A Method for the Analysis of Protein Turnover Characteristics

INDIRECT ESTIMATION OF RATES OF PROTEIN DEGRADATION

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A method for the calculation of rate constants of degradation of a specific protein during a period of change in protein amount is described. The calculation uses measurements of rates of synthesis and amount of the protein, together with one estimate of the protein half-life. The method is demonstrated by using data from measurements made on the accumulation of fatty acid synthetase in hormonally treated explants of mammary gland from mid-pregnant rabbits, and data from other authors. These calculations demonstrate a regulated programme of co-ordinated changes in rates of synthesis and degradation during a period of change in protein mass. The role of such changes in protein degradation during protein accumulation is discussed.

The accumulation or diminution of many enzymes in a variety of tissues has been shown to be due to co-ordinated changes in the rate of enzyme synthesis and the rate of enzyme degradation (Schimke & Doyle, 1970; Paskin & Mayer, 1977; Mayer & Paskin, 1978). Such changes are clearly functions of time and cannot be regarded as instantaneous changes. Hence any analysis of the roles of synthesis and degradation in a particular enzymic change must take account of the time-dependent changes in the rates of formation and destruction of the enzyme (Paskin & Mayer, 1977). In particular, it is not sufficient to invoke an n -fold increase in the rate of synthesis to account for an n -fold change in the amount of an enzyme; the time course of this change must be taken into account. Unless the half-life of the enzyme is very small, considerably more time is needed to increase the amount of enzyme by n -fold than is required to increase the rate of synthesis by n -fold.

Measurement of the absolute rate of degradation of an enzyme is often laborious and difficult to perform owing to the problem of isotopic reutilization (Poole, 1971; Goldberg & Dice, 1974). Several attempts have been made to overcome this problem by estimating the rate of degradation indirectly from measurements of synthetic rate and amount of enzyme: for example, estimates of the rate of breakdown of protein have been made from differences in measured rates of synthesis and net accumulation of protein in muscle (Millward *et al.*, 1975) or liver (Conde & Scornik, 1977). In one case a sophisticated computer model has been designed to estimate rates of degradation from measurements of pool sizes, amounts and rates of synthesis of [^3H]lysine-labelled myosin during cardiac hypertrophy (Morkin *et al.*,

1972). Such models are possible only for systems where extensive data on pool sizes etc. are readily obtainable, and these data may include several assumptions.

In the present paper we describe a method for the indirect estimation of rates of protein degradation by a simple mathematical approach, which can be easily performed on a small computer. The method uses the general principles discussed by Paskin & Mayer (1977). The application of this method is illustrated by typical calculations on our own and other authors' data (Smith & Barker, 1977; Flick *et al.*, 1977).

The advantages of a regulated programme of enzyme turnover (synthesis and degradation) indicated by these and other calculations, and the role of degradation in changes of enzyme amount, are discussed.

Experimental

Materials

The animals used were as described by Speake *et al.* (1975). Ox insulin, sheep prolactin, cortisol 21-acetate, streptomycin sulphate, sodium benzylpenicillin, NADPH, CoA, malonyl-CoA and DEAE-cellulose (DE-52) were obtained from the sources described by Speake *et al.* (1975). L-[4,5- ^3H]Leucine (specific radioactivity 52–57 Ci/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.; phenylmethanesulphonyl fluoride was from Sigma (London) Chemical Co., Kingstons, upon Thames, Surrey KT2 7BH, U.K.; Sepharose 2B was from Pharmacia (G.B.) Ltd., London W5 5SS, U.K.; glass-fibre paper (Whatman GF/A) was from Fisons Scientific Apparatus, Loughborough, Leics., U.K.;

dialysis tubing (Visking size 1:8/32) was from Medicell International Ltd., London EC4N 4SA, U.K. All other chemicals were of A.R. grade. Acetyl-CoA was prepared by the method of Smith *et al.* (1966).

Preparation of immunoadsorbent

An antiserum to rabbit mammary-gland fatty acid synthetase has been raised in sheep and shown to be monospecific (Paskin & Mayer, 1976). Immunoglobulin G was purified from this antiserum by $(\text{NH}_4)_2\text{SO}_4$ precipitation, dialysis against water and DEAE-cellulose (DE-52) chromatography (Walker & Mayer, 1977). The resulting immunoglobulin fraction was covalently linked to Sepharose 2B by imido-carbonate activation with CNBr (Porath *et al.*, 1973) under the conditions recommended for moderate activation. Under these conditions 6.2 mg of immunoglobulin G was bound per ml of Sepharose (68% of the immunoglobulin in the reaction mixture was bound to Sepharose). Immunoglobulin obtained from non-immunized animals (control serum) was similarly bound to Sepharose (7.0 mg of immunoglobulin per ml of Sepharose; 66% of immunoglobulin was bound).

Columns (0.5 ml) of antiserum-Sepharose or control-serum-Sepharose were prepared in 2 ml plastic syringe barrels by using a disc of Whatman glass-fibre paper (GF/A) to retain the Sepharose.

Preparation and culture of mammary explants

Mammary explants obtained from mid-pregnant rabbits were prepared as described by Forsyth & Myers (1971) and cultured at 37°C in O_2/CO_2 (19:1) as described by Speake *et al.* (1975) in the presence or absence of hormones (insulin, 5 $\mu\text{g}/\text{ml}$; prolactin, 1 $\mu\text{g}/\text{ml}$; cortisol, 1 $\mu\text{g}/\text{ml}$; final concentrations). The medium was renewed and gassed every 24 h.

Incorporation of radioactive isotope into explants

The rate of incorporation of L-[4,5- ^3H]leucine into immunodetectable fatty acid synthetase in mammary-gland explants was measured at various times by incubating groups of 20 explants at 37°C for 30 min in culture medium containing 25 μCi of L-[4,5- ^3H]leucine, 1.5 mM-leucine (a concentration shown to give optimum rates of incorporation in this system; L. E. Smith & R. J. Mayer, unpublished work) and O_2/CO_2 (19:1) in the presence or absence of hormones.

Preparation of tissue extracts

After incubation with L-[4,5- ^3H]leucine, explants were washed with 10 ml of 0.25 M- KH_2PO_4 (adjusted

to pH 7.0 with KOH) containing 1 mM-EDTA and 0.1 mM-dithiothreitol, at 4°C. Explants were then washed at 4°C with 4 $\times$ 5 ml of this buffer at 5 min intervals. The explants were blotted and weighed on a torsion balance, and then homogenized at 4°C in the potassium phosphate buffer (0.5 ml per 20 explants). A supernatant was prepared by centrifugation (60 min at 115 000 g_{av}) and fatty acid synthetase was assayed as described by Speake *et al.* (1975). Phenyl-methanesulphonyl fluoride was then added to the extract (approx. 2 mM final concentration) to inhibit serine proteinase activity (Paskin & Mayer, 1976). Triton X-100 was added to a final concentration of 1% (w/v).

Immunoisolation and measurement of radioactive fatty acid synthetase from tissue extracts

A sample (0.2 ml) of each tissue extract was added to a column (0.5 ml) of Sepharose 2B equilibrated in 0.25 M- NaH_2PO_4 (adjusted to pH 7.0 with NaOH) containing 1 mM-EDTA and 0.1 mM-dithiothreitol.

After 5 min, each column was washed with 2 ml of this sodium phosphate buffer and the washings were loaded on a column of either 0.5 ml of antiserum-Sepharose or 0.5 ml of control-serum-Sepharose equilibrated in the phosphate buffer. The columns were gently agitated at intervals. After 20–30 min, the columns were washed with 2 ml of the sodium phosphate buffer and the washings were discarded; then 8 M-urea (2 ml) containing 2% (w/v) sodium dodecyl sulphate was added to each column. After 10–15 min, the urea/sodium dodecyl sulphate was collected and the columns were washed with a further 1 ml of urea/sodium dodecyl sulphate. The two urea/sodium dodecyl sulphate washings were combined and extensively dialysed against water (4 litres) at room temperature (20°C) (2–3 days with five changes of water). The resulting non-diffusible material was freeze-dried, taken up in 0.05 ml of 10 mM-sodium phosphate, pH 7.0, containing 2% (w/v) sodium dodecyl sulphate and 2% (v/v) 2-mercaptoethanol, and heated at 100°C for 3 min before electrophoresis in 5% (w/v) polyacrylamide disc gels (length 6.4 cm) in the system described by Weber & Osborn (1969). Electrophoresis was carried out at 8 mA per gel until the tracking dye (Bromophenol Blue) reached the bottom of each gel tube.

Protein was fixed by immersing the gel in 10% (w/v) trichloroacetic acid for 10 min followed by rinsing in water. Gels were sliced into 2 mm sections (Adensik, 1971). The first ten slices were each solubilized in 0.5 ml of 30% ('100-volume') H_2O_2 at 55°C overnight (Cashman & Pitot, 1971). Formic acid (0.5 ml; 1%, v/v) was added to each sample, followed by 10 ml of the Triton X-100/xylene/scintillator mixture described by Speake *et al.* (1975). Radioactivity was measured in a Packard Tri-Carb

3375 liquid-scintillation spectrometer. C.p.m. were converted into d.p.m. by an external standardization method.

Radioactivity peaks were identified (normally in slices 5–9 inclusive, which contain fatty acid synthetase polypeptides in this system; N. Paskin & R. J. Mayer, unpublished work) and radioactivity (d.p.m.) in these slices was summed. Radioactivity from labelled fatty acid synthetase was estimated from (d.p.m. in gel slices containing fatty acid synthetase polypeptides from antiserum–Sepharose column)–(d.p.m. in corresponding gel slices of sample from control-serum–Sepharose column). Values of 200–5000 d.p.m. were obtained. The percentage recovery of radioactive fatty acid synthetase by this method was assumed to be constant.

In a separate series of experiments (results not shown) it was found that 75% of purified fatty acid synthetase (Paskin & Mayer, 1976) applied to the column of antiserum–Sepharose was eluted by urea/sodium dodecyl sulphate; control-serum–Sepharose was shown to bind approx. 30% of the fatty acid synthetase applied to the column, of which 70% was eluted by urea/sodium dodecyl sulphate. Hence the fatty acid synthetase measured by the technique described above represents approx. 75%–(70%×30%) = 54% of the fatty acid synthetase loaded on the columns.

Measurement of rate of degradation of fatty acid synthetase

In some experiments the rate of degradation of fatty acid synthetase in mammary explants was measured.

After incubation with L-[4,5-³H]leucine for 30 min as described above, groups of 20 explants were washed with 2 ml of culture medium containing 1.5 mM-leucine in the presence or absence of hormones at 37°C. They were then incubated in a further 2 ml of this solution for 10 min at 37°C under O₂/CO₂ (19:1). This incubation procedure was repeated three times (total of 4×10 min incubations) and the explants were replaced on grids. They were then further cultured for 6, 18 or 24 h. At the end of each period tissue extracts were prepared, and labelled fatty acid synthetase was immunisolated and determined as described above.

Calculation of rate of degradation

Principle. Changes in the amount of an enzyme with respect to time can be described by the following equation (Price *et al.*, 1962; Berlin & Schimke, 1965; Schimke, 1970):

$$\frac{dE}{dt} = k_s - k_D E$$

where dE/dt = rate of change of enzyme amount (E)

with respect to time (t), k_s = zero-order rate constant of synthesis and k_D = first-order rate constant of degradation. Given known rates of synthesis and enzyme amount at a series of times (e.g. from a series of experimental measurements), the rate of degradation at each time can be calculated by considering small but finite intervals of time; the equation above is then of the form (Paskin & Mayer, 1977):

$$\frac{\Delta E}{\Delta t} = k_s - k_D E$$

It follows from this equation that the rate of degradation (k_D) that must operate over the interval of time Δt when the rate of synthesis is k_s and the amount of enzyme changes from E to $(E + \Delta E)$ is given by:

$$k_D = k_s - \frac{\frac{\Delta E}{\Delta t}}{E}$$

Computer programs have been written to calculate any one of E , k_s or k_D as a function of time, given empirical values of the other two variables at different times (Paskin & Mayer, 1977). In such a program for the calculation of k_D , plots of k_s versus time and E versus time are effectively approximated to a series of stepwise changes at equal small intervals of time (Δt) and the equation above for k_D is applied for each interval.

The present authors use a program written in FOCAL with a Digital Equipment Corporation PDP 11/05 minicomputer. A Hydra model B medium-speed matrix printer (Hydra Corp., Mountain View, CA, U.S.A.) is used to obtain the result directly as a plot of k_D versus time, and a list of the numerical values of k_D at each interval of time is also produced.

Conversion of empirical data into compatible units. Data on rates of synthesis, rates of degradation and enzyme amount that have been obtained experimentally are reported in a variety of incompatible units, e.g. rates of synthesis may be reported as amount (d.p.m.) of label incorporated per unit weight of tissue, as mg per unit time etc., rates of degradation as percentage per h or as half-life (days or h or min) and enzyme amount from radioactivity (d.p.m.) in radioimmune assays, as activity units per unit mass of tissue (if activity is proportional to amount) etc.

To calculate k_D as described above, all such data must be converted into a consistent set of units. The following convention is used throughout this paper. (a) k_D is expressed as h⁻¹, i.e. in $2/t_{1/2}$ where $t_{1/2}$ is half-life in h. (b) Values of enzyme amount (E) (or activity, if activity is proportional to amount), are expressed as percentage of initial value [i.e. the initial amount ($t = 0$) of enzyme per unit weight of tissue is 100 and other values are expressed as percentages of this value]. (c) Rates of synthesis are expressed as percentage of initial amount/h; a 'conversion

factor' for converting reported units of synthesis into these units is obtained from data obtained in a steady state, when $dE/dt = 0$ and hence $E = k_s/k_D$; therefore k_s (percentage of initial amount/h) at the steady state $= E$ (percentage of initial amount) $\times k_D$ (h^{-1}), where E and k_D are measured in the steady state; hence the conversion factor by which all values of k_s are multiplied is given by:

$$\frac{k_s \text{ (percentage of initial amount/h) at the steady state}}{k_s \text{ (reported units) at the steady state}}$$

Where possible such a conversion factor is calculated from measurements at a steady state before the change in enzyme amount under consideration. In cases where this information is not available or where the amount of enzyme before the change is zero, the conversion factor is calculated from a steady state after the completion of the change under consideration.

The numerical values of E and k_s may be arbitrarily changed by multiplication of both by a constant without affecting the calculation of k_D . However, the numerical value of k_D used must be in absolute terms, i.e. h^{-1} .

Input of data. Values of E and k_s at successive intervals of time (Δt), which are fed into the computer for the calculation, can be obtained either by linear interpolation between measured values or by constructing a 'smoothed' curve that passes through measured values and avoids discontinuities in dE/dt or dk_s/dt . The former can give rise to unrealistic values of k_D at such sharp changes in slope; the latter may be more representative of the real way in which the variables change with time.

Presentation of results. Calculated values of k_D can be usefully presented as graphs of k_D versus time, or as numerical values for specific time points. For comparative purposes it is often useful to present calculated values of k_D as an average over a given period of time, since measured values of k_D are inherently 'time-averaged' (see the Discussion section). For example, a measured rate of degradation over a 24h period can be compared with a calculated 'average k_D ' obtained by averaging successive calculated values of k_D over the 24h period.

Results and Analysis

Fatty acid synthetase in rabbit mammary gland

Rates of degradation of fatty acid synthetase in hormonally stimulated explants of mammary gland from mid-pregnant rabbits were determined both by direct measurement and by calculation from data on activity and rate of synthesis during development. Speake *et al.* (1975) showed that changes in activity of fatty acid synthetase in rabbit mammary gland are solely due to changes in amount of the enzyme. A

conversion factor for the calculation was obtained from measurements made on explants cultured in the absence of hormones for 24h; in this situation there was little change in the specific activity of fatty acid synthetase or in the rate of synthesis of fatty acid synthetase. The results (Table 1) indicate that the rate of degradation of fatty acid synthetase is decreased during the hormonally stimulated enzyme accumulation, but increases again when hormones are removed. These results agree with those of Speake *et al.* (1976): the same qualitative pattern is obtained from both measurement and calculation. For hormones removed after 48h in culture, a good quantitative agreement is obtained for measured and calculated values. Hence an estimate of the pattern of degradation of fatty acid synthetase is obtainable by the calculation method described.

Glucose 6-phosphate dehydrogenase in rat uterus

The data of Smith & Barker (1977) on activity and synthesis of uterine glucose 6-phosphate dehydrogenase were analysed by the method described above. They were obtained from smooth curves drawn through points on the published graphs of changes in activity and synthesis [lower panel of Fig. 2 in Smith & Barker (1977)]. Two investigative analyses were carried out. (a) A conversion factor was obtained from the values of E and k_s at zero time by assuming a steady state at that period and a half-life of 111h at that time [obtained from extrapolation of loss of [$1^{14}C$]glutamate; upper panel of Fig. 2 in Smith & Barker (1977)]. (b) For the second analysis, a conversion factor was obtained from the values of E , k_s and k_D at 72h (after oestradiol treatment at 0h); at this point there is little change in either E or k_s . The results of the analyses are shown in Table 2 and Fig. 1. The assumption of a steady state at $t = 0$ (calculation a) is not valid: calculated values of k_D were negative at a large number of time points. The assumption of a steady state after cessation of oestradiol-induced changes leads to more realistic estimates of k_D . The pattern of change in rate of degradation observed by Smith & Barker (1977) is confirmed: calculation shows that degradation is slow over an initial period and then increases markedly, beginning at 33h, after administration of oestradiol (Fig. 1; Table 2), but decreases sharply from 48h onwards if oestradiol is re-administered at 48h (Table 2). The calculation method allows determination of the exact point at which degradation slows down or increases; oestradiol treatment clearly leads to a decrease in the rate of degradation in the period immediately after its administration, and a return to higher values some hours later (Fig. 1).

Fatty acid synthetase in rat liver

The data of Flick *et al.* (1977) on fatty acid synthetase in rat liver were analysed by the method

Table 1. *Degradation of fatty acid synthetase in mammary explants in organ culture*

Experimental procedure was as described in the text. Degradation was measured over 24 h periods. Values of k_D were calculated from data at 3 h intervals. Average values of k_D over 24 h periods were obtained by averaging the eight successive calculated values over the period concerned. The Table gives values of $t_± = \ln 2/k_D$. Data from two series of measurements are considered. Abbreviations: N.D., not determined; NH, no hormones in culture medium; +H, in the presence of hormones (insulin+cortisol+prolactin) from start of experiment; HR, hormones removed at start of period of measurement. * k_D slightly negative: this is assumed to represent no degradation during the period concerned (see the Discussion section).

Treatment	Period of measurement (h in culture)	$t_±$ (h)			
		Expt. 1		Expt. 2	
		Measured	Calculated	Measured	Calculated
NH	0-24	19	N.D.	29	N.D.
+H	0-24	31.5	∞^*	N.D.	∞
+H	24-48	52	150	N.D.	∞^*
+H	48-72	112	∞^*	N.D.	34.7
HR	24-48	6.9	16.5	N.D.	16.0
HR	48-72	21.3	18.0	N.D.	17.8

Fig. 1. Computer-calculated values of k_D obtained from known values of E and k_S for uterine glucose 6-phosphate dehydrogenase after zero-time administration of oestradiol (data from Smith & Barker, 1977)

The Figure represents the computer-generated graph showing E , k_S and k_D as functions of time. Values of k_D were calculated for points at 3 h intervals after oestradiol administration as described in the text, and are plotted together with the values of E and k_S at that point. The conversion factor for the data was obtained from values of E and k_S at 72 h (calculation b of Table 2). ■, k_S as percentage of initial amount of enzyme/h; ▲, E as percentage of initial amount; ○, k_D as h^{-1} .

described above, as they reported that the measured rates of synthesis and degradation account for the observed changes in enzyme amount. No steady-state measurements are reported; for investigative pur-

poses in our calculations, the values of enzyme specific activity (Fig. 1 of Flick *et al.*, 1977) and rate of enzyme synthesis (Fig. 5 of Flick *et al.*, 1977) at 66 h on a safflower-oil diet were assumed to represent a

Table 2. Analysis of the data of Smith & Barker (1977): changes in the rate of degradation of uterine glucose 6-phosphate dehydrogenase

Values given are the half-life of the enzyme as measured by Smith & Barker (1977) or as calculated from data on enzyme synthesis and activity (k_D averaged over the period of measurement). $t_{\frac{1}{2}} = \ln 2/k_D$. (Fig. 1 illustrates the calculated values of k_D used to obtain the first two values of the right-hand column.) *Calculated k_D large and negative, indicating impossible combinations of E and k_s . (a) Calculated assuming a steady state at $t = 0$ h; (b) calculated assuming a steady state at $t = 72$ h (after oestradiol treatment at 0 h).

Treatment	Period of measurement (h after initial oestradiol treatment)	$t_{\frac{1}{2}}$ (h)		
		Measured	Calculated (a)	Calculated (b)
Oestradiol at 0 h	16–36	>100	Negative*	46.2
Oestradiol at 0 h	36–84	24	21.5	13.28
Oestradiol at 0 and 48 h	48–72	∞	Negative*	346.5

steady state. At this point, enzyme activity is not changing markedly, but synthesis may be declining. If the assumption of a steady state is made, the reported combinations of enzyme activities and rates of synthesis are incompatible: large negative values of k_D at all time points are required. Clearly the assumption of a steady state at 66 h does not hold; hence analysis by the method described here is inappropriate (see the Discussion section). However, no other values approximating to a steady state are available for the analysis.

Discussion

Calculation of rates of degradation

The method of estimating rates of degradation described in this paper has several advantages over previously described methods. Problems of isotopic reutilization and other methodological disadvantages of direct measurement of the rate of degradation are minimized: although one empirical estimate of enzyme half-life is required for the calculations, this is made in a steady state when the effects of changing pool sizes are minimized. The method can be accurate: in theory, its accuracy is limited only by the accuracy of the measurements used to supply data on enzyme amount and rates of synthesis. Accurate measurements of E and k_s are much easier to make than are measurements of k_D ; it may therefore be worth compromising by investing effort to obtain one accurate steady-state measurement of k_D and then using the calculation method to obtain other values of k_D during protein accumulation or decrease, which will be more accurate than directly measured values. Although programs can be written to calculate E or k_s (Paskin & Mayer, 1977), the calculation of k_D is probably the most useful for most systems.

The method does not assume instantaneous changes of rates of synthesis and decay, as assumed by some previous calculations (e.g. Berlin & Schimke, 1965). The calculations involved are easily formalized as a simple program for small computers or program-

mable calculators, unlike more complex models (e.g. Morkin *et al.*, 1972).

Values of k_D may be obtained over short time intervals, thereby high-lighting and precisely defining changes in k_D that may be masked by 'time-averaged' measurements of rates of degradation: all direct measurements of k_D are made over a period of time and therefore reflect only the average value operative over this time (Speake *et al.*, 1975, 1976). Measured 'time-averaged' values of k_D can be compared with calculated values of k_D by the averaging procedure described. The averaging procedure has the advantage of smoothing out unrealistic variations in calculated k_D due to sharp changes in the E or k_s values used in the calculation. However, if averaged over long periods, real sustained changes in k_D occurring as part of a regulated programme of controlled turnover may be masked. For this reason it is advisable to obtain a plot of k_D versus time with small intervals of time before applying any averaging procedures. We have found that values of Δt that are less than one-eighth of the steady-state half-life give realistic values in most cases. Little improvement is then gained by making Δt smaller.

Inaccuracies in the measurement of E or k_s will be reflected in the calculations. We have found that reported combinations of E and k_s often require values of k_D that are negative at certain points (Mayer & Paskin, 1978), a representation that has no counterpart in real protein metabolism. Such values are assumed to be due to combinations of errors in measurement (overestimates of E and/or underestimates of k_s). If, by immunotitration, enzyme activity can be shown to be directly proportional to enzyme amount (i.e. any inactive enzyme is present in a constant proportion), then accurate enzyme-activity measurements can be used to obtain values of E . Cases where activity measurements are used and incompatible values of E , k_s and k_D result may reflect on this assumption of activity being proportional to amount.

Inaccuracies in the measured value of k_D used to establish the synthesis unit conversion factor will be reflected in the final profile of k_D versus time. Changes in the numerical value of steady-state k_D used will result in changes in the shape of the profile, rather than just a 'scaling-up' or 'scaling-down' of a profile of constant shape (N. Paskin, unpublished work). Inaccuracies in the measured value of k_D may arise either from experimental design (e.g. reutilization of radioactive precursor leading to an underestimate of k_D) or from measurement over a period that is not a true steady state (when $E \neq k_s/k_D$). Calculation of k_D from data on E and k_s which results in negative values can be due to two factors. (a) If the conversion factor discussed above has been accurately estimated from a true steady state then values of k_D that are negative but small may result when little or no degradation is occurring. These values will reflect the inherent errors of the measurements of E and k_s used in the calculation; if k_D is actually zero, the calculated value will fluctuate about zero. The result will be a qualitative guide to the value of k_D (i.e. little or no degradation occurring). At other points, where k_D is positive, the result can be a good quantitative guide to the value of k_D . Examples of this type of analysis are the calculations on the data for fatty acid synthetase in mammary explants (Table 1), and the data of Smith & Barker (1977) (Table 2, calculation b, and Fig. 1). (b) If the conversion factor has been estimated from data that have been wrongly assumed to represent a steady state (when in fact $E < k_s/k_D$), then values of k_D that are negative, sustained and relatively large may result. (At the other extreme, values of k_D that are positive and very large may result if a steady state has been assumed when in fact $E > k_s/k_D$.) The result is then not a good guide to the extent of degradation of the enzyme under consideration. This is the case for the analysis of the data of Flick *et al.* (1977) described above: the assumption of a steady state at 66h on safflower-oil diet leads to values that cannot account for the observed changes. Fig. 5 of Flick *et al.* (1977) suggests that synthesis at 66h has not returned to a steady state (i.e. $E < k_s/k_D$). Indeed, calculation shows that k_s must be decreased to about 8% of its value at 66h if a steady-state assumption is to explain the values given. However, unless such calculations using true steady-state measurements are made on data like these, it is clearly incorrect to state, as do Flick *et al.* (1977), that observed rates of synthesis and degradation 'suffice to account for the observed differences in enzyme levels', especially over a limited period.

The two analyses of the data of Smith & Barker (1977) (Table 2, Fig. 1) illustrate another advantage of the method of calculation described. Clearly the steady-state half-life of uterine glucose 6-phosphate dehydrogenase before the first oestradiol treatment cannot be the value of 111h obtained in the first

few hours after treatment (calculation a); the steady-state half-life before oestradiol treatment must be much less, to obtain positive values of k_D . The conversion factor used in calculation (b) predicts a steady-state half-life of 42h before oestradiol treatment. Hence the more rapid turnover of glucose 6-phosphate dehydrogenase observed after 33h appears to be a return towards an original pre-stimulated value, rather than a specific event associated with oestrogen withdrawal. This fact was not clear from measurement alone (Smith & Barker, 1977). There is evidence that in uterus (Smith & Barker, 1974) and in a transplantable tumour (Ringler & Hilf, 1975) the increase in the rate of synthesis of glucose 6-phosphate dehydrogenase may be associated with the production of inactive enzyme, which may be subsequently activated. Our calculations show (Table 2) that the computed enzyme-degradation profile is similar to that measured by Smith & Barker (1977). Therefore, whatever the proportion of inactive enzyme formed after oestradiol treatment, it must remain a constant proportion of the total (i.e. the enzyme accumulation and activity profiles must be similar), since otherwise the calculated profile would be markedly different from the measured profile.

Analyses of the type described are clearly of value in estimating qualitative and quantitative changes in protein degradation.

Experiments should be designed so that measurement of E , k_s and k_D are made in a steady state, so that calculations of k_D at all other times can be carried out. Where measurements of k_D are also made during a change, the analysis serves as a check on the accuracy of these measurements.

Role of changes in protein degradation

It is now clear that rates of protein degradation may be modulated in cells during periods of protein accumulation or diminution. This has been reported for a range of enzymes in a variety of tissues [see examples quoted in Schimke & Doyle (1970), Paskin & Mayer (1977) and Mayer & Paskin (1978)]. The question arises as to the role of such changes. The advantages of decreases in rates of degradation during protein accumulation in terms of energy requirements, rapidity of response and 'overshoot' characteristics have been discussed in a previous report (Paskin & Mayer, 1977).

It has been claimed that changes in rates of protein degradation are the single most important factor determining the increase in protein content during liver compensatory growth (Scornik & Botbol, 1976; Conde & Scornik, 1977). By contrast, changes in degradation have been assigned only a minor role in the increased myosin content during development of cardiac hypertrophy (Morkin *et al.*, 1972). Some insight into the role of degradation changes may be

obtained by a consideration of the way in which half-life determines the rate of increase in protein amount. To compare rates of increase, the amount of protein synthesized should be compared with the original pool size (Schimke, 1970). Proteins of short half-life are able to change in amount, relative to their pool size, much more quickly than those of long half-life (Schimke, 1970; Scornik & Botbol, 1976). This is highlighted by Fig. 2, which illustrates the relationships between half-life, fold change in

Fig. 2. Initial rate of change in enzyme amount predicted for proteins of various half-lives when a system in a steady state (synthesis = degradation; i.e. $k_s = k_D E$) is perturbed by an increase in synthetic rate

For ease of description, synthesis is assumed to increase by n -fold instantaneously. The initial rate of synthesis is 1; hence the initial steady-state pool size of protein is given by $E_{ss} = k_s/k_D = 1/k_D$. Numbers in parentheses indicate the value of n for each line. (1) Continuous lines. The new value of k_D (designated k_D') is assumed to become zero as k_s increases (to k_s') by n -fold. Then the initial rate of increase of enzyme amount is $dE/dt = k_s' - k_D' E_{ss} = n \cdot k_s - k_D' E_{ss} = n$ (since $k_s = 1$ and $k_D' = 0$). As a percentage of the initial pool size, this is given by:

$$P = \frac{n}{E_{ss}} \times 100 = n \cdot k_D \cdot 100 = n \cdot \frac{\ln 2}{t_{1/2}} \cdot 100\%/h$$

i.e. by $P = A - \log t_{1/2}$ for a given value of n , where A is a constant. (2) Broken lines. Degradation remains unaltered at the value k_D . Then the initial rate of increase of enzyme amount is $dE/dt = n \cdot k_s - k_D \cdot E_{ss} = n - 1$ (since $k_s = 1$ and $k_D = 1/E_{ss}$). As a percentage of the initial pool size this is given by:

$$P = \frac{n-1}{E_{ss}} \times 100 = (n-1) k_D \cdot 100 = (n-1) \frac{\ln 2}{t_{1/2}} \cdot 100\%/h$$

i.e. $\log P = B - \log t_{1/2}$ for a given value of n , where B is a constant. The initial rates of change shown represent maximum rates of change (see the Discussion section). Decreases in rates of degradation during a change result in a value intermediate between that given by the broken and continuous lines.

synthesis and the resulting rate of change in enzyme amount by the method of isopleths (Riggs, 1963). The smaller the half-life of a protein, the more important will changes in degradation (half-life) be in determining overall rate of change in amount (compare the differences between broken and continuous lines of Fig. 2 for a given value of n and several values of $t_{1/2}$). It is also clear that the higher the fold change in the rate of synthesis of enzyme the less important will changes in degradation (half-life) be in determining overall rate of change in amount (compare the differences between broken and continuous lines of Fig. 2 for a given value of $t_{1/2}$ and several values of n). Summarizing, changes in degradation will be most important in determining changes in amount of protein for those proteins of short half-life that increase in synthesis by a small amount. Since the continuous lines of Fig. 2 assume degradation to be zero, since k_s is assumed to change instantaneously and since dE/dt declines from its initial rate during accumulation (Schimke, 1970), the continuous lines of Fig. 2 represent the maximum possible rate of change of enzyme amount from a steady state for a given half-life of the enzyme and n -fold change in synthesis. Any real changes will not be instantaneous of course, and hence this maximum value is a limit that is approached but never attained in an actual accumulation. Fig. 2 may be of help in considering the roles of degradation and synthesis in a particular biological system of interest.

The debate on the role of changes in degradation in influencing changes in enzyme amount may therefore be resolved by: (a) consideration of the steady-state half-life of the protein concerned; (b) using calculations or models, such as the one described, that take into account the time-dependence of the changes involved and can be used to predict values of k_D , k_s or E .

It would appear that the approach discussed in this paper is the only one available at present that is generally applicable for the analysis of protein-turnover characteristics.

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