of the avoidance responses within each experimental period and day-to-day reproducibility confirm Sidman's results. The food curve in Fig. 2 is similar to records of pigeons and other rats after extended training on this kind of procedure. The interaction between the two schedules can be determined by manipulating each schedule in turn and observing the effect on the behavior under the control of the other schedule. For example, if the food reinforcement were not delivered at the end of the 8-min period, the rate of bar pressing would eventually fall to zero whenever the buzzer was on. Any concomitant change in the shock-avoidance behavior would be a consequence of an interaction from the food-maintained behavior. The different character of the rate changes generated by the two schedules of this experiment simplifies the identification of the interactions between them.

More complicated behavioral processes, such as discrimination procedures or other schedules of food reinforcement, could also be used alternately with the avoidance procedure to serve as base lines for the emotional by-products of the avoidance behavior or side effects of analgesic drugs.

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Filament Formation in *E. coli* Induced by Azaserine and Other Antineoplastic Agents

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Azaserine (0-diazoacetylserine) is a new antibiotic that has limited activity against microorganisms but is of great interest because of its ability to inhibit certain experimental neoplasms (*1–3*).

A remarkable effect of azaserine on the growth of *Escherichia coli* was the induction of the formation of greatly elongated filaments, as are shown (*4*) in Fig. 1. Examination of these filaments in the phase microscope indicated that they were apparently multinucleate and nonseptate. The length of the filaments varied from a few times to as much as 100 times the length of a normal cell, depending on the time of observation after exposure to the inhibitor. Short filaments could be observed only 30 min after inoculation into azaserine-containing medium.

In contrast to certain other observations on inhibitor-induced filament formation (*5, 6*), there was no critical level of azaserine at which the phenomenon

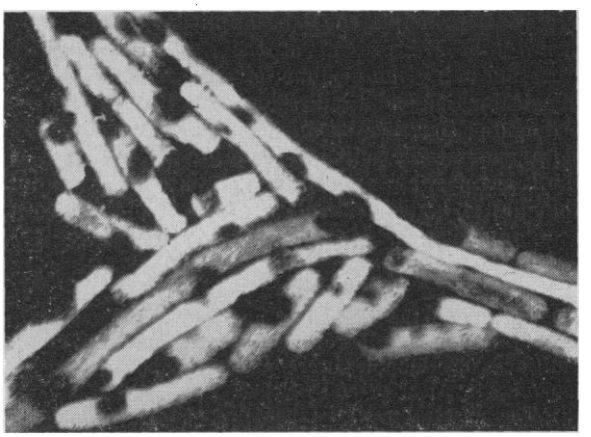

Fig. 1. Electron photomicrograph of filamentous *E. coli* after 5-hr exposure to 0.025 gamma/ml of azaserine. Several organisms of normal length may be seen (×4800).

occurred. Although detailed correlation of filament length with azaserine level has not yet been made, it was obvious that filaments appeared whether the inhibition was barely significant or almost complete.

The striking nature of this morphological aberration led to the examination of other inhibitors and antineoplastic agents. For most of the growth studies, a salts-glucose medium (*7*) was employed, but observation of filament formation could be repeated in nutrient (neopeptone) medium. *Escherichia coli* (Parke, Davis Culture Bureau No. 03489) was maintained in the synthetic medium. A 0.1-ml inoculum from an over-night growth was added to 9.9 ml of the medium at 37°C containing the desired constituents, and nephelometric determinations of cell mass were made at hourly intervals for 7 hr. Phase microscopic examinations were made of cultures that were inhibited in the range of 30 to 70 percent.

Under these conditions, the following inhibitors did not cause the formation of nonseptate filaments: chloramphenicol, chlortetracycline, oxytetracycline, streptomycin, sulfanilamide, aminopterin (*8*), a-methopterin (*8*), 8-azaguanine (*8*), glycidol, 2,6-diaminopurine, 6-mercaptopurine (*9*), ethionine, β-2-thienylalanine, 6-methyltryptophan, isobutyl diazoacetate (*9*), and diazomalonic ester (*9*). The following compounds were found to be as potent as azaserine in inducing filament formation: methyl-bis(2-chloroethyl)-amine, triethylenemelamine (*8*), and 5-diazouracil. Filaments induced by penicillin were characterized by large sphere-shaped vesicles (*10*), a feature that was never seen in filaments produced by the other inhibitors.

Since this study was made, another report has appeared with illustrations of the filaments induced by nitrogen mustard (*11*). It has been known for many years that gamma-irradiation of *E. coli* results in filament formation (*12*).

It has been observed in this laboratory, as well as in another (*13*), that inhibition of *E. coli* in synthetic mediums by low levels of azaserine may be reversed by aromatic amino acids. Higher levels of azaserine

Table 1.

Inhibitor	Level (gamma/ml)	Growth* at 7 hr	Addition	Level (gamma/ml)	Growth,* addition only	Growth,* addition + inhibitor
Azaserine	0.025	42	L-Tryptophan	2.5	100	100
Azaserine	.05	0	L-Tryptophan	5.0	100	78
Azaserine	.5	0	L-Tryptophan	50.0	100	21
Azaserine	2.0	0	L-Tryptophan	1000.0	100	8
Azaserine	0.025	52	Cytidylic acid	100	100	63
Azaserine	.025	40	Glutathione	150	100	32
Methyl-bis(2-chloroethyl) amine	1.0	40	L-Tryptophan	100	100	38
Methyl-bis(2-chloroethyl) amine	1.0	40	Cytidylic acid	100	100	36
Methyl-bis(2-chloroethyl) amine	2.0	26	Glutathione	300	84	61
5-Diazouracil	0.5	36	L-Tryptophan	100	100	65
5-Diazouracil	.5	31	Cytidylic acid	5	100	0
5-Diazouracil	.5	38	Glutathione	150	85	74

* Control growth = 100.

become increasingly refractory to this reversal, as is shown in Table 1. Sulfhydryl compounds are known to act as protective agents against the effects of radiation and radiomimetic compounds (*14*). It was discovered in the course of reversal studies that cytidylic acid *potentiated* the inhibition of *E. coli* by 5-diazouracil, although by itself cytidylic acid had no effect on growth. At the level of cytidylic acid that caused marked potentiation, adenylic, guanylic, uridylic, thymidylic, and desoxycytidylic acids had no effect. Neither uracil nor uridine, at 100-gamma/ml levels, had any reversing or potentiating effect on the inhibition by 0.5 gamma/ml of 5-diazouracil.

Advantage was taken of these effects of tryptophan, glutathione, and cytidylic acid in an attempt to discover possible interrelationships among the three filament-formers involved, with the results shown in Table 1. Both tryptophan and glutathione appeared to "reverse" partially diazouracil inhibition, indicating possible connections with the modes of action of azaserine and nitrogen mustard. The effect of cytidylic acid on diazouracil inhibition was unique, and glutathione had no effect on azaserine inhibition.

Additional information is available that emphasizes divergencies in the modes of action of azaserine and nitrogen mustard. Azaserine did not reduce the viscosity of solutions of highly polymerized desoxyribonucleic acid, nor did it inhibit rat brain cholinesterase, while nitrogen mustard is very active in both respects (*15*). On the other hand, the evidence presented here suggests that interference with aromatic amino acid synthesis or utilization may be only one aspect of azaserine activity.

Obviously, such a complex situation calls for many approaches and more penetrating analyses. On the basis of the results so far obtained, it may be concluded that filament formation under the described conditions is not peculiar to antineoplastic agents but can be indicative of a type of cytotoxicity that may or may not be extrapolated with success to mammalian systems. It is hoped that biochemical investigations in progress will make it possible to evaluate further the significance and possible utility of filament formation in the search for antineoplastic compounds.

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Sucrose Inhibition of Resorcinol Hemolysis

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In a previous electron microscopic study of the surface and interior of erythrocytes in the process of hemolysis, the observed changes suggested that osmotic hemolysis left the erythrocyte ghost elastic, while antibody and complement hemolysis produced a rigid ghost (*1*). These results were compatible with the different shape and volume changes produced in solution by low osmotic pressure on one hand and various hemolysins on the other hand (*2*, pp. 26, 82, 245). In general, the hemolysins produce a decrease in surface area followed by an increase to a critical area and volume, different for each agent, at which point hemolysis occurs. One hypothesis to explain this two-stage process would be that the hemolytic agent pro-