LETHAL EFFECT OF L-LEUCINE ON E. COLI, IN VIVO CORROBORATION OF CODING AMBIGUITY.

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Polyuridylic acid (poly U) directs not only the synthesis of polyphenylalanine, but is also known to promote the incorporation of leucine into polypeptides (Matthei et al .1962). The percentage of leucine incorporation in the presence of poly U can be quite appreciable, reaching 25 percent (Bretscher and Grunberg-Manago, 1962). The same phenomenon has also been observed in cell-free system from Chlamydomonas (Sager et al ., 1962). The incorporation of two different amino acids directed by the same codon leads to ambiguity. Whilst it's possible significance for protein synthesis in vivo is not known, it is clear that if this ambiguity should reflect also a lack of precision in the translation of the genetic information in vivo , it could lead to undesirable consequences for any organism.

An inhibitory effect of leucine which can be reversed competitively by phenylalanine was found in the case of Aspergillus mutants (Pontecorvo, 1964). In order to examine whether an excess of leucine can have a deleterious effect on living bacterial cells, we chose a multiple auxotroph of E. coli K-12 , strain 213 requiring in addition to phenylalanine also histidine and proline. This allowed us not only to observe the damage caused by possible replacement of phenylalanine by leucine, but also its specificity.

Materials and Methods. The strain of -E.coli K-12 213 used was obtained in our laboratory by crossing K-12 HfrC with a mutant requiring phenylalanine derived from strain PA417 (Ben-Gurion, 1963).

The synthetic medium was as described before (Ben-Gurion, 1963), with the addition of phenylalanine, histidine and proline, 20 µg/ml each. For viable counts the same medium supplemented with 2 % bacto agar (Difco) was used.
Cultures for use were initiated from synthetic agar slants by overnight growth in liquid medium. They were subsequently diluted tenfold in the same medium

and incubated on a rotary shaker for 3 to 4 hours until the population density reached about 1 x 10^8 cells/ml. This culture at a dilution of 1:500 was used as an inoculum for the experimental media. Unless otherwise stated in the text, the temperature of incubation was 37°C.

RESULTS AND DISCUSSION.

The effect of leucine on E. coli K-12 strain 213 was examined by incubating the organism in the synthetic medium lacking phenylalanine in the presence and absence of large amounts (200 µg/ml) of leucine. The viability was determined by plating aliquots at various intervals of time on the complete synthetic medium. In the presence of leucine the viable count decreased by more than two orders of magnitude after 8 hours and only few cells remained viable after 24 hours incubation. In the absence of leucine, after initial 10-fold multiplication a stationary phase was reached. (see Fig. 1) The limited growth of the control was probably due to residual phenylalanine. Although not in all experiments the shape of the survival curves was the same, a significant decrease in viable count after 24 hrs was always obtained. Thus we can see that incubation of

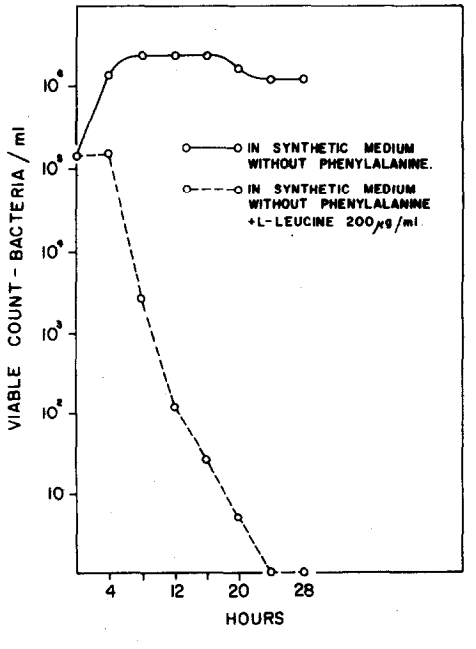

Fig. 1

E. coli cells with large amount of leucine can have a lethal action. However phenylalanine reversed this effect already at a ratio of phenylalanine to leucine 1 : 200. Isoleucine, glutamate, lysine, arginine, threonine, methionine , aspartate, alanine and glycine tested at the same high level (200 µg/ml) did not have any significant effect on survival, when they were added to the medium deficient in phenylalanine.

Table 1

Effect of amino acids of the survival of
E. coli strain 213, in synthetic medium.

Exper. No.	Amino Acid Omitted	Amino Acid Added 200µg/ml	Temp. of Incubation °C	Viable Count Initial	Viable Count After 24 hrs.
1	Phenylalanine	-	37	1.5×10^5	1.4×10^6
	Phenylalanine	Leucine	37	1.5×10^5	<10
2	Phenylalanine	-	37	1.3×10^6	7.7×10^6
	"	Leucine	37	"	<10
	"	Isoleucine	37	"	2.0×10^6
	"	-	20	"	10×10^6
	"	Leucine	20	"	4.5×10^4
	"	Isoleucine	20	"	10×10^6
3	Phenylalanine	-	37	1.5×10^5	3×10^6
	"	Leucine	37	"	4×10^4
	Histidine	-	37	"	4×10^6
	Histidine	Leucine	37	"	2×10^6
	Proline	-	37	"	$1,5 \times 10^6$
	Proline	Leucine	37	"	$1,1 \times 10^6$

The multiple nutritional requirements of the test organisms allowed us to examine the action of leucine in the absence of other essential amino acids. When histidine or proline were omitted in place of phenylalanine, addition of excess leucine did not result in appreciable lethal affect. (Table 1).

It has been shown by Szer and Ochoa (1964) that the ambiguity in coding by poly U increases at lower temperatures and other amino acids such as isoleucine are also incorporated to an appreciable extent. However under our in vivo conditions incubation of the organism at 20°C with excess of isoleucine in the absence of phenylalanine had no significant lethal action. At the same time the killing action of leucine at 20°C was less striking than at 37°C. In the cell free system the effect of lower temperature is ascribed to the increased complexing ability of the homopolymers (poly U and poly rT). We have not obtained parallel results in vivo where the directing template is the messenger RNA, whose secondary structure may be differently affected by alteration of temperature from that of poly U. In addition it can be assumed that the intracellular Mg++ concentration and other factors were not optimal for enhancement of the coding mistakes.

In the course of this work we have repeatedly isolated mutants which were insensitive to the high concentration of leucine. Similar mutants were also obtained in the case of Aspergillus (Pontecorvo, 1964). The mechanism of this resistance is being further investigated.

The above results are a strong indication that the coding ambiguity resulting in incorporation of leucine in the place of phenylalanine may also occur in vivo. The extent of such replacement, which will cause the loss of viability, has not yet been determined. Also no actual proof is available at present that the lethal effect of leucine is due to the formation of erroneous proteins. Further experiments, being conducted at present, will help to answer this question.

References

Ben-Gurion, R., J. gen. Microbiol, 30 , 173 (1963).
Bretscher, M.S. and Grunberg-Manago, M. Nature, 195 , 283 (1962).
Matthei, J.H., Jones, O.W., Martin, R.G. and Nirenberg, M.W. Proc. Natl. Acad. Sci. U.S. 48 , 666 (1962).
Pontecorvo, G. Private communication (1964).
Sager, R., Weinstein, I.B. and Ashkenazy, Y. Science, 140 , 304 (1963)
Szer, W. and Ochoa, S., J. Mol. Biol. 8 , 823 (1964).