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The relationship between the composition of the environment and the control of biosynthesis in bacteria

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In addition to defining the requirements for growth, investigations of the nutrition of micro-organisms have led directly to a greater understanding of the part played by environmental compounds in the functioning and the control of microbial metabolism. At first, attention was focused on the role of growth factors of the vitamin B group (e.g. growth factor replacement technique; Lampen, Roepke & Jones, 1946), routes of biosynthesis (e.g. tryptophan; Snell, 1943), the mode of action of growth factor analogues (e.g. sulphanilamide; Kohn, 1943) and the total activity, and hence the amounts, of various enzymes in bacteria grown in different media. In some instances, enzyme formation was dependent on the presence of the specific substrate (or a closely related analogue) in the medium (inducible enzymes and inducers) whereas the formation of other enzymes (e.g. tryptophanase, threonine deaminase) was markedly reduced if the medium contained glucose (Epps & Gale, 1942). In general, prototrophic bacteria do not excrete appreciable quantities of amino acids, purines, and pyrimidines into the medium. On the other hand, these organisms will preferentially utilize exogenous supplies of these compounds and, in some instances, endogenous synthesis then ceases (Abelson, 1954; Roberts, Cowie, Abelson, Bolton & Britten, 1955). Information of this type, together with the results of detailed studies of the routes of biosynthesis of amino acids and other substances led to the conclusion that two types of mechanism were operative in the regulation of microbial metabolism. In the first type, a substance other than the substrate can control the activity of an existing enzyme in the cell. In the second, the synthesis of enzymes (and other proteins) is directly controlled by substances such as the substrate (inducible enzymes) or the end-product* of a biosynthetic sequence (repressible enzymes). Since many examples are now known (Tables 1 and 2) in which the products 'loop back' and affect the activity and synthesis of various enzymes concerned in their synthesis, such regulatory mechanisms have been termed feedback control mechanisms. Where the synthesis of an enzyme or protein is regulated, the mechanism is termed repression: where the activity of an enzyme is controlled, the mechanism is termed feedback inhibition.

*Throughout this paper, the term 'end-product' means an amino acid, a pyrimidine or purine nucleotide or, with the glyoxylate cycle, a C_4 or C_3 acid: in other words it is the terminal product of a biosynthetic sequence of reactions.

Table 1. *Examples of end-product inhibition of biosynthesis*

Kinetics of the inhibition are denoted by C for competitive, NC for non-competitive and X for complex

End-product inhibitor	Sensitive reaction	Type of inhibition	Organism	Reference
Arginine	*N*-acetyl glutamate ⟶ *N*-acetyl-γ-glutamyl phosphate		*Micrococcus glutamicus*	Udaka & Kinoshita (1958)
Histidine	ATP + 5-phosphoribosyl pyrophosphate ⟶ phosphoribosyl-ATP	NC	*Salmonella typhimurium*	Martin (1963)
Isoleucine	Threonine ⟶ α-ketobutyrate	C	*Escherichia coli*	Umbarger & Brown (1957)
Leucine	α-keto isovalerate + acetyl CoA ⟶ α-carboxy, α-hydroxy isovalerate		*Salm. typhimurium*	Freundlich *et al.* (1963)
Lysine	Aspartate ⟶ β aspartyl phosphate	NC	*E. coli*	Stadtman *et al.* (1961)
Methionine	Homoserine ⟶ cystathionine		*E. coli*	Rowbury (1962)
Methionine*	β-aspartate semialdehyde ⟶ homoserine	X	*Saccharomyces cerevisiae*	Karassevitch & de Robichon-Szulmajster (1963)
Serine†	3-phosphoglycerate → phosphohydroxy pyruvate	X	*E. coli*	Pizer (1963)
Serine	Phosphoserine ⟶ serine	X	*E. coli*	Pizer (1963)
Threonine	Aspartate ⟶ β-aspartyl phosphate	C	*E. coli*	Stadtman *et al.* (1961)
			Sacc. cerevisiae	Patte *et al.* (1962)
Threonine	Homoserine ⟶ 4-phosphohomoserine	C	*E. coli*	Wormser & Pardee (1958)
Threonine	β-aspartate semialdehyde → homoserine		*M. glutamicus*	Nara *et al.* (1961)
Threonine‡	Aspartate → β-aspartyl phosphate		*Sacc. cerevisiae*	de Robichon-Szulmajster & Corrivaux (1963)
Tryptophan	5-phosphoshikimate ⟶ anthranilate; anthranilate + 5-phosphoribosyl pyrophosphate ⟶ ⟶ indole		*E. coli*	Moyed (1960)
Valine	Pyruvate ⟶ α-acetolactate	C	*E. coli*	Umbarger & Brown (1958*a*,*b*)
	Pyruvate + α-keto butyrate ⟶ α-aceto β-hydroxy butyrate	C		Leavitt & Umbarger (1962)
CTP	Aspartate + carbamoyl phosphate⟶ureidosuccinate	C	*E. coli*	Yates & Pardee (1956)
Phosphoenol pyruvate	Isocitrate → succinate + glyoxylate	NC	*Pseudomonas aeruginosa*	Ashworth & Kornberg (1963)

*Homoserine, serine, lysine and threonine also inhibit.
†Glycine inhibits, but is less effective.
‡Homoserine, an intermediate in the sequence, also inhibits.

All micro-organisms require essentially the same monomers, i.e. the twenty amino acids, the two purines (guanine and adenine), the three pyrimidines (uracil, cytosine and thymine) for the synthesis of the major polymers of the cell, the proteins and the nucleic acids, and in bacteria, cell-wall material. Our knowledge is most complete for monomer synthesis in *Escherichia coli*, *Salmonella typhimurium*, *Neurospora crassa*, and the yeasts Saccharomyces and Torulopsis. In such heterotrophs, the katabolism of the carbon and energy source makes energy available in the form of ATP and also provides intermediates for the synthesis of monomers. There are therefore some difficulties in nomenclature (see Davis, 1961) especially if katabolism is thought of only in terms of degradation and energy production. The terms branch point and branch-point compound have been coined to denote the point at which a biosynthetic route leads off from a main katabolic pathway.

Feedback inhibition

A key step in exploiting mutants to elucidate biosynthetic routes was to obtain accumulation of the precursors of the reactions which were blocked as a result of mutation. The accumulation of such precursors was favoured by a medium in which the essential end-product (amino acid, purine or pyrimidine) was present in amounts less than those required for optimal growth (as for example in the cross-feeding technique of Davis, 1950). Accumulation was markedly reduced when the medium contained excess of the end-product (cf. Umbarger, 1961). Thus a uracil-requiring mutant failed to accumulate the intermediate orotic acid when uracil was in excess. This failure was not due to the lack of the appropriate enzymes since the production of orotic acid began immediately the bacteria were transferred to a medium lacking uracil (Brooke, Ushiba & Magasanik, 1954). Meanwhile Abelson and Roberts and their colleagues were using radioactive precursors to trace biosynthetic routes in *E. coli*. The incorporation of ^{14}C from labelled glucose (the carbon and energy source) into an amino acid such as leucine virtually ceased immediately that amino acid was added to the medium. This technique of isotope competition showed that the endogenous synthesis of certain amino acids was completely inhibited if the bacteria could obtain their requirements preformed from the medium (Abelson, 1954; Roberts *et al.* 1955). Such experiments provided evidence that enzymes could be present in bacteria but rendered inactive by compounds derived from the environment (Adelberg & Umbarger, 1953). In prototrophic heterotrophs, the synthesis of the majority of the monomers can be controlled by feedback inhibition (Table 1). The site of feedback inhibition is usually the first enzyme after the branch point from a katabolic pathway, or the first step in the series of reactions which transforms one amino acid into another or, with aspartic acid, into a pyrimidine nucleotide. Thus in the synthesis of isoleucine, threonine is the source of the α-ketobutyrate which is then combined with pyruvate to form acetohydroxybutyrate (Fig. 1). The deamination of threonine to α-ketobutyrate is competitively inhibited by L-isoleucine (Umbarger, 1956; Umbarger & Brown, 1957, 1958*a*,*b*). Likewise in the synthesis of

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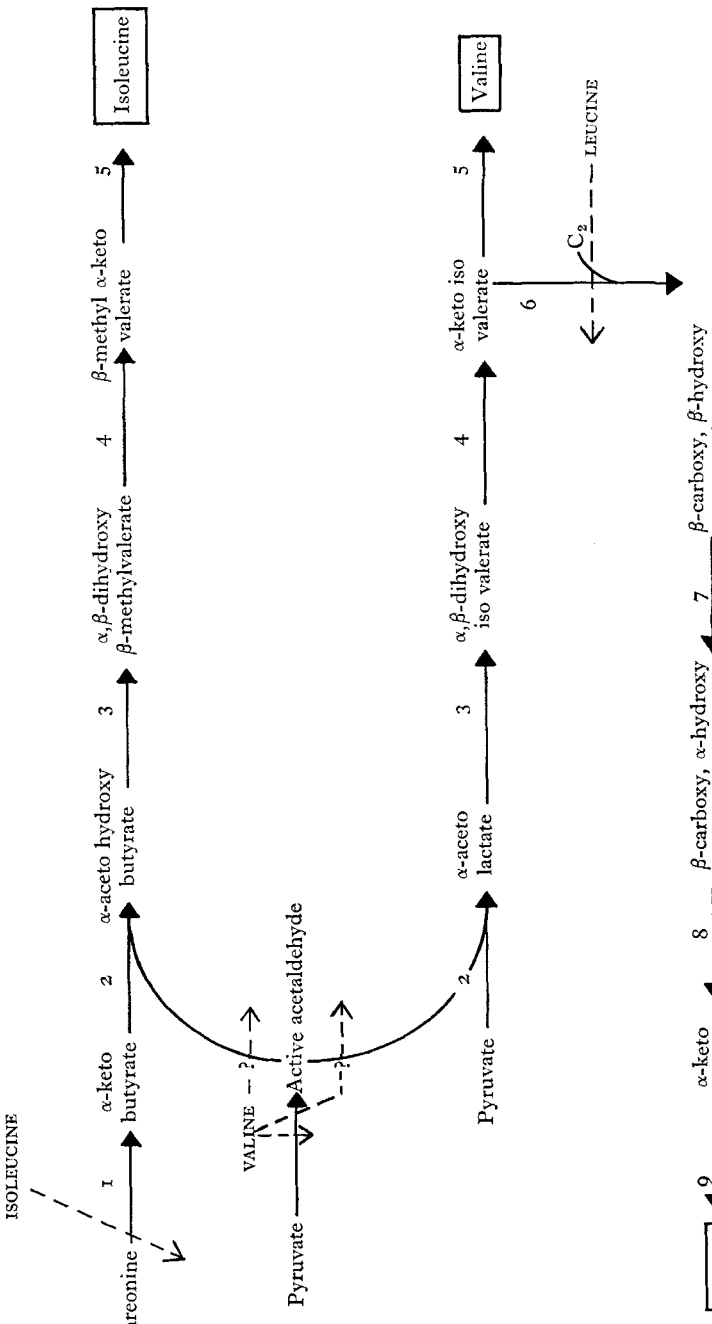

Fig. 1. Synthesis of the branched-chain amino acids in *Escherichia coli*. Products are shown in boxes and reactions 2, 3, 4 and 5 are catalysed by the same enzymes for both pathways: the transaminase for reaction 5 also catalyses reaction 9 in the synthesis of leucine. Sites of feedback inhibition are shown by broken arrows.

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histidine, the first enzyme (phosphoribosyl-ATP pyrophosphorylase):

ATP + 5-phosphoribosyl-1-pyrophosphate $\rightleftharpoons$ *N*-1, (5′-phosphoribosyl)-ATP + pyrophosphate

is inhibited non-competitively by L-histidine (Martin, 1963), whereas in serine synthesis:

$$\text{D-3-phosphoglycerate} \xrightleftharpoons{\text{DPN}} \text{phosphohydroxypyruvate} \xrightleftharpoons{\text{glutamate}} \text{phosphoserine} \longrightarrow \text{serine}$$

the 3-phosphoglycerate dehydrogenase is inhibited by L-serine (kinetics of the inhibition neither competitive nor non-competitive) (Pizer, 1963). Similarly in the set of reactions leading to the formation of uridylic acid and thence to cytidylic acid and the deoxyribose pyrimidine nucleotides, it is again the first enzyme aspartate transcarbamoylase (carbamoylphosphate: L-aspartate carbamoyltransferase):

aspartate + carbamoylphosphate $\longrightarrow$ ureidosuccinate $\longrightarrow \longrightarrow$ orotate $\longrightarrow \longrightarrow$ uridine-5′-phosphate

which is inhibited by pyrimidine nucleotides, and especially by cytidine triphosphate (CTP) (Yates & Pardee, 1956; Shepherdson & Pardee, 1960).

Feedback inhibition in branched pathways

Some amino acids (e.g. valine, leucine and isoleucine) are formed by biosynthetic routes which involve common enzymes, but different intermediates, whereas other amino acids may be derived from a common precursor amino acid and one or more of the steps in the sequence may be common to the synthesis of two or more amino acids (e.g. the aspartic acid family of amino acids, Fig. 2).

In the synthesis of valine and isoleucine (Fig. 1) control by feedback inhibition is achieved by valine regulating the formation of acetolactate (Umbarger & Brown, 1958*a*,*b*). Since the same enzyme system appears to be involved in the synthesis of acetohydroxybutyrate (Leavitt & Umbarger, 1961), inhibition by valine not only affects the synthesis of valine but also isoleucine. Such an interaction explains why some strains of bacteria are especially sensitive to exogenous supplies of valine (e.g. *E. coli* K12; Leavitt & Umbarger, 1962) and will not grow unless isoleucine is also present (see also the original observation of Gladstone (1937) with *Bacillus anthracis*). The effective inhibition of the synthesis of two end-products by only one of the end-products would seem to be a disadvantage as far as the bacteria are concerned, and perhaps separate control systems have not been evolved, since in nature valine is usually accompanied by isoleucine. When the first reaction in a biosynthetic sequence is involved in the synthesis of more than one amino acid, or the overall reaction (e.g. the deamination of threonine) also plays an essential role in katabolism and energy production, such difficulties are circumvented in some organisms by the presence of more than one enzyme carrying out the required reaction and each of these enzymes is controlled in a different and specific way (see Umbarger, 1961). Thus *E. coli* can develop two distinct threonine deaminases with very different properties—only the 'biosynthetic' one is inhibited by L-isoleucine whereas the 'katabolic' one is not formed in the presence of glucose (Umbarger & Brown, 1957).

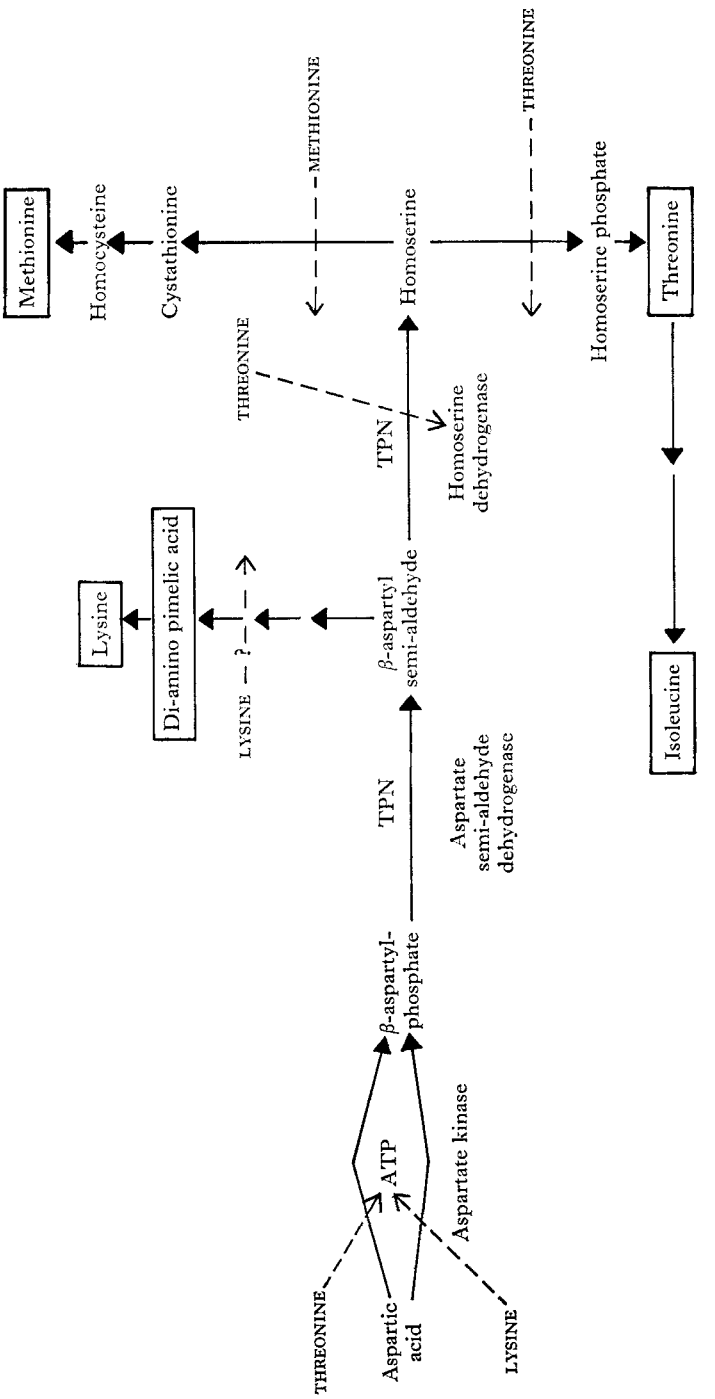

Fig. 2. Synthesis of the aspartic acid family of amino acids in *Escherichia coli*. For explanation, see legend to Fig. 1.

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In *E. coli* there are at least two different aspartate kinases (Stadtman, Cohen, LeBras & de Robichon-Szulmajster, 1961) for providing β-aspartyl phosphate, an intermediate in the synthesis of lysine, diaminopimelic acid (DAP), homoserine, threonine and methionine (the aspartic acid family of amino acids, Fig. 2). The enzymes catalyse the same reaction, but one is inhibited by lysine and the other by threonine. Hence, if the medium contains either of these amino acids, the flow of metabolites along the pathway is reduced, but not totally inhibited. Lysine and threonine together do not inhibit completely the aspartate kinase activity of extracts of *E. coli* (another aspartate kinase present?), and so aspartic acid can still give rise to methionine (and the limited amounts of DAP required for cell-wall synthesis), though not necessarily at an optimal rate. However, feedback control of one of the branch-point enzymes does not by itself provide for the channelling of the limited supply of an intermediate into the synthesis of products which cannot be obtained from the medium. It is likely that this controlled distribution is achieved by additional sites of feedback control near to or after the point where the main pathway gives rise to secondary branches leading to the synthesis of one specific amino acid. Thus in *E. coli*, homoserine is formed from β-aspartyl phosphate by homoserine dehydrogenase and this enzyme is non-competitively inhibited by L-threonine (methionine and lysine have no effect) (Patte, Le Bras, Loviny & Cohen, 1963). So, if the medium can provide threonine, supplies of β-aspartyl semialdehyde are reduced, but the synthesis of lysine rather than threonine is favoured. Since inhibition of the dehydrogenase is not complete, methionine synthesis is still possible, though it may be reduced so much that the growth rate is limited (as in the experiments of Patte *et al.* 1963). Methionine rather than threonine synthesis is favoured because threonine inhibits the conversion of homoserine to 4-phosphohomoserine, the precursor of threonine (Wormser & Pardee, 1958). In *Saccharomyces cerevisiae* in which aspartic acid is also the precursor of threonine and methionine, but not of lysine, there appears to be only one aspartate kinase, which is inhibited by threonine and not by methionine (de Robichon-Szulmajster & Corrivaux, 1963). On the other hand, the homoserine dehydrogenase is inhibited by methionine and to a lesser extent by threonine. Hence, in this yeast, threonine synthesis is controlled via the first enzyme whereas methionine synthesis is controlled at the third enzyme in the pathway (Karassevitch & de Robichon-Szulmajster, 1963).

Summary of main features of feedback inhibition

It is now possible to survey the results which have been obtained in this field and summarize the main features of feedback inhibition as follows.

(1) The regulatory enzyme for a pathway leading from main katabolic routes is the first enzyme after the branch point (e.g. in serine and histidine biosynthesis). Where an amino acid is an intermediate in the synthesis of other monomers, the amino acid becomes the branch point for the biosynthetic route and the regulatory enzyme is the one for which this amino acid is the substrate (e.g. in the synthesis of the aspartic acid family, isoleucine and pyrimidines).

(2) If the biosynthetic route has two or more subsequent branches, there may be other sites of feedback inhibition apart from the branch-point enzyme.

(3) It is the end-product of the pathway, and not a derivative, which acts as the inhibitor and the inhibitor does not react chemically with either the substrate(s) or the product(s) of the reaction: unequivocal proof is only possible when the purified enzyme is available (e.g. aspartate transcarbamoylase, Shepherdson & Pardee, 1960): in the majority of the experiments, the enzyme preparations have not been highly purified, and indeed enzymes of this type appear to be rather unstable (e.g. the first histidine enzyme; Martin, 1963).

(4) Specificity as regards the inhibitor is very high, and apart from the end-product it is usually only close analogues which show any activity.

(5) No general statement about the kinetics of inhibition can be made at present: whereas some inhibitors act competitively, others act non-competitively, and some are neither truly competitive nor truly non-competitive.

(6) The binding of the inhibitor is freely reversible, and the enzyme becomes fully active after the removal of the inhibitor.

Mechanism of feedback inhibition

Of the various microbial enzymes which are sensitive to inhibition by terminal metabolites, only one (aspartate transcarbamoylase) has so far been highly purified and crystallized, thus permitting the main features of the inhibition to be determined in precise conditions (Gerhart & Pardee, 1962). However, since comparable results have been obtained with other similar enzymes which have not been extensively purified, it has been concluded that the general features of the inhibition as exemplified by aspartate transcarbamoylase are common to the whole group of enzymes which show feedback inhibition. The basic problem is to explain how a substance of small molecular weight and bearing little or no structural resemblance to the normal substrate can nevertheless act as an effective inhibitor. There is evidence that the site which activates the substrate (the active centre) is distinct from the site which binds the inhibitor, and Gerhart & Pardee (1962) also suggested that the binding of the inhibitor brings about a deformation of the enzyme molecule. If this is so, then clearly the catalytic properties of the enzyme may be greatly altered. Monod, Changeux & Jacob (1963) have collected the available experimental evidence and have presented cogent arguments in favour of the general proposition that when the activity of an enzyme (or another type of protein) is regulated by a substance of small molecular weight, this is due to the ability of the regulatory agent (allosteric effector) to bind to the protein at a specific site (the allosteric site) and thus induce a conformational change (allosteric transition) in the protein, and so alter its catalytic properties.

The best evidence that the sites for binding the substrate and the effector are different comes from the findings that, after exposure to mild heat treatment (e.g. 2 min at 49°), mercurial substances ($HgCl_2$, *p*-chloromercuribenzoate) or ageing, aspartate transcarbamoylase (Gerhart & Pardee, 1962), the aspartate kinases (Stadtman *et al.* 1961), homoserine dehydrogenase (Patte *et al.* 1963) and the histidine first

enzyme (Martin, 1963) all become insensitive to the appropriate inhibitor, but the affinity (i.e. Km) for the normal substrate is not changed. In some circumstances, the effects of such treatments can be reversed (Martin, 1963). Three conclusions can be made from studies of the reaction kinetics of the native and desensitized enzymes: (1) the binding site for the substrate and effector are different, (2) there is more than one site for each in every native enzyme molecule and (3) the activity of each binding site is potentiated by homologous neighbouring sites (i.e. there is co-operation). Since there is more than one substrate site, the native enzyme may be a polymer of two or more sub-units and the disposition of the sub-units could be responsible for the co-operative interactions between the binding sites. Desensitization may therefore be due to disturbance of the normal dispositions and involve small displacements of polypeptide chains or even complete dissociation into sub-units. The desensitized enzymes do not show substrate co-operation effects. Though radical changes can occur, e.g. the sedimentation coefficients of native aspartate transcarbamoylase and homoserine dehydrogenase are greater than those of the desensitized enzymes, this does not always happen (e.g. the histidine first enzyme). Moreover desensitized histidine enzyme can still bind histidine and then becomes more sensitive to digestion by trypsin, implying that the histidine can still evoke a change in protein structure (Martin, 1963).

As far as microbial enzymes are concerned, the proposition that a feedback inhibitor provokes a change in the structure of an enzyme such that its catalytic properties are reduced is based at present largely on inference and by analogy with various mammalian proteins. As Monod *et al.* (1963) point out, compounds of small molecular weight profoundly alter the association of sub-units of the mammalian enzymes glutamate dehydrogenase, acetyl-CoA-carboxylase and muscle α-glucan phosphorylase, and the combination of haemoglobin with oxygen alters the crystalline form of the molecule and affects the velocity with which succeeding molecules of oxygen attach themselves to neighbouring haem groups. Though changes in enzyme activity cannot always be correlated with the state of aggregation of the sub-units, the main point is established, namely substances of small molecular weight and often totally unrelated to the substrate can alter the overall structure of a protein.

Repression and induction

The synthesis of several specific proteins in bacteria is regulated by the presence of appropriate natural metabolites in the environment (see Mandelstam, 1960). Where such a substance causes inhibition of protein synthesis, the process is termed repression: where a substance evokes the increased synthesis of a specific protein which is present in comparatively small amounts or not detectable in bacteria grown in its absence, the process is termed induction.

Repression in the biosynthesis of amino acids. Numerous examples are now known in which growth of bacteria in the presence of an amino acid causes the repression of one or more of the enzymes which participate in the biosynthesis of the exogenous amino acid (Table 2). Having found that the acetylornithine deacetylase activity of

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Table 2. *Examples of substances effecting repression of protein synthesis*

Effector	Repressible enzyme(s) or other protein	Organism	References
Arginine	Acetylornithine deacetylase, ornithine transcarbamoylase and the others in Fig. 3	*Escherichia coli*	Vogel (1957) Gorini & Maas (1957), Gorini *et al.* (1961)
Histidine	(Several)	*Salmonella typhimurium*	Ames & Garry (1959)
Isoleucine + leucine + valine	Enzymes for reactions 2, 3, 4, 5 (and 1) in Fig. 1.	*Salm. typhimurium*	Freundlich *et al.* (1963)
Isoleucine	Threonine dehydratase (deaminase)	*E. coli*	Umbarger & Brown (1958*a*,*b*)
Lysine	*meso*-diaminopimelate decarboxylase	*E. coli*	Patte *et al.* (1962)
Lysine	Aspartate kinase	*E. coli*	Patte *et al.* (1962)
Threonine	Aspartate kinase	*Saccharomyces cerevisiae*	Patte *et al.* (1962) de Robichon-Szulmajster & Corrivaux (1963)
Methionine	Cystathionine synthase	*E. coli*	Rowbury (1962)
Methionine	Cystathionase	*E. coli*	Rowbury & Woods (1961)
Methionine	Homocysteine methyltransferase	*E. coli*	Cohn *et al.* (1953) Wijesundera & Woods (1953)
Threonine	Homoserine dehydrogenase	*E. coli*	Patte *et al.* (1963)
Methionine	Homoserine dehydrogenase	*Sacc. cerevisiae*	Karassevitch & de Robichon-Szulmajster (1963)
Methionine	Homoserine dehydrogenase	*Micrococcus glutamicus*	Nara *et al.* (1961)
Tryptophan	Tryptophan synthase	*E. coli*	Cohn *et al.* (1953)
Benzoate	Three enzymes for mandelate utilization	Pseudomonas	Mandelstam (1964)
Pyruvate	Tryptophanase, D-serine dehydratase (deaminase)	*E. coli*	McFall and Mandelstam (1963)
Succinate	Isocitrate lyase	Pseudomonas	Smith & Gunsalus (1957)
Glucose	β-galactosidase	*E. coli*	Monod (1942)
Galactose	β-galactosidase	*E. coli*	McFall & Mandelstam (1963)
Uracil	Aspartate transcarbamoylase	*E. coli*	Shepherdson & Pardee (1960)
O_2*	Cytochrome *c*	*E. coli*	Wimpenny *et al.* (1963)
O_2*	Hydrogenase, formic hydrogenlyase	*E. coli*	Pichinoty (1962)

*Whether O_2 itself or a substance formed in aerobic metabolism is the true effector is not known.

bacteria grown in the presence of arginine was markedly reduced, Vogel (1957) showed that this was not due to lack of acetylornithine, the substrate of the enzyme. As long as the medium contained arginine, the bacteria did not form this enzyme, even though large amounts of acetylornithine were added to the culture. In other words, the end-product, arginine, repressed the synthesis of the acetylornithine deacetylase. On the basis of these and other experiments Vogel (1957) concluded that repression and induction were not unrelated phenomenons, but were complementary manifestations of the basic mechanism whereby the synthesis of specific proteins was regulated in bacteria. Contrary to former views, inducers were not templates for shaping the active centres of the enzymes, but were to be thought of as compounds which inhibited the action of endogenous repressor substances.

In biosynthetic pathways, repression is brought about by the end-product itself and not by a derivative. Thus repression of an enzyme can still take place even if there is a mutational block between the susceptible enzyme and the final product of the sequence. Repression is highly stereospecific and is reversible. Enzymes which can be inhibited by feedback inhibitors are often, but not always, repressible. Unlike feedback inhibition, in which the end-product only affects one or two enzymes in a sequence, it is frequently found that several enzymes in the pathway may be repressed at the same time, e.g. in the synthesis of arginine from glutamate (Fig. 3) arginine can cause the repression of seven of the enzymes (Gorini, Gundersen & Burger, 1961; Maas, 1961; Vogel, 1961). Similarly in the synthesis of histidine, the last four enzymes are repressed together (Ames & Garry, 1959). The same enzyme in different organisms may be repressed by different substances (e.g. homoserine dehydrogenase: Table 2).

Derepression. If an organism can obtain an essential monomer from the environment at a rate equal to or greater than that at which it is being utilized, then these conditions favour repression (cf. the results of Ennis & Gorini, 1961). The cell therefore benefits in that available supplies of energy and of carbon and nitrogen can be utilized in potentially more useful ways. On the other hand, what happens if the rate of protein or nucleic acid metabolism is limited because the availability of an essential monomer is not sufficient and limits the rate of growth? In such conditions, it is found that enzymes which are repressed when the metabolite is in excess are now produced in markedly greater quantities, i.e. the synthesis of these enzymes becomes derepressed (Gorini & Maas, 1957). For example, by using suboptimal amounts of arginine to limit the growth of an arginine-requiring mutant of *E. coli* in continuous culture, Gorini & Maas (1957) were able to obtain bacteria which contained up to forty times the normal content of ornithine transcarbamoylase. There are various ways in which derepression may be achieved (for list see Moyed & Umbarger, 1962) and these techniques are of great practical importance, especially to biochemists who seek a rich source of an enzyme which it is desired to purify (e.g. the aspartate transcarbamoylase content of *E. coli* has been increased 1000 times above the normal by Shepherdson & Pardee (1960)). Derepressed micro-organisms are also of commercial significance in the production of amino acids and other compounds since conditions which favour derepression are also those in which

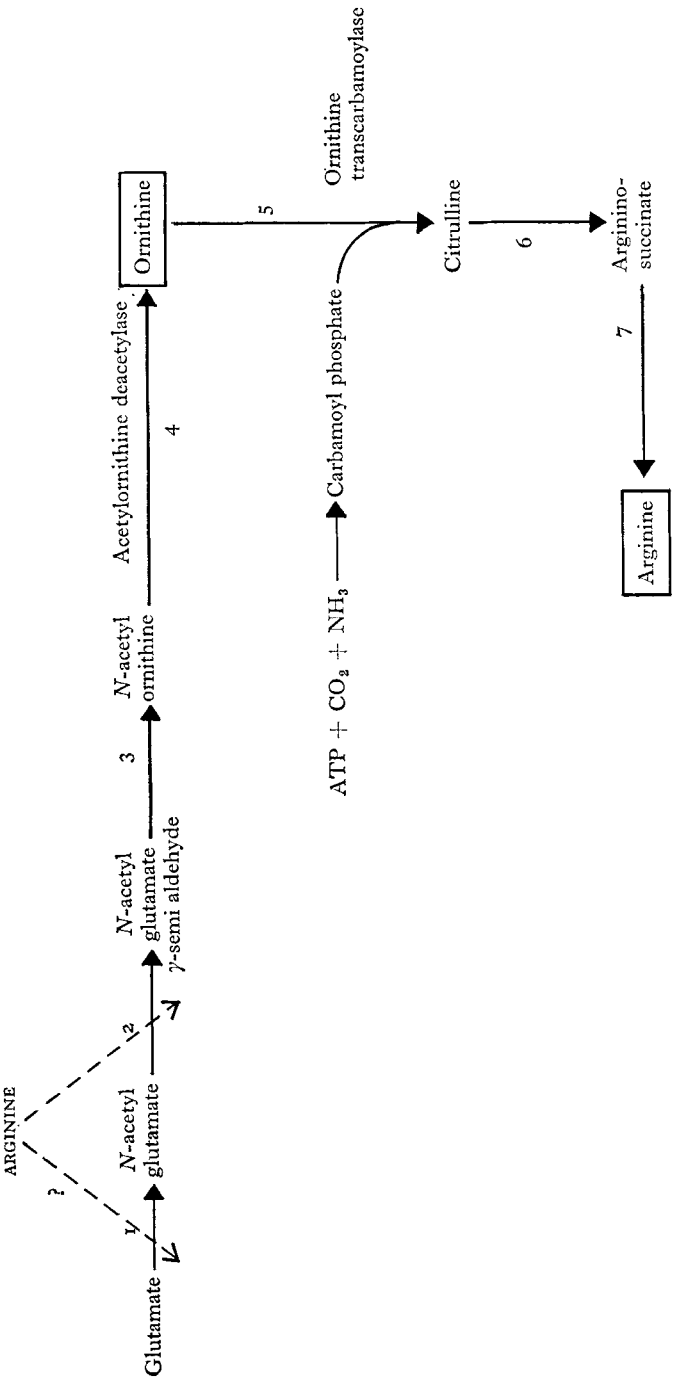

Fig. 3. Synthesis of arginine in *Escherichia coli*. For explanation, see legend to Fig. 1.

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feedback inhibition is minimal, and consequently the precursors of a genetically blocked reaction will accumulate without hindrance in the medium from which they can be isolated in relatively large amounts.

Repression in a branched pathway. When a sequence of reactions involving common enzymes leads to the biosynthesis of more than one end-product, there is evidence that since excess of only one of the products cannot totally repress any of the enzymes, overall inhibition of the whole pathway is avoided. Thus in the synthesis of the branched-chain amino acids there are several common enzymes (Fig. 1) but complete repression requires the presence of all three amino acids (isoleucine, valine and leucine) (Freundlich, Burns & Umbarger, 1963). It has been suggested that this type of control system be named 'multivalent repression' (Freundlich *et al.* 1963). It is, however, important to note that the control mechanisms in the amino acid-requiring mutants of salmonella used by Freundlich *et al.* (1963) may be different from those in the wild type parent organism (Armstrong, Gordon & Wagner, 1963; Armstrong & Wagner, 1963).

Repression of isocitrate lyase and of cytochrome c. Repression is a control mechanism which is found in other situations as well as the synthesis of amino acids and nucleotides. It also regulates the synthesis of isocitrate lyase, the synthesis of cytochrome *c* in enterobacteria and the synthesis of certain enzymes concerned with the utilization of alternative sources of carbon and energy (katabolite repression, see below).

In bacteria (*Pseudomonas aeruginosa*, *P. ovalis* Chester, *Micrococcus denitrificans*) which are growing on acetate, or compounds which give rise to acetate, as sole carbon source, the net synthesis of C_4 dicarboxylic acids (required for the synthesis of aspartic acid, pyruvate and such) is achieved by means of the glyoxylate cycle, whilst energy is made available from the combustion of acetate in the tricarboxylic acid cycle (Kornberg & Elsden, 1961). The key enzymes in the glyoxylate cycle are malate synthase, for the condensation of acetyl CoA with glyoxylate to give malate, and isocitrate lyase, which cleaves isocitrate into succinate and glyoxylate. Malate synthase activity has been found in a large number of micro-organisms irrespective of the growth conditions (i.e. it is a constitutive enzyme), whereas isocitrate lyase is usually only found when the bacteria use acetate as the sole carbon source. The presence of even small amounts of C_3 or C_4 acids (especially succinate) in the medium represses the synthesis of isocitrate lyase. The regulation of the flow of acetate to the glyoxylate cycle for the synthesis of C_4 acids (and eventually cellular constituents) and to the tricarboxylic acid cycle to provide the requisite energy appears to be accomplished by feedback control at the level of isocitrate lyase. Succinate and oxaloacetate are both strong inhibitors of the enzyme (Smith & Gunsalus, 1957; Umbarger, 1960), and more recently phosphoenol pyruvate has been shown to be an especially powerful non-competitive inhibitor (Ashworth & Kornberg, 1963).

An unexpected and unusual example of a repression effect has been found in *E. coli* and other related facultative anaerobes. Though these organisms had previously been found to contain only cytochrome b_2, Gray and his colleagues (Gray, Wimpenny, Hughes & Ranlett, 1963; Wimpenny, Ranlett & Gray, 1963) discovered

that a typical soluble cytochrome *c* is synthesized in anaerobic but not aerobic conditions. When the cultures were aerated, synthesis stopped and indeed some of the cytochrome *c* seemed to be broken down. This would appear to be an example of the synthesis of a protein being completely repressed by O_2, or perhaps more likely, by a compound formed and accumulated by bacteria metabolizing in the presence of O_2. Oxygen is also known to repress the formation of hydrogenase and formic hydrogenlyase (cf. Pichinoty, 1962).

Katabolite repression. The phenomenon of repression has so far been discussed mainly in terms of the way in which it affects enzymes in biosynthetic pathways. However, there were many much older observations which showed that the presence of certain enzymes in bacteria depended on the composition of the medium. Several of these enzymes were not formed except in the presence of the specific substrate or a chemically related analogue, i.e. they were inducible enzymes. But if glucose was also present, this assumed a dominant role and the inducible enzymes were not formed until the supply of glucose had been exhausted. One expression of this phenomenon was the two distinct growth phases (diauxic growth) shown by cultures growing in the presence of a mixture of sugars such as glucose (which was used preferentially) and lactose (which was used after the induced formation of β-galactosidase) (Monod, 1942). Enzymes which show this 'glucose effect' are katabolic enzymes, i.e. they are concerned with the utilization of alternative sources of carbon and energy, and the substrates are converted (either directly or with the aid of other enzymes) to metabolites which the bacteria can obtain independently and more readily from glucose. Explanations of the glucose effect based on inhibition of transport of the inducer, preferential synthesis of the 'glucose' enzymes, and depletion of inorganic phosphate in the internal pool, have received little or no experimental support (see Magasanik, 1961). Neidhardt & Magasanik (1956, 1957) therefore put forward the idea that the katabolites which are formed from glucose are able to repress the synthesis of enzymes which would only serve to increase the already large intracellular pools of these compounds. They proposed the term 'katabolite repression' for the inhibitory effect of glucose on enzyme formation. Support for this conclusion comes from the work of McFall & Mandelstam (1963), who studied tryptophanase and D-serine dehydratase (deaminase)—two inducible enzymes which are not produced by bacteria growing in the presence of glucose. They found that pyruvate, the product which was to be used as a source of carbon and energy, was a specific and effective inhibitor of the synthesis of these enzymes. In more recent studies of the induced formation of the sequence of enzymes which enables Pseudomonas to utilize mandelate as a source of carbon and energy, Mandelstam (1964) has shown that when an intermediate such as benzoate is added to media containing mandelate, the synthesis of enzymes which convert mandelate to benzoate, is repressed. The repressive action of benzoate can be overcome by increasing the concentration of the inducer, mandelate. Hence, when an intermediate becomes the main carbon and energy source, it can repress the formation of those enzymes which would result in its formation from other substances in the medium. When biosynthesis is much reduced, e.g. because an essential nutrient such as guanine or uracil is lacking,

the katabolism of any substance which serves as a source of carbon and energy can result in repression phenomena. Thus β-galactosidase synthesis in guanine-deficient cells is repressed by succinate, although succinate has no effect in growing guanine-supplemented cultures (Mandelstam, 1957). Hence in testing the biosynthetic capacity of bacteria in various media, it is necessary to be aware of the possibility of katabolite repression (Magasanik, 1961).

Genetic control of repression and induction. Since enzymes are proteins, the repression of enzyme synthesis is an extension of the more general problem of protein synthesis and the way in which it is controlled. Repression is an expression of the genotypic potentialities of the bacterium, since mutations are possible so that a particular enzyme can no longer be repressed by the appropriate end-product, e.g. tryptophan synthase (Cohen & Jacob, 1959) and ornithine transcarbamoylase (Gorini *et al.* 1961). The deoxyribonucleic acid of an organism contains the necessary information which can direct the synthesis of specific proteins and Jacob & Monod (1961) used available genetic data, together with experimental observations on the induction and repression of enzymes and various aspects of phage reproduction, to formulate a general scheme for the genetic control of protein synthesis (Fig. 4). The specific information determining the sequence of amino acids in a specific protein is contained in the corresponding 'structural gene'. This information can be reproduced in a special type of ribonucleic acid (messenger RNA) which in association with ribosomes and other components directs the synthesis of a polypeptide chain having the required sequence of amino acids. Whether a structural gene is permitted to make messenger RNA is determined by an adjacent genetic determinant (the operator). The genetic loci for several enzymes which function sequentially in a metabolic pathway may lie adjacent to one another and be controlled by the same operator, so that either none or all of these genes can enter into the production of messenger RNA. This functional unit of operator plus structural gene(s) is known as an operon. Whether the operator permits messenger RNA synthesis is controlled by a regulatory gene elsewhere on the chromosome. This regulator gene causes the production of a component (cytoplasmic repressor) which is specific for a particular operator and when bound to it prevents the associated structural genes from engaging in messenger RNA synthesis. Recent developments suggest that the component formed by the regulatory gene is a protein. Monod *et al.* (1963) suggest that the phenomenons of repression and induction can be explained on the basis that this protein has allosteric properties, and has two binding sites, one specific for the appropriate operator and the other for a small molecular weight effector (i.e. an end-product such as an amino acid or an inducer). In repressible systems, they propose that the regulator gene brings about the synthesis of a protein which is inactive (aporepressor) but, on combination with the end-product, there is an allosteric transition and the protein can now bind to and inhibit the appropriate operator. In inducible systems, it is proposed that the protein is active by itself and that combination with the inducer so modifies its structure that it can no longer bind to the operator: hence messenger RNA synthesis and induced enzyme formation begins (Monod *et al.* 1963). In katabolite repression, an inducer is required before any synthesis is possible, and an

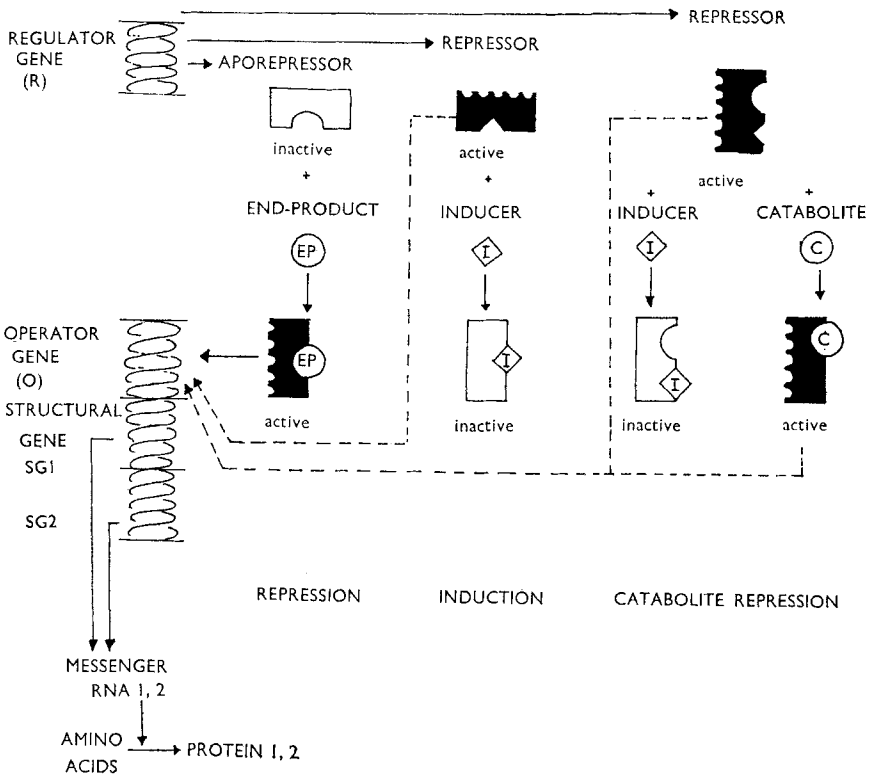

Fig. 4. Scheme for the genetic control of protein synthesis in bacteria (based on Monod, Changeux & Jacob (1963) and Jacob & Monod (1961)). The functional unit, the operon, equals the operator gene (O), plus the structural gene(s) (SG) it regulates. The cytoplasmic repressor substance produced by the regulator gene is shown in the active form (i.e. inhibits the operator) by solid symbols and in the inactive form by open symbols. Suggested mechanisms to account for repression, feedback inhibition and katabolite repression are shown.

end-product such as pyruvate can cause repression. It may therefore be proposed that in such cases the regulatory gene directs the synthesis of an active repressor, and that if this material binds the inducer it becomes inactive (induced enzyme formation possible), and if it binds an end-product, there is an allosteric transition and it becomes unable to bind the inducer and therefore remains active (no induced enzyme formation). However, much more experimental evidence is required, and especially the isolation of cytoplasmic repressors for regulating specific protein synthesis in vitro, before the details of the genetic control of protein synthesis can be established unequivocally. At the present time, it is known that an inducer promotes the formation of the specific messenger RNA and that, after this has taken place, bacteria will synthesize an inducible enzyme in the absence of the inducer (Pardee & Prestidge, 1961). Similarly, Magasanik & Nakada (quoted by Magasanik, 1963) have found that 'katabolite repression' involves inhibition of the synthesis of the appropriate messenger RNA: once the messenger RNA for β-galactosidase has been formed, the addition of a readily utilizable source of energy, such as glucose, has no effect on the synthesis of the enzyme.

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Control of nucleic acid synthesis: 'shift up' and 'shift down' phenomenons

Though the details of the systems which control the biosynthesis of amino acids and proteins are now becoming clear, information regarding the regulation of the synthesis of nucleic acid is at present rudimentary. The biosynthetic routes for the synthesis of pyrimidine and purine nucleotides have been established, and evidence has been obtained that the production of these nucleotides can be controlled by repression and feedback inhibition of one or more enzymes in the biosynthetic sequence (Yates & Pardee, 1956; Gerhart & Pardee, 1962; Magasanik & Karibian, 1960). Interest now centres on the regulation of polymerization of the nucleotides. When *S. typhimurium* and *E. coli* are transferred from a medium supporting only a low growth rate to a richer medium in which the growth rate is higher (i.e. 'shift up'), the synthesis of RNA is immediately increased and, indeed, even proceeds for a few minutes at a rate greater than that characteristic of the new medium. Meanwhile, DNA and protein synthesis continue at the preshift rate for about 20 min before there is an abrupt change to the rate which is characteristic of the richer medium (Kjeldgaard, Maaløe & Schaechter, 1958). On the other hand, bacteria going from a richer to a poorer medium, i.e. to a lower growth rate ('shift down'), show cessation of RNA synthesis for about 30 min (Kjeldgaard *et al.* 1958). These results can be correlated with the observation that when growth is controlled in a continuous-culture apparatus, the composition of bacteria is related (exponentially) to their rate of growth (Schaechter, Maaløe & Kjeldgaard, 1958). Media which are qualitatively different yet promote the same growth rate give rise to bacteria of the same size and composition but, for the same medium, increase in growth rate causes an increase in total mass, total RNA and average number of nuclear bodies per cell. The increased RNA content is mostly accounted for by an increase in the number of ribosomal particles per cell. Hence, immediately after transfer to a richer medium, the bacteria require to synthesize more RNA before their overall composition approaches the value which is characteristically produced at the higher growth rate in the richer medium. Conversely, when they are transferred from a richer to a poorer medium, there appears to be a surfeit of RNA and synthesis of RNA is halted until cellular composition is adjusted to a value which is nearer to that normally produced at the slower growth rate. Amino acid-requiring mutants of *E. coli* cannot synthesize nucleic acids in the presence of chloramphenicol (used to stop concomitant protein synthesis) unless a trace amount (10^{-6} M) of the essential amino acid is added to the experimental system (Gros & Gros, 1956). Whether the changes in the rate of synthesis of RNA during acceleration or deceleration of the growth rate (as in 'shift up' or 'shift down' conditions) are due to, and controlled by, alterations in the amounts of amino acids in the internal pools of the bacteria has not yet been established. There is some indirect evidence in favour of this theory (Kurland & Maaløe, 1962). Stent & Brenner (1961) and Kurland & Maaløe (1962) have independently suggested that amino acids may function as regulators by combining with the transfer (soluble) RNA, material which in the uncombined form may act as a repressor of ribosomal

RNA synthesis. In 'shift down' conditions, reduction in the rate of synthesis of ribosomal RNA is not due to a lack of either the nucleotide substrates or the appropriate enzymes (Neidhardt & Fraenkel, 1961).

This brief and selective review makes clear that the regulation in vivo of the synthesis of cellular materials and the activity of many enzymes is ultimately a function of the concentration of various metabolites in the internal metabolic pools of bacteria. These concentrations will be the resultant of the rate of utilization, the rate of endogenous production and, for metabolites present in the medium, the rate of entry into the cell. (Some transport systems are also subject to induction and repression, but are not discussed here.) If a rise in the concentration of a metabolite above a certain level results in repression, induction or feedback inhibition, then the end result is to enable the bacterium to make more economical use of the available sources of energy, carbon and nitrogen.

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The nutrition of certain intestinal protozoa

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The nutritional requirements of some of the free-living protozoa, such as members of the genus Tetrahymena, are now well understood (Kidder & Dewey, 1951), but the nutrition of certain non-free-living protozoa still presents many problems. The object of this review is to consider *Entamoeba histolytica* or the 'dysentery amoeba' whose habitat is the large intestine of man, and the rumen ciliate protozoa which are normally found in considerable numbers in the first compartment of the stomach of ruminants.

The nutrition of *E. histolytica* is of importance in relation to the pathogenic effect of the organism. The study of rumen ciliate nutrition, however, has been stimulated by consideration of possible beneficial contributions to the nutrition of the host. The products of rumen ciliate metabolism are passed on to the host either directly or after further breakdown by rumen bacteria and the effect of the ciliates upon the