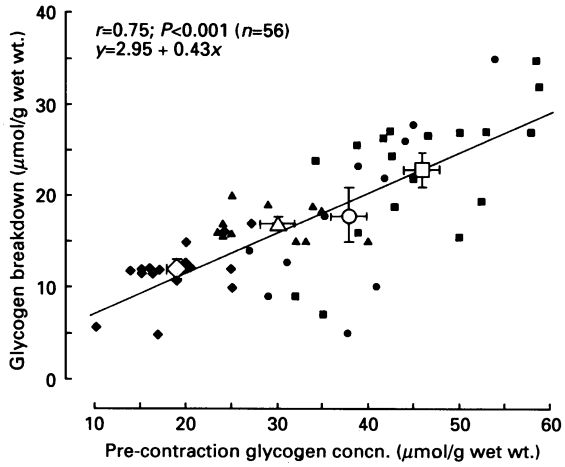

Fig. 1. Relationship between pre-contraction glycogen concentration and glycogen breakdown in perfused contracting skeletal muscle

Data points represent individual observations or group means ± S.E.M. of supercompensated rats (■, □; $n = 18$), glucose-fed rats (●, ○; $n = 11$) control rats (▲, △; $n = 11$) and glycogen-depleted rats (◆, ◇; $n = 16$) respectively. Muscle glycogen concentrations were measured in one leg before and in the other leg after 15 min of intermittent short tetanic contractions. See the Materials and methods section for further details.

Table 2. Phosphorylase activity in perfused muscle after 20 s and 2 min of contractions

Values are means ± S.E.M. of 10–11 observations: *$P < 0.05$, **$P < 0.01$ compared with corresponding value in supercompensated rats. Phosphorylase activity was measured either after 20 s or 2 min of intermittent short tetanic contractions. See the Materials and methods section for further details.

	Duration of muscle contractions	
	20 s	2 min
Total phosphorylase activity (μmol/min per g dry wt.)		
Glycogen-depleted rats	151 ± 12*	229 ± 45
Supercompensated rats	188 ± 26	247 ± 35
Phosphorylase *a* activity (% of total activity)		
Glycogen-depleted rats	31 ± 5**	10 ± 2*
Supercompensated rats	52 ± 5	19 ± 3

Muscle tissue sampled during these experiments was freeze-dried before biochemical analyses were performed, in order to exclude possible dilution effects, caused by water accumulating in the muscles during contractions. In glycogen-depleted rats, muscle glycogen concentrations were 133 ± 11 before, and 122 ± 16 and 75 ± 11 μmol/g dry wt. after 20 s and 2 min of contractions respectively. Corresponding values in supercompensated muscles were 294 ± 11, 250 ± 16 and 204 ± 15 μmol/g. Thus, in line with the above findings, muscle glycogen breakdown over 2 min of contractions was greater ($P < 0.05$) in supercompensated hindquarters (84 ± 15 μmol/g dry wt.) than in glycogen-depleted hindquarters (53 ± 8 μmol/g). This difference was entirely accounted for by a higher ($P < 0.05$) rate of glycogen breakdown during the first 20 s of stimulation in supercompensated muscles.

Muscle phosphorylase activity

Phosphorylase *a* activities in perfused muscles from glycogen-depleted and supercompensated rats during the 2 min period of electrical stimulation are given in Table 2. Throughout the stimulation period, the portion of phosphorylase existing in the *a* form was approximately twice as high in supercompensated muscles as in glycogen-depleted muscles. Total phosphorylase activity was slightly higher ($P < 0.05$) in supercompensated rats than in glycogen-depleted rats after 20 s of contractions. This difference had disappeared after 120 s of stimulation.

Muscle performance

Muscle tension at the onset and at the end of the 15 min of electrical stimulation were not significantly different between the four groups of rats, expressed either as absolute tension or as a percentage of initial tension. In the total group of rats ($n = 56$) initial muscle tension averaged 1053 ± 17 g, decreasing to 62 ± 1 % of initial tension after 15 min of electrical stimulation. We have previously shown that oxygen uptake at rest as well as during electrical stimulation is similar in hindquarters with high and low muscle glycogen concentrations [21,24].

DISCUSSION

Phosphorylase exists in the muscle cell as phosphorylase *a* and *b*, which are interconvertible via dephosphorylation and phosphorylation reactions respectively. In resting muscle, conditions are such that phosphorylase is almost completely in the *b* form, which has a very low degree of activity. When muscle contracts, phosphorylase is activated primarily by rapid conversion of phosphorylase from its less active *b* form into its much more active *a* form. This conversion is rapidly reversed when contractile activity is continued [11,12]. The present study shows for the first time that the percentage of phosphorylase in the *a* form in contracting muscle depends on the initial glycogen concentration in the muscle cell (Table 2). This finding may explain the previously [18–20,22,23] and presently (Fig. 1) observed correlation between the pre-contraction glycogen concentration and glycogenolytic rate in contracting skeletal muscle.

Studies in exercising humans more often [18–20,22,23] than not [25,26] have demonstrated a positive relationship between pre-contraction muscle glycogen concentration and the rate of glycogen breakdown for a given amount of work performed. Although there is no doubt that glycogen is not the only regulator, the question as to whether or not glycogen is a regulator of its own rate of catabolism is, however, still a matter of debate [24,27,28]. In apparent contrast with our previous findings in perfused rat muscle [21,24], in recent studies in rat [27] or human [26] skeletal muscle electrically stimulated to contract *in situ* with the circulation occluded, glycogen breakdown over 1 min of contractions was found to be independent of initial glycogen concentration. In these studies, however, the influence of extramuscular factors on glycogenolysis might well have been decreased, but not excluded, by occluding the circulation to the stimulated muscles. The present study, which re-investigated the relationship between pre-contraction glycogen concentration and glycogenolytic rate in perfused rat muscle with widely varying glycogen concentrations, points out that, under conditions of identical modulation by extramuscular factors, high pre-contraction glycogen concentrations are associated with high glycogenolytic rates, whereas low initial glycogen concentrations are associated with lower glycogenolytic rates (Table 1). Thus pre-contraction glycogen concentration was closely positively correlated with glycogen breakdown over 15 min of electrical stimulation (Fig. 1). A difference in initial glycogen concentration by a factor of approx. 2.5 (between glycogen-depleted and supercompensated rats) resulted, for an identical amount of tension development and level of oxygen uptake, in a 2-fold greater amount of glycogen breakdown over 15 min of contractions. Our findings in muscles stimulated to contract for 20 s or 2 min further showed that the difference in glycogenolytic rate is found at the beginning rather than at the end of the contraction period.

Phosphorylase catalyses the rate-limiting step in the pathway of glycogen breakdown [9]. Thus differences in glycogenolytic rate are to be explained by alterations in phosphorylase activity. Contraction-induced activation of phosphorylase includes a transient conversion of essentially inactive phosphorylase *b* into active phosphorylase *a*, in a reaction catalysed by phosphorylase kinase [9,13,34]. Interestingly, not only does the present study show glycogenolytic rate to be dependent on glycogen concentration, but at the same time it points out that, for a given degree of contractile activity, the percentage of phosphorylase existing in the *a* form is markedly higher in contracting muscles with high than in muscles with low glycogen levels. For throughout a 2 min stimulation period, phosphorylase *a* was approximately twice as high in supercompensated muscles as in glycogen-depleted muscles (Table 2). This finding at least partly explains the link between glycogen concentration and glycogenolytic rate in contracting muscle.

The present study clearly shows that glycogen concentration is a major determinant of phosphorylase activity in contracting skeletal muscle. Whether the high level of phosphorylase *a* seen in supercompensated muscle during contractions is due to elevated resting phosphorylase *a*, or to enhanced contraction-

induced activation of phosphorylase, remains to be elucidated, however. It is well known that obtaining valid measurements of phosphorylase in resting skeletal muscle is very difficult, because Ca^{2+}-induced transformation of phosphorylase *b* to *a* occurs by even the slight contractions caused by manipulating and cutting of muscles during sampling [11,12,35]. Similarly, squeezing of muscle tissue during freeze-clamping probably causes phosphorylase activation to some degree. This effect is substantial when large muscle groups (like the gastrocnemius–plantaris group in the present study) are clamped, and the inner fibres of the sample take several seconds to freeze. For this reason, the relationship between phosphorylase activity and glycogen concentration in resting muscle could not be studied by the present protocol. It is nevertheless worth mentioning that several studies *in vitro* have previously produced results that are in line with our present observations in stimulated perfused rat muscle. Early test-tube experiments performed by the group of E. G. Krebs [36,37] on Ca^{2+}-activated purified phosphorylase kinase have shown that glycogen causes a concentration-dependent activation of the enzyme. Since these early studies, considerable evidence has, however, been presented showing that the glycogen–protein–sarcoplasmic-reticulum complex [6,38] behaves as a compartmentalized multienzyme complex with functional enzymic properties that are not necessarily apparent in their isolated components (i.e. purified enzymes). Therefore, recent observations by Meinke & Edstrom [8] on isolated phosphorylase 'regulatory complexes' provide additional important information in support of the concept that glycogen by itself is a major co-regulator of phosphorylase activity in intact skeletal muscle. In their studies glycogen was, indeed, shown to stimulate markedly the Ca^{2+}-induced flash activation of phosphorylase as was first described by Heilmeyer *et al.* [38].

In conclusion, the present study shows that the percentage of phosphorylase existing in the *a* form in contracting skeletal muscle depends on the glycogen concentration in the muscle cell. High glycogen concentrations are associated with high phosphorylase *a*, whereas low glycogen concentrations are associated with low phosphorylase *a*. This finding may explain the presently and often previously observed positive relationship between pre-contraction glycogen concentration and glycogenolytic rate during muscle contractions.

Betina Bolmgreen is thanked for expert technical assistance. The study was supported by grants from the Carlsberg Foundation, Nordisk Insulin Foundation and the Danish Natural Sciences Research Council (grant 11-7766). P. H. was supported by grants from the Belgian National Research Foundation (N.F.W.O.) and from the Danish Medical Research Council (12-8870).

REFERENCES

1. Bergström, J., Hermansen, L., Hultman, E. & Saltin, B. (1967) Acta Physiol. Scand. **71**, 140–150
2. Bergström, J. & Hultman, E. (1967) Scand. J. Clin. Lab. Invest. **19**, 218–228
3. Hermansen, L., Hultman, E. & Saltin, B. (1967) Acta Physiol. Scand. **71**, 129–139
4. Saltin, B., Kiens, B. & Savard, G. (1986) in Biochemical Aspects of Physical Exercise (Benzi, G., Packer, L. & Siliprandi, N., eds.), pp. 235–244, Elsevier, Amsterdam
5. Karlsson, J. & Saltin, B. (1971) J. Appl. Physiol. **31**, 203–206
6. Entman, M. L., Keslensky, S. S., Chu, A. & Van Winkle, W. B. (1980) J. Biol. Chem. **255**, 6245–6252
7. Meyer, F., Heilmeyer, L. M. G., Haschke, R. H. & Fisher, E. (1970) J. Biol. Chem. **245**, 6642–6648
8. Meinke, M. H. & Edstrom, R. D. (1991) J. Biol. Chem. **266**, 2259–2266
9. Newsholme, E. A. & Leech, A. R. (1983) Biochemistry for the Medical Sciences, pp. 325–330, Wiley, New York
10. Chasiotis, D., Sahlin, K. & Hultman, E. (1982) J. Appl. Physiol. **53**, 708–715
11. Conlee, R. K., McLane, J. A., Rennie, M. J., Winder, W. W. & Holloszy, J. O. (1979) Am. J. Physiol. **237**, R291–R296
12. Richter, E. A., Ruderman, N. B., Gavras, M., Belur, E. R. & Galbo, H. (1982) Am. J. Physiol. **242**, E25–E32
13. Fischer, E. H., Heilmeyer, L. M. G. & Haschke, R. H. (1971) Curr. Top. Cell. Regul. **4**, 211–251
14. Aragon, J. J., Tornheim, K. & Lowenstein, J. M. (1980) FEBS Lett. **117**, K56–K64
15. Chasiotis, D. (1988) Med. Sci. Sports Exercise **20**, 545–550
16. Jansson, E., Hjemdahl, P. & Kaijser, L. (1986) J. Appl. Physiol. **60**, 1466–1470
17. Richter, E. A., Galbo, H. & Christensen, N. J. (1981) J. Appl. Physiol. **50**, 21–26
18. Galbo, H., Holst, J. J. & Christensen, N. J. (1979) Acta Physiol. Scand. **107**, 19–32
19. Gollnick, P. D., Pernow, B., Essen, B., Jansson, E. & Saltin, B. (1981) Clin. Physiol. **1**, 27–42
20. Gollnick, P. D., Piehl, K., Saubert, C. W., Armstrong, R. B. & Saltin, B. (1972) J. Appl. Physiol. **33**, 421–425
21. Hespel, P. & Richter, E. A. (1990) J. Physiol (London) **427**, 347–359
22. Hultman, E. & Sjöholm, H. (1983) in Biochemistry of Exercise (Knuttgen, H. G., Vogel, J. A. & Poortmans, J., eds.), vol. 13, pp. 63–75, Human Kinetics Publishers, Champaign
23. Jansson, E. (1980) Acta Physiol. Scand. **487** (Suppl.), 1–24
24. Richter, E. A. & Galbo, H. (1986) J. Appl. Physiol. **61**, 827–831
25. Klausen, K. & Sjøgaard, G. (1980) Scand. J. Sports Sci. **2**, 7–12
26. Ren, J. M., Broberg, S., Sahlin, K. & Hultman, E. (1990) Acta Physiol. Scand. **139**, 467–474
27. Spriet, L. L., Berardinucci, L., Marsh, D. R., Campbell, C. B. & Graham, T. E. (1990) J. Appl. Physiol. **68**, 1883–1888
28. Constable, S. H., Favier, R. J. & Holloszy, J. O. (1986) J. Appl. Physiol. **60**, 1518–1523
29. Hickson, R. C., Rennie, M. J., Conlee, R. K., Winder, W. W. & Holloszy, J. O. (1977) J. Appl. Physiol. **43**, 829–833
30. Rennie, M. J., Winder, W. W. & Holloszy, J. O. (1976) Biochem. J. **156**, 647–655
31. Berger, M., Hagg, S., Goodman, M. N. & Ruderman, N. B. (1976) Biochem. J. **158**, 191–202
32. Ruderman, N. B., Houghton, C. R. S. & Hems, R. (1971) Biochem. J. **124**, 639–651
33. Gilboe, D. P., Larson, K. L. & Nuttal, F. Q. (1972) Anal. Biochem. **47**, 20–27
34. Soderling, T. R. & Park, C. R. (1974) Adv. Cyclic Nucleotide Res. **4**, 284–333
35. Ren, J. M. & Hultman, E. (1988) Acta Physiol. Scand. **133**, 109–114
36. DeLange, R. J., Kemp, R. G., Riley, W. D., Cooper, R. A. & Krebs, E. G. (1968) J. Biol. Chem. **243**, 2200–2208
37. Krebs, E. G., Love, D. S., Bratvold, G. E., Trayser, K. A., Meyer, W. L. & Fisher, E. H. (1964) Biochemistry **3**, 1022–1033
38. Heilmeyer, L. M. G., Meyer, F., Haschke, R. H. & Fisher, E. H. (1970) J. Biol. Chem. **245**, 6649–6656

Received 30 September 1991/16 January 1992; accepted 17 January 1992