THE INTRACELLULAR TURNOVER OF PROTEIN AND NUCLEIC ACIDS AND ITS ROLE IN BIOCHEMICAL DIFFERENTIATION

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I. Introduction

After Folin's (21) classical paper on protein metabolism in 1905, it was generally accepted that the proteins of the animal organism were largely stable, and that the nitrogen excreted by an animal in nitrogen equilibrium was mainly derived from the diet, together with a small amount from breakdown of "endogenous" or tissue proteins. When isotopes became available it was possible to test this assumption and, as a result mainly of the work of Schoenheimer and Rittenberg (68), it was replaced by the concept of the "dynamic state" of body constituents. According to this theory the proteins and other nitrogenous constituents are in a constant and rapid state of turnover. More recently, several investigations on protein and ribonucleic acid (RNA) in growing bacteria suggested that these substances were, after all, stable components of the cell, and the general applicability of the dynamic theory was questioned. However, it will be seen that stability of macromolecules seems to be confined to some bacteria, and then only when they are in a state of rapid growth; otherwise turnover is found quite generally.

The term turnover is not being used here in the strict sense which would imply that every type of protein or nucleic acid within the cell is being simultaneously degraded and resynthesized. Turnover in this sense, even if it did occur, would be technically almost impossible to demonstrate, and the term will be used more broadly to mean degradation of the macromolecular material to its constituent amino acids or nucleotides and reutilization of the breakdown products for synthesis.

Protein turnover, in this wider sense, is physio-

logically important when the molecules that are being synthesized *differ* from those that are being degraded, because this provides a means whereby the enzymatic pattern of the cell can be altered in response to a change in the chemical environment. Under conditions of starvation, when no net synthesis of protein is possible, turnover is probably the only mechanism available for achieving adaptations of this type.

The process of changing the distribution of enzymes within a cell in a physiological, *i.e.*, nongenetic, manner will be referred to as biochemical differentiation.

Examples of biochemical differentiation involving turnover can be found in a wide variety of organisms. At the bacterial level it occurs when cells, having expended one substrate in the growth medium, are engaged in producing an inducible enzyme for the utilization of another. At a more complicated level it is concerned in the formation of chloroplasts by *Euglena gracilis*, in the development of slime molds from free amoebae, and in the maturation of mammalian cells.

This article is an attempt to present in outline the evidence which has led to these conclusions. It is not intended to be a complete review of the subject. For reasons which will be given, much of the work on mammalian systems is difficult to interpret and will be discussed only briefly. In addition, experiments indicating turnover of protein or nucleic acid are frequently to be found in papers dealing mainly with some other topic. In general, these have not been quoted unless they illustrate some particular point which is not otherwise dealt with.

II. Turnover and Factors Affecting Its Measurement

Simple model. It will be convenient to begin by considering an ideally simple model for protein turnover and then to go on to discuss some of the complicating factors that arise in practice. Most of the argument will apply equally to the turnover of nucleic acids.

We shall assume a homogeneous population of protein molecules P which is randomly degraded to free amino acids, a, at a rate R_1 and resynthesized at a rate R_2

$$R_1 \left(\begin{matrix} P \\ a \end{matrix} \right) R_2$$

In the simplest case $R_1 = R_2$ and the system is in a steady state. It will also be assumed, for the time being, that breakdown and resynthesis are carried out by independent biochemical systems and not by the reversible action of a single system.

In a steady state system turnover can be measured equally well by the incorporation of a labeled amino acid into the protein or by the loss from protein of a previously incorporated label. Thus, if an isotopically labeled amino acid is introduced into the amino acid pool, the proteins will become labeled at a rate determined by R_2. Removal of the labeled acid and its substitution by an unlabeled acid will then lead to disappearance of the label from the protein at an exponential rate. From the decay curve, or more easily from a semilogarithmic plot of the data, the half-life of P can be calculated. If the sizes of the pools are known one can also calculate the turnover time and the replacement rate (see Tarver (75)).

In effect, this is the method used in the well-known studies of Schoenheimer, Rittenberg, and their colleagues (68). Adult rats were fed N^{15}-labeled amino acids for a few days and the rate of disappearance of the label from the proteins of various organs was followed. The average half-life of the hepatic proteins determined in this way was about 6 days and a variety of similar values was obtained for other organs.

Some of the difficulties that arise in interpreting a result of this sort will now be discussed.

Inhomogeneous populations of protein molecules. The protein of any organ is not a homogeneous population of molecules, but a mixture of populations having possibly very different half-lives. A short exposure to labeled amino acid will lead to high specific activity in labile proteins, *i.e.*, those that have a high rate of turnover. The more stable the variety of protein, the less will it be labeled in a short exposure, and the existence of completely stable proteins will not be revealed at all by this method. Consequently it would be expected that a longer exposure would lead to labeling of the more stable proteins and hence to an apparent increase in the half-life. This, in fact, is found. For example, Swick (74) reported that the half-life of protein in rat liver varied from 1.8 to 3.8 days according to the length of exposure (1 to 8 days) to labeled CO_2.

Protein secretion; cell turnover. It has been assumed that label is lost from the cells only after protein has been degraded to free amino acids. However, label can also be lost by the

secretion of protein as such, and the amount may be substantial in such organs as liver and pancreas. Protein secretion is also a property of many bacterial species. In addition to loss by secretion, loss can also occur as a result of death and lysis of the cells, which may be followed by growth of new cells. If one is simply measuring the specific activity of protein the effect of cell turnover will be superimposed upon the actual intracellular turnover of protein.

Reincorporation. So far the tacit assumption has been made that an amino acid molecule produced through degradation of protein is efficiently trapped in the free amino acid pool and is not reincorporated. This assumption will be correct only if the pool of amino acids is large in relation to the amount of material being discharged into it, or if the intracellular pool, though small in itself, equilibrates continuously and rapidly with a larger extracellular pool. If neither of these conditions obtains, some of the amino acid released by degradation will be reincorporated and so give a low measure of the turnover rate. In the intact animal, reincorporation is also likely to occur by transfer of material from one organ to another. A striking example of this has been found in rats carrying Flexner-Jobling tumors. The animals were given a single injection of C^{14}-glycine, and it was found subsequently that, although radioactivity was lost from the proteins of the various organs examined, the total radioactivity of the tumor actually increased during the same period, because label lost from the tissue proteins was being taken up by the rapidly growing tumor (43). It would be manifestly impossible by this method to tell whether the proteins of the tumor were stable or not.

In measuring turnover of nucleic acid by incorporation there are additional problems of equilibration. It is doubtful whether nucleotides as such are taken up by most cells and it is therefore common to use P^{32}-orthophosphate or a C^{14}-labeled purine or pyrimidine base or its nucleoside. Unless these precursors equilibrate rapidly enough with the nucleotides of the pool, the rates measured will be too low because of reincorporation and it is also likely that different values for the turnover rate will be obtained, depending upon the labeling agent used. The same argument will apply if such an agent is used for trapping radioactivity released by nucleic acid breakdown. These effects are shown very clearly in the experiments of Watts and Harris (81) which will be discussed later.

To sum up, any value for turnover rate obtained by incorporation or release of labeled material will be the resultant of "true" intracellular turnover, turnover of cells, and secretion, and will depend also upon pool sizes and equilibration. The reader is referred to the review by Tarver (75), for an analysis of other factors which may obscure measurements of turnover.

III. Turnover and Exchange

It has been assumed so far that turnover of protein involves whole molecules, in other words a total degradation to free amino acids and complete resynthesis of a new molecule. One can, however, imagine that an amino acid molecule in solution could replace a corresponding residue within the protein without all the other residues of the protein being involved. Schoenheimer and Rittenberg (68) seem to have visualized the incorporation of amino acid molecules as taking place in this piecemeal fashion. "The replacement of amino acids in the protein by identical amino acids in solution requires two processes, the first of which involves the opening, the second the closing of at least two peptide linkages." Gale and Folkes (25) introduced the term "exchange" to describe the concept of piecemeal incorporation. In practice the two processes may be difficult to distinguish on the basis of an isotope experiment. Thus, assume that there is an amino acid pool containing all the amino acids, and that these can exchange with the corresponding residues in the protein molecules which become replaced at, say, 1 per cent per hour. If incorporation of a radioactive amino acid is measured, this system will give the same result as one in which protein molecules are being totally degraded and resynthesized at 1 per cent per hour.

Evidence in favor of exchange was provided by the experiments of Gale and Folkes (25). Chloramphenicol, an inhibitor of protein synthesis, did not prevent the incorporation of single amino acids—mainly glutamic acid and glycine—by suspensions of staphylococci. The amino acids incorporated could not be extracted with hot trichloroacetic acid, and this chloramphenicol-resistant reaction was assumed to be due to exchange incorporation.

In *Escherichia coli* (47) and in *Bacillus cereus* (78), amino acids are not incorporated in this way in the presence of chloramphenicol, and this

fact led to a re-examination of the experiments in staphylococci. Now, the precipitate left after extraction of the cells with hot trichloroacetic acid contains both protein and cell-wall material. The latter is composed of four amino acids and two amino sugars. It was subsequently shown that incorporation of amino acid by staphylococci in the presence of chloramphenicol occurred almost exclusively into cell wall and not into the protein, and was in fact a measure of synthesis *de novo* of cell wall (33, 49).

Exchange can also be excluded on other grounds. By definition, exchange entails that the incorporation of a certain number of molecules of an amino acid shall be balanced by the release of an equal number of molecules of that acid. In practice it has proved possible to dissociate the processes of incorporation and release. Thus, in *E. coli*, azide or chloramphenicol immediately stop incorporation but permit release of labeled amino acid molecules from protein to continue at an undiminished rate for some time (47). This dissociation of the two processes shows that the amino acid is being released by degradation of the protein molecule and not by exchange.

The possibility of exchange has been taken up again, this time in connection with RNA of isolated thymus nuclei. Allfrey and Mirsky (1) studied the incorporation and release of labeled adenosine, or orotic acid, which is incorporated mainly as uridylic acid and partly as cytidylic acid, and of uridine, which is incorporated in much the same way as orotic acid. When the nuclei were transferred to an unlabeled medium without adenosine very little of the adenosine label was lost in 3 hr, but in the presence of a trap of unlabeled adenosine about 15 per cent of the label was lost. Nucleic acid labeled with orotic acid or uridine lost radioactivity at the high rate of 20 per cent in 3 hr, and this value was doubled when a trap of unlabeled uridine was added. In these experiments the total amount of RNA remained constant and there was no loss of label from the cytidylic residues of the RNA.

Uracil, uridine 5′-phosphate, and the diphosphate did not act as trapping agents, that is, they did not augment the loss of radioactivity from the uridylic residues. In an attempt to decide whether the nucleotides could enter the nuclei, the authors showed that incorporation of C^{14}-orotic acid was reduced by about 30 per cent when the unlabeled nucleotides were added. They concluded from this competitive effect that the nucleotides had entered the nuclei and were preventing incorporation. An alternative explanation for this result is that the nucleotides prevent the entry of orotic acid even though they are themselves incapable of entering the nuclei.

The authors form the view that there is an exchange process involving the nucleotide residues of the molecule and that this explains the different rate of replacement of different nucleotides. Although this is possible, the results are equally consistent with the occurrence of degradation and resynthesis of the whole RNA molecule, with some types of trap being more efficient than others. It would, for example, be of interest to know whether label would have been conserved in the cytidylic residues if cytosine or cytidine had been used as trapping agents instead of orotic acid or uridine. It was shown too, that 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole, which inhibits the synthesis of RNA, also increases the loss of uridylic label to 40 per cent in 3 hr. If it were shown that in the presence of this inhibitor, label was still lost from the uridylic and not from the cytidylic residues, the case for exchange would be stronger: the present observations are consistent with either turnover or exchange.

To sum up, experimental evidence indicates that amino acids are not incorporated into proteins by exchange. In the case of RNA there is no evidence for exchange incorporation apart from the experiments with thymus nuclei, and these are not conclusive.

IV. Turnover of Protein in Microorganisms

A. Bacterial Systems: General Considerations

In suitable bacterial systems many of the difficulties encountered in mammalian systems can be avoided and fairly unequivocal turnover data are obtainable. It will be useful to take up in turn some of the points that have been raised earlier.

Inhomogeneous populations. Bacteria can be grown in a labeled medium from a very small inoculum. In this way it can be ensured that all species of protein become labeled and not only those having a short half-life.

Protein secretion: cell turnover. Many types of bacteria do not secrete proteins, so that loss of

label in this way is obviated. Death of cells followed by lysis and proteolysis is a factor which it is difficult to rule out. If the rate of lysis is low it will not be revealed by measurement of cell mass or cell numbers. In any case, growth of new cells may take place by reutilization of products of proteolysis so that the total number of cells remains the same. Although lysis of cells may not be preventable, its extent can be measured by making use of the fact that, for the production of most induced enzymes, the continuous presence of the inducer is necessary. If cells are first treated with an inducer which is then removed before the measurement of turnover is begun, the loss of enzyme activity or its appearance outside the cells will be a measure of lysis (47). The method depends upon choosing an enzyme which has a low rate of turnover relative to the other proteins of the cell. Fortunately this is not difficult because most induced enzymes that have been examined appear to be comparatively stable.

Reincorporation. The question of reincorporation in the presence of an inefficient trap has already been discussed. In bacteria the equilibration between externally added amino acid and the material in the pool is extremely rapid. This is evident from the fact that methionine is incorporated into the proteins of *B. cereus* at a maximal rate and without any demonstrable lag, after its addition to the medium (59). In *E. coli* the uptake of valine, leucine, and isoleucine has been shown to occur with great rapidity (14). The conclusion that equilibration is efficient enough to prevent reincorporation is also supported by the fact that comparable quantitative results are obtained by the use of methods which do not rely upon equilibration (see below).

B. Turnover in Growing Bacteria

The first experimental indication that the proteins of growing bacteria were stable was provided by the work of Cowie *et al.* (15). The proteins of *E. coli* were labeled by growing the cells in the presence of $S^{35}O_4^{=}$. The cells were then grown further in unlabeled medium containing a trap of sulfate or cystine or methionine. There was no detectable loss of radioactivity during 3 hr. In a similar experiment the growth medium was examined, and it was found that about 3 per cent of the labeled sulfur was trapped in the first half hour, after which the loss of labeled sulfur from the cells was negligible—about 1 per cent in 4 hr.

An independent approach to the problem arose through the attempt to discover whether induced β-galactosidase was formed from some macromolecular precursor or whether it was synthesized *de novo* from amino acids.

The experiments reported by Rotman and Spiegelman (67) and Hogness *et al.* (37) were carried out as follows. The proteins of *E. coli* were labeled by growing the cells with C^{14}-lactate (67) or $S^{35}O_4^{=}$ (37). The bacteria were transferred to an unlabeled medium and grown further, but now in the presence of an inducer for β-galactosidase. The enzyme formed after a short period was isolated by a combination of electrophoretic and immunological techniques and examined for radioactivity. Within the error of the experimental method it was unlabeled. These experiments not only excluded the existence of significant amounts of a specific macromolecular precursor but also the occurrence of turnover for, if there had been any degradation of proteins to free amino acids, the amino acid pool would have become labeled, and the β-galactosidase being formed at the time should have contained a proportion of this label.

It is apparent that labeled amino acids derived from protein and entering the pool would be diluted by the "new" amino acids being produced from the lactate in the medium, and the specific activity of the material in the pool would be a function of the relative rates of the two processes. From the results of the experiments it was possible to conclude that the rate of protein destruction was less than 1 per cent of its rate of synthesis, *i.e.*, less than about 1 per cent per hour.

Hogness *et al.* (37) pointed out that, if the dynamic state is to be considered physiologically important, turnover must account for a significant fraction of the total synthesis. In growing *E. coli* this is manifestly not so. Generalizing from this finding, the authors suggested that, possibly, intracellular degradation did not occur in mammalian cells either, and that the dynamic state in animals was the result of secretion or cell turnover.

Yet another method of measuring turnover in growing cells, also using an internal trap was devised by Koch and Levy (40). Their procedure depended upon the fact that when purines were added to a culture of cells, they inhibited the endogenous synthesis of purines and were

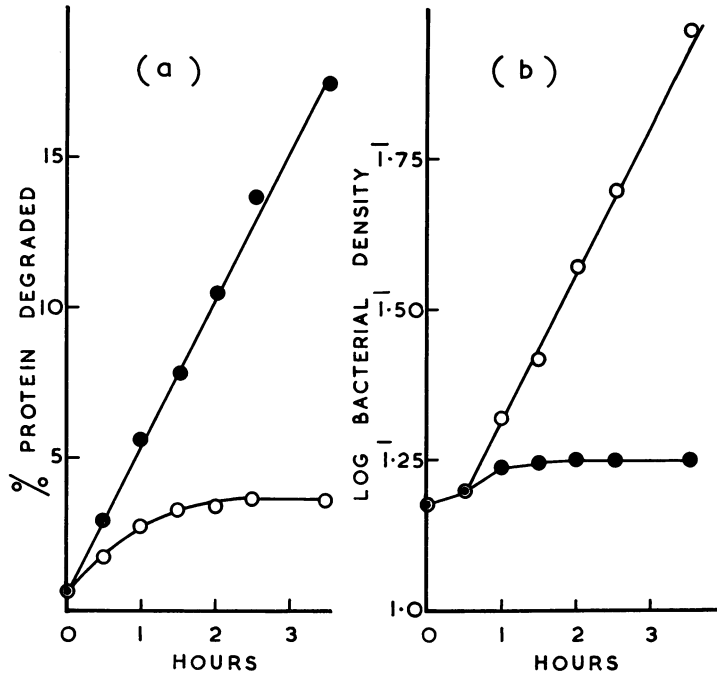


Figure 1. (*a*) Degradation of protein in growing and nongrowing suspensions of *E. coli.* (*b*) Semilog arithmic plot of bacterial density in the two suspensions. ●——●, nongrowing bacteria; ○——○, growing bacteria. In the latter, much of the breakdown occurs during the lag before establishment of exponential growth (see (45, 47)).

in consequence preferentially used for production of nucleic acids. Removal of the exogenous purines promptly released the inhibition of endogenous synthesis.

The experiment was done in two stages. In the first, the cells were grown with a labeled substance, such as carbohydrate or glycine, which could act as precursor both for nucleic acids and proteins. Unlabeled purines were present to inhibit endogenous synthesis of nucleotides. This resulted in cells which had labeled proteins and unlabeled nucleic acids.

Labeled precursors $\rightleftharpoons$ labeled proteins
Unlabeled purines $\rightarrow$ unlabeled nucleic acids. (*a*)

In the second stage the cells were transferred to an unlabeled medium, with the purines omitted, and grown further. Thus

Unlabeled precursors $\rightleftharpoons$ labeled proteins
$\searrow$nucleic acids. (*b*)

Breakdown of proteins in stage *b* would release labeled glycine which, as a purine precursor, would ultimately give rise to labeled nucleic acids. The proportions of glycine used for nucleic acid synthesis and for protein synthesis had been previously determined, and thus the rate of appearance of label in the nucleic acids could be used as a measure of protein destruction. The results indicated that about 2 per cent of the protein was degraded initially, but after 2 hr the rate of labeling of nucleic acids was negligible and indicated a half-life of 30 days or more for the proteins.

C. Turnover in Nongrowing Bacteria

A number of earlier experiments suggested that the proteins of nongrowing *E. coli* might be more labile than those of cells in rapid exponential growth. (In this context, "nongrowing" will be applied to suspensions in which there is no net synthesis of protein.)

Thus, Podolsky (58), had labeled cells with arginine and then transferred them to unlabeled medium for further growth. In a period of 30 hr covering a period of rapid growth and a long semistationary phase, about 8 per cent of the label was lost from the proteins. It had also been reported (51) that *E. coli* incubated in buffer

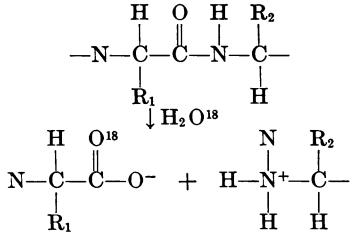
solution containing glucose and labeled methionine would incorporate label linearly at a rate corresponding to 0.03 μmoles/26 mg/hr. The concentrations of methionine used were too low to saturate the system and, at higher concentration there was about four times as much incorporation. Assuming that about 60 per cent of the cell mass is protein and that methionine is 2.2 per cent of this (60), it can be calculated that the turnover is about 5 per cent per hour.

A direct comparison of protein stability in growing and nongrowing cells was made by Mandelstam (45, 47) using a strain of *E. coli* requiring leucine and threonine. The cells were labeled with C^{14}-leucine and incubated with glucose and an excess of unlabeled leucine; this was the nongrowing suspension. To obtain growth a similar suspension was treated with threonine and ammonium salts, and the release of leucine from the protein of both suspensions was measured.

The total loss of label from the growing cells was less than 4 per cent, and over half of this took place in the first 30 min while the cells were still in lag phase. After the first hour the proteins appeared to be very stable. The slight destruction of protein at the beginning of the incubation was similar to that reported before (15, 40). By contrast protein degradation in the nongrowing cells continued linearly at about 5 per cent per hour for the duration of the experiment (see figure 1).

Resynthesis of protein was measured by incorporation of glycine in nongrowing cells and found to proceed at the same rate (4 to 5 per cent per hour). There was thus a balanced degradation and resynthesis of protein. The occurrence of cell lysis was ruled out in these experiments by the demonstration that preformed inducible β-galactosidase was quantitatively conserved in the cells.

An independent method of measuring breakdown of protein was to incubate cells in the presence of H_2O^{18} (8). Hydrolysis of a peptide linkage introduces O^{18} into one of the molecules:



Because of resonance, both oxygen atoms are equally labeled, and if the amino acid residue is reincorporated there is a 50 per cent probability that labeled oxygen will be found in the protein. These experiments, and others using D_2O as a labeling agent, indicated a degradation rate of 3 to 6 per cent per hour, which agrees with the value determined by the release of leucine. It should be noted that this method does not depend upon equilibration.

The dissimilarity in stability of protein in growing and nongrowing cells seems to be general among microorganisms. In *B. cereus*, Urbá (78) found a rate of degradation of 7 per cent per hour in nongrowing and 1.4 per cent per hour in growing cells. In the former, protein degradation proceeded at an undiminished rate for 6 hr, so that at the end of the experiment nearly half of the total cell protein had been involved in turnover.

Protein degradation in nongrowing yeast was investigated by Halvorson (30) who used two methods. The first consisted of using unlabeled cells and measuring the rate of dilution of C^{14}-glycine which had been introduced into the free amino acid pool. In the second method, the cells were labeled with C^{14}-leucine or phenylalanine and the rate of release of label was measured. These methods indicated a turnover rate of 0.75 per cent per hour, which is a minimal value since equilibration in yeast is more difficult to achieve and no external trap was used. Reincorporation could therefore have occurred. In growing cells no degradation was detectable (31).

D. Turnover in Mammalian Cells

For reasons which have already been discussed, experiments carried out with whole animals, and even with tissue slices, are difficult to assess, and there are so many of them that any attempt at a detailed consideration would make this article too long and too burdensome for the reviewer as well as the reader. This section will therefore include a description of only a few representative experiments, mainly in tissue culture, where the conditions are sufficiently defined to make the data readily interpretable.

The most thorough of these investigations was carried out by Eagle *et al.* (18). Several types of cells were grown in tissue culture in a synthetic medium with dialyzed serum as the only undefined component. Upon removal of the cells to a

medium deficient in one or more amino acids, net protein synthesis ceased, and the total nitrogen of the cells remained constant or decreased slightly during 24 to 48 hr. In this period labeled amino acids were incorporated at a constant rate of 0.75 to 1 per cent per hour, the values being roughly the same for human conjunctiva, monkey kidney, and HeLa cells. The rate of degradation was measured by first labeling the cells with C^{14}-phenylalanine or valine or lysine and then incubating them in the presence of the appropriate trap. In the absence of a trap there was negligible loss of label from the cells. In the presence of the trap, the loss was about 1 per cent per hour, so that here as in bacteria there is a balanced destruction and synthesis of protein, but in contrast to bacteria the rate of degradation was the same in growing and nongrowing cells.

The following experiment was done to show that the turnover measured was intracellular and not due to lysis of cells. A vessel containing labeled cells growing as a monolayer on the glass was connected to another containing unlabeled cells, and the system was mechanically rocked, so that the medium washed to and fro continuously. If there had been lysis and proteolysis followed by reutilization of the debris by growing cells, there should have been a transfer of label. In fact the transfer was negligible.

Another study in which lysis of cells can probably be excluded was made using Ehrlich ascites tumor cells, labeled by growth in the peritoneum of a mouse which had been injected with C^{14}-glycine or S^{35}-methionine (22). After 2 days the cells were transferred to another animal and the loss of label followed. One strain of cells lost 30 to 40 per cent of the methionine in the first 3 to 4 days of growth in the new host. Another strain of cells lost glycine label but conserved methionine. This suggests that, in this strain, liberated glycine is more efficiently trapped than methionine. Adenine, incorporated into the DNA of the cells at the same time, was almost completely conserved. If there had been lysis of cells, one would have expected adenine, glycine, and methionine labels to have been lost at the same rate.

The results were quantitatively similar to those obtained by Eagle (18), and so were the results of Moldave (52), who incubated labeled ascites cells in a cellophane sac in the peritoneal cavity of the mouse and found that the proteins of the mitochondrial, microsomal, and soluble fractions of the cells were all about equally involved in turnover.

Protein turnover has been studied in rabbit macrophages *in vitro* (34). There was no net synthesis of protein, and the cells did not multiply, but they remained alive for several days. After 12 hr, about 10 per cent of the valine in protein was labeled: again a rate of about 1 per cent per hour.

E. Mechanism of Protein Degradation

While it is apparent that protein degradation may occur at a high rate in some circumstances, it is not known to what extent all the proteins of the cell are concerned. In *E. coli* the turnover may involve as much as 25 per cent of the total protein (47) and in *B. cereus* (78) and in HeLa cells about half of the total protein can be degraded (18).

The relative stability of the protein of the ribonucleoprotein particles (ribosomes) has been compared with that of the soluble protein in nitrogen-starved *E. coli*. Both types of protein were degraded at the same rate (48). Nevertheless, it seems clear that more detailed investigations would reveal differences in the lability of different proteins. For instance, there is no detectable loss of β-galactosidase in *E. coli* at a time when the average rate of protein degradation is 5 per cent per hour (47). Indeed, most inducible enzymes that have been examined have proved to be stable under conditions where protein degradation might have been expected.

A further possibility that has to be considered is that proteins that are labile in some conditions of starvation are stable in others. An example is the alcohol-soluble protein of *E. coli* which was degraded in sulfur starvation (66) but was stable in nitrogen-starvation (Mandelstam, *unpublished data*).

The mechanism of protein degradation has been examined in tissue slices from rat liver and kidney by Steinberg *et al.* (72), who concluded that protein breakdown is not a simple catheptic reaction, but a complex one similar to protein synthesis. This was based on the finding, similar to that of Simpson (71), that inhibitors of protein synthesis are also inhibitors of degradation. The agents tested included phenylalanine analogues, 2,4-dinitrophenol, and nitrogen (*i.e.*, anaerobiosis).

In bacteria, inhibitors of synthesis are also found to inhibit breakdown, but only after a lag of nearly an hour (47). This delay suggests that the action of these inhibitors on the catabolic process is indirect, and possibly due to the fact that they cause the accumulation of metabolites which are the true inhibitors. A comparison of the kinetics of protein degradation in cells incubated with azide or chloramphenicol and in cells which have a limited nitrogen metabolism and which accumulate metabolites supports this interpretation (47).

In bacterial systems, at least, there seems to be no evidence in conflict with the assumption that catabolism of protein is achieved by simple proteolytic action of one or more enzymes. Other relevant data will be discussed in a later section.

V. Turnover of Nucleic Acids

A. RNA and DNA Turnover in Microorganisms

All investigators are agreed that in *E. coli* growing exponentially the nucleic acids are stable. Cells labeled with P^{32}-orthophosphate and then grown further in unlabeled medium conserved the label in the RNA and DNA (deoxyribonucleic acid) (24). Furthermore, conservation of label in the DNA of cells which had been grown in C^{14}-invert sugar was unaffected even in the presence of trapping agents such as thymidine, deoxyadenosine, or deoxycytidine (36). Since these deoxyribonucleotides compete favorably with glucose for incorporation into DNA they are presumably good trapping agents and the fact that they have no effect on the conservation of label is strong evidence for the stability of the DNA.

In stationary populations of *E. coli*, RNA is degraded actively. Instability of RNA, like that of protein, seems to be a consequence of stopping growth, and the actual reason for the stoppage of growth is of secondary importance. Thus, degradation of RNA follows upon starvation of a specific amino acid (26), nitrogen (48), pyrimidine (6), Mg^{++} (16, 17), or phosphate (38).

In experiments with a leucine auxotroph (26) cells were labeled with P^{32}-orthophosphate and then starved of leucine in an unlabeled medium. About 20 per cent of the P^{32} of the RNA was lost in 90 min and at least half of this was recovered from the incubation medium. There must have been a good deal of resynthesis of RNA from unlabeled phosphorus, because the total RNA decreased only 0 to 12 per cent, which is far less than the decrease in label.

The effect of phosphorus deficiency upon the stability of RNA was studied by Horiuchi *et al.* (38) who grew cells in a phosphorus-limiting medium using P^{32}-orthophosphate. The label in RNA reached a peak at the time that the phosphate in the medium was exhausted. In the next 3 to 4 hr the P^{32} label in RNA diminished about 40 per cent. Some of the material lost was used for synthesis of DNA and some was recovered in the acid-soluble fraction of the cells. When the experiment was done with cells which contained preformed β-galactosidase there was no release of enzyme to the medium, so the loss of the RNA label cannot be attributed to lysis.

In a pyrimidine-deficient mutant, starvation produced turnover of RNA, but not of DNA (6). The uridine isolated from the RNA after 90 min of pyrimidine-starvation in the presence of C^{14}-glucose contained ribose which was 9 per cent labeled; the uracil was, of course, unlabeled. This result suggests that the turnover of RNA involves recycling of the "old" uracil but now attached to "new" ribose freshly synthesized from the glucose of the incubation medium.

In yeast, too, the stability of the RNA depends upon whether the cells are growing. Halvorson (30, 31), found no detectable loss of labeled purine from the RNA of growing cells, whereas in nongrowing cells, the loss was appreciable. Turnover of RNA was noted also during formation of induced enzymes by "resting" yeast cells (11).

The only evidence for the possible occurrence of DNA turnover in normal cells comes from experiments involving the use of 5-bromouracil, which can replace thymine in the DNA molecule. When *E. coli* was cultivated in a medium containing 5-bromouracil it was found that, in the stationary phase after cessation of exponential growth, more of the analogue was incorporated than would have been expected from the net increase in DNA (86). The turnover rate indicated was of the order of 1 per cent per hour. The question that has to be considered is whether the 5-bromouracil is itself the cause of instability (85).

B. Turnover of Nucleic Acids in Phage Infection

Turnover of DNA in phage-infected cells was demonstrated by Cohen (13) who labeled cells

with P^{32} and then infected them with T2 and T4 bacteriophages. He found that 15 to 30 per cent of phosphorus in the viral DNA was derived from the host. The direct use of host DNA as precursor material has been shown by C^{14}-labeling experiments. Cells grown with C^{14}-thymidine were infected with phage$T6r^+$ in unlabeled medium (41). As much as 70 per cent of the cell thymine was transferred as such to the phage DNA.

By contrast, very little host protein is transferred (69). It was shown using C^{14}-lysine-labeled cells that only 1 to 2 per cent of the host protein was converted to $T6r^+$ protein, this being equivalent to 14 per cent of the phage protein. Far more of the phage phosphorus (26 per cent) and nucleic acid nitrogen (37 per cent) are derived from the host. In T7 infections the proportions derived from the bacterial cell are even higher: 60 to 90 per cent of the phosphorus and nucleic acid nitrogen, and about 40 per cent of the protein (63).

T2-phage infection also affects the stability of RNA in the host cell (35). One minute after infection there was a rapid incorporation of P^{32} into RNA and the molecules synthesized at this stage were in a state of rapid turnover (5) which was demonstrated by adding an excess of unlabeled phosphate after 5 min of incorporation. This resulted in the loss of about half of the P^{32} label of the RNA in 15 min. There was a corresponding appearance of P^{32} in the DNA. If degradation of RNA had proceeded beyond the nucleotide stage, incorporation of P^{32} into DNA would not have been found.

The conclusion drawn by Astrachan and Volkin (5) was that the ribonucleotides liberated by degradation were being converted to deoxyribonucleotides and then used for synthesis of phage DNA.

The general course of events in T7 infection was similar. Labeled phosphate was incorporated into RNA at a rate several times greater than the rate of incorporation into DNA and reached a peak in 10 min. The instability of the RNA was again shown by adding excess unlabeled phosphate at 9 min. A rapid loss of label from the RNA ensued, *i.e.*, about 35 per cent of the incorporated P^{32} in 30 min (80).

C. Chloramphenicol and Stability of RNA

Useful information on factors affecting the stability of RNA may also be obtained by further study of the RNA produced in the presence of chloramphenicol. Treatment of a culture of *E. coli* with chloramphenicol stopped protein synthesis almost immediately but the synthesis of RNA as measured by the incorporation of labeled adenine continued (54). If the cells were washed and transferred to a fresh medium, the ribose content and the radioactivity fell in parallel and about half of the RNA that had been synthesized in the presence of the drug was degraded in 100 min. Similar results were obtained by Hahn *et al.* (29) but not by Horiuchi *et al.* (39). The latter workers transferred the cells, after chloramphenicol treatment, to a growth medium containing amino acids and found that the RNA was stable as indicated by the retention of P^{32}. When the cells were transferred to a medium lacking an essential amino acid, about half of the RNA was degraded. These results do not necessarily conflict with the previous ones. In the richer amino acid medium, protein may be formed fast enough to stabilize the RNA in the form of ribonucleoprotein (see below). An alternative possibility is that the RNA is still degraded but that, in the richer medium, the products of RNA catabolism are reutilized for growth instead of being trapped.

D. RNA and DNA Turnover in Mammalian Cells

The literature on turnover of nucleic acid in animals is more difficult to interpret than the corresponding work on proteins, mainly because of the difficulty in devising a proper trapping method. As before, only work done with tissue cultures will be considered and it will be apparent that, even in the relatively well-defined conditions of these experiments, the measure of turnover that is obtained is likely to reflect the method used rather than the metabolic state of the organisms.

Siminovitch and Graham (70) used Earle's L-strain cells originally isolated from mouse connective tissue. The cells were labeled with P^{32}-orthophosphate and then incubated with unlabeled orthophosphate as trap. During five generations of exponential growth the label was conserved both in RNA and DNA.

In a similar experiment the nucleic acids of L-cells were labeled with C^{14}-formate (76). The cells were grown further at a slow rate (doubling time of 72 hr) in medium with unlabeled formate. After seven generations, the adenine and guanine of the RNA had lost about 80 per cent of their

radioactivity, whereas DNA showed complete conservation of the label. If, however, unlabeled thymidine was also present in the medium there was an appreciable fall in the C^{14}-thymine retained in the DNA. Retention of adenine and guanine in the DNA was unaffected by thymidine. The result suggests that, in these cells, the DNA as well as the RNA was turning over, but that the breakdown products were reincorporated before they had equilibrated with the C^{14}-formate.

A thorough investigation of nucleic acid turnover in rabbit macrophages was carried out by Watts and Harris (81). The cells remained alive and motile *in vitro* for at least 12 hr, but did not multiply or grow; that is, the total RNA, DNA, and protein remained constant. The cells incorporated labeled adenine into RNA linearly and, after 12 hr, about 20 per cent of the adenine in RNA was labeled. DNA was not significantly labeled, and the authors concluded that it was stable. The experiments on the retention of labeled adenine are especially interesting. The RNA of the macrophages was labeled during 4 hr incubation with C^{14}-adenine. The cells were then incubated with a variety of trapping agents. The result was found to depend upon the ease with which the trapping substance could displace a labeled intermediate and prevent it from being reincorporated. With adenosine, the most effective of the compounds tested, the apparent half-life of the RNA involved in turnover was 2 hr; with adenine or the 2′-, 3′-, or 5′-phosphate of adenosine it was about 12 hr, whereas with inosine 5′-phosphate no turnover was detectable at all.

These experiments, together with those of Allfrey and Mirsky (1) which have already been described, illustrate clearly the difficulties encountered in obtaining a meaningful result in measurements of nucleic acid turnover. In the case of nucleic acids, even more than with proteins, a change in the rate of incorporation or loss of label does not necessarily reflect a change in the rate of synthesis or degradation of the macromolecule, but may mean merely a change in the rate of equilibration of the exogenous precursor with the nucleotide pool. The results of isotope experiments should be interpreted with caution unless effective equilibration has been demonstrated by comparing the specific activities of the exogenous precursor and the nucleotide pool.

VI. The Control of Protein and Nucleic Acid Degradation

A. Ribosomes and the Stability of Proteins and Nucleic Acids

From the evidence already discussed it is clear that while the proteins and RNA of the mammalian cell are in a state of turnover, whether the cells are growing or not, those of microorganisms are controlled by the physiological state of the cells, being relatively stable during active growth and rapidly degraded when growth is stopped. The actual factor which causes growth to stop seems to be immaterial, since, as we have already seen, deficiencies of leucine, pyrimidine, or phosphorus are equally effective in initiating degradation of RNA, whereas degradation of protein in *E. coli* will take place when the cells are deprived of carbon, nitrogen, or a specific amino acid. On the other hand, DNA, except in phage infection, has appeared to be stable in most experiments. It should be borne in mind that the failure to detect DNA turnover could be technical and that DNA may turn out to be less stable than the present data indicate.

Coming now to the mechanism of protein degradation we have to consider the possibility that growing cells in fact do not possess the necessary enzymatic mechanism for degrading protein, and that it is synthesized only after the cells stop growing. The fact that protein degradation begins without lag in starved cells (47) argues against this, but does not rule it out. The alternative is to assume that there is always a potential ability to degrade protein but that in growing cells it is, for some reason, not expressed.

In fact, it has been shown that *E. coli* contains proteolytic activity which becomes manifest only after the cells have been broken in a Hughes press (10). The proteins of the cell-free preparation are then broken down at 3 to 4 per cent per hour at 37 C, a value corresponding with that found *in vivo* in nongrowing cells.

The stability of the RNA in *E. coli* during growth appears also to depend upon the fact that the ribonuclease of the cells is inactive while the cell structure is left intact but, if the organisms are sonically disrupted or lysed by phage, they exhibit ribonuclease activity (50). It has more recently been shown that the ribonuclease is associated almost entirely with the 30S ribosome particles, and becomes active when

the structure of the ribonucleoprotein is disrupted (20).

The ribosomes of *E. coli* contain about 90 per cent of the total RNA of the cells (77) and about 30 per cent of the protein. When intact they are very stable and resistant to the action of ribonuclease. However, their integrity *in vitro* depends upon the concentration of Mg^{++} (77). Lowering the Mg^{++} leads to a reversible disintegration of the larger particles into smaller ones but beyond a certain point the particles break up altogether. A similar effect of Mg^{++} has been shown *in vivo* by Dagley (16, 17) during starvation. In cells starved of carbon and nitrogen the 40S particles disappeared in 2 hr and the material was partly accounted for by an increase in the number of 20S particles; the remainder was presumably solubilized. A very similar instability of particles was produced in yeast by starvation (4). Sufficient Mg^{++} could prevent these changes in *E. coli* (16, 17).

These results support a much earlier report (73) showing that, if *E. coli* which had been grown in tryptic digest was transferred to bicarbonate buffer containing lactate, the cells lost 60 per cent of their RNA and 20 to 30 per cent of their DNA in 3 hr. Magnesium chloride stabilized the nucleic acids.

B. A Possible Control Mechanism for Nucleic Acid and Protein Stability

The occurrence of ribonuclease and proteolytic enzymes in an inactive state, coupled with the facts known about ribosome instability make it possible to formulate a simple hypothesis for the control mechanism involved in protein and RNA breakdown which appears to be consistent with the known facts.

When the cells are in active growth, the ribosomes maintain their integrity, and the ribonuclease and protease on the particles are inactive. Under conditions of starvation, the ribosomes disintegrate, either because there is a fall in the concentration of intracellular Mg^{++} or for some other reason. The solubilization of the ribonucleoprotein has two consequences. The RNA which was protected in the particulate state becomes exposed to attack and at the same time the degradative enzymes which were bound on the particles are now liberated.

Support for this scheme is provided by observations on the stability of ribosomal RNA *in vitro* (Mandelstam, *unpublished data*). Ribosomes from *E. coli* were suspended in tris buffer (0.01M pH 7.4) in the presence and absence of Mg^{++} (0.01 M). When Mg^{++} was present no significant degradation of RNA occurred during 6 hr incubation at 35 C. In the absence of Mg^{++} there was, as *in vivo*, a linear breakdown of RNA which was indicated by the appearance in the acid-soluble state of material absorbing at 260 mμ. At the end of 1 hr incubation, about 25 per cent of the ribosomal RNA had been rendered acid-soluble. This result supports the observations of Bolton *et al.* (7a), who showed that activation of ribonuclease could be achieved by adding the chelating agent, ethylenediaminetetraacetic acid, or sodium chloride. In 0.125 M sodium chloride, all the RNA became acid-soluble in about 20 min. The authors ascribed this effect to the displacement of $Mg.^{++}$ A similar degradation has been demonstrated *in vivo* in nitrogen-starved cells (48), but the rate at which the process occurs is much lower (about 5 per cent per hour).

It is to be expected that when cells are restored to a growth medium, conditions for ribosomal stability will also be restored. This will stop the production of soluble, *i.e.*, labile ribonucleoprotein from the particles and automatically bring the degradation of RNA and ribosomal protein to an end. The reason why the breakdown of the soluble proteins should also cease is more obscure. These proteins, in starved *E. coli*, are degraded at the same rate as the ribosomal proteins (48). When growth is resumed, the proteolytic enzymes which were active in the stationary cells must presumably be inhibited, perhaps by readsorption on to ribosomes or otherwise. In any case the reaction is fairly slow, since as can be seen (figure 1) breakdown of protein continues at a detectable rate for at least an hour after the cells have begun to grow.

C. Control of DNA Stability

Some of the factors affecting the stability of DNA in *E. coli* have been elucidated through studies of phage infection. The lysate obtained after T2 infection was shown to contain appreciable deoxyribonuclease activity (12). This enzyme was also found in T6 broth lysates by Kozloff (41), who went on to examine some of its properties. Extracts of uninfected bacteria contained an inhibitor of the deoxyribonuclease which was specific in that it did not inhibit pancreatic deoxyribonuclease. It was assumed to be an RNA. In support of this conclusion, it was

shown that a bacterial extract from uninfected cells, treated with ribonuclease, developed deoxyribonuclease activity similar to that found in the phage lysates. At the same time the extract lost its ability to inhibit the deoxyribonuclease of the lysates. The deoxyribonuclease, like the ribonuclease, seems to be attached to the ribosome (19).

It is therefore possible that the stability of DNA depends upon the stability of the RNA, or at least of one component of the RNA, and that the inhibitor is lost in the early RNA turnover which follows phage infection. Destruction of the inhibitor is then followed by the extensive degradation of DNA which is always found in phage-infected cells.

VII. Turnover and Biochemical Differentiation

A. Induction and Repression of Enzymes

From what is now known about the control of enzyme production it is clear that a cell, even in a state of active growth, will not synthesize a certain proportion of the enzymes it is potentially capable of synthesizing or, to be more exact, it will produce them in trace quantities only. This applies to the whole class of inducible enzymes, which are not produced when the inducer is not in the medium and which sometimes may not be produced even when it is (see below). In addition, many of the enzymes concerned in the synthesis of intermediary metabolites can be classified as repressible (79), their synthesis being inhibited by the metabolite which is an end product of the reaction sequence. For example, in *E. coli*, uracil represses the formation of at least three enzymes concerned in the synthesis of pyrimidines (84), arginine represses the formation of two of the enzymes concerned in its synthesis (27, 79), and histidine represses the synthesis of no less than four enzymes involved in its formation (3). These and other examples have recently been reviewed (56).

In principle the advantages of having the twin mechanisms of induction and repression are clear. A bacterial cell which has its synthetic capacities concentrated upon the production of those enzymes that are functional in the environment at the time, can be expected to grow faster and to be at a selective advantage in comparison with a cell producing the full complement of enzymes which it is potentially able to produce.

It will be useful to consider what is involved in the case of *E. coli* where we now have sufficient information to attempt a slightly more quantitative approach. The total number of repressible enzymes may turn out to be quite large. Thus, it was shown some years ago (66) that at least nine amino acids inhibited their own synthesis, and several more instances have been discovered since. In two cases, arginine and histidine, more than one enzyme in the biosynthetic pathway is repressible, and there is no reason to think that these cases are in any way exceptional. So, in cells growing in a rich medium containing purines, pyrimidines, and amino acids, the number of repressed enzymes can be roughly estimated at 20 to 50. This seems reasonable in view of the fact that three metabolites, arginine, histidine, and uracil, will together repress at least nine enzymes. As for inducible enzymes, the number is not less than 10 and is probably much more. Induction and repression mechanisms may thus together control the rates of synthesis of something like 50 to 100 enzymes.

There is not enough information to allow a calculation of what difference this number of enzymes will make in terms of protein economy, but an indication can be obtained from what is known about a few enzymes. Thus, from data on purified β-galactosidase (37) it can be calculated that this enzyme alone can account for 3 to 5 per cent of the protein of *E. coli*. The repressible enzyme, alkaline phosphatase, accounts for about 7 per cent of the total protein synthesized when *E. coli* is grown in a medium where phosphate is limiting, so that the repression normally observed is released (44). Since we do not know how many inducible enzymes any organism has, nor to what extent its biosynthetic enzymes are already repressed, it is not possible to assess what the wastage of protein would be if there existed neither induction nor repression, and all enzymes were produced constitutively and at fixed relative rates. The two examples quoted suggest that the wastage might easily amount to more than 10 per cent and that an organism which functioned in this way would be relatively inefficient.

However, repression and induction would not be useful properties in themselves unless the cells also had a mechanism for rapidly producing repressed or induced enzymes when the chemical environment changed. Protein turnover appears to be the mechanism employed in such a situation,

and its role will be made clearer by considering a few examples.

B. Induced Enzyme Formation

Biochemical differentiation, defined as a change in the enzymic pattern of a cell, can be seen at a simple level in induced enzyme formation in bacteria. The example we shall consider is the sequence of events in a culture of *E. coli* growing in a medium containing glucose and lactose. Enough is now known about this system to permit a fairly comprehensive description of what occurs.

Growth takes place in two phases (53). In the first, the cells grow at the expense of the glucose and the lactose is not utilized. This may be either because glucose prevents the uptake of lactose into the cell by the galactoside permease (64) or because glucose utilization causes metabolites to accumulate which inhibit the production of inducible β-galactosidase (55). Whatever the reason, the cells exhaust the glucose and enter a state of lag possessing only traces of β-galactosidase (65).

At this stage one can envisage two different ways in which the β-galactosidase that is required for growth on the lactose could be provided. There could be a fairly specific channelling of the contents of the very small (46) free amino acid pool into synthesis of β-galactosidase, *i.e.*, a preferential synthesis of the enzyme. This interpretation proposed by Rickenberg and Lester (65), seemed reasonable at the time because, during the lag period, there was no net synthesis of protein, but there was a big increase in the β-galactosidase of the cells (65). The alternative is the breakdown of protein to free amino acids, of which a proportion is used for synthesis of β-galactosidase. Preferential synthesis would have entailed a very elaborate system of specific repression; that is, the synthesis of virtually all proteins would have had to be inhibited except the one that is required. Turnover does not require such a highly evolved control system for protein synthesis and appears to be the mechanism used. Thus, the cessation of growth immediately initiates breakdown of protein, and a proportion of the amino acids so made available is used for synthesis of β-galactosidase. It was also shown that this proportion is the same in the lag period as it is in growth (45). In other words, the system which synthesizes the enzyme appropriates the same proportion (about 1 per cent) of the free amino acids passing through the pool.

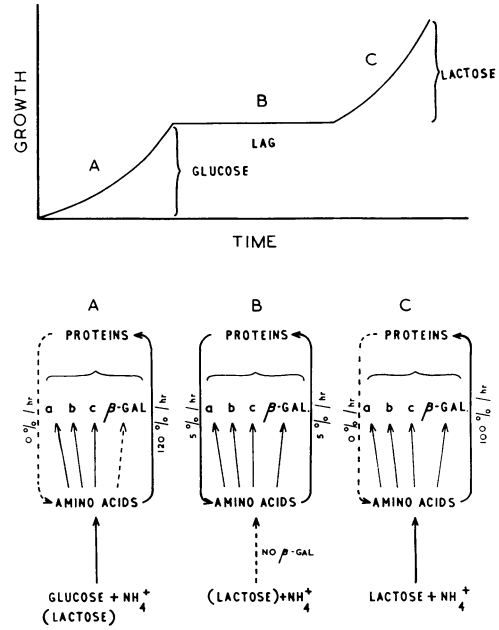


Figure 2. Changes induced in adaptation of *Escherichia coli* from growth on glucose to growth on lactose. (*A*) Phase of exponential growth on glucose; protein breakdown is inhibited during growth; glucose inhibits formation of β-galactosidase. (*B*) Lag phase: cessation of growth (because of glucose exhaustion) initiates protein breakdown; synthesis of β-galactosidase takes place. (*C*) Phase of exponential growth on lactose; protein breakdown is again inhibited.

The adaptation process is summarized in figure 2.

In this instance protein turnover seems to be an essential part of the biochemical differentiation. The types of lag period encountered when cells are transferred from a rich medium to a simpler one have not as yet been studied in detail, but it seems likely that they are characterized by synthesis of previously repressed enzymes from turnover material. Thus, it has been shown that if pyrimidine-requiring *E. coli* is starved of its requirement, there is an apparently preferential synthesis of three of the enzymes in the sequence synthesizing pyrimidines (84).

C. Sporulation and Germination of Bacillus Species

Foster and Perry (23) have presented evidence that the changes which accompany sporulation of *Bacillus mycoides* in water include extensive

turnover of protein. Breakdown and resynthesis of protein were measured by release or incorporation of S^{35}-methionine. The sequence of events after suspending the cells in water was as follows. Protein breakdown took place very rapidly to begin with, about 75 per cent of the label becoming soluble in the first 4 to 5 hr. This was turnover material and, unless a trap of unlabeled methionine was present, most of it was reincorporated into the protein. Resynthesis of protein lagged somewhat behind, reaching a peak at 5 to 8 hr, and being followed by spore formation at 9 to 12 hr. These transformations were considered to be entirely intracellular (57), although it has been claimed by other workers that sporulation in water may involve considerable lysis of cells (61).

Germination of spores of *Bacillus megaterium* and *Bacillus subtilis* is also accompanied by gross biochemical changes involving degradation of macromolecules. About 30 per cent of the dry weight was lost to the external medium as nitrogen-containing material. Much of this was dipicolinic acid. The remainder consisted of a mixture of thirteen amino acids. In addition there was also cell-wall peptide characterized by glucosamine, muramic acid, and diaminopimelic acid (62). This extensive breakdown of protein and cell wall is paralleled by the loss of heat resistance and the appearance of heat-sensitive enzymes, which were presumably stabilized in the dormant state by adsorption onto protein and other macromolecular materials (see Halvorson and Church (32)).

D. Formation of Chloroplasts

Another example of intracellular biochemical reorganization produced in response to a change in the environment is provided by the flagellate *Euglena gracilis*. The cells are photosynthetic when grown in light, but lose their chlorophyll when grown in the dark. Brawerman and Chargaff (9) transferred dark-grown cells to a resting medium (phosphate buffer containing glucose and Mg^{++}) and exposed them to light. This led to a synthesis of chlorophyll which slackened after 60 hr. At this stage the cells resembled normal light-grown cells, appeared undamaged and had unaltered motility. There was no cell multiplication during the experiment and the total RNA and protein remained constant.

The exposure to light was shown to have produced the following changes. The chloroplast fraction of the cells increased in protein content and contained 43 per cent of the cell protein at the end of the experiment compared with 26 per cent at the beginning. The RNA of this fraction increased from 11 per cent to 18 per cent of the total. These changes represented a transfer of material from the mitochondrial and soluble fractions, the microsomes retaining their protein and RNA. The formation of chloroplasts appeared to have been achieved by means of a thorough turnover of protein, the incorporation of leucine being 0.4 μmoles per mg protein. If we assume that the protein contains about 6 per cent leucine, the observed incorporation indicates that about 90 per cent of the cell protein had been degraded and resynthesized. The turnover of RNA as measured by the incorporation of adenine was proportionately large.

E. Biochemical Differentiation in Slime Molds

At a more complex level we have the biochemical differentiation underlying the life cycle of the slime mold. These organisms can exist as vegetative independent amoebae. When they are starved for some hours they aggregate to form multicellular units, the pseudoplasmodia, each containing about 10^5 cells. This takes place in the absence of cell division. Further differentiation then takes place; a portion of the cells comprising the multicellular organism become a stalk and the rest a mass of spores. The maturation is accompanied by considerable production of cellulose. A brief summary of the cycle and references to the original work have been given by Wright and Anderson (82, 83), who have investigated some of the changes in enzymatic pattern that occur, and shown that they are a prelude to polysaccharide synthesis.

During the whole transition from free cells to the final stage of sporulation, the cells lose about 50 per cent of their total protein content. This represents an almost quantitative conversion to carbohydrate since the net loss in weight is only about 7 per cent, some of which could be due to the secretion of ammonia into the medium (28).

At the same time the residual protein shows a considerable rearrangement in enzymatic pattern (82, 83). During the first 16 to 20 hr, covering the period of aggregation, but before formation of stalk and spores, the total protein remains constant or tends to fall slightly. It is at this stage that the major enzymatic transformations occur. Five of the enzymes examined increased

10- to 30-fold. Among these were glucose 6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase, and uridine diphosphoglucose synthetase. Another six enzymes showed smaller (up to 3-fold) increases. The enzymes generally reached a peak at about 20 hr and then began to disappear, some much more rapidly than others.

The enzymatic rearrangement was followed by the extensive breakdown of protein and the formation of polysaccharide which accompany the formation of the mature stalk and spores.

F. Biochemical Differentiation in Mammalian Cells

Development of the mammalian organism from a single cell is characterized by a series of biochemical differentiations, in the course of which some potentialities become expressed, such as the ability to make digestive enzymes or hemoglobin while others disappear (see below) until finally cells emerge with very dissimilar biochemical activities.

In such a complex environment it is not clear how much should be attributed to intracellular rearrangement. Thus, the loss of a particular enzymatic activity in an organ of a developing embryo could be due to active destruction of the enzyme, or it could be due to cessation of synthesis followed by successive dilution of the existing enzyme at each cell generation. Similarly, the appearance of a new enzymatic activity or the accentuation of one which already exists could be the result of intracellular protein turnover or alternatively the enzyme might be synthesized from "new" materials carried by the blood stream. For instance, it is difficult to know whether the development of the tail of the maturing sperm cell is carried out at the expense of existing cell material or whether it represents synthesis *de novo*. Again, it is not clear whether the lack of RNA in spermatozoa is due to intracellular degradation or simply to exclusion of cellular RNA from the head and tail portions when they develop. All that is definitely known is that the spermatozoa differ from the cells they are derived from. They have acquired motility, but they have no RNA and have largely lost their ability to synthesize proteins, although incorporation of amino acids can still take place at a slow rate (7). During spermatogenesis there is also a progressive loss of alkaline phosphatase (42).

An example of biochemical differentiation which is more definitely intracellular is provided by the development of the erythrocyte from the reticulocyte and its further aging. In this instance the change in enzymatic pattern results from the loss, presumably by degradation, of enzymatic functions and not from their development.

Allison and Burn (2) compared the levels of cholinesterase, glyoxalase, and catalase in reticulocytes and erythrocytes, which were separated by differential centrifuging of blood. The reticulocytes contained three times as much of each enzyme as the erythrocytes. Changes in the aging red cell were studied by transfusing a group A subject with group O blood. At intervals, samples were assayed for activity after differential agglutination to separate A and O cells. As would be expected, the cells of the recipient had constant levels of enzyme activities, but the donor cells lost activity at an exponential rate. An erythrocyte at 120 days had about 10 per cent of the amount of enzyme it had had as a reticulocyte.

VIII. Conclusions

In conclusion it will be useful to consider in more general terms the part of turnover in the physiology of the cell. It hardly needs to be stressed that the process is important only when the turnover material is a substantial proportion of the total synthesis. Consequently a low rate of degradation can be disregarded when the rate of net synthesis is high. This is the case in growing bacterial cultures. In nongrowing bacteria, the turnover rate for protein (and RNA), is about 5 per cent per hour, so that in the course of, say, 6 to 8 hr about a third of the cell's protein will have been cycled through the free amino acid pool. This is sufficient to allow extensive formation of inducible or repressible enzymes.

In mammalian cells the turnover rate is lower (1 per cent per hour) but so is the growth rate. Under optimal conditions of tissue culture, the doubling time of cells is about a day, representing an increase of 3 per cent per hour. Even with this high growth rate, turnover material would contribute about 25 per cent of the total protein being synthesized. When the doubling time is greater than 4 days, turnover will account for the bulk of the protein being synthesized at any time.

In this sense a growing mammalian cell corresponds rather to a nongrowing bacterial cell, being dependent upon turnover for achieving

biochemical differentiation. A turnover rate of 1 per cent per hour would still circulate about 25 per cent of the cell protein through the amino acid pool in a day, so that even at this rate the enzymatic pattern of the cell could still be altered considerably in a relatively short time.

This review has been restricted to a consideration of intracellular turnover, that is, to events that could, in principle, take place within a single cell. In practice, however, we are almost always concerned with large populations of cells where intercellular turnover can occur by death of some of the cells and the utilization of the lytic products by the survivors. This would allow not only biochemical differentiation, but also the selection of mutant cells. The population would consequently be biochemically more flexible than one in which there was only intracellular turnover.

Turnover in this sense could be a major factor in the selection of phage-resistant and drug-resistant strains of microorganisms from sensitive populations, and also in more complex types of differentiation such as those which occur in the insect chrysalis or the developing embryo.

IX. Acknowledgments

I wish to thank many of my colleagues in the National Institute for Medical Research for much helpful discussion and criticism, and I am particularly indebted to Drs. M. R. Pollock and H. J. Rogers.

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