CHAPTER IV

DISCUSSION
The widespread consumption of arecoline by human beings through their betel nut chewing habits and its usage for anthelmintic and cholinergic therapeutic effects have prompted the investigation of its adverse effects on experimental systems. Main focus was made on its carcinogenic, mutagenic and teratogenic potency (Rao's ICMR Project Report, 1985), apart from its pharmacological effects mainly on nervous system (IARC Report, 1985). As far as immunotoxicity of this compound is concerned only rudimentary and fragmentary knowledge is available (Shahabuddin, 1978; Shahabuddin and Rao, 1980; Casale et al., 1983). The present study was undertaken with a view to determine dose related effects on immune organs as well as immune response to T-dependent antigen, in vitro effect on mitogen induced lymphocyte blastogenesis and the possible mechanisms of immunosuppression.

The acute subcutaneous L.D.50 for arecoline in both sexes of Swiss albino mice determined in the present study was 97 mg/kg bw. This value is in conformity with the value already reported for mice (Merck Index, 1983). Though L.D.50 values of both oral and i.p. routes in mice showed sex related variations, the difference was more pronounced in the former (Table II.1). Female mice showed greater mortality when compared to male mice at similar oral
dose of arecoline. This may be due to differences in the liver drug detoxifying mechanisms. The L.D.$_{50}$ values of arecoline for oral and i.p. routes in mice have not been reported in the literature so far. However, the time course of cholinergic symptoms and violent stimulation of peristaltic movement of the intestine, which results in diarhoea as a late effect, were similar to those given in earlier reports (Mujumdar et al., 1979).

The present study deals with the immunotoxic effects of arecoline administered (subcutaneously, s.c.) to murine in vivo system. Results obtained after chronic arecoline treatment, at sub-lethal doses, and at various time intervals, revealed a significant reduction in growth rate (body weight, bw) of animals only at a dose of 20 mg/kg bw. The lower doses showed less pronounced effects. Looking into absolute and relative organ weights of spleen, mesenteric lymph nodes, liver and kidney, the general observation was that arecoline treatment, at a high dose (20 mg/kg bw), caused moderate reductions. The exceptions were the pronounced reduction of thymus weight and a mild increase in the weight of adrenals (Tables II.2a, II.2b, and II.2c; Fig.II.1).

Histological examination revealed that thymus atrophied with a severe reduction in the cellularity of
cortical region at 20 mg/kg bw dose of arecoline (Fig. II.1). Furthermore, a mild reduction in the cellularity of medullary region was also evident. Besides thymus, the spleen showed a less number of cells in the periarteriolar lymphatic sheaths with inconspicuous germinal follicles (Fig. II.2) and mesenteric lymph nodes showed a mild decrease in cellularity of the medulla and paracortical regions (Fig. II.3). Adrenal cortex showed an increase in size of cortex with zona fasciculata hypertrophy (Fig. II.4). Some other drugs (e.g., caffeine and corticosteroids) and chemicals (e.g., TCDD and organotin compounds) also cause similar effects (Luster et al., 1982; Saxena et al., 1984).

An earlier preliminary report from our lab (Shahabuddin, 1978) showed that arecoline treatment decreases number of RBCs. However, no observations were made on WBCs. The present study shows that WBCs and RBCs decline in their number in a dose-dependent manner (Table II.3).

In thymus, spleen and mesenteric lymph nodes, arecoline treatment affected cellularity without causing much influence on cell viability (Table II.6a, II.6b and II.6c). These changes were seen at a dose which induced changes in weight of these organs and in their
histomorphology. These results depict that higher dose of arecoline affect the lymphoid organs markedly. Earlier reports show that some other plant alkaloids have similar effects. For example, vinblastine depresses number of bone marrow cells resulting in acute leukopenia (DeConti and Creasey, 1975).

Blood/serum biochemical parameters such as total protein, albumin, glucose, acid phosphatase and hemoglobin showed no appreciable alterations following arecoline treatment. The changes seen in SGOT and SGPT levels indicate that the liver function is moderately affected (Table II.4). However, histologically liver appeared normal.

In concordance with the zona fasciculata hypertrophy of adrenals, corticosterone levels in serum increased depending on the dose of arecoline (Table II.5). This is in agreement with the work of Overstreet et al. (1986) who have shown that arecoline (4 mg/kg bw; single dose) enhanced the serum level of corticosterone 3-5 fold after 15 min of treatment in rats (Flinders Sensitive Line). Considering these changes it is likely that arecoline effect on lymphoid system could be indirect, since increased corticosterone in the blood plasma causes 1) lymphocytopenia or decreased circulating lymphocytes, 2) thymus involution
and 3) related loss of tissue mass of the spleen and peripheral lymph nodes (Claman, 1972; Gillis et al., 1979; Riley, 1981). It is important to note here that such an elevation was not seen in the saline treated animals and hence it nullifies stress-mediated physiological consequences due to animal handling.

In order to look into the biochemical mechanism of action of arecoline on immune cells, change in oxygen consumption was studied. Since arecoline treatment exerted maximum damage to thymus cells, rat thymocytes were used for the evaluation. The animal model was changed so as to obtain large number of cells for the present study. Normal oxidative metabolism is an essential feature for the proper functioning of lymphoid cells (Subba Rao and Glick, 1977; Lee and Park, 1979). The sensitivity of the respiration rate of isolated lymphoid cells from different lymphoid organs has been employed as an in vitro test system to elucidate the molecular sites of chemical action by earlier workers (Lee and Park, 1979; Lee et al., 1979; Penninks and Seinen, 1980). Penninks and Seinen (1980) may perhaps be the first to study the respiration in isolated thymocytes as a model system to assess the cytotoxic potential of organotin compounds and demonstrated a dose dependent inhibition of respiration of thymocytes in response to
dialkyltin compounds. The value of such studies is enhanced with investigations which are carried out under different conditions such as the whole cells in the presence or absence of substrates (Penninks and Seinen, 1980).

In the present study, when thymocytes were incubated with arecoline in the absence of any exogenously added substrates, a biphasic change in the rate of oxygen consumption was observed (Table II.7). The marked rise in the rate of oxygen consumption at sub-toxic concentrations of arecoline (10^{-7} M and 10^{-5} M) may be due to uncoupling of oxidative phosphorylation as has been demonstrated by many workers in isolated mitochondria exposed to chemical agents (Fukami, 1976; Moreland, 1980; Nizami and Hellingworth, 1980; Manring and Moreland, 1981), due to catabolic degradation of arecoline or due to the inactivation of some other mechanism which controls oxygen consumption. At a higher concentration (10^{-3} M) there was a decrease in the rate of oxygen consumption. The viability of rat thymocytes was not affected during the 10 min incubation period at any concentration of arecoline. Therefore, the decrease in the rate of oxygen consumption at a high concentration of arecoline is not due to cell death. Respiratory rate in the cells, both in vivo and in vitro, is controlled by a highly complex mechanism involving the interplay of several enzymes.
of glycolysis, and tricarboxylic acid cycle (TCA), mono and divalent ions, several cofactors and the respiratory chain (Dupraw, 1968; Leninger, 1975; Mayes, 1977). The rate of respiration is highly susceptible to even minor changes in the permeability of membranes, the transport of ions, and the availability of substrates and co-factors essential for oxidative metabolism. However, it is difficult to localize the site of action of arecoline with the available data. Other xenobiotics like organo chlorinated pesticides and cyclodiene affect respiration through inhibition of several enzymes of TCA cycle and the respiratory chain (Pardini et al., 1980).

In an attempt to simulate some of the in vivo conditions arecoline effect on oxygen consumption was seen in the presence of various respiratory substrates. With substrates in the medium, the oxygen consumption of thymocytes was higher than that of the controls, and the magnitude of the increase dependent on the nature of the substrate used. The increase seen in oxygen consumption of thymocytes with glucose, pyruvic acid, and lactic acid is consistent with the work of Penninks and Seinen (1980). Interestingly, all substrates eliminated the fall in the oxygen consumption induced by $10^{-3}$M arecoline. Thus, these substrates seem to counteract the arecoline induced
interference in the regulatory mechanisms which otherwise resulted in inhibition of respiration. Furthermore, lower concentrations of arecoline increased oxygen consumption, which was further elevated by various substrates. This may be due to increased utilization resulting from catabolic degradation of arecoline itself independent of the oxygen consumption resulting from substrate utilization. It appears that the inhibition of oxygen uptake by $10^{-3}$M arecoline could be alleviated by the exogenous supply of respiratory substrates. Hence, arecoline at higher concentration could have interfered with normal utilization of endogenous substrates by thymocytes. To confirm this, further studies on consumption of various substrates and activity of enzymes of the respiratory pathway have to be done.

In the second phase of present immunotoxicity testing of arecoline, a battery of immunological tests (delayed type hypersensitivity reaction to SRBC, the assay of host susceptibility to endotoxin shock, plaque forming cell assay, circulating antibody titers and hemolysis titers against SRBC, total IgG by ELISA) were carried out in mice to investigate the changes in cell mediated, humoral and indirect immunity. Also in vitro effect of arecoline on mitogens induced proliferative response was assessed using $^3$H-thymidine incorporation assay.
Results obtained from various immunological tests have indicated that arecoline affects the functional parameters of immunity. Among the cell-mediated immune tests, delayed type hypersensitivity reactions to SRBC revealed treatment-related changes. At 20 mg/kg bw dose of arecoline, given at various treatment periods, the DTH response was reduced by 27-45%, whereas at 10 mg/kg bw, the decrease was 13 to 32% (Tables III.A.1a and III.A.1b).

The antibody plaque forming cell response to SRBC is currently considered to be a sensitive assay to examine the immunotoxicity of a test compound, since it requires a functional response and cell cooperation between macrophage, T- and B-cells (Vos, 1977; Luster et al., 1982). In order to correlate observed reduction of cellularity of spleen with clonal expansion of lymphocytes against SRBC, plaque forming cell (direct PFC) assay was used. Treatment of arecoline continuously for a week decreased PFC response in a dose-dependent manner, reaching a maximum reduction of 28% at 20 mg/kg bw arecoline (Table III.B.1).

In addition, the concentration of a specific antibody in serum could be used as a measure of the functional status of all three developmental phases of the humoral immune response - antigen recognition, activation
and expression (Silkworth and Loose, 1981). Arecoline treatment reduced both HA and HL titers to SRBC in a dose-dependent manner. Exposure of mice to 20 mg/kg bw of arecoline resulted in 29-43% decrease in HA titer and 35-49% decrease in HL titer to SRBC. Arecoline treatment at 10 mg/kg bw brought about a decrease in HA titer in the range of 11-19% and in HL titer in the range of 13-25%. Arecoline at 5 mg/kg bw did not affect antibody production to SRBC (Table III.B.2a). In concordance with these results primary total IgG, as evaluated by competitive ELISA, also showed dose-dependent change with maximum suppression (34%) at 20 mg/kg bw dose (Table III.B.2b). The decrease in CMI and HI responses to SRBC was not duration-dependent. Hence, the total IgG content was evaluated in sera samples obtained after three weeks treatment only. It is important to note here that a single dose of arecoline, given one day prior to antigen inoculation, did not elicit any change in DTH or HA and HL antibody titer responses (Data not shown). Thus, it may be concluded that continuous treatment of mice for certain period of time with arecoline is necessary for its immunosuppressive action. A preliminary study from our laboratory has shown earlier (Shahabuddin and Rao, 1980) that arecoline, on twice daily treatment with 0.5 mg/mouse, reduced both HA antibody titer and delayed type hypersensitivity to bovine serum albumin (BSA, a soluble T-
dependent antigen). The present study showed that this action of alkaloid is clearly dose-dependent, with no effect at the dose of 5 mg/kg bw dose. Study with caffeine showed that at low dose level, it causes immunostimulation (Saxena et al., 1984). Some other alkaloids like vincristine, vinblastine and colchicine are also reported to suppress immune responses. The vinca alkaloids inhibited both the formation of antibodies to serum albumin and delayed hypersensitivity in rats (Aisenberg, 1963). Both colchicine and vincristine inhibited the secretion of IgM antibody (Teplitz et al., 1975).

Lack of influence of duration of treatment at any given dose level may either be due to the absence of cumulative influence of arecoline (which is rapidly metabolized in the body) or due to cell renewing ability of bone marrow. Here, it is interesting to note the work of Overstreet and Jamal (1986), who have showed behavioural tolerance to arecoline in rats. They have also shown that tolerance is dependent on dose of arecoline.

In order to examine whether arecoline induced immunosuppression also affects memory cells, HA and HL titers and total IgG content were evaluated after three weeks exposure. Here again, there was dose-dependent suppression of humoral immune response (Table III.B.2c and
III.B.2d). The responses at 5 mg/kg bw dose of arecoline was similar to that of controls.

The value of results of endotoxin hypersensitivity merits emphasis here as the host resistance parameters are becoming increasingly important in the assessment of immunotoxicity of drugs and chemicals (Dean et al., 1979; Luster et al., 1982). Results of endotoxin hypersensitivity experiments in the present study indicated an insignificant increase in the susceptibility of arecoline treated animals to endotoxin shock (Table III.A.11).

Other xenobiotics such as TCDD, HCB, lead, cadmium, cobalt sulfate and sulfur dioxide, are also known to compromise the immune system resulting in the increased susceptibility of the animals to infectious agents (Loose et al., 1978b; Koller and Vos, 1981). This dysfunction or loss of resistance would place the host in jeopardy unless another component of the immune system is sufficiently augmented to provide a compensatory effect (Spyker-Cranmer et al., 1982). Though arecoline caused thymic atrophy with depletion of cortical thymocytes, this was not reflected in any increase in susceptibility to endotoxin shock.

There are several factors which are known to influence the results of endotoxin sensitivity assay. One
such factor is the pathomorphological alterations of liver parenchymal cells, since participation of this group of cells is considered to be important in the metabolic expression of endotoxin shock. Carbon tetrachloride is a good example to illustrate this point (Loose et al., 1978a, 1978b). Since no major pathological alteration of liver was seen after arecoline treatment, it is unlikely that endotoxin sensitivity is mediated by indirect effects.

During senescence, the immune system undergoes striking alterations, associated with progressive decline of immune responsiveness to exogenous antigens and increasing incidence of autoimmune phenomena (Cooper, 1984; Schultz, 1984). Both the humoral immune capacity (B-cells) and the cellular immune capacity (T-cells) are reduced in older animals as compared with adolescent or juvenile animals. Thus, possible cell population changes leading to immune dysfunction in aging are likely to be restricted to B-cells, T-cells and macrophages. Several studies have been carried out on these cell populations and many conflicting reports could be quoted. It is generally agreed, however, that aging affects mainly cell function rather than absolute cell number (Walford et al., 1981). Hence, experiments were designed to investigate arecoline effect on immune response in aged female mice. Though aged mice showed individual
variations to a certain extent, arecoline treatment did suppress delayed type hypersensitivity and HA and HL antibody titers to SRBC (Table III.A.3 and III.B.4). Chronic stress increases adrenal weights in aged mice and thereby increase in the level of corticosteroids would bring about immunosuppression (Strausser and Fiore, 1984). Thus, cholinergic action of arecoline might have caused further lowering the immune responses to SRBC in old mice. Possibility of direct action of arecoline also cannot be ruled out.

Experiments designed to investigate reversibility of the arecoline effect have shown that immunological effects are reversible in nature and that this could be demonstrated within one week after withdrawal of arecoline exposure (Table III.A.4 and III.B.5). These results are expected since arecoline is biodegradeable by mammals and it is not stored in the body.

Since lymphocyte function is integral to the development of an immune response, it is appropriate to delineate its role in xenobiotic-induced immune dysfunction. The cytotoxic potential of arecoline, as evaluated in cell culture experiments showed concentration dependent effect 24 h after treatment (Table III.A.5). Cytotoxic effects of
arecoline are slow as we could not detect appreciable change in viability at 6 h, at the concentration of arecoline tested. These observations may account for the noted less marked in vivo cytotoxic ability of arecoline.

Depending on the nature of the foreign substance, initial recognition of the antigen may occur through a combination of direct and/or indirect interactions with receptors on the lymphocyte surface membranes. Polyclonal mitogens such as con A and PWM are thought to activate lymphocytes by binding to glycoprotein receptors on the cell surface (Edelman, 1973). The measurement of mitogen induced $^{3}$H-thymidine incorporation in lymphocytes is an assessment of the activation phase of the immune response with the advantage of bypassing specific antigen initial recognition (Katz, 1977). Hence, it was worthwhile to assess the effect of arecoline on mitogen-induced lymphocyte activation.

In order to select a suitable range of sub-toxic concentrations of arecoline and to observe arecoline effect on spontaneous proliferative response, spleen cells were exposed to arecoline at various concentrations ($10^{-8}$ - $10^{-4}$M). The 72 h culture was used to measure $^{3}$H-thymidine incorporation. Results revealed that only at $10^{-4}$ concentration, there was a remarkable suppression (32%) in
the \(^3\)H-thymidine incorporation. In the range of \(10^{-8}\) to \(10^{-5}\)M, arecoline did not elicit any appreciable change in spontaneous proliferative response (Table III.A.6). This is not in tune with the in vitro cytotoxic effect of arecoline in spleen cells where it reduced cell viability in a dose-dependent manner. The incorporation of \(^3\)H-thymidine may be also due to unscheduled DNA synthesis in the surviving cells, since arecoline is shown to be mutagenic in bone marrow cells and spermatids, in vivo (Rao's ICMR Project Report, 1985). Such a postulation is possible, since Sinha and Rao (1985b) have shown that arecoline increases unscheduled DNA synthesis in the early spermatids of mice, exposed in vivo.

The results obtained from in vitro effect of arecoline on con A- and PWM-induced spleen cell blastogenesis showed that concomitant exposure with arecoline, at sub-toxic concentrations (\(10^{-6}\) and \(10^{-5}\)M), decreased con A-induced blastogenesis more than that of PWM-induced response (Tables III.A.7 and III.B.6). The per cent of inhibition of con A-induced blastogenesis, in the presence of arecoline at \(10^{-6}\) and \(10^{-5}\)M concentrations, were 13% and 25%, respectively, whereas, PWM-induced blastogenesis was affected (17%) only at \(10^{-5}\)M. Results obtained from \(10^{-4}\)M concentration of arecoline are not taken into account here.
since this dose suppressed spontaneous blastogenesis of spleen cells by 32%.

To exclude the possibility that arecoline is interfering with con A/PWM binding with the cell surface, arecoline was given at 12 h after mitogen stimulation. Results suggest that arecoline induced inhibition of blastogenesis may not be through interference with mitogen binding the cells. It seems from the observations that arecoline is slightly more inhibitory when added at 12 h than concomitantly with mitogens (Tables III.A.8 and III.B.7). These results suggest that activated cells are more sensitive to arecoline.

The mechanism by which arecoline exerts suppression of mitogen-induced blastogenesis is difficult to explain. The interaction of a specific antigen or a non-specific mitogen at a restrictive receptor site on the cell surface of an immunocompetent cell results in subsequent biochemical changes and cell division (Cook et al., 1983). Hence, it would be possible that arecoline would have affected any one of the segments to depress blastogenesis. There are limits to any methodology using an in vitro system to examine drug and lymphocyte interactions. These include the drug concentration used, persistence of drug and its metabolites, cell populations and the level of mitogen used.
There have been several studies done questioning the correlation of $^3$H-thymidine incorporation and cellular response. Rogers (1976) presented data suggesting that there is a high likelihood that a specific part of the genome is being replicated rather than the entire genome. However, according to Bernheim et al. (1978), the most likely explanation for Roger's findings could be due to cellular death. Depending on culture conditions of lymphocytes they found discrepancies in the correlation between cultures with, and without, drug would seem to reflect differences in rate of transformation. Since the two mitogens used were thought to stimulate different populations of cells (Caron, 1966), the interaction between mitogens and arecoline seems to transcend different cell populations and is most likely related to a more basic cellular mechanism.

The induction of proliferation of competent T-lymphocytes by lectins, such as con A, involves a series of events (Smith and Ruscetti, 1981). Initially a subset of lymphoid cells, when exposed to the stimulus, produce interleukin-2 (IL-2), while other T-cells become activated, acquire IL-2 specific receptors, and then proliferate in response to this newly produced IL-2. In this way, IL-2 can drive this immune response with the degree of proliferation
dependent upon the amount of IL-2 available to fuel this reaction. Therefore, one explanation for a hyporeactive con A response as seen above may be due to decreased production of IL-2 by the stimulated cells in the presence of arecoline. Hence, the possibility of arecoline interfering with IL-2 production and with IL-2 action on IL-2 dependent cytolytic T-cell Line (CTLL) were evaluated.

Looking into the effect of arecoline on IL-2 production the results showed that at $10^{-6} \text{M}$, $10^{-5} \text{M}$ and $10^{-4} \text{M}$, the depression in IL-2 dependent CTLL proliferative response was 12, 21 and 26%, respectively (Table III.A.9). It is interesting to note that supernatants of arecoline alone treated spleen cells did not cause any appreciable effect on CTLL blastogenesis at any given concentration of arecoline. Hence, reduction in the level of IL-2 may be mediated through arecoline effect on activated lymphocytes. These observations suggest that arecoline mediated inhibition of IL-2 production would be possibly responsible for depressed con A induced blastogenesis of spleen cells.

Present study reveals that concomitant exposure of CTLL to arecoline and rat IL-2 has no influence on IL-2 induced blastogenesis (Table III.A.10). This may suggest the non-interference of arecoline with IL-2 receptors through which IL-2 acts on lymphocytes.
The stem cells of lymphoid cells undergo a controlled program of differentiation during which they become immunocompetent. Mature, immunocompetent T- and B-lymphocytes are small relatively inactive cells arrested at the $G_0$ stage of the cell cycle. Lymphocyte proliferation occurs when specific cells reactive against foreign antigens are activated and amplified. We studied whether arecoline interferes with cell mediated and humoral immune response in phase specific manner. The results showed that DTH response, primary HA and HL antibody titers and total IgG were reduced in a dose-dependent manner causing maximum effect at 20 mg/kg bw dose of arecoline in animals which were treated continuously for 4 days following immunization (Table III.A.2c, III.B.3b and III.B.3c). The animals which received arecoline at 12 h following immunization showed moderate to significant reduction at 10 and 20 mg/kg bw doses (Table III.A.2b, 2c; III.B.3a, 3b). The animals which were treated at 1st, 2nd or 4th day following immunization did not elicit appreciable change in DTH response, HA and HL antibody titers, and total IgG content. The groups which received 5 mg/kg bw showed response as that of control (Table III.A.2a). The possible reason for suppression is likely to be cholinergic action of arecoline. The link between cholinergic stimulation and suppression of humoral immune response has been postulated by Casale et al. (1983).
They have shown that IgM plaque forming cells (PFC) response to SRBC was reduced in mice treated with cholinergic organophosphate pesticides (malathion, parathion and dichlorvos) and cholinomimetic arecoline. Their results on the effect of continuous release of arecoline from silastic implants given on 2nd day following SRBC immunization showed 50% suppression of IgM PFC response over the control. On the contrary, arecoline given as a single dose (65 mg/kg bw) on 2nd day did not appreciably affect IgM PFC response. Our results are in conformity with their observations, except that we have seen moderate suppression in CMI and HI responses when arecoline was given at the early phase of activation by antigen (12 h after immunization).

They have postulated that the link between cholinergic stimulation and suppression of the PFC response by organophosphates is possibly mediated by a decrease in the level of cholinesterase resulting in increase in the level of acetylcholine which, in turn, would affect the immune system. In support of this view, cholinergic receptors have been identified on lymphocytes and macrophages (Richman and Arnason, 1979; Whaley et al., 1981) apart from nerve cell junctions. However, arecoline treatment (single or continuous exposure) did not inhibit cholinesterase level (Casale et al., 1983). Since arecoline
is a acetylcholine/muscarinic agonist it may possibly bind with acetylcholine receptors (AchR) on lymphocytes or macrophages and thereby bring about the immunosuppression.

An alternative hypothesis as mentioned earlier is that the observed immunosuppression might be mediated by glucocorticosteroids released in response to the toxic chemicals stress associated with the cholinergic crisis. The present study in mice and some other study in rats (Overstreet et al., 1986) have clearly shown that arecoline causes increase in the level of corticosterone. The work of Szot and Murphy (1970), who have demonstrated elevated plasma corticosteroid concentration in rats given sublethal doses of parathion (a cholinergic compound) further strengthen our observations. Several investigators have shown that the addition of glucocorticoids to lymphocytes, during early phases of either mitogenic or antigenic stimulation, severely suppressed activated lymphocyte proliferation (Elves et al., 1964; Tormey et al., 1967; McIntyre et al., 1969). However, delayed addition of steroid resulted in normal ligand-induced replication. Some other studies with antigen and mitogen-stimulated lymphocytes and with antigen specific CTLL cells have indicated that activated lymphocytes contain increased number of glucocorticoid receptors and are as sensitive to
glucocorticoid-induced metabolic inhibition as are unstimulated lymphocytes (Smith et al., 1977; Gillis et al., 1979). Hence, the suppression of immune responses caused by the treatment of arecoline as a single dose at 12 h after immunization may be due to indirect effect. The maximum immunosuppression observed in mice which received arecoline continuously for 4 days possibly suggest direct and/or indirect effects.

Since immune responses are good bioindicators of the physiologic state of the host, studies of this kind may provide valuable information regarding the adverse health effects due to xenobiotics.

In view of the wide distribution of chemicals in the environment, and the belief that current knowledge about adverse health effects resulting from immunotoxic xenobiotics may represent only "tip of the iceberg", the possibility that exposure to such substances may play a greater role in disease causation than previously suspected should be investigated as a matter of priority. Since a good number of people chew betel nut in several oriental countries including India, the possibility of occurrence of arecoline induced-immunosuppression and subsequent increased susceptibility to infections cannot be ruled out.