DISCUSSION

Carcinogenic action per se does not always operate at optimal efficiency. A large number of variables have a major influence on the carcinogenic response (Berenblum, 1974, 1978; Sivak, 1979; Kouri, 1980; Miller and Miller, 1981; Farber, 1984). The variables may augment/accentuate the process or they may inhibit/retard the process. Hence, the need to identify such variables (modulators) which ultimately determine the susceptibility of an individual to carcinogens is of paramount importance.

In the present study following a two-stage protocol, the modulating influence of mustard oil, garlic oil and MPG, hypothermia and hyperthermia, on the DMBA-induced skin carcinogenesis has been investigated.

The results (Table-I) clearly revealed that mustard oil did not enhance the tumor response in terms of tumor incidence and tumor yield. It was also not effective to retard or arrest the tumor response, unlike some other members of Cruciferae plants such as Cabbage and Cauliflower (Wattenberg, 1977; Wattenberg and Loub, 1978). Benzyl isothiocyanate and phenethyl isothiocyanate which occur in Cruciferous plants have been reported to have
inhibitory influence in animal models against BP and DMBA-induced neoplasia in three organs, i.e. fore stomach, lung and mammary gland, when isothiocyanates were administered prior to initiation (Wattenberg, 1977). There was no inhibitory influence when isothiocyanate was given 4 hr after the DMBA. In the present experimental model, the target site was treated with mustard oil prior to as well as subsequent to initiation with DMBA and no inhibition in tumor response was noticed. The absence of the anticarcinogenic property of mustard oil poses two questions -

(a) Is mustard devoid of benzyl isothiocyanate and phene thyl isocyanate present in other Cruciferous plants? (b) Is it that these natural constituents, are lost during the process of obtaining oil from mustard seeds? However, on the basis of the findings of this investigation (there was no augmentation in the tumorigenic response) mustard oil could be labelled as a "non-risk" or "non-precipitating factor."

The absence of cocarcinogenic and anticarcinogenic property of mustard oil in other organ systems needs experimental verification.

The results (Table 3.1) showed that MPG in the present experimental format had no inhibitory influence on
the initiation stage. The finding does not preclude the possibility of different results under different treatment schedules. The hyperthermic and hypothermic (48 hr before and 48 hr after initiation) did not interfere with the mechanism of initiation. The only visible influence of the cold stress was the prolongation in the latent period. The delay in the appearance of the overt tumor might be related to the depression in the mitotic activity of the cells due to severe cold (Bullough, 1950; Baker, 1977).

Garlic oil inhibited significantly \( (P < 0.01) \) the tumor response, presented as the percent of mice with tumors (Table 1.2) when it was topically applied to the target site, thrice prior to initiation and thrice subsequent to initiation with DMBA, the initiating dose of DMBA being 102 \( \mu \)g. In the second series of experiments, the initiating dose of DMBA was reduced to 51.26 \( \mu \)g and garlic (the dose remaining same as in series-I), was applied prior as well as subsequent to initiation. The inhibition (presented as the % of mice bearing tumor) was increased from 52.94% (Table 1.2) to 61.14% (Table 2.2) with decreased initiating dose, in agreement with the suggestion of Slaga et al (1974). Pursuing the line of reasoning that the inhibitory effect of thermomodulators is both dose and time dependent, (Slaga and Bracken, 1977; DiGiovanni et al, 1980), the treatment schedule of garlic
was split (Expt. series-II) into (a) Pre-initiation application, (b) Post-initiation application, (c) Concomitant application, in addition to, the pre and post application (in combination) so as to find out which one of these applications was effective in inhibiting the tumor response. Results (Table 2.2) revealed that the prior treatment caused 45.5% inhibition in the percent incidence, which was statistically significant ($P < 0.05$) whereas, the subsequent treatment of garlic (2 hr, 24 hr and 48 hr after initiation) did not bring about significant inhibition (22.17%). The inhibition was the least when garlic was applied concomitant with DMBA. Inhibition was more pronounced (61.14%) and statistically more significant ($P < 0.01$) when the regimen of garlic oil consisted of 3 pre-initiation doses and three post-initiation doses (in combination). Inhibition, in terms of tumor yield by the pre and post treatment of garlic was less significant ($P < 0.05$).

The inhibitory influence of garlic oil on DMBA-induced tumorigenesis may be traced to its various constituents. Apart from its main substance, Alliin, ($C_{6}H_{11}O_{3}NSH_{2}O$) garlic contains (Table 5) a number of
vitamins (see Raghavan et al., 1982).

Table 5. Vitamins in Garlic

<table>
<thead>
<tr>
<th>Vitamins</th>
<th>Quantity in mg/100 g (fresh garlic)</th>
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</thead>
<tbody>
<tr>
<td>Nicotinic acid</td>
<td>0.12-0.29</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.29-0.482</td>
</tr>
<tr>
<td>Vitamin 'A'</td>
<td>284 IU</td>
</tr>
<tr>
<td>Thiamin and Riboflavin</td>
<td>32 μg</td>
</tr>
<tr>
<td>Biotin</td>
<td>22 μg/g</td>
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Trace metal selenium has also been detected in garlic oil. Almost all the constituents present in garlic such as Vitamins 'A', 'C', and selenium, independently, have been reported to possess antineoplastic properties. The antineoplastic property of sulphur containing compounds against BP-induced neoplasia has been reported (Wattenberg, 1974). The anticarcinogenic effect of selenium is also well documented (Shamberger, 1970; Jacobs, 1977). Reduced tumor formation was observed (Shamberger and Rudolpe, 1966) when sodium selenide was applied concomitantly with croton oil. The prophylactic effect of Vitamin 'A' has been well established by various workers like Sporn (1977), Bollag (1971), Verma et al. (1977, 1979), Bollag and Matter (1981),
Beems (1984). Similarly, the preventive action of Vitamin 'C' has been demonstrated by Mergens et al (1978); Cameron, (1979); Mirvish (1981); Scanlan, (1983). But the controversy (Abul Hajj and Kelliher, 1982) over the use of Vitamin 'C' for treatment of malignant disease as well as prevention of tumor induction is still going on.

The prime objective of the present experimental format was to evaluate the modifying potential of some variables and not to delve into the mechanism of inhibition. The mechanisms of action of most inhibitors reported so far are poorly understood. This lack of information makes it difficult to organize them into cohesive pattern. With a view to providing an organizational framework, Wattenberg, (1983) classified inhibitors according to the time in the carcinogenic process at which they are effective.

According to that classification, garlic can be placed under the category of "blocking agents" - because it brought about significant inhibition when it was topically applied to the target site prior to initiation. The classification of inhibitors into "blocking agents" and "suppressive agents" has brought considerable order to the confusing variety of inhibitors. The significant
(P < 0.05) inhibition caused by the application of garlic oil, prior to initiation leads to the presumption that garlic might have inhibited or blocked the formation of DNA-adduct; formation of DNA-adduct is considered to be the primary single event mechanism of action for initiation (Kouri, 1980; Miller and Miller, 1981). Studies by Mintz and Illmensee (1975), Dipple et al (1984) suggest that tumor initiation is a two or multi-event mechanism. Some other non-genetic changes other than DNA binding are necessary to complete the process of initiation. In the light of these observations, the inhibition brought about by the prior application of garlic oil can be attributed to inhibition of adduct formation and/or inhibition of the second epigenetic/non-genetic event(s).

Several mechanisms have been proposed to explain the process of inhibition of the multi-event mechanism of initiation. They are:

(a) Inhibition of the activation of DMBA to its ultimate carcinogenic metabolites (decreased activation),

(b) enhancement of the activity of the carcinogen detoxification systems,
(c) combination of decreased activation and increased detoxification, and
(d) Increased scavenging of the reactive critical metabolites (Figure 8).

The failure of the subsequent treatment (2 hr, 24 hr and 48 hr after initiation) of garlic oil to cause significant inhibition could be related to the early enzymatic activation of DMBA which gets completed within 12 hrs after initiation (Gelboin, 1962; Gelboin et al., 1972). It has been demonstrated (Gelboin, 1967) that inhibitors operate at a very early time. The regimen of garlic, which consisted of three applications prior to initiation and three applications subsequent to initiation brought about pronounced (61.14%) inhibition as if the prior and post-treatments had acted by summation. The reason for the increase in inhibition is not known. These results suggest that garlic may be capable of inhibiting the carcinogenic process "at more than one time point". Existence of such inhibitors capable of "acting at more than one time point" has been proposed by Wattenberg, (1983).

It is indicated (Wattenberg, 1983) that most of the blocking agents (inhibitors) that enhance carcinogenic
POSSIBLE SITE OF ACTION

Fig. 8 - A simplified scheme depicting the four possible sites (A, B, C and D), at which the inhibitors (Blocking agents) can intervene.
detoxification systems induce an increase in activity of multiple enzymes. The studies of Siddiqui and Pawar, (1984) indicate that oral administration of garlic oil for 7 days to adult wistar male and female rats decreased the levels of hepatic microsomal cytochrome P-450 and the activity of cytochrome c-reductase.

Thus, the inhibitory/blocking capacity of garlic on the initiation component of chemically induced tumorigenesis, inspite of being an inhibitor of enzymes, leads one to speculate that the inhibition observed in the present studies is not due to enhancement of the carcinogen detoxification system(s). Possibly garlic exerts its inhibitory influence via a different mechanism or mechanisms.