Effect of Microtubular or Translational Inhibitors on General Cell Protein Degradation

EVIDENCE FOR A DUAL CATABOLIC PATHWAY

By JOSEPH S. AMENTA,* MARY J. SARGUS* and FRANCESCO M. BACCINO†

*Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, U.S.A.,
and †Istituto di Patologia Generale, Torino, Italy

(Received 29 March 1977)

Rat embryo fibroblasts were grown in Eagle's minimal essential medium with 10% serum and cell proteins prelabelled with L-[1-¹⁴C]leucine, followed by a 24 h chase. When transferred to medium deprived of serum these cells showed a 2–3-fold increase in the production of trichloroacetic acid-soluble radioactivity during a 4 h observation period. The microtubular poisons vinblastine, vincristine and colchicine partially inhibited this induced proteolysis, but had no effect on the proteolytic rate of cells maintained in medium with 10% serum. A similar discriminating effect on induced proteolysis was observed with cycloheximide, puromycin and insulin. The inhibitory effects of cycloheximide and vinblastine were not additive. These data support the hypothesis that, in addition to the basal turnover of cell proteins, a second mechanism of protein degradation involving cytoplasmic autophagy can be activated by nutritional step-down and is selectively inhibited by agents that interfere with microtubular function and protein synthesis.

Although turnover of cell proteins is a well-documented tenet of biology, the cellular mechanisms by which these proteins are degraded have not been established. Agents that inhibit the formation of ATP or protein synthesis decrease the basal rate of degradation of cell proteins (Simpson, 1953; Steinberg & Vaughan, 1956; Brostrom & Jeffay, 1970; Hershko & Tomkins, 1971), indicating that, at least under some conditions, energy and protein synthesis are necessary for cell protein catabolism. Alternatively, conditions thought to effect a 'nutritional step-down' enhance the rate of cell protein degradation (Hershko & Tomkins, 1971; Poole & Wibo, 1973; Woodside *et al.*, 1974; Epstein *et al.*, 1975). This enhancement is largely prevented by inhibitors of protein synthesis, which nonetheless have little or no effect on basal proteolysis (Hershko & Tomkins, 1971; Epstein *et al.*, 1975; Woodside, 1976), thereby suggesting that more than a single mechanism may account for the degradation of cell proteins. However, these data provide no information as to the sites or processes involved.

Lysosomes, containing the necessary enzymes for complete degradation of cell proteins (Barrett, 1972), have been proposed as the degradative site for cell proteolysis (Gordon, 1973; Wibo & Poole, 1974; Segal *et al.*, 1974; Dean, 1975), although most of the supporting evidence is circumstantial. More direct

are experiments showing that the enhanced proteolysis observed in liver-perfusion studies is associated with the development of autophagosome-like bodies in hepatocytes and increased rates of protein breakdown in isolated lysosomes (Neely *et al.*, 1974). The demonstration of a rapid increase in the number of autophagosomes and secondary lysosomes in cell cultures exposed to an acute nutritional step-down (Mitchener *et al.*, 1976) further supports a lysosomal role in cell proteolysis under conditions of accelerated catabolism. These reports, however, leave unresolved whether the vacuolar system makes a significant contribution to the general turnover of cell proteins under basal conditions.

The present study was designed to assess the functional role of secondary lysosomes in enhanced cell proteolysis by utilizing the known inhibitory effects of microtubular poisons on the functioning of the vacuolar system (Malawista, 1971; Neve *et al.*, 1972; Ekholm *et al.*, 1974; Williams, 1976). These studies support the hypothesis that the vacuolar system, when activated by nutritional step-down conditions, provides in cultured fibroblasts a quantitatively significant proteolytic mechanism for a brief time period. However, such an acute proteolytic response and the basal turnover of cell proteins may be operated by distinct mechanisms (Amenta *et al.*, 1976, 1977a; Baccino, 1977).

Experimental

Chemicals

Cycloheximide, colchicine and chloroquine were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.; vinblastine (Velban) and vincristine (Oncovin) were from Eli Lilly and Co., Indianapolis, IN, U.S.A.; insulin was from E. R. Squibb and Sons, Princeton, NJ, U.S.A.; NaF was from Fisher Scientific Co., Pittsburgh, PA, U.S.A.; puromycin dihydrochloride was from ICN Life Sciences Group, Cleveland, OH, U.S.A.; cytochalasin B was from I.C.I. Research Laboratories, Macclesfield, Cheshire, U.K. L-[1-¹⁴C]Leucine (>50 mCi/mmol) and L-[4,5-³H]leucine (40–60 Ci/mmol) were obtained from Amersham/Searle Corp., Arlington Heights, IL, U.S.A. Foetal calf serum and Eagle's minimum essential medium (cat. no. F-11) were from Grand Island Biological Co., Grand Island, NY, U.S.A.

Measurements

The methods used have been previously described (Amenta *et al.*, 1976). Rat embryo fibroblasts from strain F344 (Hilltop Laboratories, Scottsdale, PA, U.S.A.) were grown in Eagle's minimum essential medium supplemented with 10% foetal calf serum and L-[1-¹⁴C]leucine; 24h before each experiment, fresh unlabelled medium was added as a chase. To start the experiment, fresh basal (10% serum) or step-down (no serum) medium was substituted. The various inhibitors were added directly to the media in the concentrations listed in Table 1. Samples of the media were removed at 0, 1, 2 and 4h to determine the amount of trichloroacetic acid-soluble radioactivity released from the cells. Total cell protein was measured by the method of Lowry *et al.* (1951), with bovine albumin as standard.

Results

Inhibition of the acute proteolytic response

In control cells removal of serum from the medium increased the rate of proteolysis from 0.9 to 2.5%/h. Microtubular as well as protein-synthesis inhibitors selectively interfered with this enhanced proteolysis (Table 1).

Vinblastine (10 μ M) inhibited the induced proteolysis by 60% in the experiments shown (Table 1, A, B, C). A small inhibiting effect of vinblastine could still be detected at a concentration of 1 μ M, but no effect was seen at 0.1 μ M. With 100 μ M-vinblastine increased loss of cells from the monolayer and increased permeability to Trypan Blue indicated that at this concentration the drug significantly affected cell viability. Preincubating the cells for 4h with 10 μ M-vinblastine, before placing them in serum-free medium containing vinblastine, did not increase the inhibiting effect. By contrast, vinblastine did not inhibit protein turnover in control cells.

Table 1. *Inhibitors of cell proteolysis*

Fibroblasts prelabelled with [¹⁴C]leucine were incubated for 4h in Eagle's minimal essential medium either with 10% serum (basal) or without serum (step-down). Values are the means $\pm$ s.d. for the acid-soluble ¹⁴C found in the medium, expressed as percentage of total ¹⁴C. Numbers in parentheses are numbers of flasks in each group. Probability of the significance of differences versus controls with no inhibitor added, by Student's *t* test: **P* < 0.05, ***P* < 0.01.

Inhibitor	Acid-soluble ¹⁴ C released (%/4h)	
	Basal	Step-down
A. None	4.8 $\pm$ 0.3 (6)	13.5 $\pm$ 1.2 (6)
Vinblastine (10 μ M)	4.7 $\pm$ 0.4 (6)	8.5 $\pm$ 0.7 (6)**
Vinblastine (1 μ M)	5.4 $\pm$ 0.3 (6)	11.6 $\pm$ 0.8 (6)*
Cycloheximide (0.4 mM)	4.2 $\pm$ 0.6 (6)	7.8 $\pm$ 0.3 (6)**
B. None	3.3 $\pm$ 0.2 (6)	10.0 $\pm$ 0.6 (6)
Vincristine (10 μ M)	3.2 $\pm$ 0.3 (6)	7.7 $\pm$ 0.5 (6)**
Colchicine (25 μ M)	3.1 $\pm$ 0.2 (6)	7.8 $\pm$ 0.3 (6)**
Cytochalasin B (1.2 μ g/ml)	4.0 $\pm$ 0.4 (6)**	8.6 $\pm$ 0.7 (6)*
C. None	3.5 $\pm$ 0.4 (6)	10.5 $\pm$ 0.8 (6)
Vinblastine (10 μ M)	3.9 $\pm$ 0.5 (6)	6.8 $\pm$ 0.6 (6)**
Cycloheximide (0.4 mM)	3.3 $\pm$ 0.3 (6)	5.5 $\pm$ 0.4 (6)**
Cycloheximide/vinblastine	3.2 $\pm$ 0.4 (6)	5.5 $\pm$ 0.5 (6)**
D. None	3.0 $\pm$ 0.5 (6)	7.7 $\pm$ 0.5 (6)
Puromycin (1 mM)	2.8 $\pm$ 0.3 (6)	3.5 $\pm$ 0.4 (6)**
Puromycin (0.2 mM)	2.9 $\pm$ 0.4 (6)	4.7 $\pm$ 0.4 (6)**
E. None	3.7 $\pm$ 0.4 (7)	7.3 $\pm$ 0.6 (7)
Insulin (100 munits/ml)	3.4 $\pm$ 0.4 (10)	4.9 $\pm$ 0.3 (10)**
Chloroquine (10 mM)	2.9 $\pm$ 0.3 (7)**	3.8 $\pm$ 0.3 (7)**
NaF (10 mM)	2.1 $\pm$ 0.2 (6)**	4.0 $\pm$ 0.4 (6)**

To determine whether other inhibitors of microtubular function had similar effects on cell proteolysis, we treated cells with colchicine (25 μ M) or vincristine (10 μ M). As with vinblastine, these agents affected only the step-down-induced proteolysis, decreasing the rate by approx. 35% in the experiment shown (Table 1, B). On the other hand, cytochalasin B, which is known to bind to microfilaments and cell membrane, caused both a slight increase in basal proteolysis and a moderate decrease in step-down proteolysis (Table 1, B).

We also measured the effect of inhibitors of protein synthesis on both basal and enhanced proteolysis. When cells were incubated with 0.4 mM-cycloheximide, no significant decrease in basal proteolysis was detected, but, in marked contrast, the induced proteolysis was decreased by 65% (Table 1, C). On the other hand, when fibroblasts were preincubated with 0.4 mM-cycloheximide for 4h, then placed in

either serum-supplemented or serum-free medium with cycloheximide for an additional 4h, both basal and induced proteolysis were inhibited. It appears that cycloheximide, unlike vinblastine, given sufficient time can also inhibit basal proteolysis, thus showing both an immediate inhibitory effect on enhanced proteolysis and a delayed effect on basal proteolysis.

Since cycloheximide may affect cell functions other than protein synthesis (Kimberg *et al.*, 1973; Rez & Kovacs, 1973; Mitranic *et al.*, 1976), we also treated cultures with puromycin (Table 1D). At concentrations of 1 mM and 0.2 mM the enhanced proteolysis was decreased by 85% and 40%, whereas the same concentrations had no significant effect on the basal proteolytic rate.

To ascertain whether the inhibition observed with either microtubular or protein-synthesis inhibitors was on the same catabolic mechanisms, cells were exposed to both cycloheximide and vinblastine simultaneously. The resulting inhibition of the induced proteolysis was not greater than that observed with cycloheximide alone (Table 1, C), indicating that the effects of these two inhibitors were not additive. As observed with cycloheximide alone, there was a slight decrease in the rate of proteolysis in cells incubated in basal media with the combined inhibitors, although again this was of small magnitude and not statistically significant.

To establish the similarity of the present fibroblast cultures to experimental systems described by others (Hershko & Tomkins, 1971; Poole & Wibo, 1973; Neely *et al.*, 1974; Wibo & Poole, 1974; Knowles & Ballard, 1976), we treated these cells with substances that have been shown in other cell systems to inhibit either induced proteolysis alone or both induced and basal proteolysis (Table 1, E). NaF, which decreases ATP concentrations in the cell and directly inhibits lysosomal proteinases (Hershko & Tomkins, 1971; Poole & Wibo, 1973; Knowles & Ballard, 1976), inhibits basal proteolysis by 45% and induced proteolysis by 60%. Chloroquine had similar inhibitory action, whereas insulin inhibited only the induced proteolysis by 60%. The inhibitory effects of these agents on basal and enhanced proteolysis in our rat fibroblasts closely parallel those observed in cultured hepatoma cells (Hershko & Tomkins, 1971; Knowles & Ballard, 1976) and in perfused rat liver (Neely *et al.*, 1974).

Morphological observations

As observed by phase-contrast microscopy, during the 4h incubation period many of the fibroblasts placed in the serum-deficient medium progressively retracted their cytoplasm toward the nucleus, leaving elongated portions of the peripheral cytoplasm attached to the culture flask and conferring a refractile stellate outline on the retracting cells. Many of these would eventually withdraw such cytoplasmic pro-

cesses, resulting in rounded cells only loosely attached to the flask surface. We could detect no consistent effect of any of the microtubular inhibitors on these step-down-induced morphological changes, although in some flasks it appeared that the number of cells responding to the serum-free medium may have decreased.

In sharp contrast, cytochalasin B (1.2 μ g/ml), when added to both basal and serum-deficient media, produced within 30min a retraction of all cells into a sharply defined highly refractile stellate form, which eventually rounded, and floated free in the media. The morphological changes produced by puromycin resembled the response seen with cytochalasin B: both basal and serum-deficient media containing puromycin induced a rapid retraction of cytoplasm in virtually all cells. Cycloheximide, in contrast, had little discernible effect, producing a cell response similar to that observed with the microtubular inhibitors. These observations suggest that the changes in cell morphology on exposure to serum-free medium and the proteolytic response are not interdependent phenomena.

With electron microscopy, we have observed a marked increase in the number of autophagic vacuoles and secondary lysosomes in cells exposed to step-down medium for 4h. Cells treated with vinblastine while in step-down medium showed an alteration in the location of these vacuoles. Instead of a random distribution within the cell cytoplasm, the vacuoles appeared to be clustered together in isolated groups within the cytoplasm. We were not able to ascertain whether vinblastine decreased the number of vacuoles in the cells in step-down medium.

Discussion

The demonstration that under some experimental conditions the degradation of cell proteins requires concurrent protein synthesis (Steinberg & Vaughan, 1956; Brostrom & Jeffay, 1970) supports the contention that a complex energy-requiring process (Simpson, 1953; Hershko & Tomkins, 1971; Poole & Wibo, 1973) is herein involved, which at least in some conditions is regulated by steps or reactions depending on protein synthesis. However, complicating the situation are observations that under other experimental conditions inhibition of protein synthesis may have no effect on protein degradation (Hershko & Tomkins, 1971; Poole & Wibo, 1973; Woodside *et al.*, 1974). Our data with inhibitors of protein synthesis, demonstrating an almost immediate inhibition of step-down-induced proteolysis, while at the same time effecting very little change in the basal turnover of cell proteins, provides a possible clue to reconcile these discrepancies. Cells undergoing enhanced proteolysis as a result of nutritional step-down or other possible factors are likely to

show an immediate curtailment of protein breakdown on protein-synthesis inhibition, whereas cells maintaining only the basal proteolysis would barely show any effect, at least in the short time period. We suggest that the observation that protein-synthesis inhibitors promptly decrease the rate of general protein breakdown in an experiment should raise the suspicion of a possible enhancement of this process in the experimental situation under investigation. We believe that all these observations support best the hypothesis that step-down-induced proteolysis is dependent on cell structures requiring energy and protein synthesis for their integrity and activity (Brostrom & Jeffay, 1970; Mortimore & Mondon, 1970; Amenta *et al.*, 1976, 1977a). On the other hand, basal proteolysis may be less critically dependent on rapidly produced structural modifications in the cell, but more basically controlled by the rate of synthesis of proteins (enzymes, others) necessary for maintenance of protein turnover in the cell. (Such a mechanism would presumably require longer times to produce effects; Amenta *et al.*, 1977b.)

In view of the accumulating evidence associating lysosomes with the breakdown of cell proteins, a causal relationship appears likely at least under some experimental conditions involving a nutritional step-down. Lysosomes are actively involved in the autophagic processes underlying the enhanced proteolysis that occurs in a liver perfusion system, as demonstrated by the increased degradation of endogenous proteins in isolated lysosome-rich fractions (Barrett, 1972). Likewise, isolated rat hepatocytes display both enhanced protein catabolism (Seglen, 1975) and striking autophagic activity (Seglen & Reith, 1976), accompanied by increased lysosome fragility (Grammeltvedt & Berg, 1976). Both cycloheximide and insulin inhibit this induced proteolysis in the liver perfusion system (Mortimore *et al.*, 1973; Neely & Mortimore, 1973; Woodside, 1976), observations that compare well with the effects of these same agents on cultured cells exposed to serum deprivation, either fibroblasts, as in the present work, or hepatoma cells (Gelehrter & Emanuel, 1974; Epstein *et al.*, 1975; Gunn *et al.*, 1976). Electron-microscopic observations on HeLa cells placed in a serum-free medium have shown a dramatic increase in the number of autophagic vacuoles and secondary lysosomes within 3 h (Mitchener *et al.*, 1976). We have also seen an increase in the number of autophagic vacuoles and secondary lysosomes in serum-lacking fibroblasts at this time (J. S. Amenta, M. J. Sargus, S. Venkatesan & H. Shinozuka, unpublished work), corresponding to the period of maximal proteolysis that we have observed with biochemical measurements (Amenta *et al.*, 1976, 1977a). These data provide strong support for the hypothesis that this induced proteolysis results from activation of cytoplasmic autophagy.

Since microtubules are necessary for optimal functioning of the vacuolar system, we would predict that agents interfering with microtubular functions would inhibit any proteolytic mechanism in the cell involving secondary lysosomes. That these agents impair the degradation of exogenous proteins is well established (Malawista, 1971; Neve *et al.*, 1972; Ekholm *et al.*, 1974; Williams, 1976). We have previously shown (Amenta *et al.*, 1976) that the enhancement of endogenous proteolysis occurring in serum-deprived fibroblasts is inhibited by vinblastine. The present paper extends those observations to other drugs known to inhibit microtubular function. Although different microtubular poisons may affect other cell processes (Williams, 1976), they all inhibit both microtubular functioning and enhanced proteolysis, favouring the hypothesis that this latter inhibition is via impairment of microtubules and of the activity of the vacuolar system. Hoffstein *et al.* (1977) have observed that microtubular poisons produce a disorganization of the autophagic vacuoles—secondary lysosomes in cells and inhibit hydrolysis of exogenous proteins by approx. 50%. We have observed similar morphological alterations in the location of these vacuoles within the cytoplasm of our rat fibroblasts and a comparable degree of inhibition of enhanced endogenous proteolysis. Apparently a disorganized vacuolar system is able to function as a proteolytic mechanism, albeit at a significantly decreased rate, and this applies to both hetero- and auto-lytic processes.

That autophagy, as observed with the electron microscope, is a quantitatively significant mechanism in the turnover of cell proteins has been frequently accepted without supporting biochemical data (Gordon, 1973; Pfeifer, 1976). Our studies support the hypothesis that, at least under step-down conditions, the increased number of vacuoles reflects an increase in their formation and results in an increased breakdown of cell protein. Microtubular inhibitors, by disrupting the organization of the vacuolar system, seem to interfere with this mechanism.

To summarize most of the present findings, cultured rat embryo fibroblasts are able to operate the degradation of their own protein constituents at different rates, quickly shifting from the basal to an enhanced rate on removal of serum from the culture medium. Although energy is required for both basal and enhanced proteolysis, the latter is distinguished in that it is promptly suppressed by inhibitors of protein synthesis as well as of the microtubular function, whereas the basal process is not. Quite obviously, consistent with these data would be the dualistic hypothesis that the general degradation of cell proteins in cultured fibroblasts and conceivably other cell systems involves two mechanisms. One corresponds to a promptly activated rather indiscriminate proteolytic process which is effected by a rapid

structural alteration of the vacuolar system (autophagy); this acute proteolytic response is maintained for a short interval (hours) in the fibroblast system on serum deprivation (Amenta *et al.*, 1976), yet it may well operate over a longer time range in other situations. A second mechanism, accounting for the steady basal rate of protein turnover in the cell, may also involve lysosomes to some extent, but in a manner more selective than the autophagic mechanism and subjected to a different range of controls (Knowles & Ballard, 1976; Amenta *et al.*, 1977a). Regulation of the activities of some fast-turnover cell proteinases appears to be at least part of the controlling mechanism for basal proteolysis in the cell.

Finally, the present experiments were only designed to investigate the degradation of cell proteins with low turnover rates (owing to the 24 h chase between cell labelling and the experimental period). Nonetheless it should be mentioned that evidence has been provided suggesting that the breakdown of proteins turning over rapidly or slowly is controlled separately, at least in part (Poole & Wibo, 1973; Epstein *et al.*, 1975; Bradley *et al.*, 1976; Knowles & Ballard, 1976). Moreover the enhanced degradation by step-down and its suppression by translational inhibitors or insulin appear to be phenomena involving mainly, though not exclusively, slowly turning-over proteins (Hershko & Tomkins, 1971; Poole & Wibo, 1973; Epstein *et al.*, 1975; Knowles & Ballard, 1976).

References

- Amenta, J. S., Baccino, F. M. & Sargus, M. J. (1976) *Biochim. Biophys. Acta* **451**, 511–516
- Amenta, J. S., Baccino, F. M. & Sargus, M. J. (1977a) in *Intracellular Protein Catabolism II* (Turk, V. & Marks, N., eds.), pp. 27–42, Plenum Press, New York
- Amenta, J. S., Sargus, M. J. & Baccino, F. M. (1977b) *Biochim. Biophys. Acta* **476**, 253–261
- Baccino, F. M. (1977) in *Biochemical Mechanisms of Tissue Injury* (Slater, T. F., ed.), Academic Press, London and New York, in the press
- Barrett, A. J. (1972) in *Lysosomes: a Laboratory Handbook* (Dingle, J. T., ed.), pp. 46–135, North-Holland, Amsterdam
- Bradley, M. O., Hayflick, L. & Schimke, R. T. (1976) *J. Biol. Chem.* **251**, 3521–3529
- Brostrom, C. O. & Jeffay, H. (1970) *J. Biol. Chem.* **245**, 4001–4008
- Dean, R. T. (1975) *Nature (London)* **257**, 414–416
- Ekholm, R., Ericson, L. E., Josefsson, J.-O. & Melander, A. (1974) *Endocrinology* **94**, 641–649
- Epstein, D., Elias-Bishko, S. & Hershko, A. (1975) *Biochemistry* **14**, 5199–5204
- Gelehrter, T. D. & Emanuel, J. R. (1974) *Endocrinology* **94**, 676–684
- Gordon, A. H. (1973) in *Lysosomes in Biology and Pathology* (Dingle, J. T., ed.), vol. 3, pp. 89–137, North-Holland, Amsterdam
- Grammeltvedt, R. & Berg, T. (1976) *Hoppe-Seyler's Z. Physiol. Chem.* **357**, 977–981
- Gunn, J. M., Ballard, F. J. & Hanson, R. W. (1976) *J. Biol. Chem.* **251**, 3586–3598
- Hershko, A. & Tomkins, G. M. (1971) *J. Biol. Chem.* **246**, 710–714
- Hoffstein, S., Goldstein, I. M. & Weissmann, G. (1977) *J. Cell Biol.* **73**, 242–256
- Kimberg, D. V., Field, M., Gershon, E., Schooley, R. T. & Henderson, A. (1973) *J. Clin. Invest.* **52**, 1376–1383
- Knowles, S. E. & Ballard, F. J. (1976) *Biochem. J.* **156**, 609–617
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Malawista, S. E. (1971) *Blood* **37**, 519–529
- Mitchener, J. S., Shelburne, J. D., Bradford, W. D. & Hawkins, H. K. (1976) *Am. J. Pathol.* **83**, 485–492
- Mitranic, M., Sturgess, J. M. & Moscarello, M. A. (1976) *Biochim. Biophys. Acta* **421**, 272–279
- Mortimore, G. E. & Mondon, C. E. (1970) *J. Biol. Chem.* **245**, 2375–2383
- Mortimore, G. E., Neely, A. N., Cox, J. R. & Guinivan, R. A. (1973) *Biochem. Biophys. Res. Commun.* **54**, 89–95
- Neely, A. N. & Mortimore, G. E. (1973) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **32**, 299
- Neely, A. N., Nelson, P. B. & Mortimore, G. E. (1974) *Biochim. Biophys. Acta* **338**, 458–472
- Neve, P., Ketelbant-Balasse, P., Williams, C. & Dumont, J. E. (1972) *Exp. Cell Res.* **74**, 227–244
- Pfeifer, U. (1976) *J. Cell Biol.* **70**, 206a
- Poole, B. & Wibo, M. (1973) *J. Biol. Chem.* **248**, 6221–6226
- Rez, G. & Kovacs, J. (1973) *Virchows Arch. B* **12**, 123–132
- Segal, H. I., Winkler, J. R. & Miyagi, M. P. (1974) *J. Biol. Chem.* **249**, 6364–6365
- Seglen, P. O. (1975) *Biochem. Biophys. Res. Commun.* **66**, 44–52
- Seglen, P. O. & Reith, A. (1976) *Exp. Cell Res.* **100**, 276–280
- Simpson, M. V. (1953) *J. Biol. Chem.* **201**, 143–154
- Steinberg, D. & Vaughan, M. (1956) *Arch. Biochem. Biophys.* **65**, 93–165
- Wibo, M. & Poole, B. (1974) *J. Cell Biol.* **63**, 430–440
- Williams, J. A. (1976) *Endocrinology* **98**, 1351–1358
- Woodside, K. H. (1976) *Biochim. Biophys. Acta* **421**, 70–79
- Woodside, K. H., Ward, W. F. & Mortimore, G. E. (1974) *J. Biol. Chem.* **249**, 5458–5463