that afferent pathways from the diencephalic reticular ascending system induce, via hypothalamic centers, neurohumoral mechanisms participating in stress situations, including increased liberation of adrenalin. The lesion of the thalamic mid-line nuclei apparently interrupts this neurohumoral reflex arc which participates in metabolic preparatory reactions.

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Synchronization of Division in Escherichia coli

Abstract. The smallest cells of a culture in the logarithmic phase, when isolated by a single rapid filtration through 1.2-μ Millipore paper, show good synchronization and reproducibility through the first division cycle. The method minimizes metabolic shock and provides a culture in which an easily reproducible initial state is established at a known time.

Tests of the Maruyama and Yanagita technique (*1*) for isolating the largest cells of an *Escherichia coli* culture led us to the conclusion that temperature shock, introduced by extensive manipulations at room temperature, was probably responsible for the erratic results obtained. Subsequent experiments led to a synchronizing technique which is simple and fast and involves no operations likely to disturb the normal metabolism of the organism.

Our procedure for *E. coli* strains K12 (λ) and B is as follows (*2*). A 100- to 150-ml batch culture is grown in synthetic medium (*3*), with continuous aeration, to a density of the order of 10^8 cells per milliliter. Without other manipulation, the culture is filtered quickly through a single sheet of grade RA Millipore paper, pore size 1.2 μ, standard Millipore equipment being used for vacuum filtration (*4*), with a 25-liter bottle as "vacuum reservoir." Filtration is interrupted when a few milliliters of culture remain on the filter or when clogging reduces the rate of filtration appreciably. Filtration is completed in 2 to 3 minutes. The filtrate, containing 1 to 2 percent of the total cell population, is aerated through a sintered glass wand immediately after filtration. The entire process is carried out in the constant-temperature cabinet in which the initial culture is grown. No significant improvement resulted from repeated filtration through single sheets of Millipore paper or from the use of stacks of two or more papers.

Figure 1 shows the results of a typical experiment in which the filtrate was sampled periodically and assayed for cell count by triplicate plating on nutrient agar. Dilutions were adjusted to yield about 100 colonies per plate at minimal density. Zero time is taken at the mid-point of the filtration interval. The results of all our tests, with the two strains of *E. coli* and temperatures ranging from 25° to 37°C, may be summarized as follows. (i) Through the first growth cycle the $n(t)$ curve (cell count–time curve) is flat for about 70 percent of the mean generation time. The doubling time approximates the mean generation time closely. Contrary to common experience with temperature shock methods, the doubling is real rather than nominal, supporting the conclusion that quick filtration without separation of the cells from the original medium involves little if any disturbance of normal growth. (ii) Under close temperature control the second division cycle is well marked, but synchronization deteriorates in this cycle. Beyond 1.5 times the mean generation time, reproducibility of the $n(t)$ curve has not been good enough to warrant selection of a typical curve. Relaxation of temperature control is reflected in a deterioration of synchrony and reproducibility in the interval from 0.7 to 1.5 times the mean generation time. (iii) Beyond the second cycle the $n(t)$ curve assumes a form which approximates the normal growth curve.

As additional grades of Millipore paper become available, improvement in cell-size resolution, with consequent sharpening of synchronization, will probably be possible. It should be remarked, however, that if the standard deviation in generation time characteristic of *E. coli* is as large as 0.3, as was recently estimated (*5*), a cell population strictly homogeneous in age at filtration would show rapid deterioration of synchrony after the first cycle. Attempts made in our laboratory by W. V. Morgan and one of us

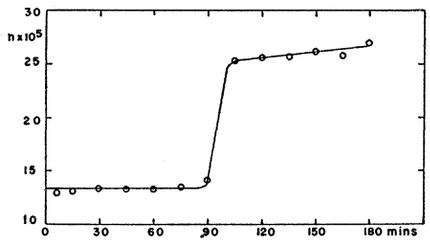

Fig. 1. Cell count-time characteristic for *E. coli* K12 (λ) filtered at zero time. *N*, cells per milliliter; *t*, 30°C; mean generation time, 105 minutes. Grade RA Millipore paper was used as filter.

(P.A.A.) to impose synchronized division on continuously cultured *E. coli* by programmed temperature cycling gave disappointing results which are probably attributable to this factor.

From the preparative standpoint, the quick-filtration method would appear to have the marked advantage of supplying a culture for which the initial state and succeeding growth curve are sharply defined on the time axis and hence determinable before rather than after the event (*6*).

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References and Notes

1. Y. Maruyama and T. Yanagita, *J. Bacteriol.* **71**, 542 (1956).
2. We are indebted to Max Delbrück for the K12 and to W. Szybalski for the type B slants.
3. This is the C-medium of Roberts, Abelson, Cowie, Bolton, and Britten [*Carnegie Inst. Wash. Publ. No. 607* (1955)]. Glucose to 0.1 percent was added via UF filter after autoclaving.
4. The equipment is manufactured by the Millipore Filter Corp., Bedford, Mass.
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Utilization of Organic Carbon by a Marine Crustacean: Analysis with Carbon-14

Abstract. An isotope-dilution technique using carbon-14 was employed to determine quantitatively the carbon budget of a filter-feeding crustacean. The amount of carbon ingested ranged between 0.044 and 0.139 mg. Incorporation of carbon varied between 11.3 and 73.6 percent per day per organism, with an average of 32.5 percent for the animals tested. Values for oxygen consumption are given as they relate to carbon intake and utilization.

Energy and feeding interrelationships have been experimentally determined for some zooplankters. For example, Richman (*1*) determined a calorimetric budget for the fresh water crustacean