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-

Table 1. Resorcinol hemolysis of human erythrocytes—effect of sucrose and glucose.

2.5-percent suspension of RBC in:			Hours*							
Resorcinol *M*	NaCl *M*	Sugar *M*	0.5	1	1.5	2.5	3.5	4.5	7	23
0.032	0.15		0	0	+	+	+	+	+	+
0.124	0.15		±	±	++	++	++	++	+++	++++
0.124	0.15	0.28 glucose	±	±	+	+	+	+	++	++
0.124	0.15	0.15 sucrose	0	0	0	0	0	0	±	+
	0.15	0.28 glucose	0	0	0	0	0	0	±	±
	0.15	0.15 sucrose	0	0	0	0	0	0	±	±
	0.15		0	0	0	0	0	0	±	±

* At 25°C for 7 hr, then at 3°C for next 16 hr.
± indicates a trace, and ++++ indicates maximal hemolysis.
An isotonic solution = 0.15*M* NaCl = 0.3*M* sucrose or glucose = 10 percent sucrose = 5 percent glucose.

duces an increase in the intermolecular surface forces that results in shrinking or folding of the "membrane" with an increased rigidity. The second stage, swelling to the point of rupture, could be explained by an increase in internal osmotic pressure. This can be tested (*3*) by attempting hemolysis in a solution made hypertonic with nonpenetrating molecules such as sucrose. Resorcinol was chosen as the test hemolytic agent because it allows a volume increase before hemolysis greater than that produced by most other agents, including antibody and complement (*2*, p. 240).

In the first of these preliminary experiments, varying concentrations of resorcinol (1,3-benzenediol) were added to 2.5-percent suspensions of washed human red blood cells. The NaCl concentration was maintained at 0.15*M* (isotonicity). The degree of hemolysis was estimated from 0 to ++++ by the volume of sedimented red cells and the color of the supernate. Results typical of a series of tubes are shown in Table 1. Human red cells are hemolyzed by resorcinol in a manner suggested by the general S-shaped hemolysis-time curves discussed by Ponder (*2*, p. 176). At low resorcinol concentrations, a small amount of hemolysis occurred in 1.5 hr and did not progress further during the experiment. Larger concentrations produced more rapid and more extensive hemolysis. Resorcinol hemolysis is completely inhibited by 5 percent sucrose (0.15*M*) in isotonic saline. Five percent glucose (0.3*M*) under similar conditions showed only moderate inhibition, which could be explained by the specific active transport of glucose into human red cells (*4*).

In the next experiments, a few representative results of which are shown in the upper half of Table 2, it was found that hypertonic sucrose solutions would also prevent resorcinol hemolysis of rabbit red cells. Sodium chloride at the same osmotic pressure does not prevent resorcinol hemolysis but does slow it. This partial inhibition by a slowly penetrating ionic substance is compatible with an osmotic effect and suggests that the sugars do not need to act by virtue of their chemical similarity to one another and to resorcinol. Lowering the temperature from 26° to 3°C delays hemolysis.

In the third set of experiments, the sucrose concentration was varied. The results of two of these are shown in the lower half of Table 2. Most solutions of 1 percent sucrose (0.030*M* or 0.1 isotonic) in isotonic NaCl and higher concentrations of sucrose completely inhibited resorcinol hemolysis of rabbit erythrocytes in the experimental period; 0.025*M* sucrose and gradually decreasing concentrations down to 0.005*M* permitted increasing rates of hemolysis.

The results are entirely compatible with the original

Table 2. Resorcinol hemolysis of rabbit erythrocytes—effect of temperature, hypertonic NaCl, and sucrose concentration.

2.5-percent suspension of RBC in:			Hours						
Resorcinol *M*	NaCl *M*	Sucrose *M*	0.16	0.5	1	1.5	2	2.5	3
0.124*	0.15		0	0	0	+	++	+++	++++
0.124	0.15		0	±	++	++++	++++	++++	++++
0.124	0.07	0.6	0	0	0	0	0	0	0
0.124	0.37		0	0	±	±	+	++	+++
0.124	0.15		0	0	0	+	++	++++	++++
0.124	0.15	0.010	0	0	0	±	+	++	+++
0.124	0.15	0.030	0	0	0	0	0	0	0

* At 3°C; the other results are at 25 to 26°C. *p*H of resorcinol tubes 6.8 to 6.9.
Controls with equivalent amounts of sucrose and NaCl showed no hemolysis.
The last three rows were determined with a different suspension of red cells at a different time.

hypothesis, although other effects of the hemolytic agent on the structure of the cell cannot be ruled out. Inhibition of saponin or bile salt hemolysis by various sugars in isosmotic solutions has been described and the possible influence of reduced ionic strength discussed (*2*, p. 274). The sucrose inhibition of the present experiments is effective even with the ionic strength maintained constant. An increased internal osmotic pressure produced by a hemolytic agent might be due to (i) an increase of ions in the interior of the cell consequent to the loss of differential membrane permeability, or (ii) dissolving normally insoluble or structurally bound internal macromolecules. Experimental support of the first factor has been advanced by several investigators (*5, 6, 7*). However, a low concentration of resorcinol will allow marked cation shifts with only a little swelling (*2*, p. 244). The second possibility has apparently not yet been investigated, but it is noteworthy that the excess osmotic pressure, 0.1 isotonic, of the lowest sucrose concentration completely inhibiting resorcinol hemolysis in these experiments corresponds exactly to the osmotic pressure calculated for hemoglobin in red cells by Wilbrandt (*7*). Further work is being done to elucidate these mechanisms with quantitative volume and percentage hemolysis determinations.

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An Earlobe Algesimeter: A Simple Method of Determining Pain Threshold in Man

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A dependable and reproducible method for determining pain threshold is essential in the quantitative evaluation of analgesic drugs. Thermal radiation and electric stimulation of the tooth pulp, two commonly used methods, have recently been criticized (*1–3*), because the results obtained have not been uniformly reproducible even in trained subjects. Faradic stimulation, for the determination of pain threshold, was first clinically investigated by Martin *et al.* in 1913 (*4–7*). Macht and his associates (*8*) in 1915 used an inductorium to study quantitatively the analgesic action of opium alkaloids in trained subjects. It is the purpose of this paper to describe the use of the inductorium for the determination of pain thresholds in untrained subjects and to introduce the earlobe as the site of stimulation.

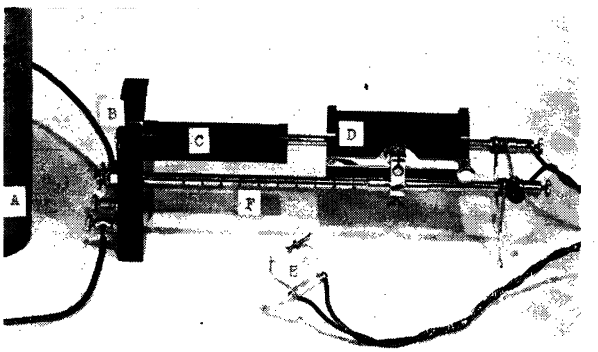

Fig. 1. Inductorium for determination of pain thresholds: *A*, battery; *B*, circuit breaker; *C*, primary coil; *D*, secondary coil; *E*, earpiece; *F*, calibrated scale.

The apparatus (Fig. 1) consists of a standard inductorium connected to a 1½-v dry cell battery. The primary and secondary coils are identically wired. A simple key is interposed between the battery and inductorium. Direct interrupted current is obtained through an electromagnetic circuit breaker. With the help of an electrocardiograph, the circuit breaker was regulated to produce faradic current of 60-cy/sec frequency. An adjustable earpiece is connected to the secondary coil of the inductorium.

The investigation was carried out on 12 male and 12 female untrained volunteers whose ages ranged from 21 to 36 yr. The subject lay on a bed in a quiet room and was allowed to rest for 20 min before the testing began. The earpiece was then applied to one of the earlobes and the screw was adjusted until good contact without discomfort was obtained. The secondary coil was then moved at a uniform slow speed toward the primary coil, and the subject was familiarized with the sensations of vibration, prickling, and sudden sharp pain (the end-point), which occur successively as the secondary coil is advanced. The volunteer was instructed to signify immediately the onset of the sharp pain. After this preliminary trial, 10 tests were carried out at 3-min intervals on each subject. The position of the secondary coil at the moment of response was read from the calibrated scale; this figure was taken to represent the pain threshold. After the 10 tests, one similar test was carried out on the other earlobe. During the experiments, the subjects could not see the markings on the scale, nor were the results discussed with them. All the tests were performed by the same investigator (M.S.).

Table 1 shows the means and standard errors of pain thresholds obtained in the male and female groups. The single test on the contralateral ear fell