Disappearance of Isocitrate Lyase Enzyme from Cells of *Chlorella pyrenoidosa*

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1. When acetate-adapted cells of *Chlorella* are suspended in nitrogen-free medium and supplied with glucose, isocitrate lyase activity disappears from the cells at a rate of about 9%/h. This loss of activity is shown to be accompanied by loss of isocitrate lyase protein. 2. When isocitrate lyase activity is assayed in intact cells after freezing and thawing, the rate of loss of activity after addition of glucose approaches 20%/h. 3. It is shown, by using ^{35}S , that the rate of turnover of isocitrate lyase protein is somewhat lower than that of other major soluble proteins; general protein turnover during nitrogen starvation, and after glucose addition, is too slow to account for the rate of loss of isocitrate lyase protein. 4. Disappearance of isocitrate lyase activity must result from a mechanism that allows degradation of this specific protein under conditions of limiting nitrogen supply.

The unicellular green alga *Chlorella pyrenoidosa*, like many other micro-organisms, makes the enzyme isocitrate lyase (EC 4.1.3.1) when forced to use acetate as sole carbon source (Syrett, Merrett & Bocks, 1963). When fully adapted to acetate, the isocitrate lyase protein in cells of *Chlorella pyrenoidosa* strain 211/8p constitutes about 7% of total soluble cell protein and can be readily seen as a discrete band after separation of the soluble proteins by polyacrylamide-gel electrophoresis (John & Syrett, 1968a). After transfer of acetate-adapted cells of *Chlorella* to a medium containing glucose, the activity of isocitrate lyase falls. The fall is particularly rapid in nitrogen-free medium; other enzymic activities, e.g. that of malate dehydrogenase, are stable under similar conditions (Morris & Syrett, 1965). We have now investigated further the factors leading to loss of isocitrate lyase activity and show that the loss is correlated with a fall in quantity of isocitrate lyase protein.

EXPERIMENTAL

Culture of organism. *Chlorella pyrenoidosa* Cambridge Culture Collection strain 211/8p was grown autotrophically in medium containing (per litre of distilled water) 0.75 g of NH_4NO_3 , 0.4 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 5.8 g of K_2HPO_4 ,

4.53 g of KH_2PO_4 , 5 mg of Fe as a Fe^{3+} -EDTA complex and 1 ml of A5 trace-element solution (Arnon, 1938). The phosphates were autoclaved separately and mixed with the other ingredients after cooling. The pH of the medium was 6.7. Cultures were grown at 25°C, aerated vigorously with air containing 0.5% CO_2 and illuminated with day-light fluorescent tubes giving a light intensity of $28000 \text{ erg cm}^{-2} \text{ s}^{-1}$ at the surface of the culture vessels. Cultures were adapted to acetate after reaching a density not exceeding 3.4×10^7 cells/ml. Adaptation was achieved by adding a sterile solution of sodium acetate to give a final concentration of 0.2% anhydrous salt; cultures were then aerated in darkness at 25°C for 24, 36 or 48 h. The longer adaptation periods required another addition of acetate after 24 h equivalent to a further 0.1% anhydrous sodium acetate in the culture. Nitrogen-free medium lacked NH_4NO_3 . Low-sulphur medium contained only 0.025 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ /l but also contained 0.1 g of MgCl_2 /l.

Preparation of cell-free extracts. Cells were harvested by centrifugation, washed and suspended in an appropriate buffer at 4°C to give a dense suspension, which was passed through a French pressure cell at 4°C. The exudate was centrifuged at 10^5g for 90 min at 3°C and the supernatant taken. Protein content was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

Assay of isocitrate lyase activity. Isocitrate lyase activity was assayed in intact cells whose semipermeability had been destroyed by freezing and thawing (Syrett, 1966). Activity in cell-free extracts was assayed by measuring glyoxylate formed from isocitrate after a 10 min incubation period (John & Syrett, 1967).

Electrophoresis. Proteins in the 10^5g supernatant were separated by polyacrylamide-gel electrophoresis (Ornstein & Davis, 1961). A 50-250 μg sample of protein was applied to each gel. After electrophoresis proteins were

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fixed and stained by immersing the gels for 1 h in 1% Naphthalene Black in 7% (v/v) acetic acid. Excess of Naphthalene Black was washed out with 7% acetic acid, the final wash containing about 0.5% (w/v) Dowex 1 (X8) resin. The density of stained protein bands was determined by using a Chromoscan densitometer (Joyce, Loeb and Co. Ltd., Gateshead, U.K.). John & Syrett (1968a) showed that the area under the Chromoscan trace was directly proportional to quantity of protein under the conditions used.

Radioactivity measurements. [^{35}S]Sulphate (carrier-free) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Cell-free extracts containing proteins labelled with ^{35}S were subjected to polyacrylamide-gel electrophoresis. After staining, individual bands in segments 2 mm in length were cut from the gels and squashed between the ground faces of 25 mm-diam. glass planchets. The planchets were dried and the radioactivities counted with a thin-end-window Geiger-Müller tube, two planchets being derived from each segment. With this counting procedure self-absorption was considerable (about 75%), but tests with segments of gels into which known quantities of ^{35}S had been incorporated showed that results were reproducible and counts from segments of equal size were directly proportional to the amount of ^{35}S introduced into them.

^{35}S in total protein in the cell-free extract was also determined. A sample of extract containing about 2 mg of protein was diluted to 3 ml and 1 ml of 20% (w/v) trichloroacetic acid solution was added. The tubes were immersed in a boiling-water bath for 15 min and cooled, and the mixture was centrifuged. The protein pellet was washed with cold 6% (w/v) trichloroacetic acid, dissolved in 0.5 ml of 0.1 M-NaOH and diluted to 3.0 ml. Protein in samples was determined by the method of Lowry *et al.* (1951), and 0.05 ml portions were dried in triplicate on ground-glass planchets and the radioactivities counted with a thin-end-window Geiger-Müller tube. Self-absorption was shown to be negligible. Results were then expressed as c.p.m./ μg of protein.

RESULTS

Disappearance of isocitrate lyase activity from acetate-adapted cells after the addition of glucose. Fig. 1 shows the disappearance of enzyme activity from acetate-grown cells after the addition of 0.5% glucose to the culture. Loss of enzyme activity was fastest in cells suspended in nitrogen-free medium (Fig. 1b); one-fifth of the initial enzyme activity disappeared during the first hour in this experiment. Fig. 2 shows that enzyme activity was lost within 10 min of the addition of glucose. The addition of cysteine (5 $\mu\text{mol}/\text{ml}$) or methionine (5 $\mu\text{mol}/\text{ml}$), either separately or together, made no difference to the loss of enzyme activity in nitrogen-free medium. These amino acids were added in the turnover experiments described below.

Correlation of loss of enzyme activity with loss of enzyme protein. John & Syrett (1968a) showed that cell-free extracts from acetate-adapted cells contain a protein that during electrophoresis on 7.5% poly-

Fig. 1. (a) Changes in isocitrate lyase activity in acetate-adapted cells suspended in nitrogen-containing medium after the addition of acetate (●) or glucose (▲). (b) Change in isocitrate lyase activity after the addition of glucose to cells suspended in nitrogen-free medium. Cultures adapted to acetate for 48 h were harvested and the cells were washed and suspended in medium with or without NH_4NO_3 . Cell density was 10000 cells/mm³. Samples (40 ml) of suspension in the dark at 25°C were aerated with air containing 0.5% CO_2 . Sodium acetate (final concn. 0.2% anhydrous salt) or glucose (final concn. 0.5%) was added at zero time. Samples (5 ml) were removed at the times shown and rapidly frozen in ethanol-solid CO_2 . After storage at -15°C and thawing, the samples were assayed for isocitrate lyase activity by the frozen-cell method.

acrylamide gel moves at a rate corresponding to approx. 0.35 mobility unit. This protein is a major component in the soluble proteins of acetate-adapted cells but is absent from autotrophically grown cells. Isocitrate lyase activity is associated with this protein band and its position on the gels coincides with that of purified isocitrate lyase protein (John & Syrett, 1967). John & Syrett (1968a) showed that the quantity of this isocitrate lyase protein could be accurately determined from densitometer traces of stained gels when the total quantity of protein applied to gels was less than 250 μg .

An experiment was carried out to correlate the

Fig. 2. Time-course of the initial disappearance of isocitrate lyase activity. Experimental conditions were as described in the legend to Fig. 1 except that the cell density was 4000 cells/mm³. Glucose (final concn. 0.5%) was added at zero time. Enzyme assays were by the frozen-cell method. ●, Cells in medium containing nitrogen; ▲, cells in nitrogen-free medium.

loss in isocitrate lyase activity after addition of glucose to acetate-adapted cells with loss of isocitrate lyase protein, the quantity of protein being determined from densitometer traces of stained polyacrylamide gels (Fig. 3a). The loss of isocitrate lyase protein and loss of enzyme activity as determined by assay in cell-free extracts correlate well (Fig. 3b). When determined by the frozen-cell method, enzyme activity decreases more rapidly than does activity determined in cell-free extracts from broken cells (Fig. 3b). Possible reasons for this discrepancy are discussed below.

Rate of incorporation of ³⁵S into soluble proteins. The rate of incorporation of ³⁵S, supplied as sulphate, into isocitrate lyase protein was compared with its rate of incorporation into other major soluble proteins. The cells were adapted to acetate in the dark for 48 h and were growing with acetate as a carbon source when a pulse of [³⁵S]sulphate was supplied. After incubation with [³⁵S]sulphate for 1–2 h soluble proteins were isolated from the cells and separated by polyacrylamide-gel electrophoresis. After staining, selected protein bands, including isocitrate lyase protein, were removed and the radioactivity in them was determined. To allow for possible differences in the sulphur content of the proteins studied and in the amounts of each protein, the same proteins were isolated from acetate-adapted cells uniformly labelled with ³⁵S. The radioactivity incorporated into the protein bands after a short incubation of cells with [³⁵S]sulphate was then expressed as a proportion of the ³⁵S present in the corresponding protein band from uniformly labelled cells. When ³⁵S content expressed in this way is plotted against time, the relative rates of synthesis *de novo* of the major soluble proteins in *Chlorella* can be compared.

Fig. 3. Correlation of the disappearance of isocitrate lyase activity and loss of isocitrate lyase protein. (a) Chromoscan traces of polyacrylamide gels showing the disappearance in cells incubated with glucose for the times shown, of the protein peak due to isocitrate lyase protein (indicated by arrow). (b) ○, Isocitrate lyase protein on polyacrylamide gels estimated from Chromoscan densitometer traces similar to those shown in (a); ●, isocitrate lyase activity assayed in cell-free extracts; ▲, isocitrate lyase activity in cell samples assayed by the frozen-cell method. Acetate-adapted cells were harvested, washed and suspended (20000 cells/mm³) in 1500 ml of nitrogen-free medium. The suspension was placed in darkness at 25°C and aerated vigorously with air containing 0.5% CO₂. At zero time glucose (final concn. 1%) was added. Samples (200 ml) and quadruplicate 0.2 ml samples were taken at the times shown, frozen and stored at -15°C. After thawing the small samples were assayed for isocitrate lyase activity by the frozen-cell method. Cells in the large samples were broken in a French pressure cell and 10⁵ g supernatants containing 200 μg of protein were subjected to polyacrylamide-gel electrophoresis. The stained gels were scanned with a Chromoscan densitometer (a), and the amount of isocitrate lyase protein was determined (b).

Results (Fig. 4) showed that the rate of incorporation of ³⁵S was highest into band B and lowest into the isocitrate lyase band.

Fig. 4. Rate of incorporation of ^{35}S into isocitrate lyase protein and other major soluble proteins of *Chlorella* after a pulse of ^{35}S sulphate. Cells were grown autotrophically in low-sulphur medium and adapted to acetate by incubation for 48 h at 25°C in the dark in the presence of sodium acetate (0.2%). Cells were then harvested and resuspended (13000 cells/mm^3) in 500 ml of acetate medium containing 0.35 mCi of carrier-free ^{35}S sulphate. Portions (250 ml) were removed after incubation for 1 h and 2 h and the cells were removed by centrifugation, washed and broken at 2°C in 0.02 M-tris-HCl buffer, pH 7.5. The suspension was centrifuged at 10^5g for 1 h. A volume of supernatant containing $130\mu\text{g}$ of protein was applied to gels, in triplicate, for standard electrophoresis. After electrophoresis and staining of the gels, the bands A, B, C, D and E were cut out in 2 mm segments and their radioactivities determined. Another culture was grown autotrophically in 250 ml of low-sulphur medium containing 0.6 mCi of ^{35}S sulphate. When this culture contained 15000 cells/mm^3 it was darkened, sodium acetate (final concn. 0.2%) was added and it was aerated for 48 h at 25°C . During this period the cell number increased to 20000 cells/mm^3 . The proteins of these cells were considered to be uniformly labelled with ^{35}S . Cell-free extracts were prepared from these cells and their proteins separated electrophoretically as described above. The radioactivity in the protein bands, separated after the pulse experiment, is expressed as a proportion of the radioactivity in the corresponding protein band after uniform labelling. At the top is a diagram of a gel stained after electrophoresis showing the position of the bands isolated and whose radioactivities were counted. Band C is isocitrate lyase protein; A corresponds to 'fraction 1' protein from green leaves and contains ribulose diphosphate carboxylase activity. B, D and E are proteins of unknown function. Band E runs with the Bromophenol Blue front during electrophoresis and probably consists of degraded protein. ^{35}S incorporation: ▼, into band A; ○, into band B; ●, into band C (isocitrate lyase); ▲, into band D; ■, into band E.

Fig. 5. Incubation of ^{35}S -labelled *Chlorella* in nitrogen-free medium with glucose. (a) Disappearance of ^{35}S -labelled protein (▲) and isocitrate lyase activity (●). (b) Release of non-sulphate ^{35}S into the medium. A culture (2 litres) was grown autotrophically in low-sulphur medium containing 2 mCi of ^{35}S sulphate to a density of 18000 cells/mm^3 . The culture was darkened and sterile sodium acetate (0.2% final concn.) was added. After 24 h aeration at 25°C a further addition of sodium acetate, equivalent to 0.1% (w/v) final concn., was made and aeration in the dark continued for a further 24 h. The cell number was then 24000 cells/mm^3 . The cells were harvested, washed and suspended in nitrogen-free medium containing 0.5 mM-L-methionine, 0.5 mM-L-cysteine hydrochloride and 0.5% glucose. The suspension was aerated in the dark at 25°C , 500 ml samples were removed after 0, 3, 9 and 24 h and the cells were separated from the medium. 0.38 M- BaCl_2 (0.1 ml) was added to 2 ml of medium, the precipitated BaSO_4 was removed by centrifugation and 0.1 ml portions were dried for counting of the radioactivity of the non-sulphate ^{35}S . Cell-free extracts were prepared from the cells as described in the legend to Fig. 3 and ^{35}S in total soluble protein was determined as described in the Experimental section. The isocitrate lyase activity was assayed in the cell-free extracts.

Comparison of the rate of breakdown of isocitrate lyase protein with that of other proteins. Cells uniformly labelled with ^{35}S and adapted to acetate

were transferred to and incubated in nitrogen-free medium containing 0.5% glucose. These conditions favour the disappearance of isocitrate lyase. The

Fig. 6. Loss of ^{35}S from the major soluble proteins during incubation of *Chlorella* in nitrogen-free medium with glucose. The cell-free extracts prepared during the experiment (details in legend to Fig. 5) were subjected to polyacrylamide-gel electrophoresis. Soluble protein ($270\text{ }\mu\text{g}$) from each sample was applied to gels in triplicate. After staining, the major protein bands (see Fig. 4) were excised in 2mm segments and radioactivity in them was determined. Results are expressed as a percentage of the radioactivity present in each band at zero time. Loss of ^{35}S : ▼, from band A; ○, from band B; ●, from band C (isocitrate lyase); ▲, from band D; ■, from band E.

suspending medium also contained 0.5mM-L-methionine and 0.5mM-L-cysteine; it was previously shown that the addition of these amino acids did not affect disappearance of isocitrate lyase activity. Samples of cells were removed at intervals, the cells broken and the proteins in the cell-free extracts separated by electrophoresis. Total protein ^{35}S precipitated with trichloroacetic acid from the cell-free extracts and the release of non-sulphate ^{35}S into the medium were also measured (Fig. 5).

Isocitrate lyase activity disappeared at a rate of about 9%/h, whereas the rate of loss of ^{35}S from total soluble protein occurred at a rate of about 1.2%/h and was accompanied by a linear increase of non-sulphate ^{35}S in the medium. This increase of non-sulphate ^{35}S presumably resulted from the release of sulphur-containing amino acids resulting from protein degradation. Similarly, when the loss of ^{35}S from the major proteins was compared (Fig. 6) it is clear that ^{35}S in the isocitrate lyase band declined steadily over the period of incubation whereas that in the other bands either declined more slowly (bands A and B) or after a fairly rapid decline remained constant (bands C and D).

DISCUSSION

Isocitrate lyase is required for *Chlorella* to grow with acetate as sole carbon source; it is not necessary for growth on glucose. Addition of glucose to acetate-adapted cells of *Chlorella* results in a rapid loss of isocitrate lyase activity, particularly when the cells are deprived of nitrogen. In Fig. 5 isocitrate lyase activity was lost from cell-free extracts at a rate of about 9%/h and in Fig. 3 the loss, although slower, was of similar order. Fig. 3 shows, too, that the loss of activity from cell-free extracts is paralleled by loss of isocitrate lyase protein, so we conclude that loss of enzymic activity is a consequence of protein degradation rather than of enzyme inactivation. Isocitrate lyase activity can also be assayed in intact cells after their semi-permeability has been destroyed by freezing. Isocitrate lyase activity assayed in this way disappears even more quickly after addition of glucose and at a rate that may approach 20%/h (Fig. 2). The reason for the apparently greater rate of loss of activity is not clear. It could arise either from a progressive underestimation of isocitrate lyase activity in the frozen-cell assay or from an initial underestimation of enzyme activity in cell-free extract assays. Underestimation of the initial amount of isocitrate lyase in cell-free extracts could occur in two ways. Enzyme activity in cell-free extracts is related to soluble protein concentration, whereas the frozen-cell assays give amount of enzyme/unit volume of experimental suspension (this is equivalent to enzyme activity/unit number of cells in these experiments since cell division is negligible in nitrogen-free medium). Hence if the amount of soluble protein/cell progressively fell the rate of isocitrate lyase disappearance would appear less in cell-free extract assays. This explanation is untenable since the amount of soluble protein/*Chlorella* cell does not decrease significantly during such periods of nitrogen starvation (I. Morris, personal communication). The alternative possibility is that part of the enzyme is particulate, and so not found in the 10^5g supernatant, and that the proportion of soluble enzyme increases progressively during incubation of the cells in nitrogen-free medium. This possibility was tested and eliminated experimentally (Thurston, 1970). If cell-free extract assays do not initially underestimate the quantity of isocitrate lyase present, frozen-cell assays must progressively underestimate isocitrate lyase activity. The properties of frozen cells suggest that the semipermeable properties of the cell membranes have been destroyed, rendering the cells permeable to small molecules such as isocitrate, but that the intact cell wall still prevents the passage of molecules as large as NAD (P. J. Syrett, unpublished work). Consequently lower apparent activity of

isocitrate lyase in the frozen-cell assay could result if, after glucose addition, an inhibitor of isocitrate lyase was formed that was unable to leave the frozen cells. Such an inhibitor would be greatly diluted when a cell-free extract was prepared. However, if this is the explanation the inhibiting substance is not one of the small metabolites, phosphoenolpyruvate, pyruvate, succinate or oxaloacetate, known to inhibit *Chlorella* isocitrate lyase (John & Syrett, 1968b), since any one of these compounds, when added to frozen cells, inhibits isocitrate lyase activity in the cells to the same extent as isolated enzyme (Thurston, 1970). Consequently these substances must move freely into and out of frozen cells.

The simplest explanation of the loss of isocitrate lyase protein from acetate-adapted cells after the addition of glucose is that it is a consequence of protein turnover. In acetate-adapted cells the isocitrate lyase activity might represent a balance between rate of synthesis and rate of breakdown of the enzyme protein. The addition of glucose prevents isocitrate lyase synthesis in *Chlorella* (Syrett, 1966). Therefore if breakdown continued loss of isocitrate lyase protein could occur. This hypothesis requires a rate of protein turnover of about 9%/h to account for the rate of loss of isocitrate lyase protein. The growth rate of *Chlorella* with acetate as carbon source is only about 1.2%/h (Syrett *et al.* 1963), so a general rate of protein turnover of 9%/h would be remarkable; moreover, calculations based on the data of Bassham & Kirk (1964) for *Chlorella* make it unlikely. It is possible, however, that isocitrate lyase turns over much faster than the bulk of proteins in *Chlorella*. Suggestions that individual proteins might vary in rates of turnover have been made by Pine (1965) and Willetts (1967). Therefore the rates of ^{35}S incorporation into isocitrate lyase and into other major soluble proteins in acetate-adapted *Chlorella* in steady state conditions were compared. Isocitrate lyase protein constitutes some 7% of total soluble protein and hence is easily discernible as a heavy distinct band after electrophoresis (John & Syrett, 1968a). If isocitrate lyase were turning over faster than the other proteins, the incorporation of ^{35}S into it should be significantly faster than into other protein bands. The results (Fig. 4) showed differences in the rates of incorporation of ^{35}S into the various protein bands; incorporation into band B was almost three times as fast as incorporation into isocitrate lyase. Individual proteins therefore probably do turn over at different rates, but of those measured isocitrate lyase had the lowest rate and the hypothesis that isocitrate lyase disappearance is a consequence of a particularly high turnover rate for this protein must be rejected.

This experiment was a comparative one and gave

no information about absolute rates of protein turnover. It is possible that, under conditions of nitrogen starvation and in the presence of glucose, protein turnover in general in *Chlorella* is greatly increased. There is evidence that during nitrogen starvation of bacteria protein turnover increases (Mandelstam, 1960). When acetate-adapted cells uniformly labelled with ^{35}S were suspended in nitrogen-free medium and supplied with glucose, ^{35}S was lost from total soluble proteins at a rate of about 1.2%/h (Fig. 5a). Major proteins, with the exception of isocitrate lyase, showed average losses of ^{35}S of 1–2%/h over 24 h; isocitrate lyase showed a considerably greater loss. These values may be underestimates of the absolute values of protein turnover since not all the ^{35}S -labelled sulphur-containing amino acids liberated by protein breakdown may be trapped by the unlabelled sulphur-containing amino acids that were added to the medium. Nevertheless they suggest that turnover is not as fast as 9%/h. Moreover, they show clearly that under these conditions ^{35}S is lost more rapidly from isocitrate lyase than from other soluble proteins.

We therefore conclude that the loss of isocitrate lyase activity after the addition of glucose to acetate-adapted cells of *Chlorella* is due to the breakdown of isocitrate lyase protein and that this is not a result of general protein turnover or of a particularly high rate of turnover of isocitrate lyase protein. Rather, some mechanism must come into operation that allows the degradation of a specific protein no longer being used for metabolic reactions. The mechanism could involve either an increase in the activity of intracellular proteolytic enzymes or an alteration in the susceptibility of isocitrate lyase protein to proteolytic attack.

There are several reports of similar rapid disappearance of enzyme activities. In yeast, galactozymase, which is induced by galactose, disappears on addition of glucose when the nitrogen supply is limiting (Spiegelman & Reiner, 1947). High malate dehydrogenase activities in yeast resulting from growth on acetate rapidly decrease when glucose is added (Witt, Kronau & Holzer, 1966; Ferguson, Boll & Holzer, 1967). Enzymes of the maltose-utilizing pathway disappear when yeast is transferred to nitrogen-free medium containing glucose (Robertson & Halvorsen, 1959). Ornithine transcarbamoylase activity disappears from yeast after the addition of an exogenous supply of arginine (Bechet & Wiame, 1965). In rat liver tryptophan pyrrolase disappears in the absence of an exogenous supply of tryptophan (Schimke, Sweeney & Berlin, 1965) and serine dehydratase disappears after glucose is supplied (Jost, Khairallah & Pitot, 1968).

In *Escherichia coli* β -galactosidase is stable in growing cells after removal of the inducer (Hogness,

Cohn & Monod, 1955), but in non-growing cells it disappears in the absence of inducer (Willetts, 1967). During differentiation enzymic activities can disappear with amazing rapidity particularly in slime moulds (Sussman & Sussman, 1969).

Sufficient work has been done to make it clear that different mechanisms underlie these instances of disappearance of enzymic activity. Ornithine transcarbamoylase activity in yeast is lost by irreversible inactivation of the enzyme that is not accompanied by disappearance of enzyme protein (Bechet & Wiame, 1965; Messenguy & Wiame, 1969). The same appears to be true of the loss of glutamine synthetase activity from *E. coli* (Holzer, 1967, 1969). The loss of malate dehydrogenase from yeast is readily reversible (Duntze, Neumann & Holzer, 1968; Ferguson *et al.* 1967); this probably indicates reversible enzyme inactivation not involving protein disappearance. In most other instances it is uncertain whether enzyme protein disappears or not. However, disappearance of tryptophan pyrrolase and serine dehydratase from rat liver has been shown (in both cases immunologically) to be accompanied by disappearance of protein (Schimke *et al.* 1965; Jost *et al.* 1968).

It is noteworthy that several instances of rapid disappearance of an enzymic activity, including that of isocitrate lyase, from a micro-organism occur under non-growing conditions and the enzymic activity that disappears is no longer essential for cellular metabolism. In many micro-organisms high rates of protein turnover have been observed under non-growing conditions (Mandelstam, 1960), and massive turnover of protein also occurs during differentiation in slime moulds (Sussman & Sussman, 1969) and in sporulating bacteria (Mandelstam & Waites, 1968). Probably enzyme disappearance by protease degradation is a response to an intracellular shortage of material for synthesis of new protein. One aspect of this is an increased rate of protein turnover; another is the complete degradation of specific proteins such as isocitrate lyase in *Chlorella*. But this raises the question of which mechanisms allow certain proteins to be degraded but not others; at present little is known about this.

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