Prolonged Effect of Insulin on Glucose Uptake by Rat Skeletal Muscle

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The fate of insulin, as it relates to its action on skeletal-muscle glucose uptake, was studied in non-cyclically perfused rat hindlimbs. Insulin (1 m-i.u./ml) with and without ^{125}I -labelled insulin was infused intra-arterially for 5 or 6 min. Net glucose uptake and the release of ^{125}I -labelled insulin into the venous effluent were evaluated by arteriovenous-difference measurements for an additional 24–32 min. The infusion of insulin for 5 min promoted glucose uptake, an effect that persisted throughout a subsequent 25 min of perfusion in the absence of insulin. The addition of insulin antibody to the perfusate in the presence of insulin blocked the action of insulin on glucose uptake, but it failed to alter insulin action if the muscle tissue had been exposed to insulin before addition of antibody. When ^{125}I -labelled albumin was infused for 6 min, venous effluent radioactivity decayed rapidly and remained HClO_4 -insoluble and there was no significant tissue retention of radioactivity. Comparable experiments in which ^{125}I -labelled insulin was infused for 6 min revealed that the venous effluent radioactivity decayed more slowly, a significant amount of the ^{125}I -labelled insulin appeared as fragments (HClO_4 -soluble) and there was a significant retention of radioactivity in the tissue. Radioactivity in muscle tissue biopsies obtained 28 min after infusion of ^{125}I -labelled insulin was associated largely with intact insulin and a peptide of mol.wt. 2400. The total radioactivity retained in the muscle at this time was 7% of the amount infused. An insulin bolus (1 i.u.) failed to increase the discharge of this tissue-associated radioactivity. These results suggest that insulin and a product of insulin metabolism persists in muscle tissue long after the arterial presence of insulin ends. This tissue residence and processing of insulin may be important components of insulin's prolonged action on glucose uptake by skeletal muscle.

The first step in the action of insulin on a target cell appears to be the interaction of the intact insulin molecule with a receptor site on the plasma membrane of the cell. The characteristics of this interaction have been extensively investigated (for reviews see Pilkis & Park, 1974; Goldfine, 1978), but exactly how the interaction is translated into a physiological response is unknown. Evidence suggests that the binding of insulin to its receptor may be associated with the metabolism of the insulin molecule (Terris & Steiner, 1975, 1976) and with the uptake of insulin or products of its metabolism into the cell (Goldfine *et al.*, 1978; Carpentier *et al.*, 1979). Indeed it has been postulated that the cellular uptake of insulin or the products of its metabolism may be important for some of insulin's biological effects (Steiner, 1977).

The effect of insulin on glucose utilization by

rat diaphragm muscle has been shown to persist long after the hormone has been removed from the incubation medium (Stadie *et al.*, 1949; Pastan *et al.*, 1966). The molecular basis of this persistent effect of insulin is unknown, although it may be related to the retention in or on the muscle cells of insulin or products of its metabolism.

In this study we have used an isolated perfused rat hindlimb preparation to explore the duration of insulin's action on glucose uptake by skeletal muscle. This preparation was used because of its reproducible sensitivity to insulin (Lewis *et al.*, 1977). We have used ^{125}I -labelled insulin to assess the metabolic fate of the hormone in skeletal muscle as it relates to glucose uptake. A similar procedure has been used by others to study the fate of insulin in the liver (Terris & Steiner, 1976) and in adipose tissue (Kahn & Baird, 1978).

Experimental

Animals

Male Sprague-Dawley rats weighing 140–200 g were starved overnight after being fed Purina rat chow *ad libitum*.

Perfusion technique

The methods used have been described in detail previously (Schultz *et al.*, 1977; Lewis *et al.*, 1977). In brief, after pentobarbital anaesthesia of the rat the vessels not supplying the hindlimbs were ligated and the aorta was cannulated. The perfusion pump was started and immediately thereafter the inferior vena cava was cannulated and the entire venous effluent was collected.

The perfusate consisted of 33% washed bovine erythrocytes suspended in Krebs bicarbonate buffer containing 3% (w/v) bovine serum albumin and 10 mM-glucose. The rate of non-cyclic flow through the hindlimb ranged from 3 to 3.3 ml/min.

Tracer proteins, porcine insulin and guinea-pig anti-insulin serum were infused just proximal to the aortic cannula.

The infusion rate of 0.05 ml/min was maintained by a syringe infusion pump (model 906; Harvard Apparatus Company, Millis, MA 02054, U.S.A.). This system provided precise onset and cessation of infused substances. Quadriceps-muscle tissue biopsies of approx. 2.5 g were rapidly dissected from the thigh at the end of each perfusion, weighed and their radioactivity was determined.

Perfusate substrates

Insulin for the tracer studies was prepared by iodination with Na¹²⁵I (Union Carbide, Tuxedo, NY 10987, U.S.A.) of pure porcine insulin by standard techniques (Hunter & Greenwood, 1962) and was iodinated to a specific radioactivity of 120 μ Ci/ μ g. ¹²⁵I-labelled insulin was added to non-labelled insulin to give a specific radioactivity of 0.14 μ Ci/m-unit (15×10^4 c.p.m./ml) in the arterial infusate and the tracer radioactivity was at least 97% precipitable by HClO₄ (3 M). Arterial and venous perfusate samples were collected and immediately chilled in an ice bath and centrifuged at 4°C for 5 min at 2500 g to remove the erythrocytes. The glucose in the samples was measured by a model 23A glucose analyser (Yellow Springs Instruments Corporation, Yellow Springs, OH, U.S.A.).

HClO₄ precipitation of ¹²⁵I-labelled insulin

Perfusate (1 ml) free of erythrocytes was added to a glass test tube (10 mm $\times$ 75 mm) and the proteins were precipitated by the addition of 0.1 ml of HClO₄ (3 M). Trichloroacetic acid (5%) and 0.3 M-HClO₄ were shown to be equally effective in separating

radioactive insulin into soluble and precipitable fractions. The precipitated proteins were removed by centrifugation for 5 min at 2500 g. HClO₄-precipitable radioactivity was considered as relatively intact insulin molecules, whereas HClO₄-soluble radioactivity was considered to represent fragments of ¹²⁵I-labelled insulin (Izzo *et al.*, 1967). ¹²⁵I-labelled albumin (8×10^4 c.p.m./ml of perfusate; purchased from Abbott Laboratories, Chicago, IL 60064, U.S.A.) was infused as a control in experiments parallel to those with insulin tracer.

Experimental design

Insulin and insulin antibody experiments. Hindlimbs were perfused for 30 min after a 5 min washout period. Insulin was infused for either 0–5 min or 0–30 min in the presence and absence of insulin antibody. Insulin antibody (lot no. 614 from P. H. Wright, University of Indiana Medical Center, Indianapolis, IN, U.S.A.), when added, was infused from 5 to 30 min or 0 to 30 min and was present at a concentration five times that necessary to neutralize the insulin present in the perfusion medium. The measurement of flow rates and the concentration of glucose in the arterial and venous samples permitted calculation of glucose disappearance (Lewis *et al.*, 1977).

Insulin, ¹²⁵I-labelled insulin, ¹²⁵I-labelled albumin and bolus experiments. Additional experiments were performed to evaluate the fate of infused insulin. Hindlimbs were perfused for 30 or 34 min after a 5 min washout period. In these experiments hindlimbs were initially infused for 0–6 min with insulin (1 m.i.u./ml) in the presence and absence of ¹²⁵I-labelled insulin. ¹²⁵I radioactivity was monitored in the venous effluent every 2–4 min. Radioactivity was determined in the hindlimb muscle at the end of the experiment. ¹²⁵I-labelled albumin infusions were used as a marker for the fate of molecules largely confined to the extracellular space.

In further experiments, 18 min after ¹²⁵I-labelled insulin infusion, a bolus of unlabelled insulin (1 i.u.) was given in an attempt to enhance release of ¹²⁵I radioactivity from the hindlimb.

Chromatographic analysis of tissue radioactivity

Quadriceps-muscle tissue biopsies of approx. 2.5 g were rapidly dissected from the thigh at the end of each perfusion, weighed and homogenized at 4°C in 0.8 M-HCl/ethanol (1:3, v/v; 10 ml/g of muscle) in a Tissuemizer (Tekmar Company, Cincinnati, OH 45222, U.S.A.). The extract was centrifuged at 3000 g for 10 min at 4°C. The pellet was rehomogenized as described above and the supernatants were pooled. This procedure solubilized over 95% of the tissue-associated radioactivity. The supernatants were freeze-dried and the resulting extract was dissolved in 6 M-urea/3 M-acetic acid/0.1%

Triton X-100 and applied to a column (1.5 cm × 25 cm) of Sephadex G-25 and eluted with urea/acetic acid/Triton. Fractions were collected (1 ml) and their radioactivity was determined.

Determination of radioactivity

¹²⁵I radioactivity of iodinated compounds was measured in an Auto-Gamma Scintillation Spectrometer (Packard Instrument Co., Downers Grove, IL 60515, U.S.A.), with a counting efficiency of 50%.

Statistical analysis of results

Levels of significance of difference between mean values were assessed by the Student's paired *t* test (Snedecor, 1956). All results are expressed as the mean ± S.E.M. where appropriate.

Results

Effect of insulin on glucose uptake

The onset and duration of the action of insulin on glucose uptake by the perfused hindlimb are depicted in the upper portion of Table 1. In control experiments glucose disappearance remained constant for the 30 min of study (1.10–1.30 μmol/min per 100 g of rat). Continuous infusion of insulin enhanced net glucose disappearance within the first 5 min, and the glucose disappearance continued to increase steadily to a value of 4.10 μmol/min per 100 g of rat by the 25–30 min interval. This rate of glucose disappearance is significantly higher (*P* < 0.01) than that observed during the initial 5 min of perfusion. Infusion of insulin from 0 to 5 min only, increased the rate of glucose disappearance throughout the entire 30 min of perfusion, although the rate of net glucose uptake for the 25–30 min interval was lower than that for the continuous-insulin-infusion rates.

Action of insulin antibody

As shown in Table 1, guinea-pig anti-insulin serum added after infusing insulin for 0–5 min failed to alter insulin-stimulated glucose disappearance. However, when the same amount of neutralizing antibody was infused with insulin there was no stimulation of net glucose uptake.

Venous ¹²⁵I-labelled insulin during and after brief arterial infusion of ¹²⁵I-labelled insulin

The upper curve of Fig. 1 illustrates the rapid rise and slower fall of HClO₄-precipitable radioactivity during and after short-term infusion with ¹²⁵I-labelled insulin. The decay of the HClO₄-soluble radioactivity is shown in the lower curve of Fig. 1.

Muscle tissue biopsies obtained 28 min after cessation of insulin and ¹²⁵I-labelled insulin (from nine

Table 1. Effects of insulin and insulin antibody on glucose uptake by the perfused rat hindlimb

Rat hindlimbs were non-cyclically perfused for 30 min as described in the text. Portions from the six 5 min venous effluent collections were assayed for glucose. Antibody and/or insulin were infused during the time period indicated. The values are means ± S.E.M. for six observations (* indicates *P* < 0.01 compared with control).

Addition to perfusate	Time period of addition (min)	Venous collection intervals (min)	Glucose disappearance (μmol/min per 100 g of rat)					
			0–5	5–10	10–15	15–20	20–25	25–30
Control	—	...	1.25 ± 0.35	1.10 ± 0.30	1.18 ± 0.15	1.25 ± 0.10	1.26 ± 0.10	1.30 ± 0.15
Insulin	0–30	...	2.40 ± 0.20*	2.60 ± 0.23*	3.35 ± 0.20*	3.40 ± 0.30*	4.25 ± 0.25*	4.10 ± 0.15*
Insulin	0–5	...	2.75 ± 0.34*	2.93 ± 0.30*	3.10 ± 0.35*	3.25 ± 0.20*	3.25 ± 0.18*	3.10 ± 0.28*
Insulin + antibody	0–5	...	3.70 ± 0.45*	2.78 ± 0.69*	3.24 ± 0.28*	3.03 ± 0.36*	3.14 ± 0.45*	3.00 ± 0.11*
Insulin + antibody	5–30	...	1.19 ± 0.54	1.36 ± 0.55	1.47 ± 0.37	1.46 ± 0.43	1.75 ± 0.30	1.58 ± 0.07
Insulin + antibody	0–30	...	1.19 ± 0.54	1.36 ± 0.55	1.47 ± 0.37	1.46 ± 0.43	1.75 ± 0.30	1.58 ± 0.07

Fig. 1. Venous radioactivity during and after arterial infusion of ^{125}I -labelled insulin in the perfused rat hindlimbs

Rat hindlimbs were non-cyclically perfused as described in the text for 6 min with ^{125}I -labelled insulin (15×10^4 c.p.m./ml of perfusate) and unlabelled insulin (1 m.i.u./ml of perfusate) and then for a further 28 min in the absence of insulin. HClO_4 -soluble (O) and insoluble (●) radioactivity in the venous perfusate plasma was measured and expressed as c.p.m./ml of plasma. The ordinate (logarithmic scale) indicates ^{125}I radioactivity found during the venous sampling interval of 2–4 min depicted on the abscissa (linear scale). Each point is the mean $\pm$ S.E.M. for nine observations.

separate studies) contained 4366 ± 1180 c.p.m./g of muscle. Chromatographic separation of this radioactivity on Sephadex G-25 indicated that it could be separated into three distinct species, which ran in positions corresponding to intact insulin, insulin A chain (mol.wt. 2400) and iodotyrosine (Fig. 2). The relative distribution of radioactivity in these three fractions was 28, 56 and 16% respectively.

Effects of insulin bolus on venous ^{125}I -labelled insulin after brief arterial infusion of ^{125}I -labelled insulin

In four experiments a bolus of 1 i.u. of insulin was infused 18 min after cessation of ^{125}I -labelled insulin. The bolus did not increase the radioactivity in the venous effluent, although glucose uptake increased within 10 min from 2.26 ± 0.06 $\mu\text{mol}/\text{min}$ per 100 g of rat to 4.69 ± 0.37 $\mu\text{mol}/\text{min}$ per 100 g of rat.

Fig. 2. Gel chromatography of muscle radioactivity after arterial infusion of ^{125}I -labelled insulin in the perfused rat hindlimbs

Muscle tissue biopsies were taken from the thigh at the end of each perfusion and the tissue-associated radioactivity was solubilized as described in the text. The soluble extract was subjected to gel chromatography on a column (1.5 cm $\times$ 25 cm) of Sephadex G-25 eluted with 6 M-urea/3 M-acetic acid/0.1% Triton X-100. Fractions (1 ml) were collected and their radioactivity was determined.

Fig. 3. Venous radioactivity during and after arterial infusion of ^{125}I -labelled albumin in the perfused rat hindlimbs

The experimental procedure was as described in Fig. 1, except that ^{125}I -labelled albumin (8×10^4 c.p.m./ml of perfusate) was present in place of insulin. Radioactivity was measured in the venous perfusate plasma. The ordinate (logarithmic scale) depicts ^{125}I -labelled albumin radioactivity found during venous sampling intervals of 2–4 min depicted on the abscissa (linear scale). Each point is the mean $\pm$ S.E.M. for four observations.

Venous ^{125}I -labelled albumin during and after brief arterial infusion of ^{125}I -labelled albumin

At 2 min after cessation of the arterial infusion of ^{125}I -labelled albumin, radioactivity in the venous effluent peaks and subsequently rapidly declines (Fig. 3). At 24 min after cessation of the ^{125}I -labelled albumin infusion only 121 ± 73 c.p.m./g of muscle per 100 g of rat remains in quadriceps-muscle biopsy. More than 99% of ^{125}I -labelled albumin-radioactivity in all fractions collected was precipitable with HClO_4 .

Discussion

The results of this study show that brief exposure to insulin at 1 m-i.u./ml has a prolonged effect on glucose uptake by the non-cyclic perfused rat hindlimb. An insulin concentration of 1 m-i.u./ml was chosen for this study, since it has previously been shown to produce a marked but submaximal effect on glucose uptake in this system (Lewis *et al.*, 1977). The effect of insulin was found to persist for at least 28 min beyond its arterial presence. This observation of sustained insulin action differs from that of glucagon in its activation of hepatic glycogenolysis, where glucagon rapidly stimulated glucose release within minutes and cessation of this signal rapidly restores the stimulated metabolic function to baseline values (Park & Exton, 1972). In addition, constant insulin infusions at 1 m-i.u./ml steadily increase glucose disappearance over the initial 20 min of study. This augmentation of insulin action on net glucose uptake also differs from the spike decline pattern of hepatic glycogenolysis observed for constant infusion of glucagon (Bomboy *et al.*, 1977) and suggests fundamental differences in the mechanism of action of these two hormones on their target tissues.

Insulin antibody was used to see whether the binding of insulin to antibody would decrease glucose disappearance to control values. The presence of antibody failed to alter glucose uptake when the antibody was infused after insulin exposure; however, when infused with insulin, the antibody inhibited insulin action on glucose uptake. These results are similar to those of Wohltmann & Narahara (1967) and suggest either that insulin interacts with hindlimb tissues in a way that denies antibody-hormone interaction or that the prolonged effect of insulin on glucose uptake might be the result of a series of events set in motion by an initial interaction of insulin with the muscle cell membrane. It has been postulated that the cellular uptake of insulin or the products of its metabolism may be important for some of insulin's effects (Steiner, 1977), and evidence has been presented for the cellular uptake of insulin or products of its metabolism (Goldfine *et al.*, 1978; Carpentier *et al.*,

1979). To explore these possibilities ^{125}I -labelled insulin tracer was used to investigate the metabolic fate of insulin as it relates to the persistent effect on glucose uptake. Many reports have indicated that insulin iodinated to the specific radioactivity used in this study is a valid tracer for native insulin (Freychet *et al.*, 1971; Cuatrecasas, 1971). Since the ^{125}I is almost exclusively in the A-chain of insulin, these studies reflect the status of the A-chain only (Hamlin & Arquilla, 1974). The results of experiments with ^{125}I -labelled insulin were compared with similar experiments in which ^{125}I -labelled albumin was used as a tracer, since albumin is known to be confined to the extracellular space and not to be metabolized by muscle tissue. When ^{125}I -labelled albumin was infused for 6 min, venous effluent radioactivity decayed rapidly and was near the background value within 24 min. The radioactivity in the venous effluent remained 99% HClO_4 -insoluble, and there was no significant tissue retention of radioactivity. Comparable experiments in which ^{125}I -labelled insulin was infused for 6 min revealed that the venous effluent radioactivity decayed more slowly and that there was significant retention of radioactivity in the tissue (4366 c.p.m./g of muscle). In addition, a significant fraction of ^{125}I -labelled insulin was fragmented to HClO_4 -soluble material by hindlimb metabolism, and was released into the venous effluent. Presumably, enzyme systems at tissue sites responsible for insulin degradation process insulin to these small fragments (Brush & Kitabchi, 1970; Brush, 1971).

Boluses of insulin infused intra-arterially 18 min after the infusion of ^{125}I -labelled insulin failed to increase venous effluent radioactivity, even though the insulin boluses were effective in enhancing glucose uptake. This observation suggests that processing of radioactively labelled insulin may occur at a site distal to muscle membrane bathed by extracellular fluid. This observation is also consistent with the demonstration of intracellular translocation of insulin (Gorden *et al.*, 1978) to a site where it cannot be displaced by a large extracellular concentration of insulin and with the intracellular localization of the enzymes thought to be involved in insulin metabolism (Freychet *et al.*, 1972; Varandani, 1973).

The nature of the radioactivity present in quadriceps-muscle biopsies obtained 28 min after the arterial infusion of ^{125}I -labelled insulin had been discontinued was investigated by gel chromatography, and was shown to be associated with intact insulin, a species of mol.wt. 2400, which corresponds to insulin A-chain and free iodotyrosine. Such a distribution of radioactivity could be explained as resulting from a process responsible for insulin degradation and inactivation. Thus studies to date suggest that the metabolism of insulin in a variety

of tissues proceeds initially by a reductive cleavage into the A- and B-chains followed by extensive proteolytic cleavage of the separated chains (Brush, 1971; Chandler & Varandani, 1975; Phelps *et al.*, 1979). However, it is conceivable that the metabolism of insulin may be an important component of the mechanism of action of the hormone (Steiner, 1977; Goldfine, 1978) and that an initial cleavage into A- and B-chains is part of this process. Evidence in favour of this suggestion has come from studies that show weak but definite insulin-like activity of peptides derived from regions of the insulin molecule (Weitzel *et al.*, 1971, 1973). These findings taken together suggest that insulin binds to muscle, has a long residence in this tissue and slowly leaves the hindlimb partly intact and partly in small fragments. The small but significant concentration of intact insulin and insulin fragment present in muscle tissue may be of critical importance in explaining the prolonged action of insulin on glucose uptake, although it is possible that they may simply represent stages in the degradation and inactivation of insulin by muscle tissue.

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References

- Bomboy, J. D., Lewis, S. B., Lacy, W. W., Sinclair-Smith, B. C. & Liljenquist, J. E. (1977) *Diabetes* **26**, 177–184
- Brush, J. S. (1971) *Diabetes* **20**, 140–145
- Brush, J. S. & Kitabchi, A. E. (1970) *Biochim. Biophys. Acta* **215**, 134–144
- Carpentier, J. L., Gorden, P., Freychet, P., LeCam, A. & Orci, L. (1979) *J. Clin. Invest.* **63**, 1249–1261
- Chandler, M. L. & Varandani, P. T. (1975) *Biochemistry* **14**, 2107–2115
- Cuatrecasas, P. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1264–1268
- Freychet, P., Roth, J. & Neville, D. M. (1971) *Biochem. Biophys. Res. Commun.* **43**, 400–408
- Freychet, P., Kahn, R., Roth, J. & Neville, D. M. (1972) *Biochem. Biophys. Res. Commun.* **247**, 3953–3961
- Goldfine, I. D. (1978) *Life Sci.* **23**, 2639–2648
- Goldfine, I. D., Jones, A. L., Hradek, G. T., Wong, K. Y. & Mooney, J. S. (1978) *Science* **202**, 760–763
- Gorden, P., Carpentier, J. L., Freychet, P., LeCam, A. & Orci, L. (1978) *Science* **200**, 782–784
- Hamlin, J. L. & Arquilla, E. R. (1974) *J. Biol. Chem.* **249**, 21–32
- Hunter, W. M. & Greenwood, F. C. (1962) *Nature (London)* **194**, 495–496
- Izzo, J. L., Bartlett, J. W., Roncone, A., Izzo, M. J. & Bale, W. F. (1967) *J. Biol. Chem.* **242**, 2343–2355
- Kahn, C. R. & Baird, K. (1978) *J. Biol. Chem.* **253**, 4900–4906
- Lewis, S. B., Schultz, T. A., Westbie, D. K., Gerich, J. E. & Wallin, J. D. (1977) *Horm. Metab. Res.* **9**, 190–195
- Park, C. R. & Exton, J. H. (1972) in *Glucagon: Molecular Physiology, Clinical and Therapeutic Implications* (Lefebvre, P. J. & Unger, R. H., eds.), pp. 77–108, Pergamon Press, Oxford and New York.
- Pastan, I., Roth, J. & Macchia, V. (1966) *Proc. Natl. Acad. Sci. U.S.A.* **56**, 1802–1809
- Phelps, B. H., Varandani, P. T. & Shroyer, L. A. (1979) *Arch. Biochem. Biophys.* **195**, 1–11
- Pilkis, S. J. & Park, C. R. (1974) *Annu. Rev. Pharmacol.* **14**, 365–388
- Schultz, T. A., Lewis, S. B., Westbie, D. K., Wallin, J. D. & Gerich, J. E. (1977) *Am. J. Physiol.* **233**, E514–E518
- Snedecor, G. W. (1956) *Statistical Methods*, 5th end., p. 534, The Iowa State College Press, Ames
- Stadie, W. C., Havgaard, N., Marsh, J. B. & Hills, A. G. (1949) *Am. J. Med. Sci.* **218**, 265–274
- Steiner, D. F. (1977) *Diabetes* **26**, 322–340
- Terris, S. & Steiner, D. F. (1975) *J. Biol. Chem.* **250**, 8389–8398
- Terris, S. & Steiner, D. F. (1976) *J. Clin. Invest.* **57**, 885–896
- Varandani, P. T. (1973) *Biochem. Biophys. Res. Commun.* **55**, 689–696
- Weitzel, G., Eisele, K., Guglielmi, H., Stock, W. & Renner, R. (1971) *Hoppe-Seyler's Z. Physiol. Chem.* **352**, 1735–1738
- Weitzel, G., Eisele, K., Schulz, V. & Stock, W. (1973) *Hoppe-Seyler's Z. Physiol. Chem.* **354**, 321–330
- Wohltmann, H. J. & Narahara, H. T. (1967) *Biochim. Biophys. Acta* **135**, 170–173