on the present day surface, of elements of groups C and D, together with occasional implements in iron. The exact relationship of these artifacts will only be established by careful excavation of *in situ* material.

The main flow of the Mukutan river was diverted towards the north by the Njemps tribesmen 100 or more years ago to irrigate the wash plain for agriculture.

Raw materials

An ongoing study is concerned with identification of the various rock types used by makers of the tools in groups A–D. The earliest tool kits reveal great petrological variety including different trachytes, phonolites and welded tuffs, together with occasional basalts. The Acheulian raw materials of group B are restricted to trachyte with some phonolite and a few small pieces in welded tuff. Group C is almost entirely based on water rounded blocks and cobbles of phonolite.

Various volcanic rocks occur in the area surrounding the artifact sites. Thus it is possible to carry out petrological and geochemical investigations, including trace element analyses, to identify the source rocks of the artifacts. This will permit an estimate of the minimum ranges over which the early stone technologists travelled to collect their raw materials.

The fact that two more individuals of robust australopithecines have been found from horizons yielding abundant artifacts (others are known from Olduvai Gorge and East Rudolf), poses the question: "Who made the tools?"

This work forms part of a programme of research financed by the Natural Environment Research Council, the Boise Fund of the University of Oxford, the Wenner-Gren Foundation, New York, and the L. S. B. Leakey Foundation. We thank Dr S. Weaver, and R. Foley for help with field work and Dr Mary Leakey for valuable discussion. Assistance in the field also came from Mr and Mrs A. Cooper and Mr M. Fulton. The Kenya Ministry of Natural Resources made research permits available, and Mr R. E. F. Leakey and the Trustees of the National Museums of Kenya provided facilities in Nairobi. J. W. K. Harris of the Department of Anthropology, University of California provided a preliminary analysis of the samples from group A.

Received July 30; accepted October 10, 1975.

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Significance of autogenously regulated and constitutive synthesis of regulatory proteins in repressible biosynthetic systems

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The functional implications of the different modes of regulation have been examined systematically. The results lead to certain predictions. The regulatory protein in repressor-controlled systems is constitutively synthesised. In activator-controlled systems synthesis of the regulatory protein is autogenously regulated. There is favourable agreement between these predictions and published experimental evidence.

REGULATION of specific enzyme synthesis has been most thoroughly characterised in the inducible catabolic systems of enteric bacteria[1,2], and in the temperate bacteriophage λ (ref. 3). Regulation of enzyme synthesis in the biosynthetic systems of bacteria has remained more obscure. Among studies on systems of the latter type, those on the tryptophan biosynthetic system in *Escherichia coli* are probably most advanced. Regulation of this operon clearly involves the classical mechanism proposed by Jacob and Monod[4]. Trytophan, the end product of the pathway, is a corepressor that can combine with an aporepressor molecule to form a "repressor" that binds to the tryptophan operator and blocks transcription[5–10]. Arginine regulation in *E. coli* may also involve a classical aporepressor[11,12], although the evidence is less extensive.

In contrast to the tryptophan and arginine systems, there is no evidence of a classical aporepressor for the histidine and isoleucine–valine biosynthetic systems, although these systems also have been investigated extensively. Consequently, basic assumptions about repressor function have been re-examined critically and other mechanisms have been proposed.

Histidyl-tRNA, rather than histidine itself, has been clearly implicated in repression control of the histidine operon in *Salmonella typhimurium* (see review by Brenner and Ames[13]). Ames and his coworkers[13–15] suggested that a complex of histidyl-tRNA synthetase (aporepressor) and aminoacylated $tRNA^{His}$ (corepressor) may be a repressor of the histidine operon. In contrast to this suggestion that the synthetase is a negative element in control, Wyche *et al.*[16] reported evidence that the synthetase is a positive element. There is evidence for yet another type of model in which the first enzyme in the histidine biosynthetic pathway (N-1-[5′-phosphoribosyl] adenosine triphosphate: pyrophosphate phosphoribosyltransferase, EC 2.4.2.17) has a role in repression control. Goldberger and coworkers[17–19] proposed that the first enzyme acts as a negative element in the control. But involvement of the first enzyme as a positive element in the control of the histidine operon also has been suggested[19,20].

These and other possible models now can be tested with purified (or partially purified) components in an *in vitro* transcription system and in a coupled transcription–translation system. Blasi *et al.*[21] reported that the first enzyme blocks *in vitro* transcription of the histidine operon but, surprisingly, histidyl-tRNA was not required for this result. In contrast, Artz and Broach[22] obtained evidence that a positive element is required for expression of the histidine operon in the coupled transcription–translation system. Their studies also suggest that transcription of the histidine operon requires translation of its specific mRNA. One of several explanations consistent with these observations is that translation produces a positive factor required for transcription[22]. Thus, the results so far from these *in vitro*

systems are consistent with the notion that a product of the histidine operon is involved in regulating its own synthesis. In one case, however, this product is thought to be the first enzyme and a negative element in control, whereas in the other, this product is an unspecified positive factor. A combination of the two is also a possibility.

Similar developments have occurred in the study of the isoleucine–valine (*ilv*) biosynthetic system. This system involves multivalent repression[23,24] in a branched set of pathways[25] and is, therefore, more complex than the histidine system. As is the case for the histidine operon, no classical aporepressor has been identified for the isoleucine–valine system. On the basis of biochemical data Hatfield and Burns[26] suggested a model of repression control for the *ilvADE* operon in *S. typhimurium* that involves the first enzyme of the isoleucine pathway (threonine deaminase, EC 4.2.1.16), leucyl-tRNA, valine, threonine and isoleucine. According to this model, which now has been modified and extended to cover regulation of the other *ilv* operons in *E. coli* and *S. typhimurium*[27], the first enzyme is an aporepressor and the multivalent corepressors are isoleucyl-tRNA, leucyl-tRNA and valyl-tRNA. On the other hand, Levinthal *et al.*[28] have described a regulatory mutant of *E. coli* in which the expression of the *ilvADE* operon is diminished and unresponsive to variations in the branched chain amino acids. This phenotype results from a single point mutation in the structural gene for the first enzyme of the isoleucine pathway. Based on these and other genetic results, Levinthal *et al.*[28] suggested that the first enzyme is a positive element controlling expression of the isoleucine–valine system.

A defective transducing phage harbouring the *ilv* genes[29] is being used for *in vitro* transcription and coupled transcription–translation assays, to study the molecular events in the regulation of the isoleucine–valine biosynthetic system of *E. coli*.

As this brief introduction indicates, there are repressible biosynthetic operons whose regulation involves a classical, constitutively synthesised aporepressor. There also are examples of such systems that involve autogenous regulation, whereby a regulatory protein directly modulates expression of its own structural gene[30]. Although in no case has it been demonstrated that either of these is the sole mechanism of control, the question arises: what are the functional implications of these two established modes of regulation? This question cannot be answered by comparing directly two representative systems (for example, tryptophan and histidine) because there may be still other elements involved in their control and because the systems are different in many ways that are irrelevant to a comparison of the two modes of regulation *per se*. An answer requires a more controlled comparison in which the two systems are identical in every respect except for the difference in a regulatory mechanism. This is difficult to do experimentally. At present a more practical approach is to compare mathematically models that accurately represent the different types of regulation. Such comparisons have been made and the details, unnecessary here, are available[31]. Before presenting the results it will be useful to enumerate the criteria for functional effectiveness that have been used in comparing the various models.

Criteria for functional effectiveness

Just as numerous chemical and physical criteria make possible characterisation of the molecular components of biological systems, well defined criteria make possible characterisation of the functional behaviour of an integrated system. For simplicity, in the analysis that follows I deal specifically with the regulation of unbranched pathways for the biosynthesis of amino acids. The following criteria for functional effectiveness can be formulated for these systems. It should be noted that none of these criteria assumes anything about the type of regulatory mechanism involved.

(1) Minimisation of the change in the level of end product as it shifts from one steady state to another in response to a change in demand for end product. This implies that the end product will not be depleted to such an extent that its rate of utilisation is limited when the demand is increased.

(2) Responsiveness to change in the availability of initial substrate. This is essential for the redistribution of common intermediary metabolites that occurs in response to a changing environment when the available carbon is limited.

(3) Reduction of enzyme synthesis when the end product is supplied exogenously. Since the biosynthetic enzymes become superfluous when the end product is available preformed in the environment, the importance of this criterion for cellular economy is obvious.

(4) Stability. Presumably one of the prime functions of pathways synthesising amino acids is to provide a relatively constant supply of their end products for protein synthesis. An effective system would not oscillate wildly, starving the organism for end product during one phase and overproducing and wasting it in the next. The impaired growth of mutants exhibiting such instability is readily apparent[32].

(5) Temporal responsiveness to change. The regulatory mechanism should enable the system to respond quickly to changes in its environment.

(6) Insensitivity to perturbations in the structure of the system itself. Small changes in the system may be the result of mutations in structural or regulatory genes, errors in transcription or translation of the genetic information, or physical influences such as temperature shifts. These changes tend to exert a deleterious effect on the cell and, as Sonneborn suggested[33], cells "buffered" against such harmful effects are likely to have a selective advantage.

Repressor-controlled systems

Models of classical and autogenous regulation are represented schematically in Fig. 1. In the classical Jacob–Monod model the structural gene for the regulatory protein is located in a separate transcriptional unit and is itself unregulated, whereas in the model of autogenous regulation one of the protein products of the operon is the regulator, which therefore directly controls expression of its own structural gene. For purposes of comparison these models are assumed to be equivalent except for their differences in regulatory interaction.

These two models can be compared for a wide variety of conditions, and because there are several criteria to consider, a meaningful tabulation of all the results presents a problem. Figure 2, however, is a rather simple diagram that conveniently summarises many of these results. In this plot the vertical axis represents the strength of the contribution of end product to the regulation while the horizontal axis represents the strength of the autogenous contribution. Each point in this two-dimensional space represents a different system with distinctive properties. Nevertheless, this space can be divided into subclasses of systems that have similar properties.

In repressible systems that utilise a repressor mechanism, the end product has a negative effect on transcription, and the autogenous contribution is also negative. Since both contributions are negative, only the lower left hand quadrant of Fig. 2 needs to be considered. The vertical axis in this quadrant is the locus of points representing systems with constitutively synthesised repressor, since the autogenous contribution is zero. Line *b* represents another important classification. The position of this line is determined by analysing the local stability of these models. All systems represented by points above line *b* are stable (if perturbed momentarily, they will return to their predisturbance condition), whereas all systems represented

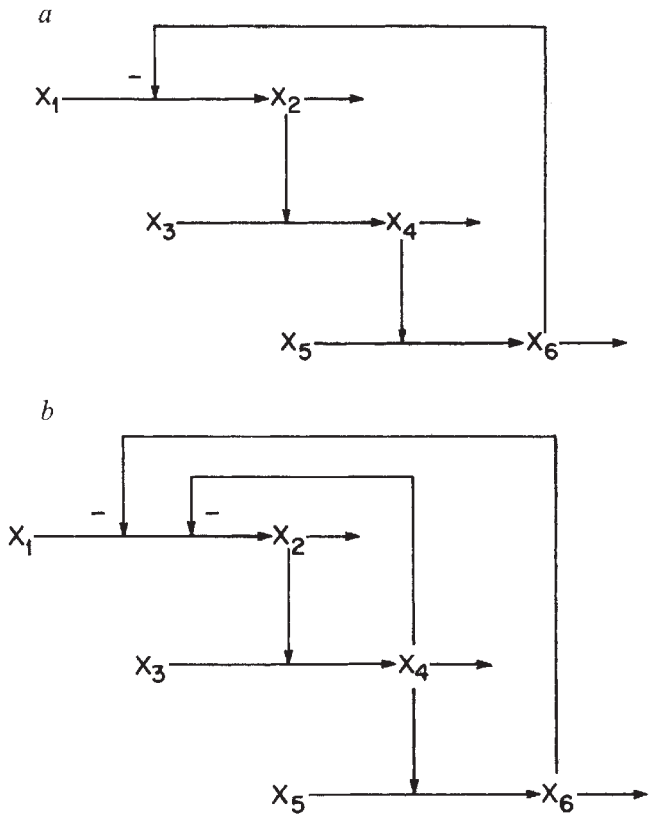

Fig. 1 Schematic models of repressible biosynthetic systems. Model with constitutive synthesis of the regulatory protein, which is not explicitly indicated. *b*, Model with autogenously regulated synthesis of the regulatory protein. X_1, Weighted sum of the nucleotide pools; X_2, specific messenger RNA; X_3, weighted sum of the amino acid pools; X_4, enzymes of the biosynthetic pathway (plus regulator in the case of autogenous regulation); X_5, substrate, and X_6, product/modifier. The horizontal arrows indicate chemical transformations at the mRNA, enzyme and metabolite levels. The vertical arrows represent modifier or catalytic influences (the sense of the influence is positive unless otherwise indicated). Except for the differences in regulation these models are assumed to be identical.

below this line are unstable (they will not return to their predisturbance condition).

Lines, such as *c*, *d* and *e*, that radiate from a single point on this plot are lines of equivalence with respect to the first three criteria for functional effectiveness. All systems whose regulatory parameters have values that determine points on a given line of equivalence have: (1) the same steady-state response to a change in demand for the end product, (2) the same steady-state response to a change in substrate availability and (3) the same decrease in enzyme synthesis for a given increase in the concentration of extracellular end product. The slope of the line determines the magnitude of the above three responses. Systems represented on a steeper line of equivalence, function more effectively according to the first and third criteria but less effectively according to the second.

Systems for which the second criterion is of primary importance are represented by lines of equivalence with low slope, for example, line *c*. As previously noted, all systems on such a line have identical responses according to the first three criteria. Comparisons on the basis of stability, the fourth criterion, can be made by noting the relative distances of points on line *c* from the boundary of instability, line *b*, in Fig. 2. Since lines *c* and *b* diverge to the left, systems with greater strengths of autogenous regulation are represented further from the boundary of instability than is the equivalent system with constitutive synthesis of repressor. Consequently, this latter system is the least stable of all the systems represented on the line of equivalence *c*.

Systems for which the first three criteria are of equal importance are represented by lines of equivalence with intermediate slope, such as line *d* in Fig. 2. All the systems represented on this line have identical responses according to the first three criteria. They also have the same degree of stability since line *d* is parallel to the boundary of instability, line *b*.

Similarly, systems for which the first and third criteria are of primary importance are represented by lines of equivalence with steep slope, for example, line *e* in Fig 2. These systems are equivalent as judged by the first three criteria, but the systems with autogenously regulated synthesis of repressor must be considered inferior to the equivalent one with constitutive synthesis of repressor according to the fourth criterion because autogenous regulation now is clearly a destabilising influence.

The two types of repressor-controlled systems are examined in Fig. 3 on the basis of temporal responsiveness, the fifth criterion for functional effectiveness. The three panels in this figure correspond to the three different classes of equivalence represented by lines *c*, *d* and *e* in Fig. 2. In the top panel (*c*) the response labelled 0.0 corresponds to the system with constitutive synthesis of repressor. The system is in a steady state with concentrations normalised to unity before time zero. At $t=0$ the concentration of extracellular end product is increased and then maintained at an elevated level throughout the experiment. The concentration of intracellular end product is plotted as a function of time. Similar responses are plotted for a series

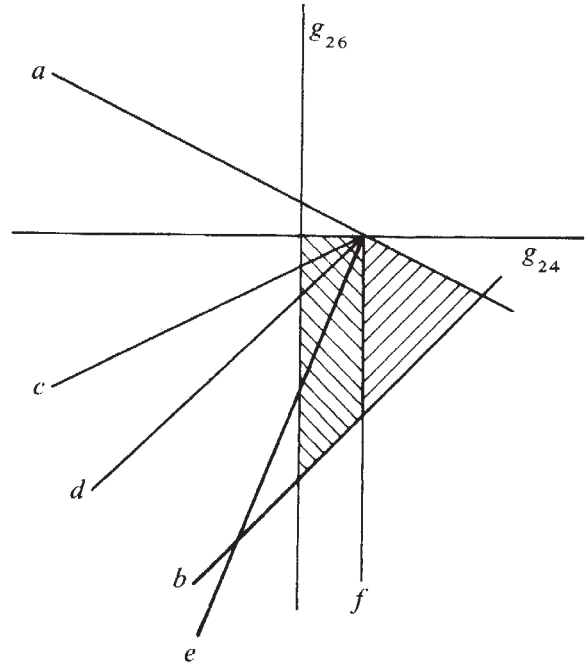

Fig. 2 Graphical comparison of systems with alternative types of regulation. The vertical axis represents the strength of the product's contribution to the regulation of transcription and the horizontal axis represents the strength of the autogenous contribution to this regulation. *a* and *b*, Boundaries of instability. *c*–*f*, Lines of equivalence with respect to the first three criteria for functional effectiveness. The vertical axis is the locus of points representing systems with constitutive synthesis of either activator or repressor. The shaded areas to the right of this axis are the locations of points representing stable systems with autogenously regulated synthesis of activator. Similarly, the area above line *b* in the lower left hand quadrant represents stable systems with autogenously regulated synthesis of repressor. (See text for further discussion.)

of systems with different strengths of the autogenous contribution to the regulation. Clearly, autogenous regulation decreases the time required for the system to settle into the new steady state. The same is true in Fig. 3*d*. In Fig 3*e*, however, autogenous regulation actually increases the response time.

A comparison on the basis of the sixth and final criterion for functional effectiveness shows that the sensitivities to external perturbations of the systems with autogenously regulated synthesis of repressor are less than or equal to those of the system with constitutive synthesis of repressor.

On the basis of the comparisons discussed above one can draw the following conclusions. If the second criterion for functional effectiveness is of primary importance, then systems with autogenously regulated synthesis of repressor will have a selective advantage. The behaviour of these systems is superior to that of systems with constitutive synthesis of repressor on the basis of the second, fourth, fifth and sixth criteria, but inferior with regard to the first and third. But if the first and third criteria for functional effectiveness are of primary importance, as seems more reasonable for biosynthetic systems, then systems with constitutive synthesis of repressor will have the selective advantage. The behaviour of these systems is superior to that of systems with autogenously regulated synthesis of repressor on the basis of criteria one, three, four and five, but inferior with regard to two and six. These conclusions will be discussed in relation to available experimental evidence in a later section.

Activator-controlled systems

The models represented in Fig. 1 also can be used for the comparisons in this section. Systems with constitutive synthesis of an activator protein can be represented directly by the model in Fig. 1*a*, whereas systems with autogenously regulated synthesis of activator can be represented by the model in Fig. 1*b* provided the negative sign previously associated with the repressor is changed to a positive one, implying activation. Since the autogenous contribution to the regulation now is positive, only the lower right hand quadrant of Fig. 2 need to be examined. The systems with constitutive synthesis of activator are represented along the vertical axis. As in the previous section, line *b* represents the lower boundary of stability. In addition, there is an upper boundary of stability given by line *a*. As in the previous section, lines, such as *c*, *d*, *e* and *f*, that pass through a common point on this plot are lines of equivalence with respect to the first three criteria for functional effectiveness.

Systems for which the second criterion is of primary importance again are represented by points on line *c*. These systems are equivalent with respect to the first three criteria, but since line *c* diverges to the left from the boundary of instability, line *b*, the system with constitutive synthesis of activator is more stable than the equivalent systems with autogenously regulated synthesis of activator. Systems for which the first three criteria are of equal importance are represented by points on a line of equivalence with intermediate slope, such as line *d*. All the systems represented on this line have identical responses according to the first three criteria, and according to the fourth criterion as well, since lines *b* and *d* are parallel. Similarly, systems for which the first and third criteria are of primary importance are represented by a steeper line of equivalence, such as *e* in Fig. 2. These systems are also equivalent by the first three criteria, but those with autogenously regulated synthesis of activator must be considered superior to the equivalent one with constitutive synthesis of activator according to the fourth criterion because autogenous regulation is a stabilising influence. Thus, the effect of autogenous regulation on the stability of activator-controlled systems is just the opposite of that found to be true for repressor-controlled systems.

The results in Fig. 4 show that autogenous regulation also has opposite effects on the response times of repressor-controlled and activator-controlled systems. The three panels in this figure correspond to the three different classes of equivalence represented by lines *c*, *d* and *e* in Fig. 2. The response time is increased by autogenous regulation in Fig. 4*c* and *d*. In Fig. 4*e*, however, the response time is decreased by autogenous regulation.

A comparison on the basis of system sensitivity, the sixth criterion, shows that the sensitivities to external perturbations of the system with constitutive synthesis of activator always are less than or equal to those of the equivalent systems with autogenously regulated synthesis of activator.

The following conclusions can be drawn from the comparisons in this section. If the second criterion for functional effectiveness is of primary importance, then systems with constitutive synthesis of activator will have a selective advantage. These systems are superior to systems with autogenously regulated synthesis of activator by the second, fourth, fifth and sixth criteria, but inferior according to the first and third. If, however, the first and third criteria for functional effectiveness are of primary importance, as seems more reasonable for biosynthetic systems, then systems with autogenously regulated synthesis of activator will have the selective advantage. These systems are

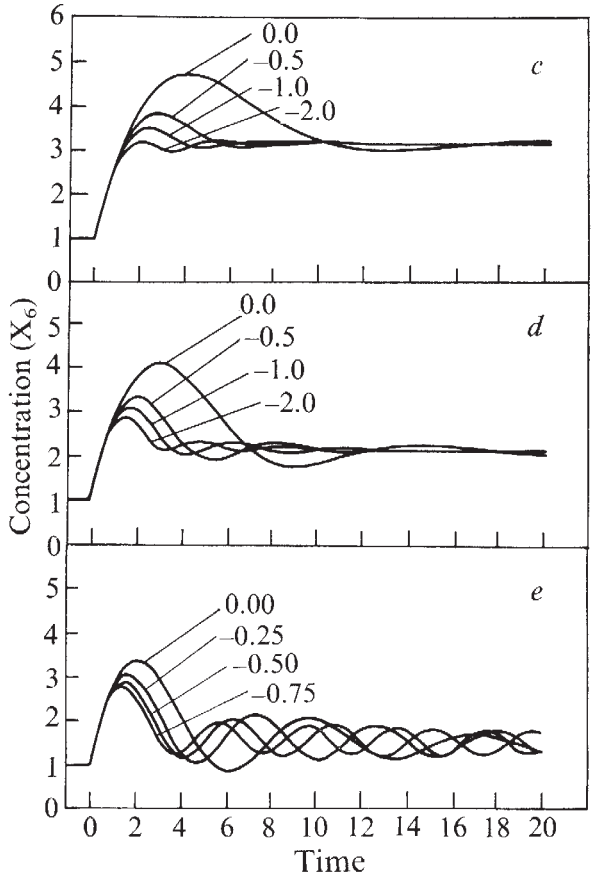

Fig. 3 Temporal responsiveness of systems with autogenously regulated synthesis of repressor. *c*, *d* and *e* correspond to systems in the three different equivalence classes represented by lines *c*, *d* and *e* in Fig. 2. The numbers associated with the individual curves are values of g_{24}, representing the strength of the autogenous contribution to regulation. The corresponding values for the parameter g_{26} are chosen so that the points representing the different systems within a class all lie on the same line of equivalence. Curves with $g_{24} = 0$ represent systems with constitutive synthesis of repressor. (See text for further discussion.)

superior to systems with constitutive synthesis of activator on the basis of criteria one, three, four and five but inferior with regard to two and six.

(Re-examination of the lower right-hand quadrant in Fig. 2 shows that only a portion of the allowable systems have been considered in the preceding comparisons. The remaining systems with autogenously regulated synthesis of activator exhibit two classes of behaviour for which there is no counterpart among the other types of regulatory systems considered here. Line *f* represents the class of systems for which there is zero depletion of end product in steady state after an increase in demand for end product. The class of systems represented by points within the shaded triangular region to the right in Fig. 2 have the unique property of responding to an increased demand for end product by increasing the level of that end product—a response to demand that is the inverse of the usual one. Whether such behaviour has biological significance is unclear and it will not be discussed further here. A more detailed analysis is given in ref. 31.)

Predictions and observations

As previous sections of this article show, the effectiveness of activator-controlled and repressor-controlled operons is changed in opposite ways if the regulator protein is, in addition, autogenously regulated. In systems for which the first and third criteria for functional effectiveness are most important, as is likely for repressible biosynthetic systems, autogenous regulation of the regulator protein improves the performance of activator-controlled systems and degrades that of repressor-controlled systems. Thus, biosynthetic systems governed by a repressor are not expected to use autogenous regulation, while those governed by an activator can be predicted to involve such regulation. These predictions were deduced from differences inherent in the classical and autogenous modes of regulation, other things being equal. Present information concerning actual, specific operons is insufficient to yield such generalisations through induction from individual cases. Nevertheless, the experimental evidence is consistent with the above predictions in the few cases where data that can be analysed are available.

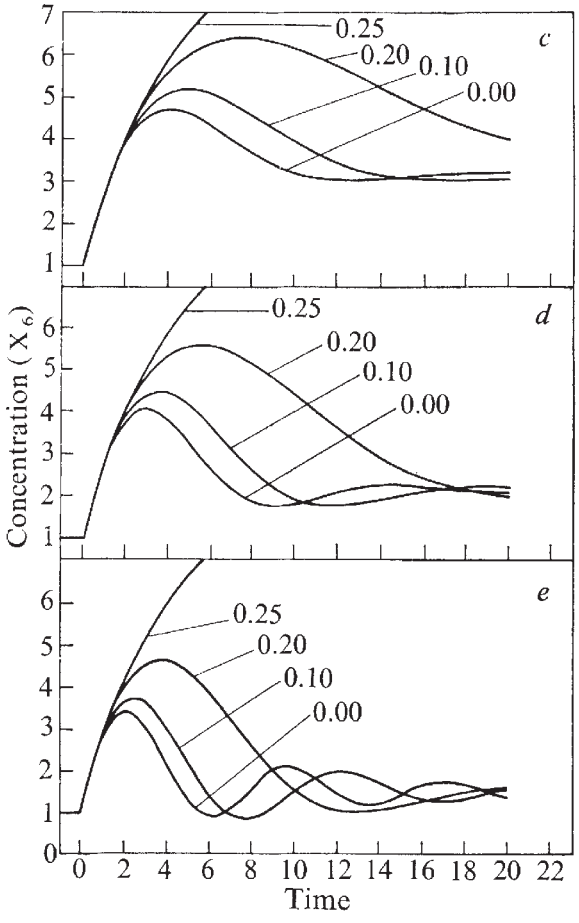

Fig. 4 Temporal responsiveness of systems with autogenously regulated synthesis of activator. *c*, *d* and *e* correspond to systems in the three different equivalence classes represented by lines *c*, *d* and *e* in Fig. 2. The numbers associated with the individual curves are values of g_{24}, representing the strength of the autogenous contribution to regulation. The corresponding values for the parameter g_{26} are chosen so that the points representing the different systems within a class all lie on the same line of equivalence. Curves with $g_{24} = 0$ represent systems with constitutive synthesis of activator. (See text for further discussion.)

In the case of the tryptophan operon in *E. coli*, the best documented example of a biosynthetic system involving control by a repressor, there is no evidence for autogenous regulation of repressor. The structural gene for the tryptophan repressor is located outside the known transcriptional units under its control[34], so the simplest form of autogenous regulation is excluded. Nevertheless, a more complex, regulon model, in which the repressor modulates expression of its own structural gene in parallel with that of the tryptophan operon, cannot be excluded rigorously since repressor levels have not been compared in strains with the tryptophan operon repressed and derepressed.

In the case of the arginine system in *E. coli*, which also seems to involve control by a repressor, there is no evidence for autogenous regulation of repressor. Although the arginine system itself constitutes a regulon with several unlinked transcriptional units regulated in parallel[35], the structural gene for the repressor is not linked to any of these[34] and there is no evidence that its expression is subject to modulation by the level of arginine within the cell.

On the other hand, the clearest example of a biosynthetic system involving control by an activator is the isoleucine–valine system in *Saccharomyces cerevisiae* and in this case the activator is autogenously regulated[36,37]. With an extensive collection of mutants in the structural gene for the first enzyme of the isoleucine pathway (threonine deaminase), Bollon[37] has constructed a fine-structure map and demonstrated that this protein is multifunctional; mutations affecting its activator function overlap with mutations affecting its catalytic function.

For all three operons discussed above the evidence is consistent with the predictions based on the analysis of autogenous regulation. For other biosynthetic systems the experimental evidence is more ambiguous. For example, in *E. coli* there is strong experimental evidence that the first enzyme of the isoleucine pathway is involved in the autogenous regulation of the isoleucine–valine system, but whether it functions as repressor or activator is unclear. The prediction based on the analysis in the preceding sections is that some product of the operon is an activator, in agreement with the data of Levinthal *et al.*[28]. Similarly, in *S. typhimurium* there is evidence consistent with the idea that some product of the histidine operon is involved in autogenous regulation of the system, but whether this product is an activator or a repressor is controversial. Again, the prediction is that this product is an activator, in agreement with the data of Artz and Broach[22]. (The conflicting evidence for the nature of the regulators of these operons, which was referred to in the introduction, might be reconciled more easily if they were in fact biregulators rather than activators. It is easier to conceive of a biregulator changing physical state, as a result of mutation or environmental conditions, and exhibiting the properties of either repressor or activator than to imagine an activator doing this. Nothing in the analysis and predictions given above would change substantially if activators

were considered biregulators analogous to the arabinose *C*-gene product, which is primarily an activator in function but also has repressor attributes. The arguments are given in ref. 31.)

The tryptophan and arginine biosynthetic systems have been described in terms of a classical, Jacob–Monod model with constitutive synthesis of regulator. On the other hand, the histidine and isoleucine–valine biosynthetic systems have been described in terms of an autogenously regulated model with an enzyme/regulator directly involved in modulating expression of its own structural gene. These two models, however, need not be mutually exclusive. A combination of the two modes of regulation might exist, possibly with one or the other mode playing a dominant role in some systems. The results in the preceding sections suggest that the most promising of such combinations would be constitutive synthesis of a repressor and autogenously regulated synthesis of an activator. Is there any experimental evidence for such a combination?

There is evidence for multiple control mechanisms in most of the biosynthetic systems that have been well studied. Mutants with a chain-termination codon early in the structural gene for the first enzyme or with this gene deleted have been described in the histidine[19] and isoleucine–valine[38] biosynthetic systems of enteric bacteria. In each case, the system continues to be regulated in an apparently normal manner, at least by the usual all-or-none tests for regulatory function. Thus, mutations that eliminate the autogenous mode of regulation permit another, possibly the classical mode, to become manifest.

A better example is provided by the tryptophan operon of *E. coli*. In this system, where there is clear evidence for the classical mode of regulation involving a constitutively synthesised repressor, there also is evidence suggesting that the first enzyme of the tryptophan pathway (anthranilate synthetase, EC 4.1.3.27) is involved in regulating expression of the operon [39]. Somerville and Stetson[40,41] reported additional evidence for involvement of the first enzyme in regulation of the tryptophan operon and suggested that this enzyme is normally an activator or positive element in the control, which agrees with the above prediction. When the structural gene for the first enzyme is deleted, the tryptophan operon continues to be regulated in an apparently normal manner, again by the usual all-or-none tests for regulatory function[42].

In addition to the two modes of regulation discussed above there are indications of novel control mechanisms[43–45], at least some of which involve sites on the DNA between the promoter–operator region and the first structural gene of the operon[44,45].

In systems for which the first and third criteria for functional effectiveness are relatively unimportant, the results are just the reverse of those discussed above. Autogenous regulation of the regulator protein improves the performance of repressor-controlled systems and degrades that of activator-controlled systems. Although it seems unlikely that any repressible biosynthetic system would fall into this particular class, there are systems of other types that might be considered non-biosynthetic, that do fit this category. For example, Sompayrac and Maaløe[46] have proposed a model for regulating the concentration of the protein(s) believed to be involved in the initiation of DNA replication. The regulator protein in this model is autogenously regulated and functionally autonomous, that is, it does not require the participation of an effector molecule in its regulatory function. Although not a repressible biosynthetic system in the conventional sense of the term, formally this model is a special case of that represented in Fig. 1*b* with $g_{36} = 0$. Accordingly, such systems would be represented along the horizontal axis in Fig. 2. Sompayrac and Maaløe[46] pointed out several advantages for their model involving a repressor whose synthesis is autogenously regulated. The results in the preceding sections suggest that the behaviour of such systems is optimal with respect to stability, temporal responsiveness and insensitivity to perturbations in the structure of the system itself. Dennis and Nomura[47] have indicated that in *E. coli* a similar mechanism of autogenous regulation might be involved in control of ribosomal protein genes.

Perhaps it should be re-emphasised here that the predictions made throughout this article are based on a particular weighting of the different criteria for functional effectiveness. If this choice were incorrect, then the predictions could be wrong, but clearly for reasons that have nothing to do with the general validity of the approach used.

In conclusion, the expression of biosynthetic operons in enteric bacteria is undoubtedly controlled by several different mechanisms acting in concert. This has made it difficult to study regulation *in vivo* where all the elements may interact or contribute in various degrees to produce the end result. The identity of the elements participating in the regulation and the nature of their roles at the molecular level can only be established rigorously *in vitro*. An understanding of the intact regulatory system, however, will require knowledge of the functional implications at the physiological level for each molecular mechanism and, eventually, integration of the individual mechanisms into an accurate model of the intact system. With progress being made in each of these areas, the understanding of repressible biosynthetic operons should soon be as thorough as it is of the simpler inducible catabolic operons.

I thank Robert F. Goldberger and Stanley W. Artz for valuable suggestions during preparation of the manuscript. This research was supported in part by grants from the National Science Foundation and an institutional research grant to the University of Michigan from the American Cancer Society.

Received July 25; accepted September 17, 1975.

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letters to nature

Soft X-ray search of centre of Cygnus Loop

THE Mullard Space Science Laboratory equipment on the Copernicus satellite has been used to search, with negative results, for evidence of a compact object in the centre of the Cygnus Loop supernova remnant.

Rocket measurements[1] indicate that a central object exists, and contributed, at the time of observation, about 4% of the X-ray flux from the entire loop. This object may be variable[1,2] in intensity, and may even be pulsing[1]. Reanalysis of earlier rocket data[3] gives statistically more significant evidence for pulsation, but at a different frequency, which would be consistent with a compact object which is not a pulsar. The same data require, however, that the pulsing object be roughly equal in brightness to the integrated brightness of the rest of the nebula at the relevant energy, 0.28 keV, at the time of observation, while other rocket data[4,5] show that this is certainly not the case at particular other times. The availability of a Channel Electron Multiplier (CEM) detector, sensitive at the relevant energy (0.28 keV), on board the Copernicus satellite, has provided the opportunity to observe the centre portion of the Cygnus Loop and to test whether a central object does, from time to time, radiate as strongly at 0.28 keV as rocket data[3] suggest.

The CEM has been described by Margon *et al.* (see ref. 6 and references therein). The field-of-view of the CEM is 15′ full width at half maximum. Since the exact position of the central object is not known, a set of nine observing sections, centred on the rocket[1] position, were chosen so that there was a slight degree of overlap. These nine positions are given in Table 1.

The observations were made on May 19, 1974. Three spacecraft orbits were required to observe the nine sections. Sections, 1, 2, 3 were covered from 1349 to 1422 UT; 6, 5, 4 from 1530 to 1600 UT; and 7, 8, 9 from 1709 to 1739UT. The observations could only be made while the satellite was in the shadow of the Earth since the CEM is also sensitive to Lyman alpha (Lα) radiation. During the periods when the satellite was in sunlight, the CEM was turned off. (Margon *et al.*[6] note that in spite of the Lα background, the telescope is known to respond normally to low energy X rays; in particular Sco X–1 has been observed.) Each data acquisition interval lasted 62.5 s. From six to ten observations were made in each section.

Figure 1*a* presents plots of the raw data as received from the satellite. Three points are immediately apparent: (1) each plot displays a characteristic parabolic shape, due to CEM sensitivity to geocoronal Lα radiation; (2) a systematic decrease in this background occurs on successive orbits, clearly due to instrumental effects; and (3) no sharp break occurs between any of the three sections on each of the three orbits, as would be expected if a strong source of 0.28 keV X rays were present. If the central source were present, at the greatest intensity observed[3], we would expect that one of the sections would display a 'discontinuity' of about 735 counts over the Lα background, given the known sensitivity[6] to X rays of the CEM. A lower flux would result in a smaller discontinuity. The data show that if such a source was present in the field of view, it was not of high intensity at the time of observation.

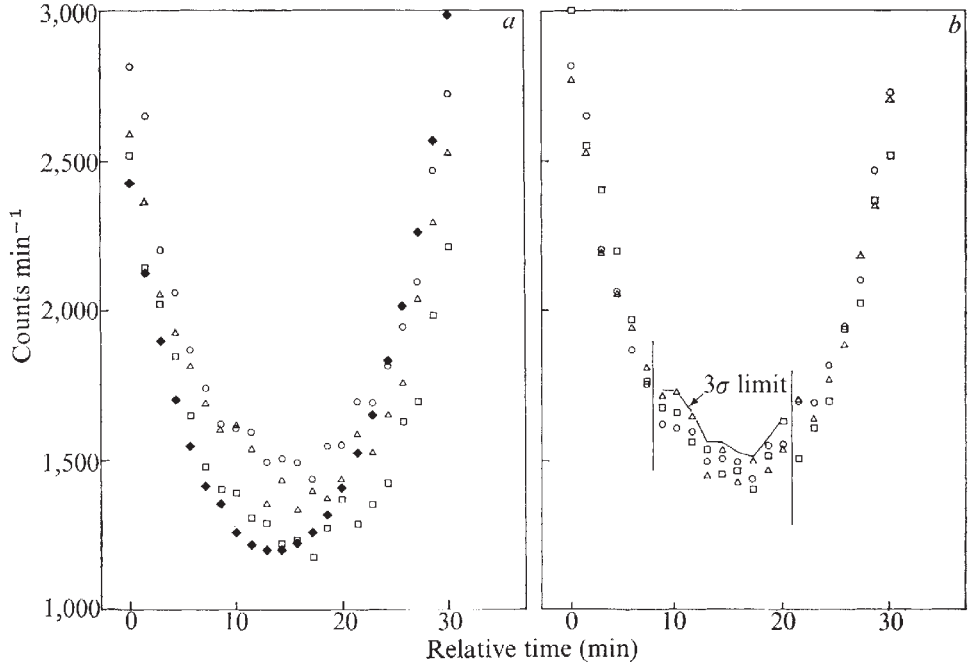

Fig. 1 *a*, Signal received from directions near the centre of the Cygnus Loop nebula on three orbits of the Copernicus satellite. The data are shown in raw form, and give no evidence for any soft X-ray emission. Also shown is the relative expected background of Lα radiation, calculated by Dr R. Meier of the US Naval Research Laboratory. *b*, The data of *a* are shown, processed to remove instrumental effects. This permits the determination of a 3σ upper limit on any possible X-ray source, which is about 80 times smaller than the highest reported observed intensity. ○, First orbit; △, second orbit; □, third orbit; ◆, theoretical Lα.