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Protein turnover and proliferation

Failure of SV-3T3 cells to increase lysosomal proteinases, increase protein degradation and cease net protein accumulation

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The contrasting control of lysosomal proteinases, protein turnover and proliferation was studied in 3T3 and SV-3T3 (SV-40-virus-transformed 3T3) cells. 1. In 3T3 cells, net protein accumulation proceeded from 5%/h (doubling time, $T_d = 14$ h) in growing cells to 0%/h as cells became quiescent. SV-3T3 cells never ceased to gain protein, but rather decreased their protein accumulation rate from 6–7%/h ($T_d = 10$–12 h) to 2%/h ($T_d = 35$–40 h) just before culture death in unchanged medium. 2. In both cell types the rates of protein synthesis per unit of protein (*a*) were proportional to the initial serum concentration from 0 to 6%, and (*b*) declined under progressive depletion of undefined serum growth factors. In depleted growth medium, leucine incorporation per unit of protein in 3T3 and SV-3T3 cells declined to almost equal synthetic rates while the 3T3 cell existed in a steady state of zero net gain, and the SV-3T3 cell continued to gain protein at a rate of 2%/h. 3. Whereas a large fraction of the control of 3T3-cell net protein accumulation can be accounted for by an increase in degradation from 1%/h to 3%/h, the SV-3T3 cell did not exhibit a growth-related increase in degradation appreciably above 1%/h. 4. Thus, by using first-order kinetics, the continued net protein accumulation of the transformed cell can be accounted for by a failure to increase protein degradation, whereas fractional synthesis can be made to decline to a rate similar to that in the quiescent non-transformed cell. 5. Upon acute serum deprivation, both cell types similarly exhibited small rapid increases in proteolysis independent of cell growth state or lysosomal enzyme status. 6. The 3T3 cell increased its lysosomal proteinase activity in conjunction with increase in the growth-state-dependent proteolytic mechanism; however, the SV-3T3 cell failed to increase lysosomal proteinases or the growth-state-dependent proteolytic mechanism.

Cell growth accompanies an unbalanced state of protein turnover, and the failure of the neoplastic cell to cease growth involves the failure to equalize synthesis and degradation. It has been suggested that the growth control of some cells is not accompanied by sustained alterations of bulk protein degradation (Bradley, 1977; Baxter & Stanners, 1978; Amenta *et al.*, 1980), and that neoplastic transformation is not accompanied by appreciable differences in the control of intracellular proteolysis (Bradley, 1977; Baxter & Stanners, 1978). In disagreement with the former suggestion, the preceding paper indicated that under certain culture conditions the control of non-transformed 3T3-cell net protein accumulation is accompanied by almost equal synthetic and degradative alterations (Lockwood *et al.*, 1982). As distinguished by the time course of kinetic alterations, there are two proteolytic mechanisms in 3T3 cells. A previously reported rapid mechanism (Hershko *et al.*, 1971; Poole & Wibo, 1973; Hendil, 1977; Gunn *et al.*, 1977; Warburton & Poole, 1977) increased and decreased protein degradation promptly in response to the absence or presence of serum or insulin in the

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culture medium. A slowly changing proteolytic mechanism was associated with changes in DNA synthesis and growth state, and induction of lysosomal proteinases.

One major subdivision of intracellular protein degradation is lysosomal and non-lysosomal [reviewed by Dean (1980) and Goldberg *et al.* (1981)]. In contrast with 3T3 cells, virally transformed SV-3T3 cells completely fail to increase acid proteinases as they proliferate suicidally into the death of the culture (Lockwood & Shier, 1977). Thus the present study of 3T3 and SV-3T3 cells provides a comparison of protein turnover kinetics and proliferation in the presence and absence of the control of lysosomal proteinases.

Experimental

A31-3T3 and SV-3T3 cells (Aaronson & Todaro, 1968) were supplied by Dr. George Todaro to the M.I.T. cell-culture centre and cultured as previously described (Lockwood *et al.*, 1982). Other methods have been previously described (Lockwood *et al.*, 1982).

Results

Proliferation and net protein accumulation as 3T3 and SV-3T3 cells depleted the growth medium

The kinetics of proliferation and protein accumulation in 3T3 cells have been described previously (Lockwood *et al.*, 1982). In contrast with 3T3 cells, SV-3T3 cells continued to proliferate suicidally after depletion of the medium or attainment of a high cell density (Aaronson & Todaro, 1968; Paul *et al.*, 1974; and see below). Under equal initial culture conditions, subconfluent SV-3T3 cells attained a peak rate of protein accumulation, K_a, approx. 30–40% higher than that of subconfluent 3T3 cells (Fig. 1). Both cell types then decreased the rate of protein accumulation in association with some combination of increasing cell density and/or depletion of the medium by progressive growth (Fig. 1). However, unlike the 3T3 cell, the SV-3T3 cell never attained a zero net protein gain, nor existed in stable, non-proliferative, culture for extended periods. Regardless of initial cell density, the net protein accumulation rate decreased to approx. 2%/h (doubling time, T_d, of 35–40 h) as cultures were dying (Fig. 1). Measurements of protein accumulation

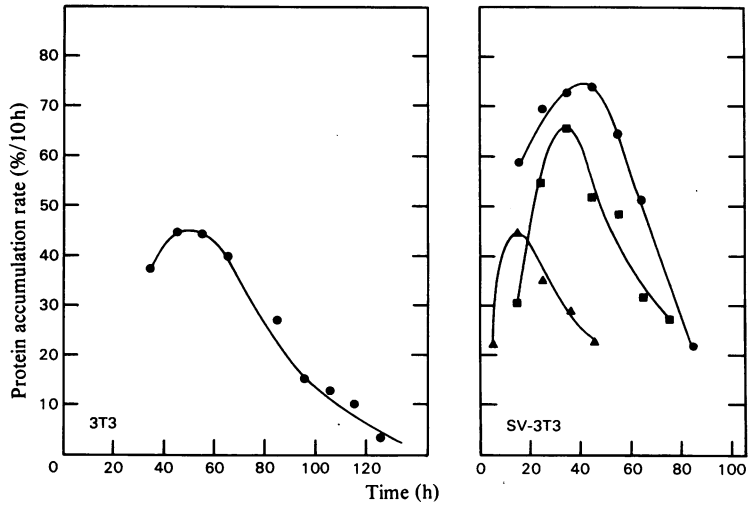

Fig. 1. *Net protein accumulation rates in 3T3 and SV-3T3 cells*
The medium of 3T3 and SV-3T3 cultures containing 6% serum was changed at zero time when both cell types were at the same low initial cell density of 2×10^4 dish (●). Also shown are two additional experiments in which the medium of SV-3T3 cells was changed at an initial cell number of 2×10^5 cells per dish (■) and 2×10^6 cells per dish (▲). The rates of culture protein accumulation (%/10h) were calculated as the average for a 10h period as previously described (Lockwood *et al.*, 1982). At low cell numbers, harvests were concentrated by freeze-drying. By 120h, 3T3 cells were almost quiescent at 10^6 cells per dish. At the last points shown, SV-3T3 cultures had attained high densities of approx. 4×10^6–7×10^6 cells per dish for all three experiments. All values shown are from cultures that were 99% viable at the last points shown. SV-3T3 cells began to die approx. 10–20h after the last indicated points. By measuring detachment of previously radiolabelled cell protein as described in Fig. 4, it was determined that protein detachment contributes an insignificant error.

below 2%/h could not be observed, due to the beginning of cell death and detachment at either subconfluent or confluent density when growth was slowed to this accumulation rate. When plated at very low density, SV-3T3 cells grew in an uneven, patchy distribution, resulting in a high cell density at low numbers of cells per dish. Thus it is not possible to separate clearly growth limitation due to increasing density from limitation due to serum depletion. Use of a semi-logarithmic representation of SV-3T3 cell growth can mistakenly imply a cessation of growth at high density; however, a linear growth curve reveals a sharp point of culture death and no growth plateau (see below, Fig. 5).

Protein synthesis per unit of protein as 3T3 and SV-3T3 cells depleted the growth medium

If the identical radioisotope solution is used to pulse-label 3T3 and SV-3T3 cells at a high leucine concentration (4mM), then the rate of leucine incorporation per unit of protein can be directly compared between cell types in a simultaneous experiment. After an initial lag period, due to the change from conditioned to unconditioned growth medium at low cell density, fractional protein synthesis in both cell types attained peak maximal rates (Fig. 2). As 3T3 cells depleted limiting growth factors from 10% serum, they decreased the peak fractional rate of protein synthesis by 40–50% and attained a sustained constant synthetic rate (Fig. 2, and Lockwood *et al.*, 1982). Similar to the higher peak rate of net protein accumulation in SV-3T3 cells under these conditions (Fig. 1), the transformed cell also attained a 30–40% higher rate of fractional protein synthesis as compared with the 3T3 cell. After the maximum, the protein-synthesis rate of SV-3T3 cells declined toward a value similar to that in quiescent 3T3 cells (Fig. 2). However, despite the similar synthesis rates, the SV-3T3 cells gained protein at a rate of approx. 2%/h just before culture death, whereas the 3T3 cell existed in a state of zero net gain with a balanced turnover rate of 3%/h (Lockwood *et al.*, 1982; and see below).

The decline in protein synthesis per unit of protein at subconfluent density (Fig. 2) could be attributable to a depletion of either the synthetic component(s) of the growth medium or the growth factor(s) of serum (Holley & Kiernan, 1974*a*,*b*). Thus a further comparison of the serum-dependence of 3T3 and SV-3T3 cell protein synthesis was studied with a double-isotope technique that yields results identical with the measurement of leucine incorporation per mg of protein (Lockwood *et al.*, 1982). At higher cell density, the transient lag in protein synthesis due to unconditioned medium (Fig. 2) is greatly decreased (T. D. Lockwood, unpublished work), and the synthesis rates were approximately maximal at 8h. The fractional rates of protein synthesis in both 3T3

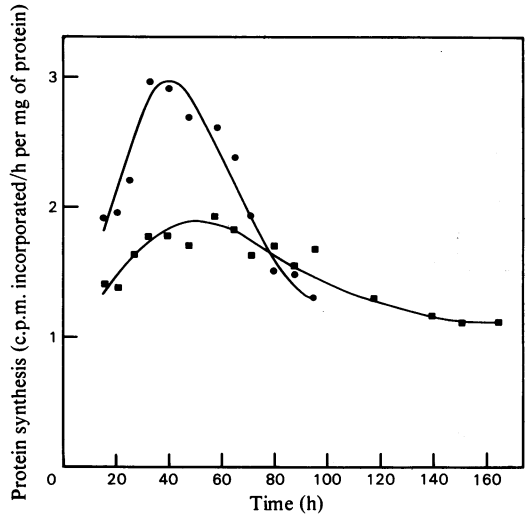

Fig. 2. *Progress of 3T3 and SV-3T3 cell protein synthesis per unit of protein versus culture time in unchanged medium*

Protein synthesis was determined in the two cell types in a parallel experiment using the identical initial medium and isotopic labels as previously described (Lockwood *et al.*, 1982). The experiment was begun by change of medium at zero time when cells were at a density of 3×10^4 per dish. These initial conditions correspond approximately to the traces in Fig. 1 describing net protein accumulation beginning at 2×10^4 cells per dish. The 3T3 cell cultures (■) at 160h were quiescent at 10^6 per dish and gained no protein; however, the SV-3T3 cell cultures (●) at 60–80h exhibited a protein-doubling time of approx. 35–40h (Fig. 1) at a density of 5×10^6 cells per dish.

and SV-3T3 cells exhibited a dependence on initial serum concentrations from 0.25 to 6% as measured 8h after the change of medium (Fig. 3). Any particular serum concentration supported an approx. 30% higher maximal rate of fractional protein synthesis in the transformed cell at 8h. The experiment was designed and monitored microscopically so as also to measure fractional protein synthesis at the approximate time of the attainment of quiescence of 3T3 cells and just before the death of SV-3T3 cells at high density. At 24h after the change of medium, the rates of protein synthesis per unit of protein in both cell types were similar over the entire range of serum concentrations. The combined results of Figs. 2 and 3 indicate clearly that the rates of fractional protein synthesis in the SV-3T3 cell as well as the 3T3 cell are responsive to (*a*) the initial serum concentration and (*b*) the progressive consumption of unknown limiting serum growth factors.

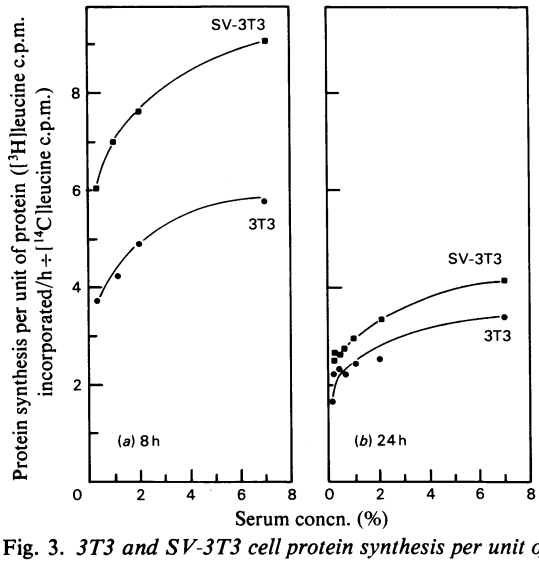

Fig. 3. *3T3 and SV-3T3 cell protein synthesis per unit of protein as a function of serum concentration*

3T3 and SV-3T3 cells were previously grown over a 50-fold increase in cell number in the identical batch of medium containing [^{14}C]leucine so as to label uniformly and identically total cell protein of both cell types. The experiment was designed such that both 3T3 and SV-3T3 cultures were just sub-confluent at approx. 2×10^6 cells per 5 cm dish and still growing at zero time. The ^{14}C-labelled media (6% serum) in which both cell types were grown were changed to those containing the indicated serum concentrations and the identical [^{14}C]leucine specific radioactivity. The rates of [^{3}H]leucine incorporation were determined at 8 h (*a*) and 24 h (*b*) after the change of medium in a parallel experiment using the identical label for both cell types, as previously described (Lockwood *et al.*, 1982). At the lower serum concentrations some cells had detached by 24 h; however, those remaining anchored were >99% viable as determined by Trypan Blue dye exclusion, and cell detachment does not affect the measurement.

Growth-state-dependent mechanisms and prompt mechanisms of 3T3 and SV-3T3 cell protein degradation

In confirmation of the previous paper (Lockwood *et al.*, 1982), the growing 3T3-cell protein degradative rate, K_d, increased from 1.0–1.2%/h ($t_{\frac{1}{2}} \simeq 60$–70 h) to 2.7–3.0%/h ($t_{\frac{1}{2}} \simeq 23$–26 h) as cells ceased proliferation (Figs. 4*a* and 4*b*). The rate of protein degradation in the growing SV-3T3 cell was 0.9–1.1%/h ($t_{\frac{1}{2}} \simeq 65$–80 h), and the transformed cell did not increase this degradative rate in unchanged depleted medium (Fig. 4*c*). In separate experiments the observed degradation period was initiated at the beginning, middle and end of the SV-3T3-cell growth curves at low, medium and high cell densities respectively. As shown by the integral representations of total culture protein content (Fig. 4*d*) or the differential plots of separate data (Fig. 1), the SV-3T3-cell protein-accumulation rates varied considerably in response to depletion of serum factors, attainment of high cell density, or some combination. However, the actual variations in the rate of protein accumulation (%/h) (Fig. 1) cannot be discerned from a logarithmic representation of the integral total culture protein content (Figs. 4*a*–4*e*). Thus large variations in the rate of SV-3T3-cell protein synthesis (Figs. 2 and 3) and net protein accumulation (Figs. 1 and 4*d*) are not accompanied by a discernable increase in protein degradation even into the death of the SV-3T3 cell cultures. However, similar to the 3T3 cell (Lockwood *et al.*, 1982), a small, prompt increase in SV-3T3-cell protein degradation of approx. 0.5–1.0%/h could be superimposed upon the 1%/h basal SV-3T3-cell degradative rate in either low-density or high-density cells after serum deprivation (Table 1).

The error contributed by detachment of labelled protein from the monolayer was determined by simultaneous harvest of the growth medium (Lockwood *et al.*, 1982). The amount of labelled detached protein degraded by proteinases in serum was determined to be negligible. In the measurement of protein degradation by the present method, the

Table 1. *Protein degradation in growing 3T3 and SV-3T3 cells*

Protein degradation was measured as the release of previously incorporated leucine from uniformly labelled cell proteins into the medium over 4 h as previously described (Lockwood *et al.*, 1982). Protein degradation in maximally growing 3T3 cells (5×10^5 per dish; *a*) and SV-3T3 cells (4×10^5 per dish; *b*) is not significantly different. All elevations in protein degradation on serum deprivation are highly significant ($P < 0.025$, Student's *t* test). However, the SV-3T3-cell degradative rates in low-density cultures (*a* and *b*) and high-density culture (*c* and *d*; 3×10^6 cells per dish) are not significantly different.

Cells ...	Protein degradation (%/h) 3T3	SV-3T3
(*a*) Low-density + 10% serum	1.2 ± 0.11 ($n = 5$)	1.1 ± 0.10 ($n = 5$)
(*b*) Low-density − serum	1.8 ± 0.18 ($n = 6$)	1.5 ± 0.12 ($n = 6$)
(*c*) Confluent + 10% serum	*	1.2 ± 0.14 ($n = 6$)
(*d*) Confluent − serum	*	1.6 ± 0.08 ($n = 6$)

* See Lockwood *et al.* (1982).

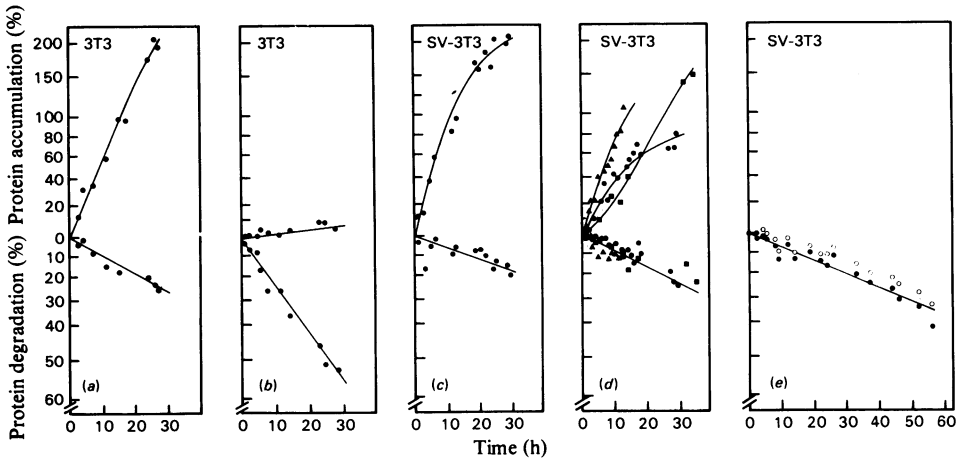

Fig. 4. *3T3 and SV-3T3 cell net protein accumulation and protein degradation in fresh and depleted media*
Cells were previously grown over at least a 40-fold increase in cell number in media containing [^{3}H]leucine so as to label cell protein uniformly. At zero time, cultures were rinsed and changed to non-radioactive media containing 6mM-leucine. Methods were as previously decribed (Lockwood *et al.*, 1982). Values shown in panels (*a*)–(*d*) have not been corrected for cell detachment, and an indication of the percentage error resulting from such is shown in panel (*e*). Values for half-lives and degradative rates are presented as a range owing to actual slight variations in degradative rates under undefined differences between batches of cultures. Slight variations in degradative rates were also observed in other experiments corrected for cell detachment as in panel (*e*). (*a*) Before the degradative period, 3T3 cells had been growing maximally at a low cell density of 10^5/dish in radioactive medium containing [^{3}H]leucine and 10% serum. To label uniformly 98% of cell protein, stock cells had been previously grown in identical radioactive medium before plating. A similar procedure was used for the SV-3T3 cultures described below. At the beginning of the degradative period, the radioactive medium was replaced with non-radioactive medium containing 10% fresh serum and 6mM-leucine. The average half-life of trichloroacetic acid-precipitable radioactivity remaining in the dish was 60–70h in four such experiments, and the degradative rates ranged between 1.0 and 1.2%/h. (*b*) Labelled 3T3 cells were quiescent at 2×10^6 cells per dish after 5 days of growth in unchanged medium containing 6% serum. Non-radioactive replacement medium containing 6% serum was previously depleted by the growth of unlabelled cultures (Lockwood *et al.*, 1982). The half-life of quiescent 3T3-cell protein was 23–26h, and the degradative rate was 2.7–3.0%/h in five experiments. (*c*) Similarly to the experiment described for (*a*), SV-3T3 cells were growing maximally at a density of 10^5 per dish in radioactive medium containing 10% serum. At zero time, the medium was changed to non-radioactive medium containing 10% fresh serum. The half-life of cell protein in three such experiments was 65–80h, and the degradative rate was 0.9–1.1%/h. (*d*) Similarly to conditions described in (*b*), SV-3T3 cells were grown without change of medium in 6% serum and, at various times in the growth curve, partially depleted radioactive media were replaced with non-radioactive media from cultures grown in parallel. The half-lives of SV-3T3 cell protein were 65–80h at the beginning (■), middle (▲) and end (●) of growth curves at initial cell densities of 5×10^4, 5×10^5 and 3×10^6 per dish respectively. (*e*) Protein degradation was determined in SV-3T3 cells at an initial density of 10^5 per dish under 3% fresh serum as in (*a*)–(*d*). To the total amount of trichloroacetic acid-precipitable radioactivity in the monolayer (●) was added that macromolecular radioactivity recovered from the growth medium to indicate the combined radioactivity in monolayer and medium (○).

percentage error contributed by cell detachment is greatest at low degradative rates; thus the results of Fig. 4(*e*) represent the maximal error of the present procedure. Detachment of protein was considerably greater under some other culture conditions; however, no significant differences were observed between 3T3 and SV-3T3 cell protein detachment in the present experiments.

Protein accumulation, protein turnover and lysosomal proteinase activities in 3T3 and SV-3T3 cells

As described in the previous paper (Lockwood *et al.*, 1982), a comparison of changes in the rate of protein synthesis per unit of protein with simultaneous changes in the rate of net protein accumulation provided an indication of increased rate of protein degradation as cells ceased growth. A similar single experiment performed simultaneously on 3T3 and SV-3T3 cells permits comparison of the contrasting control of proliferation, protein turnover and lysosomal proteinase activities in these cell types when both are grown from the same initial cell density in the identical batch of medium (Fig. 5). The use of unchanged medium containing 6% serum provided results that were similar to those with 10% serum (Lockwood *et al.*, 1982); however, 6% serum permitted more reliable experimental control of the

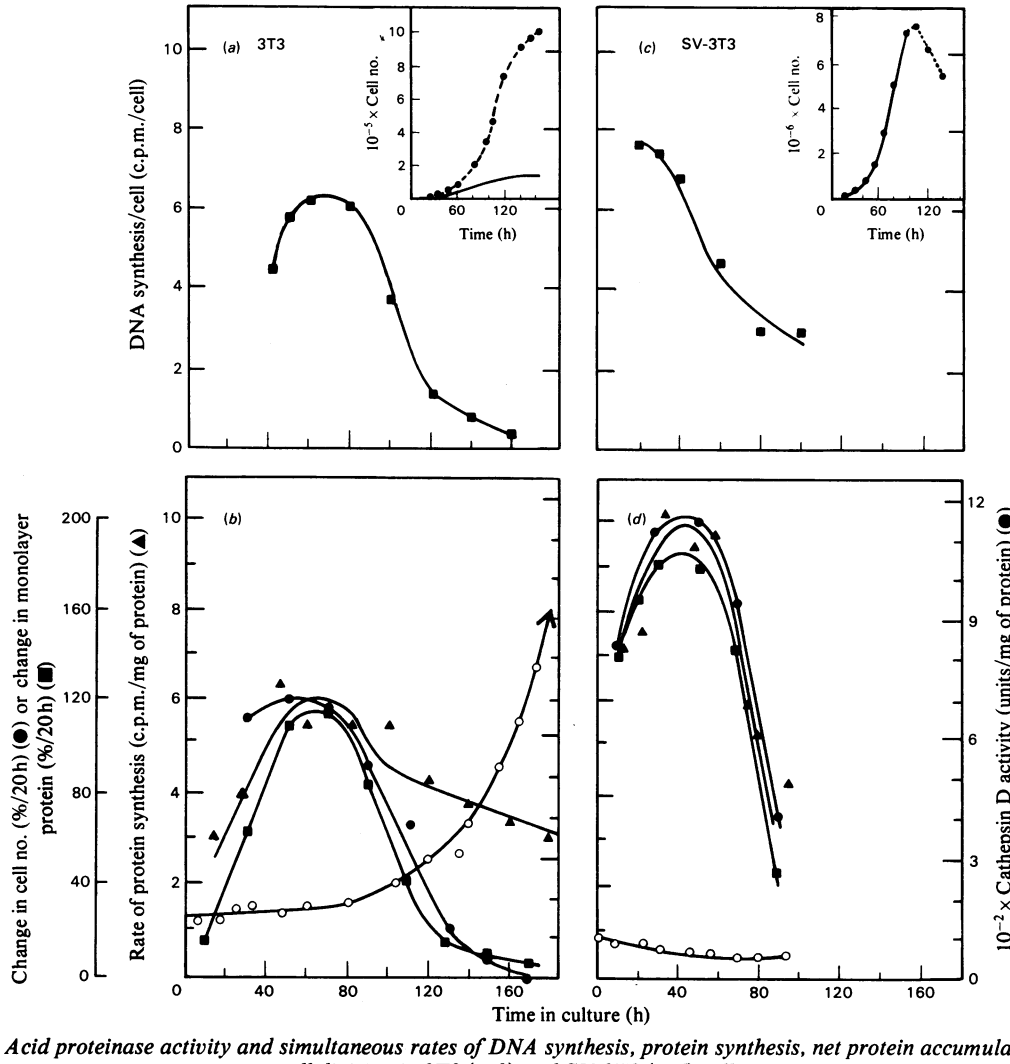

Fig. 5. *Acid proteinase activity and simultaneous rates of DNA synthesis, protein synthesis, net protein accumulation and cell division in 3T3 (a, b) and SV-3T3 (c, d) cells*

Measurement of parameters for 3T3 and SV-3T3 cells was begun by changing the medium of growing cultures at cell densities of 5×10^4 per dish. Cultures were grown without change of medium in the identical batch of medium containing 6% serum. All measurements were undertaken in parallel with the identical radioisotopes in a single simultaneous experiment with the same batch of cultures. Experimental details were similar to those described in Figs. 1–4 and in the preceding paper (Lockwood *et al.*, 1982). A more precise indication of kinetic parameters is presented therein. The final 3T3 cell number was 10^6 per dish; however, the cell growth curve (panel *a*, inset) is also shown expanded by one order of magnitude (dashed trace). Panels (*a*), (*c*): thymidine incorporation/cell (■); panels (*b*), (*d*): rate of protein synthesis/mg of protein (▲); percentage change in cell no./20h (●); percentage change in monolayer protein/20h (■); acid proteinase activity [units (μg of haemoglobin hydrolysed/h)/mg of protein] (○). Insets show cumulative cell numbers.

final cell density so as to prevent overcrowding and a partial decline in steady-state turnover (Lockwood *et al.*, 1982).

The initial growth lag resulting from change of medium at low cell density was of lesser duration in the SV-3T3 than in the 3T3 cultures. 3T3 cells proliferated to a slightly subconfluent density and became quiescent at 5–6 days. SV-3T3 cells slowed the rate of proliferation, but continued to grow suicidally until the beginning of culture death at 3–4 days. As previously described (Lockwood *et al.*, 1982), the 3T3-cell increase in protein degradation

from 1%/h to 3%/h led to a divergence between changes in the rates of protein synthesis and net protein accumulation as cells decreased DNA synthesis and became quiescent (Figs. 5*a* and 5*b*). However, because the SV-3T3 cell never sustained an increase in protein degradation appreciably above 1%/h under suicidal proliferation (Fig. 4), it would be expected that changes in the rate of net protein accumulation would be controlled by changes in the rate of protein synthesis (see the Discussion section). Accordingly, the SV-3T3 cell maintained approximately proportional changes in the rates of protein synthesis per unit of protein and net protein accumulation into the death of the culture (Figs. 5*c* and 5*d*). The possibility of small changes in SV-3T3-cell protein degradation within the approximate range of 0.9–1.2%/h would not be disproved by the experimental accuracy of the results of Figs. 4 and 5.

Maximally growing 3T3 and SV-3T3 cells exhibited similar low acid proteinase activity (Figs. 5*b* and 5*d*). The 3T3 cell increased lysosomal proteinase activity in association with the increase in protein degradation and decrease in DNA synthesis. However, the SV-3T3 cell completely failed to increase lysosomal proteinase activity in association with its failure to increase the growth-dependent proteolytic mechanism and failure to cease net protein gain (Figs. 5*b* and 5*d*).

The increase in 3T3-cell haemoglobinolytic activity at pH 2.8, which is largely cathepsin D, was indicative of a general increase in acid proteinase activities from pH 2 to 6. Conversely, the failure of the SV-3T3 cell to elevate the activity measured at pH 2.8 was accompanied by a co-ordinate failure to elevate any other acid activity detected by this method (results not shown).

The present conclusions were qualitatively similar whether enzyme activity was expressed in terms of cell number or units of protein. However, due to changes in cell protein content during growth transitions, the relationship between proteinase activity and protein turnover was extremely variable and imprecise when 3T3-cell proteinase activity was expressed in terms of cell number.

Discussion

Protein turnover and protein accumulation in 3T3 and SV-3T3 cells

As described previously (Lockwood *et al.*, 1982), the relationship $K_a = K_s - K_d$ can be employed as a crude kinetic model to characterize the changes in the synthetic rate, K_s, and the degradative rate, K_d, that are associated with changes in the net protein accumulation rate, K_a. The previously described turnover kinetics of the 3T3 cell are 5%/h = 6%/h − 1%/h during maximal growth, and 0%/h = 3%/h − 3%/h during quiescence in depleted medium at uncrowded cell density.

Although the peak rate of SV-3T3-cell protein accumulation depended on the culture conditions (Fig. 1), when these cells were plated at a low density of 10^5 per dish and permitted to grow in unchanged medium containing 6–10% serum, a transient peak protein accumulation rate of 6–7%/h was characteristically observed. In contrast with the 3T3 cell, the SV-3T3 cell never attained a steady state of protein turnover with zero net gain before the death of the culture (Fig. 1). Thus a typical transition in SV-3T3-cell protein accumulation which must be accounted for in terms of turnover is the transition from a variable peak accumulation rate of approx. 6–7%/h to a net protein gain of 2%/h ($T_d \simeq 35$–40 h) just before culture death and detachment. Because the SV-3T3 cell did not exhibit a sustained protein-degradation rate above approx. 1%/h (Fig. 4), the changes in net protein-accumulation rate (Figs. 1 and 5) are contributed almost entirely by the observed variations in synthetic rate (Fig. 2) as cells depleted the growth factors of serum (Fig. 3). Because the measured degradation rate remained at 1%/h (Fig. 4), the implied synthetic rate at any point is equal to the measured accumulation rate plus 1%/h. Thus the approximate kinetic description of SV-3T3-cell net protein accumulation during maximal growth is 6%/h = 7%/h − 1%/h. Although the magnitude and time course of the attainment of the maximal synthetic and accumulation rates varied in relation to culture conditions (Figs. 1 and 2), the final kinetic description of turnover approached 2%/h = 3%/h − 1%/h just before culture death. The small, rapid elevations in SV-3T3 cell proteolysis after serum deprivation [Table 1; see also Gunn *et al.* (1977) and Hendil (1977)] do not appear to contribute appreciably to the sustained bulk control of net protein accumulation in unchanged depleted medium (Figs. 4 and 5).

If the 3T3 and SV-3T3 cells were to share the same limited nutritional-hormonal system, the transformed cell could exist in a state of positive nitrogen balance even at the same synthetic rate as the quiescent non-transformed cell. Moreover, even at the same fractional rates of synthesis, the transformed or growing cell could hypothetically parasitize amino acids from proteins of the non-transformed quiescent cell. Speculatively, such a mechanism could also contribute to the positive nitrogen balance of the growing cells of an embryo under maternal nutritional insufficiency.

Lysosomal proteinases and the kinetics of protein degradation in 3T3 and SV-3T3 cells

The elevation in growth-dependent proteolysis and acid proteinases in the 3T3 cell, and absence of

both from the SV-3T3 cell, could be associated with increased lysosomal function in quiescent non-transformed cells and the failure of such function in transformed cells. Further evidence for a lysosomal involvement in growth-dependent proteolysis is provided by separate studies (Goldberg *et al.*, 1981; T. D. Lockwood & A. L. Goldberg, unpublished work). Low concentrations of inhibitors of lysosomal function exert little effect on protein degradation in rapidly growing 3T3 cells. However, the same concentrations of these agents do inhibit a large fraction of quiescent-cell proteolysis. Nonetheless, the functional control of the compartmentalized acid proteinases might not be rate-limited by the cellular content of the measured activity. Moreover, the observation that alterations in the rapidly changing proteolytic mechanism were independent of lysosomal enzyme status does not rule out a possible involvement of lysosomal compartmentation in this mechanism. Finally, a possible growth-state or transformation-state dependence of ubiquitous extralysosomal proteolytic systems such as the Ca^{2+}-activated proteinase and the ATP-dependent proteinase are currently unknown (Dean, 1980; Holzer & Heinrich, 1980; Goldberg *et al.*, 1981), as is the fraction of cell protein degraded by such systems.

Acid hydrolases are secreted by cultured cells and are readily detectable in mammalian serum (review by Hasilik & Neufeld, 1980; Erickson *et al.*, 1981; T. D. Lockwood, unpublished work). Although the secretion–recapture hypothesis of lysosomal enzyme translocation remains controversial, it has been conclusively demonstrated that extracellular lysosomal hydrolases are bound to high-affinity cell-surface receptors and internalized in functional form via coated pits (reviewed by Pastan & Willingham, 1981). With regard to the present results, the possible implications of direct (Olsen *et al.*, 1981) or indirect (Robbins & Myerowitz, 1981) lysosomal enzyme transfer and/or differential uptake are obvious; however, a possible hormonal role of these enzymes has not been investigated. Uptake of the extracellular serum proteinase inhibitor α_2-macroglobulin has also been demonstrated in cultured cells (Pastan & Willingham, 1981).

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