of mutant colonies in these two groups were similar. No mutants were recovered from colonies not exposed to thymidine-H^3 (tubes 7 and 8). The number of mutants isolated can be accounted for by supposing that thymidine-H^3 acts as a selective lethal agent for cells that have grown in its presence.

On theoretical grounds, it does not seem likely that much of the selective killing of wild-type could be caused by radiation from thymidine-H^3 in the surrounding solution. The emitted beta particle has an average energy of 5.7 kev and a range in water of about 1 μ. In these experiments the ratio of fluid volume to bacterial volume was about 10^4, and almost all the radiation originating in the surrounding fluid would fail to reach the bacterial cells. This argument is supported by the results of the reconstruction experiment, in which the presence of thymidine-H^3 did not accelerate killing of the histidine auxotroph.

In addition, aliquots from tubes 1 to 8 of the mutant isolation experiment were diluted sixfold after the period of incubation and stored at 5°C. Almost as many mutants were isolated from these samples as from the undiluted tubes. This suggests that thymidine-H^3 is incorporated into the cells in a form unable to diffuse out during cold storage.

In a third experiment, the bacterial suspension was diluted considerably, so that the count at the end of incubation was only 10^6 per milliliter. Killing was rapid, presumably because the amount of thymidine-H^3 per cell was high, and after 5 days of cold storage the yield of colonies which were mutants ranged from 10 to 50 percent.

Among the auxotrophic mutants isolated by this procedure the following requirements have been identified: arginine, cystine, glycine, histidine, isoleucine, leucine, methionine, proline, serine, threonine, tryptophane, tyrosine, valine, isoleucine plus valine, and multiple aromatic compounds. These experiments show that thymidine-H^3 is an efficient selective agent for the isolation of auxotrophic bacterial mutants. The method may be applicable to other species of cells.

MARTIN LUBIN
Department of Pharmacology, Harvard Medical School, Boston, Massachusetts, and Marine Biological Laboratory, Woods Hole, Massachusetts

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Mutagenic Effect of Azaserine in Relation to Azaserine Resistance in Escherichia coli

Abstract. For demonstration of the mutagenic effect of azaserine (mutation from streptomycin dependence to nondependence), higher concentrations of this antibiotic are required with azaserine-resistant *Escherichia coli* than with the sensitive, parental strain. At barely toxic concentrations of azaserine, however, the mutagenic response of the resistant strain is many times higher than that of the sensitive strain.

The suitability of azaserine as a chemical mutagen in elucidating the mutagenic process in *E. coli* has been evaluated and described in an earlier publication (*1*). Like most other mutagens, azaserine induces mutations with increasing frequency at concentration levels that are increasingly bactericidal. Nevertheless, it is one of the few known chemical mutagens which is appreciably mutagenic at levels relatively nonbactericidal. This poses the important question as to whether the process of azaserine-induced mutagenesis is related to the bactericidal property or whether the two phenomena are independent of each other. With this question in mind, a comparative study of the mutagenic response in mutants of a streptomycin-dependent strain of *E. coli*, showing varying degrees of azaserine resistance, was initiated (*2*).

From the parent Sd-4 strain (A) (*3*), mutants (B, C) resistant to varying levels of azaserine (*4*) were derived by methods based on the gradient plate principle (*5*). These were maintained on nutrient agar containing 100 μg of streptomycin per milliliter and concentrations of azaserine increasing from 0 to 1000 μg/ml. Washed cells from agar slants were used as inocula for the experiments. Details of the methods were the same as those described in an earlier paper (*1*). The effect of exposure to varying concentrations of azaserine for a period of 2 hours at 32°C on the survival of azaserine-sensitive (A) and azaserine-resistant (B, C) strains and on the mutation rate to streptomycin independence was studied.

The results, selected as representative of several independent experiments, are presented in Fig. 1. They suggest that azaserine is able to induce mutations in azaserine-resistant strains of *E. coli* only at concentration levels much above that required for mutagenesis in the parent, sensitive strain. It is apparent that, in strains resistant to azaserine, an increase in the mutation rate comparable to that produced in the more sensitive parental culture is obtained only at higher concentrations of the mutagen. Comparable results were obtained with several additional, independently isolated azaserine-resistant mutants. A closer analysis of the data, however, reveals that the resistant strains treated at barely bactericidal concentrations of azaserine show a higher mutagenic response than the sensitive parent under comparable conditions.

The data presented suggest that although the mutagenic and bactericidal effects of azaserine are correlated, the quantitative responses are not identical. If the bactericidal effects of azaserine on sensitive and resistant cells are regarded

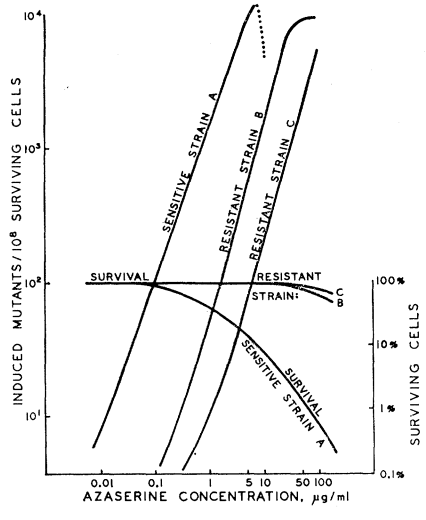

Fig. 1. Effect of azaserine concentration on the number of induced mutants (mutation from streptomycin dependence to independence) and on the percentage of survivors, assessed with azaserine-sensitive strain Sd-4 of *E. coli* and two azaserine-resistant mutants derived from that strain. Two hours' exposure in distilled water at 32°C.

as separate lethal mutations, the differential effect of this mutagen on survival and on suppressor mutations to streptomycin independence could be considered analogous to the differential mutagenic effect of a mutagen on different genes, as observed by Demerec (*6*).

V. N. IYER
W. SZYBALSKI
S. B. Garda College, Navsari, Bombay, India, and *Institute of Microbiology, Rutgers University, New Brunswick, New Jersey*

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Measurement of Regional Blood Flow by Indicator Dilution

Abstract. Appropriate selection of injection and collection sites permits quantification of certain regional blood flows by the single-injection, indicator-dilution method. Quantifying formulas are derived, and application of the method to several regional beds is described.

The indicator-dilution method (*1*) is used extensively to measure cardiac output but has had limited application to regional flow. Following rapid venous or central injection of an indicator, successive curves of rising and falling indicator concentration are inscribed at an artery, representing initial circulation and recirculations. If the amount of indicator injected (I) is known, and if the area (A), which is the product of the average concentration (C) and the duration of the curve (T), is determined for the extrapolated semilogarithmic graph of the initial curve, then the flow (Q) is calculated from the following conventional formula:

$$Q = I/CT = I/A \qquad (1)$$

It follows that if, after central injection, the amount of indicator entering a given vessel were known, flow through that vessel could be calculated. Thus, for the vessel R:

$$I_R = Q_R C_R T_R \qquad (2a)$$

and

$$Q_R = I_R/A \qquad (2b)$$

where A is the area under the local curve ($C_R T_R$) or under any simultaneous peripheral dilution curve, all such areas being equal.

If R is the single afferent or efferent for a region, knowledge of I_R permits calculation of the regional flow. I_R is proportional to the unknown flow and has no other physiologic determinants. If, however, a natural or designed measurable supplement to Q_R produces a measurable alteration in A, solution is possible by means of simultaneous equations.

If any vessel or chamber V receives blood from several sources, among them the complete flow for region R, and if I_R is delivered prior to indicator I_X from other sources, an early curve, representing Q_R, will be inscribed, following mixing, at sites distal to V. The average concentration of the indicator (C_V) during inscription of this early curve is a function of the indicator (I_R) and the flow ($Q_R + Q_X$) that traverses V during the time interval T. Thus:

$$I_R/(Q_R + Q_X)T_V = C_V \qquad (3)$$

Combining Eqs. 2 and 3, we have:

$$Q_R A = (Q_R + Q_X) C_V T_V \qquad (4)$$

$C_V T_V$ is the area under the early curve. Therefore:

$$Q_R A = (Q_R + Q_X) A_V \qquad (5a)$$

or

$$Q_R = \frac{(Q_R + Q_X)}{A/A_V} = \frac{Q_X}{(A/A_V) - 1} \qquad (5b)$$

A is the area under a conventional arterial curve. Therefore, whenever a discrete A_V is obtained, the Q_R responsible for it can be quantified as a function of Q_X or of $Q_R + Q_X$. The latter, moreover, is a measurable quantity whenever V is a cardiac chamber, since $Q_R + Q_X$ must then become the output of one of the ventricles, and, in certain instances, Q_X may be a measurable fraction of a ventricular output. Thus, one can measure flows which empty into a cardiac chamber to produce a dilution curve distinct from those of general circulation and recirculation.

This principle is applicable to at least four systems:

1) With left-to-right shunts—through atrial septal defects, for example—shunted blood (Q_R) joins Q_X to produce pulmonary flow, which can be estimated from a peripheral arterial dilution curve after injection into the pulmonary artery, while Q_R produces an early curve from the right ventricle. The ratio of the area of the early right ventricular curve to the area of the systemic arterial curve equals the fraction of pulmonary flow traversing the shunt.

2) After proximal aortic injection, the earliest curve expected from the left ventricle, in the absence of valvular regurgitation, is that of the indicator completing its first circulation. However, physiologic shunts exist in the form of systemic-pulmonary (chiefly bronchopulmonary) communications through which a portion of the left ventricular output returns to the left atrium without traversing systemic great veins and the right heart. This pulmonary collateral flow (Q_R) plus right ventricular output (Q_X) equals systemic flow, which is estimated from the peripheral arterial dilution curve, while Q_R produces an early curve from the left ventricle. The ratio of the area of the early left ventricular curve to that of the systemic curve equals the fraction of the left ventricular output traversing pulmonary collateral channels.

3) The first indicator to appear at the pulmonary artery after left atrial injection should be that traversing the short, rapid, low-volume pathway through the coronary sinus into the right atrium. The systemic output is estimated from a peripheral arterial curve, while the early pulmonary artery curve is proportional to coronary sinus flow, and the ratio of the area of the latter curve to that of the former expresses coronary flow as a fraction of systemic output.

4) With mitral regurgitation the flows converging upon the left atrium are the forward flow (Q_X) and the backflow (Q_R). After left ventricular injection, the early curve from the left atrium will be proportional to Q_R, while the conventional systemic arterial curve will be proportional, not, as in the situations cited above, to the sum of Q_R and Q_X, but to Q_X alone, the forward flow. Since the measured general flow is a fraction of, and not the entire, ventricular output, the version of Eq. 5 required is that in which Q_R equals the general flow divided, not by the ratio of the general to the regional area, but by that ratio minus 1.

In addition, one can infuse Q_X exogenously, as a known volume, into the arterial inflow to any part. If, then, following central injection and mixing of the indicator, a turbulence exists at or distal to the infusion site sufficient to produce mixing between the arterial blood and the infusate, a collection downstream will reveal a proportionally altered dilution curve. The ratio of the area of the latter curve to that obtained at another peripheral vessel will give the fraction of systemic output traversing the local bed. This has obvious implications for measurement of flows in organs and limbs.

Although the early appearance of dye at sites upstream from the site of injection has been used qualitatively to detect the presence of left-to-right shunts and mitral regurgitation (*2*), quantification of these or of other flows by comparison of the areas of modified regional curves with general systemic curves has not, to our knowledge, been reported.

The practical applicability of this