

Table 1. Influence of pH, oxygen tension, dinitrophenol (DNP), and temperature on the effect of mitomycin C on root-tip cells of *Vicia faba*. All roots were treated with 0.001 percent mitomycin C; the recovery period was 72 hours; 200 cells were scored for each treatment.

Treatment conditions	Normal (%)	Abnormal (%)	Deletions (%)	Isochromatids (%)	Exchanges (%)
<i>Influence of pH; roots treated at 20°C in air</i>					
pH 8.5	70	30	0	32	21
pH 6.8	66	34	0	36	17
pH 4.5	62	38	0	55	15
<i>Influence of oxygen tension; roots treated at pH 6.8 at 20°C</i>					
0% O ₂ (N ₂)	54	46	0	39	21
20% O ₂ (air)	55	45	0	56	16
100% O ₂	58	42	0	44	19
<i>Influence of 1 × 10⁻⁴ M dinitrophenol; roots treated at pH 6.8 at 20°C in air</i>					
DNP present	55	45	0	58	16
DNP absent	58	42	0	36	16
<i>Influence of temperature; roots treated at pH 6.8 in air</i>					
Temp. 10°C	52	48	0	61	15
Temp. 25°C	55	45	0	56	16

ring within 48 hours. A concentration of 0.0005 percent for 1 hour produced no lethal effect, some inhibition of mitosis (few division figures were apparent 24 hours after treatment), and a low frequency of chromatid aberrations 48 and 72 hours after treatment. A 0.001-percent concentration of mitomycin C with a 1-hour treatment period proved to be the most efficient combination for examining the effect of this compound on chromosomes. After treatment with 0.001-percent mitomycin C there was a marked inhibition of mitosis. Few division figures or aberrations occurred 24 hours after treatment. The graph (Fig. 1) illustrates the frequencies of isochromatid aberrations obtained at different fixation times after treatment. The highest frequency was obtained at 72 hours and the frequency decreased thereafter, but the level of isochromatid aberrations was still high 96 hours after treatment. It would be very difficult to determine the mitotic stage that is most sensitive to mitomycin C, since mitosis is inhibited to such an extent after treatment. The highest frequency of aberrations coincided with the highest frequency of division (at 72 hours), but the rate was maintained at a constantly high level from 48 through 96 hours. These data suggest that at least most interphase stages are sensitive to mitomycin C.

Since many of the chemical mutagens produce different results in different environments, it was of interest to determine how such alterations of the environment as pH changes, variations in oxygen tension, elimination of adenosine triphosphate, and changes in temperature altered the effect of mitomycin C. The results of experiments designed to determine the nature of the influence of these environmental changes on the effects of mitomycin C are listed in Table 1. The results seem to indicate that varying the environment

during treatment with mitomycin C does not appreciably alter the ability of this mutagen to induce breaks.

Another interesting property of many radiomimetic compounds is their ability to produce breaks in one or more specific areas along the lengths of the chromosomes of *Vicia faba*. In contrast to breaks induced by x-rays, which seem to be distributed more or less at random (8), breaks induced by 8-ethoxycaffeine are concentrated in the nucleolar constriction (6, 9), those induced by maleic hydrazide occur in segments of heterochromatin on either side of the centromere of the long chromosome, and breaks induced by beta-propiolactone, nitrogen mustard, di(2,3-epoxypropyl) ether, and potassium cyanide occur in heterochromatic segments in the middle of the short chromosomes (8, 10, 11). Also, when breaks are distributed in somewhat random fashion, the rates obtained by dividing the total number of breaks in the short chromosomes by the total number of breaks in the long chromosomes (*S/L* ratio) is approximately 2.5 (11). The *S/L* ratios observed after treatment with 8-ethoxycaffeine or maleic hydrazide are less than 1 (11), whereas those observed after treatment with nitrogen mustard or di-epoxide are greater than 2.5 (11).

Over 800 breaks (in 450 cells) induced by mitomycin C were scored as to location. The *S/L* ratio was observed to be about 1.5, indicating some specificity for the long chromosomes. Approximately 80 percent of the breaks observed in the long chromosomes were situated in the nucleolar organizer constriction, about 15 percent being located in the heterochromatic segments on either side of the centromere. Most of the other breaks occurred in the heterochromatic segments of the short chromosomes. In all, about 93 percent of the breaks were located in heterochromatin.

Mitomycin C, like most radiomimetic compounds, has unique qualities. For example, like beta-propiolactone, nitrogen mustard, di-epoxide, and several others, it seems to act independently of oxygen tension, temperature, and pH; but the breaks induced are mainly concentrated in the long chromosomes as are those induced by 8-ethoxycaffeine and maleic hydrazide, which, however, are known to exhibit a strong oxygen and temperature dependency.

Because mitomycin C is of interest as a carcinostat, it is desirable to know whether its anticarcinogenic characteristics can be maintained through its action on DNA synthesis at the same time that its capacity to break chromosomes is inhibited; conceivably, its inherent toxicity to human beings would be reduced by such inhibitions. It is of interest, therefore, to know to what degree its action on DNA synthesis is related to its ability to break chromosomes, and to what degree both properties are related to its action as an anticarcinogen (12).

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Sensory Deprivation and

Pain Thresholds

Abstract. Four days of sensory deprivation produced a significant lowering of thresholds for electrically induced pain.

Eighteen male adult subjects served in a study to determine the effects of sensory deprivation upon cutaneous pain thresholds (1). Each subject spent a minimum of 1 hour per day in the deprivation chamber, during which time pain thresholds were measured. These periods were considered to be practice periods. Most subjects served in this manner for 3 or 4 days before their thresholds became sufficiently stable to

Table 1. Mean pain thresholds (in milliamperes).

Test No.	Sensory deprivation group	Control group
1	0.290	0.240
2	0.182	0.221
Difference	0.108	0.019

be considered reliable. The criterion of reliability was taken to be variability of less than 10 percent for an entire practice period. If a subject did not meet this criterion in the course of four practice periods he was eliminated from the study. The practice periods were discontinued at the conclusion of the period in which the criterion was met.

The sensation of pain was elicited by applying an electrical current of a frequency of 1000 cy/sec through small dry electrodes clamped to either side of the lobe of the right ear. The electrodes were brass disks, 7 mm in diameter, which were firmly clamped to the ear lobe but caused no discomfort. The weight of the electrodes, of the clamping device, and of the connecting wires was supported in such a manner that there was no sensation of weight or pull at the ear. The stimulus, which originated from an oscillator, was amplified by a constant-current amplifier. The intensity of the stimulus delivered to the subject was controlled by an attenuator. The wave was constantly monitored by an oscilloscope to see that it was sinusoidal.

Pain thresholds were determined by a modified method of limits, the 50-percent value being calculated. The method of limits was modified so that stimuli of an intensity very much in excess of threshold were not used. High-intensity stimuli of this nature are not only extremely unpleasant for the subject but they also produce such a persistent aftereffect that they mask subsequent stimuli. Even a stimulus of threshold intensity produced what was characteristically described as "stinging, nasty pain."

On the day after the last practice period, each subject was brought back for an additional session, when his pain threshold was determined. This usually required about 100 presentations of the electrical stimuli.

After the pain threshold had been determined, nine of the subjects were exposed, one at a time, to 4 days of sensory deprivation. A detailed description of the conditions of the confinement can be found in Vernon *et al.* (2). For the present, it is sufficient to say that sensory deprivation consisted of confinement in a small lightproof and soundproof cubicle. The cubicle was barely large enough to contain a

single bed, upon which the subject lay. At the end of the 4 days of confinement and before he left the deprivation chamber, the subject's pain threshold was determined a second time.

The remaining nine subjects were not exposed to sensory deprivation, and thus they provided a control group. Four days after their pain thresholds had been determined they were called back to the laboratory, where a second determination of their pain thresholds was made. The pain thresholds for all subjects were determined in the sensory deprivation chamber under identical conditions. The major difference between the two groups was the manner in which they spent the 4 days which intervened between the first and second determinations of their pain thresholds. The experimental group spent this period under conditions of sensory deprivation, while the control group spent it in the normal manner.

The data for these two groups, presented in Table 1, reveal that, on the average, the pain threshold for the confined group dropped 0.108 ma after 96 hours of sensory deprivation. This change is statistically significant at better than the 1-percent level of confidence (3). All the experimental subjects showed a lowered pain threshold on the second determination. The control group showed a slight drop in pain threshold which averaged 0.019 ma—a change that is not significant. About half of the control group showed a rise in threshold, the other half, a drop. Thus, it can be seen that sensory deprivation not only has a uniform effect upon pain thresholds but also produces an increase in sensitivity. This is not the first instance of an increase in sensitivity resulting from isolation. Doane *et al.* (4) found the two-point limen to be significantly lowered after 48 and 72 hours of confinement.

One explanation of this finding is that a contrast phenomenon is produced by sensory deprivation. Another and more involved explanation can be formulated in terms of the action of the reticular formation of the brain stem. Under normal circumstances neural inputs from sensory departments can be blocked or partially inhibited at the level of the reticular formation. The blocking is produced in the descending tracts under cortical excitation aroused by any sensory stimulation. Thus it may be that sensory deprivation, by drastically reducing the amount of sensory input, minimizes the activity in the descending tracts of the reticular formation. If there is less inhibition to overcome at that level, then perhaps sensitivity is accordingly increased. In the case of pain, the neural impulses resulting from the pain stimuli would encounter less opposition and would

register at a lower level of intensity. Or, to state it more accurately, registration would occur with impulses of lower frequency.

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References and Notes

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Selective Viral and Rickettsial Serum Antibody Absorption by a Chromatographic Column

Abstract. Serum antibodies behave as cations at neutral pH and thus have low affinity for cellulose anion-exchange columns. Antigens of small size derived from adenovirus, influenza virus, and typhus rickettsiae, however, readily adsorbed to such columns. These adsorbed antigens specifically removed antibodies from antisera. This simple method permits antibody absorption by antigens ordinarily sedimented with difficulty.

Absorption of serum antibodies with particulate antigens has been a useful approach to the analysis of antigenic structure and antibody populations. This report presents a new method for antibody absorption which employs a chromatographic column to which "soluble antigens" are attached (adsorbed). Chromatographic columns, binding antigens covalently to resins or nonspecifically to other substances, have been employed to absorb and purify antibodies (1). Difficulties associated with this use of columns have been nonspecific antibody adherence to columns, antigen damage during chemical manipulations, and impair-

Table 1. Adenovirus type 7 serum (human, convalescent) before and after absorption by column antigens.

Column antigen	Serum CF titer with antigens*		
	Type 3	Type 4	Type 7
None	40	40	40
Adenovirus, type 7	<10	<10	<10
Adenovirus, type 4	<10	<10	<10

* Figures represent the reciprocal of the highest dilution of serum-fixing complement in the presence of four units of antigen.