arbitrary. Furthermore, the occurrence of a solar eclipse must not be confused with its visibility which would be the all-important factor for Neolithic man. It is well known that solar eclipses are comparatively rare events at a particular point on the Earth's surface. Examination of the Nautical Almanacs for the years 1858–1955 shows that 35 such winter solar eclipses occurred, but of these only three were visible or partially visible at Greenwich and hence at Stonehenge. Neolithic man could not therefore have seen any pattern in solar eclipses near the winter solstice, and any search for a periodic pattern of eclipses must be restricted to those of the Moon.

The Neolithic astronomer at Stonehenge would see a random distribution of about 90 per cent of the lunar eclipses shown in Fig. 1, and it can readily be verified that the winter Moon is not "eclipsed in repetitive fashion based on a 56 year cycle"[3]. The histogram in Fig. 2 shows the frequency of the intervals between any lunar eclipse and all the other eclipses in Fig. 1. The positions of the frequency peaks are given by 18·61 $n/2$ for successive integral values of n, and 18·61 yr is the period of rotation of the nodes. There is, however, no regular separation between eclipses, and therefore the regular occurrence of eclipse seasons would not in itself be sufficient for Neolithic man to predict lunar eclipses in any particular year. It is noteworthy that the peak near 56 yr is one of the smallest, indicating that 56 yr is a particularly poor period to use when attempting to predict eclipses.

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PROFESSOR G. S. HAWKINS writes: The sentence referred to in the preceding article is badly worded, and, indeed, taken by itself can lead to a misinterpretation of the Stonehenge theory. The sentence was an attempt to summarize the more detailed astronomical discussions in the earlier section of the paper, and I apologize for its poor appositeness.

It is difficult in a few words to summarize these time patterns and I refer the reader to my original discussion of the lunar periodicities[1] and the alignments[2]. Hoyle[3] and Newham[4] have confirmed the alignments and the basic relation of the number 56 to the azimuthal swing of the Moon and eclipses.

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Catabolite Sensitive Site of the *lac* Operon

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A partial deletion in the *lac p* region makes the *lac* operon insensitive to catabolite repression.

ALTHOUGH the phenomenon of catabolite repression of the *lac* operon is well known[1], no genes directly involved in the process have been identified so far. As far as is known the *lac* system comprises the three structural genes specifying three proteins involved in lactose metabolism and their three control elements, the *i* gene which specifies repressor, the operator (*o*) locus which binds repressor and the promoter region (*p*) which facilitates transcription of the operon. An unlinked gene that was previously reported to alter specifically the susceptibility of the *lac* operon to catabolite repression[2] is now known to affect repression of other enzymes as well[3]. Evidence has suggested that catabolite repression, like repression caused by the protein repressor product of the *i* gene, reduces the rate of transcription of the *lac* operon[4]. Nevertheless, mutations in the *i* gene and in the target of the *i* repressor, the *o* locus which specifically binds *lac* repressor, do not show an altered susceptibility to catabolite repression[2,5]. In fact, catabolite repression can be observed in cells devoid of *lac* repressor produced by the *i* gene[5]. We would therefore expect that a genetic site so far undiscovered, which is part of the *lac* operon, determines the response of the *lac* genes to catabolite repression.

We decided to examine strains in which the genes of the *lac* region had been fused to an operon known to be insensitive to catabolite repression. Such a system is available in the *trp-lac* fusions first isolated by J. R. B., Signer and Epstein[6]. The results of our study show that the *lac* genes do not respond to catabolite repression in strains in which the *lac* operon has been fused to the *trp* operon, and that partial deletion of the promoter region of the *lac* operon is sufficient to render the *lac* operon insensitive to catabolite repression.

The strains we used are catalogued in Table 1. We used a medium containing glycerol as a major carbon source to measure unrepressed enzyme synthesis, and a medium containing glucose-6-phosphate as a major carbon source to measure enzyme synthesis under catabolite repression[8]. The effect of catabolite repression on a normally functioning *lac* operon is shown in experiment 1 of Table 2. In the strain used in this experiment, the *lac* region has been transposed, but only the *i* gene, and not the structural genes of the *lac* operon, has been fused with the *trp* operon. Because of the deletion fusing the *i* gene to *trp*, β-galactosidase synthesis is constitutive.

Table 1. PARENTAL STRAINS

Strains	Genetic composition
W 1	Deletion I*
W 2	Deletion II + L1*
W 2(−L1)	Deletion II only*
X 8047	L1 deletion in transposed position*
3.300	*lac* constitutive i^-z^+ from 3.000†
CA 8001	3.000 derivative with promoter deletion L1‡
CA 8003	3.000 derivative with promoter point mutant L8‡
CA 8019	3.000 derivative with promoter point mutant L29‡

* These strains are streptomycin resistant, carry a *lac-pro* A,B deletion, and are *ara*⁻ in R^+ *trp* and *ara*⁺ in R^- *trp*. Their construction will be described elsewhere[9] and they are illustrated in Fig. 1.

† Luria stocks.

‡ Ippen *et al.*[7]; Miller *et al.*[14].

Table 2. CATABOLITE REPRESSION IN FUSED STRAINS

Experiment	Strain	*trp* Regulation	Promoter character	Anthranilic synthetase Glycerol	Anthranilic synthetase Glucose-6-PO_4	β-Galactosidase Glycerol	β-Galactosidase Glucose-6-PO_4
1	W 2(−L1)	R^+	+	1·1	1·1	20,000	1,100*
2	W 2	R^+	deleted	3·3	3·8	360	352
3	W 2	R^-	deleted	469	212	2,710	1,330
4	W 1	R^-	deleted	—	—	3,770	5,510

Cells were grown in Ozeki-minimal medium[11] with B1 (2·5 μg/ml.), L-proline (80 μg/ml.), L-tryptophan (80 μg/ml.), and the carbon source indicated, and were collected in exponential phase at about 5×10^8 cells/ml. The cells were concentrated a hundred-fold and then treated with 250 μg/ml. of lysozyme, 50 μg/ml. of DNase, in *tris* buffer (0·05 M, *p*H 7·5) with Mg^{++} (0·005 M) (personal communication from S. Yankofsky). The cells were then frozen and thawed twice (methanol–dry ice) and the cell debris was spun down.

Anthranilic synthetase was assayed as recommended by Yanofsky[12], except that increasing fluorescence caused by anthranilic acid formation was followed in a Turner model 111 recording spectrofluorometer. The units given are expressed as Turner units/min/mg extract. β-Galactosidase was measured as described by Loomis and Magasanik[5], and units are expressed as units/mg of protein. R^+ is sensitive to tryptophan repression. R^- is constitutive.

* The greater sensitivity of this strain to repression by glucose-6-phosphate compared with strain 3.300 (Table 4) seems not to be related to the *lac* operon but to the general genetic background of the strain.

It can be seen that the level of β-galactosidase in the cells grown on glucose-6-phosphate is only one-twentieth that found in the cells grown on glycerol. In the strains used in experiments 2 and 3 of Table 2, β-galactosidase synthesis is controlled by the *trp* system. Thus in the R^+ *trp* derivative of W2, in which the enzymes of tryptophan biosynthesis are subject to repression by tryptophan, the concentrations of anthranilate synthetase and of β-galactosidase are much lower than in the corresponding R^- *trp* derivative, which is constitutive for the *trp* enzymes. In experiment 2, no repression by glucose-6-phosphate was observed. In experiment 3, the concentration of β-galactosidase is somewhat reduced by glucose-6-phosphate, but not more so than the concentration of anthranilate synthetase. In experiment 4 of Table 2, another fused strain, constitutive for *trp* enzyme synthesis, was used (Table 1). Here the concentration of β-galactosidase in the glucose-6-phosphate medium was slightly higher than in the glycerol medium. We conclude from these experiments that the usual strong repression of β-galactosidase by glucose-6-phosphate does not occur in strains the β-galactosidase synthesis of which is under the control of the *trp* system.

It is possible that deletions causing the fusion of the *trp* and the *lac* regions have removed a gene responsible for the synthesis from glucose-6-phosphate of a catabolite repressor substance. This, however, is very unlikely. In strain W2(−L1) (Table 1 and Fig. 1), the deletion of the region between *trp* and the transposed *i* gene (Fig. 1) has not eliminated sensitivity to catabolite repression (experiment 1, Table 2). It is still possible, however, that

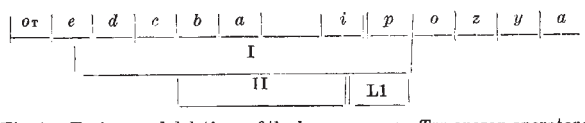

Fig. 1. Fusions and deletions of the *lac* operon. o_T, *Trp* operon operator; *e*, *d*, *c*, *b*, *a*, tryptophan structural genes[10]; *i*, *lac* repressor gene[7]; *p*, promoter site[7]; *o*, operator site[7]; *z*, *y*, *a*, *lac* structural genes[7]; L1, promoter deletion[14]; I, II, *trp-lac* fusion deletions: construction as we used before[9].

the L1 deletion itself removes such a gene. In experiments 2, 4 and 6 of Table 3, addition of an episome carrying an intact *i* and *p* region does not restore sensitivity to catabolite repression of the chromosomal *lac* genes. The deletions fusing the two operons therefore have not removed a gene responsible for producing the catabolite repressor from glucose-6-phosphate.

In spite of earlier indications[5], it is also possible that the *i* gene repressor product is essential for catabolite repression. The L1 deletion removes a portion of the *i* gene, as well as the promoter region *p*, and this may account for the disappearance of sensitivity to repression by glucose-6-phosphate. By providing the fused strains of Table 2 with an intact *i* gene carried on an episome (experiments 2 and 4 of Table 3), we have further confirmed that the *i* gene repressor is not involved in catabolite repression. In the parent strains, β-galactosidase synthesis proceeds at the same rate in the presence or absence of IPTG, an inducer of the lactose operon. In the strains carrying the i^+z^- episome, the rate of β-galactosidase synthesis is reduced by omission of IPTG. In other words, the rate of enzyme synthesis is controlled by the repressor product of the *i* gene carried in the episome. In no case does the presence of glucose-6-phosphate significantly reduce the rate of β-galactosidase synthesis. Because the presence of the intact *i* region has not restored catabolite repression, we conclude that the deletion of the *i* gene is not responsible for the insensitivity of the fused strains.

An objection to this view can, however, be made. In the fused strains carrying the i^+ episome, there is only a two to three-fold difference between the induced and uninduced levels of β-galactosidase. Thus, in these strains, the *i* repressor is not nearly as effective in repressing the *lac* operon as in the normal i^+ strain, where it causes a six-hundred-fold reduction in the rate of enzyme synthesis. This observation is, at present, unexplained, but it may be a consequence of the greater distance between the initiating site in the *trp* operon and the *lac* operator[9]. If the *i* repressor has restored sensitivity to catabolite repression this effect could have been too small for observation.

Moreover, if the fusion with the *trp* operon by itself can alter the control of the *lac* genes whose operator is intact[9], the possibility that the fused strains contain a normal catabolite sensitive site has not been completely excluded, but the degree of repression is too small for detection.

To answer these objections we examined β-galactosidase synthesis in a strain the *lac* region of which has been transposed but not fused with the *trp* region, and the *i* gene and promoter site of which is partially deleted (experiments 5 and 6, Table 3). This strain, X8047, produces β-galactosidase at a slow but measurable rate. Introduction of an episomal *i* gene reduces the rate of constitutive

Table 3. INABILITY OF *i* GENE PRODUCT TO RESTORE CATABOLITE REPRESSION

Experiment	Strain	IPTG (10^{-3} M)	β-Galactosidase carbon source: Glycerol	β-Galactosidase carbon source: Glucose-6-phosphate
1	W-1, R^-	+	2,700	2,600
		−	2,800	2,500
2	W-1/$F'I^+Z^-$ R^-	+	2,300	2,400
		−	1,200	1,200
3	W-2, R^-	+	1,600	1,700
		−	1,500	1,400
4	W-2/$F'I^+Z^-$ R^-	+	2,100	1,900
		−	590	540
5	X 8047 R^-	+	190	180
		−	150	140
6	X 8047/$F'I^+Z^-$ R^-	+	170	130
		−	4	4

Cells were grown as described in Table 2, except that diploid strains did not require proline for growth. Experiments with IPTG were carried out with cells pre-incubated in IPTG. β-Galactosidase was measured on exponentially growing whole cells as described by Loomis and Magasanik[5]. The units given are slopes of lines plotting enzyme units (UZ)/ml. against Klett multiplied by 1,000.

Diploid strains were constructed by mating the indicated parents, each containing a *lac-pro* A,B deletion, with a streptomycin sensitive donor, E 7089, containing a *lac* (i^+z^-)-*pro* A,B episome, and selecting for streptomycin resistant proline prototrophs.

Table 4. CATABOLITE REPRESSION IN UNTRANSPOSED STRAINS

Strain	β-Galactosidase carbon source: Glycerol	Glucose-6-phosphate
3.300	23,800	6,450
CA 8003	933	195
CA 8019	828	190
CA 8001	140	159

The growth medium in all cases contained 10^{-3} M IPTG. Assays and extracts are as described in Table 2.

synthesis of the enzyme fifty-fold, but glucose-6-phosphate fails to reduce the rate of enzyme synthesis irrespective of the presence or absence of the *i* gene product.

Thus it seems that the lack of response to catabolite repression is in all cases a result of the partial deletion of the *lac p* region. This view is confirmed by the observation that, in a strain with this deletion, CA 8001, the *lac* region of which has not been transposed, β-galactosidase is insensitive to catabolite repression (Table 4). On the other hand, in strains CA 8003 and CA 8019 with point mutations of the transition class[13] in the promoter site, β-galactosidase is as sensitive as usual to catabolite repression. Because in both the point mutants and the deletion mutant the concentration of β-galactosidase is much lower than in the parent strain, 3.300, it is evident that it is not simply the restriction in the rate of β-galactosidase synthesis that has caused the escape from catabolite repression.

In summary, our results show that a partial deletion in the *lac p* region renders the *lac* operon insensitive to catabolite repression. The deletion might remove the target site for a specific catabolite repressor protein the structural gene of which has not yet been discovered. Such a repressor would interact with a small molecule, a product of glucose catabolism, and then combine with the target site to reduce messenger production by the *lac* operon.

On the other hand, we need not propose an undiscovered repressor protein. Catabolite repression of the *lac* operon could be the result of the nonspecific catabolite interacting with the complex of RNA polymerase and the *lac* specific promoter. This catabolite could thus reduce initiation of RNA synthesis by the polymerase. With fusions of the *lac* operon to the *trp* operon, the polymerase and the *trp* promoter would form a complex insensitive to the catabolite. Deletions in the promoter such as L1 might alter the complex so that it is insensitive to the catabolite. In these cases, the synthesis of β-galactosidase would not be subject to catabolite repression.

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Sequence of the *in vitro* Transcription of T4 DNA

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The sequence of *in vitro* transcription of T4 DNA seems to parallel the *in vivo* process.

THE products of *in vitro* transcription of several DNA templates by purified *E. coli* RNA polymerase have been shown to resemble their *in vivo* counterparts in a number of ways—most strikingly with respect to asymmetry and selection of the transcribing strand[1–8]. It was suggested in 1965 that such fidelity of selection must be established at the initiation step of RNA synthesis[4,5]. This proposal has never been tested; the most direct demonstration would involve *in vitro* RNA polymerization on a single long transcription unit tested for the sequential appearances of promoter–proximal and distal polynucleotide sequences in the RNA product. We have instead used the entire mature T4 DNA molecule as template.

The appearance of viral RNA during the first minutes after bacteriophage T4 infection of *E. coli* has been analysed in detail by hybridization-competition, which identifies messages as hybridizable polynucleotide without regard to contiguity. This analysis reveals a transient period during the first minutes after infection, in which both the number of different RNA sequences, and the relative abundance of each sequence, change. The result of an experiment modelled after the work of A. Bolle, R. H. Epstein and W. Salser (unpublished) which confirms the existence of such a sequence *in vivo* is shown in Fig. 1 and Table 1. These observations do not, by themselves, distinguish whether the observed transcriptive sequence arises from (*a*) sequential, non-synchronous initiation of different transcription units or (*b*) propagation of transcription along synchronously initiated transcription units of appreciable length or (*c*) a combination of these. Because the average size of T4 RNA molecules increases during the first minutes after infection (contrary to previous observations[9]), however, a part of the observed transcriptive succession must arise from the finite transit time for viral transcription units.

We have examined the RNA synthesized *in vitro* for a corresponding sequence. Labelled RNA, synthesized *in vitro* at 15° C for various times (Table 2), has been

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