Bacterial Mutant with Impaired Potassium Transport and Methionine Biosynthesis

Abstract. *A mutant of* Escherichia coli *has been isolated in which potassium transport and methionine biosynthesis are both impaired. Studies on the nutritional requirements of this organism suggest that the cystathionine cleavage enzyme is impaired. Experiments in low-potassium medium showed that the mutant was unable to maintain the usual high cell potassium concentration. Studies that were made of the mutant and its revertants suggested that a single genetic event was responsible.*

Mutant forms of *Escherichia coli*, deficient in their capacity to transport potassium against a concentration gradient, have previously been isolated both in this laboratory by Schultz and Solomon (*1*) and by Lubin and Kessel (*2*). In view of the possibility that the potassium-transport system might also serve other metabolic pathways, we have modified the selection technique to seek a mutant with a double defect. The purpose of this communication is to report the isolation of an auxotroph of *E. coli* B which is defective both in its ability to transport potassium and in methionine biosynthesis.

The present mutant ($H^-M^-\Phi^-_K$, strain BL 2) was derived from a histidine-marked auxotroph (H^-) of *E. coli* B (*3*). The parent auxotroph was similar to the wild type, being able to grow in a low-K medium in the absence of all amino acids but histidine. It was isolated by Lubin's method (*4*) of recycling through penicillin selections (*5*) with the modifications of Gorini and Kaufman (*6*). Cells in the log phase of growth were irradiated with a single dose of ultraviolet light sufficient to reduce the viable count to less than 1 percent of the original value. The entire suspension was collected on a 0.45μ Millipore filter and grown overnight in the K-rich medium A of Davis and Mingioli (*7*), supplemented with 18 amino acids including histidine. A portion of the suspension was diluted to a cell density of about 6×10^7 cells per milliliter and a penicillin selection was then performed in K-poor media ("sodium A," which is similar to medium A except that the K concentration is reduced to 0.021m*M* by a mole-for-mole replacement of K with Na) supplemented with 18 amino acids. The entire penicillin-treated suspension was then collected on 0.45μ Millipore filters, washed, and grown overnight in K-rich medium A with amino acids. If a mutant was not isolated, a portion of this last suspension was subjected to the recycling procedure, which consisted of repeating the process of penicillin selection and harvesting.

The present mutant was isolated on the third cycle. It was streaked on complete media and a single clone was selected for further studies. This auxotroph did not grow either on K-rich plates (medium A) supplemented with histidine, or on K-poor plates (sodium A) supplemented with 18 amino acids. Good growth was achieved on K-rich plates supplemented with 18 amino acids. Thus the auxotroph differed from its progenitor in requiring both high K and other amino acids in addition to histidine.

Studies of growth on K-rich plates indicated that methionine was the amino acid required. In studies designed to localize the defect, growth was obtained with methionine and homocysteine, but not with cystathionine, homoserine, cysteine, or serine. Thus the defect would appear to lie in the cleavage of cystathionine to homocysteine and pyruvate (*8*). In order to determine whether the defect is associated with the enzyme in this reaction, it would be necessary to show that the present auxotroph is permeable to cystathionine. This has been shown to be the case for another methionine auxotroph of *E. coli* B (*9*) which is defective in cystathionine synthesis. That this organism is permeable to cystathionine is evidenced by growth in the presence of $10^{-3}M$ cystathionine, a concentration in which $H^-M^-\Phi^-_K$ is unable to grow. This suggests that the methionine defect is associated with the cystathionine cleavage enzyme.

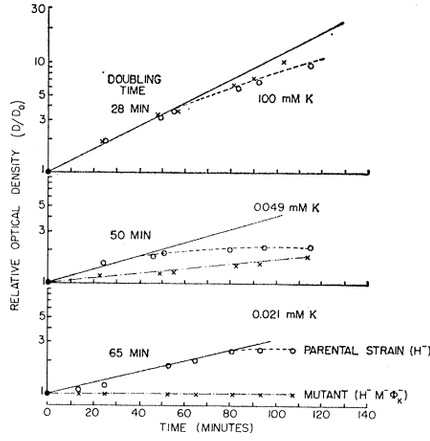

Fig. 1. Growth of H^- and $H^-M^-\Phi^-_K$ as measured by relative optical density. The origin has been shifted so that the initial point is at the beginning of the log phase. On the absolute scale growth fell off from logarithmic rate at an optical density of about 0.1 in 0.021m*M* K, 0.25 in 0.049m*M* K, and about 0.55 at 100m*M* K. In the media of low K concentration this has been attributed to exhaustion of K in the medium (*11*).

Figure 1 shows the effect of K concentration in the medium on growth. Auxotroph and parent strain were grown with constant shaking at 37°C to log phase in sodium A medium enriched with 18 amino acids and 5m*M* KCl. The cells were collected on 0.45μ Millipore filters, washed with sodium A, and transferred to fresh medium containing 18 amino acids and varying concentrations of K from 0.02m*M* to 100 m*M*. Growth was measured turbidimetrically by a Klett spectrophotometer. At the lowest K concentration, mutant growth was entirely suppressed. Growth began in 0.049-m*M* K, though the rate was still much slower than that of the parent strain. When the K concentration was increased to 100m*M* there appeared to be no difference between the growth rate of the parent strain and the mutant. Figure 1 also illustrates an unexpected finding, the effect of the K concentration in the medium on the growth rate of the parent strain, H^-. In the parent strain the doubling time increased from 28 to 65 minutes as the K concentration was lowered from 100m*M* to 0.021m*M*.

Experiments were next carried out to demonstrate that the failure of $H^-M^-\Phi^-_K$ to grow in a low-K medium was a consequence of a defect in the K-accumulation system. During the log phase of growth in sodium A medium, enriched with 18 amino acids and 5m*M* KCl, cells were collected by centrifugation at 1400*g* for 20 minutes. The K concentration, measured as described by Schultz and Solomon (*10*), was 217 ± 3 (S.E.) mmole per liter of cell water for H^- and 239 ± 2 mmole per liter of cell water for $H^-M^-\Phi^-_K$. After two washings in sodium A medium, these harvested cells were resuspended in the same medium except that the K concentration was reduced to 0.021-m*M*. The initial sample of H^- cells

was then characterized by a K concentration of 128 mmole per liter of cell water. During the incubation at 37°C, the cells took up K so that, at the end of a 15-minute period, the K concentration had risen to 212 mmole per liter of cell water, which approximates the K concentration in the unwashed harvested H^- cells. On the other hand, the initial K concentration in $H^-M^-\Phi^-_K$ had fallen to 60 mmole per liter of cell water, the lower value presumably arising because the mutant cell was unable to maintain its K concentration during the washing and centrifugation. In this case the cells lost so much K during incubation that no K could be detected in the cell by our analytical methods after 15 minutes at 37°C. Thus, it appears that $H^-M^-\Phi^-_K$ is unable to maintain the high K concentration ratios which can be attained by the parent strain.

It therefore appears that $H^-M^-\Phi^-_K$ is defective in its ability to concentrate K as well as in ability to synthesize methionine. In order to see whether these are expressions of a single defect, experiments were carried out on revertant clones. Thirteen such clones, selected at random from plate cultures on low-K medium which contained the 18 amino acids, were streaked on low-K medium plates, which lacked all amino acids but histidine. All 13 transport revertants had simultaneously reverted their biosynthetic defect since they grew well on these plates. Similarly four clones selected for reversion from their amino acid requirement were also transport revertants since they grew well on low-K medium supplemented with histidine. One amino acid revertant was isolated which retained its transport defect and would not grow on the low-K medium.

We also considered that all methionine-requiring auxotrophs might have defects in their K-transport system or that K might be necessary for methionine transport. Neither appeared to be the case. Several methionine-requiring auxotrophs of *E. coli* B (*9*) were investigated, including one that can grow in the presence of homocysteine but not cystathionine. All were able to grow on low-K medium, indicating that their methionine defects were unrelated to a K requirement.

In sum, we have observed a mutant with defective K transport and a methionine requirement. Although the possibility that we are dealing with two genetic defects has not been ruled out by our experiments, the indication is strong that only one genetic event is involved in the observed mutant and its revertants. If this is indeed the case, we would expect K transport in this organism to be metabolically related to methionine biosynthesis.

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References and Notes

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Auditory Flutter-Driving of Visual Flicker

Abstract. *Changes in the physical flutter rate of a clicking sound induce simultaneous changes in the apparent flicker rate of a flashing light. For example, for one observer a flicker with a frequency of 10 cycles per second was driven downward to as low as 7 cycles per second and upward to as high as 22 cycles per second by changing the rate of initially synchronous auditory clicks. The reverse does not occur—changes in the flicker rate do not induce changes in apparent flutter rates.*

The sense of the lateral position of objects is mediated largely by vision. Moreover, sounds will be sensed as originating from the most reasonable visible sources perceived to be acting in synchrony with the sounds. The ear, on the other hand, is an organ of eminently superior temporal resolution. A healthy young ear has a critical flutter frequency (the frequency at which a clicking sound appears steady) which is much greater than the critical flicker frequency (the frequency at which a flashing light appears steady) of an eye in the same head. Thus it seemed reasonable to try to obtain an index of the perceived rate of visual flicker by asking the subject to match this to a perceived rate of auditory flutter.

Up to the present time, the critical flicker frequency is the only aspect of visual temporal resolution that has been studied in detail, primarily because phenomenal flicker rates are very difficult to measure.

Unfortunately, such flicker rates are not satisfactorily measured by rates of flutter either. The interesting intersensory fact, first noted by Gebhard and Mowbray, is that the flutter appears to "drive" the flicker (*1*). Suppose that a whitish light is set to flicker at 5 cy/sec and a whitish sound (approximately but not necessarily of the same waveform) is set to flutter (approximately but not necessarily in phase) at exactly the same rate. Then the flutter rate is gradually turned up or down in frequency. The perceived flutter rate will, of course, go up or down accordingly; but quite unexpectedly, so also will the apparent flicker rate. The reverse does not occur—we can drive the flicker by the flutter but we cannot drive the flutter by the flicker. Since this driving is compelling, auditory flutter rates cannot be used to measure visual flicker rates. On the other hand, though flicker rate changes do not drive the flutter frequencies, they nevertheless cannot be used to measure subjective flutter rates because, in the presence of a fixed flutter rate, the change of flicker rate is quite simply not seen. The flicker stays put, locked in apparent synchrony with the flutter.

An experiment was therefore conducted to determine, first, over what flicker-flutter ranges driving takes place, and, second, how far up and down a change in flutter can drive an initially synchronous flicker. The parallel dimensions of brightness—loudness, color—pitch, binocularity—binaurality, laterality—contralaterality, waveform, phase, on-off ratio, and modulation depth can be examined only after the basic phenomenon itself is described.

The effect of sound on the critical flicker frequency has been studied