Multiple dimethylbenz(a)anthracene (DMBA) and 3-methylcholanthrene (MCA) treatments, and radiation exposures were used for the induction of leukemia in the ICRC and Swiss female mice. ICRC mice showed higher incidence of lymphoblastic leukemia in comparison to the Swiss strain.

Splenectomy and thymectomy, before multiple DMBA and MCA treatments and prior to the radiation exposures in the ICRC mice, significantly influence the incidence of leukemia as well as the latent period—the interval needed for the induction of leukemia after the start of the treatment. Thymectomy prior to the radiation exposures was much more effective in the induction of leukemia (decrease in leukemia incidence) than when followed by DMBA and MCA administration in ICRC mice, whereas reverse was true for splenectomy. Latent period was delayed after both splenectomy and thymectomy.

The role of spleen and thymus—the active sites of hemopoietic and leukemogenic activities—in the leukemogenesis following DMBA and MCA administrations, and radiation exposures have been discussed.

Synergism of radiation/DMBA & MCA and of the steroid hormones (estradiol, progesterone)/DMBA & MCA in the induction of leukemia was also studied. Radiation, specially
when administered in small split doses, enhanced the incidence of leukemia though the latent period was somewhat delayed; the higher incidence is attributed to the enhanced cellular proliferation owing to the radiation exposures which in turn is congenial for the action of DMBA and MCA. Similarly, estradiol enhances the incidence of leukemia following DMBA and MCA treatments in ovariectomized ICRC mice; progesterone, however, proved ineffective in this respect. Ovariectomy alone lowered the incidence of leukemia induction in the ICRC mice. Ovariectomy, estradiol and progesterone treatments failed to influence the incidence of leukemia in the Swiss strain.

Leukemia induced by DMBA, MCA and radiation in the present studies is invariably accompanied by the loss in the body weight and depletion of the erythrocytes in the peripheral blood. Increase in the total number of lymphoblasts in the peripheral blood was quite apparent in the leukemic animals. Marked increase in weight of the spleen and higher activity of acid and alkaline phosphatases in the spleen and liver from the leukemic mice is considered indicative of infiltration and active proliferation of the leukemic cells in these organs. This is substantiated since the spleen, liver and kidney also revealed infiltration of the leukemic cells. Thymus too was enlarged (due to thymus tumor) specially in the animals.
where leukemia was induced following radiation exposures.

The lymphocyte response to phytohemagglutinin (PHA) in culture, was more pronounced in the ICRC mice than in the Swiss ones. This accounts for the higher incidence of leukemia in the ICRC mice and is also considered suggestive of some common factor in these two processes (viz. leukemogenesis \textit{(in vivo)} and blastogenesis \textit{(in vitro)}. The response of the lymphocytes to PHA also indirectly indicate the immunological status of the animals. Leukemic serum from the ICRC mice was also studied for its influence on \textit{in vitro} transformation of the lymphocytes to lymphoblasts in the presence of PHA and was found to inhibit the process. Inhibitory capacity of the leukemic serum was found to increase with the progression of leukemia. In the present investigations, the factor(s) responsible for the inhibition of PHA-induced lymphocyte transformation in the leukemic serum is (are) non-dializable, suggesting component(s) involved is (are) large sized molecules. This nondializable fraction is heat resistant. Mechanism of the action of these inhibitors have been discussed in the light of the previous reports and their possible role in the regulation of immune system suggested.

Serum, peripheral blood lymphoblasts, bone marrow and spleen from the leukemic animals were analysed for various
enzymes (viz. acid and alkaline phosphatases, adenosine triphosphatase (ATPase), 5'-nucleotidase, lactic dehydrogenase (LDH), succinic dehydrogenase (SDH), acid deoxyribonuclease (acid DNase), adenosine deaminase (ADA), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) and the results compared with the corresponding tissues/cells from the control animals.

Alteration in these enzymes in the serum of the leukemic animals in comparison to the controls, has been discussed in the light of altered pathophysiology of hemopoietic system. Both acid and alkaline phosphatases registered decrease in the leukemic serum whereas LDH, GOT and GPT levels were significantly higher than in the controls.

Activities of acid and alkaline phosphatases, ATPase, LDH and ADA in the leukemic lymphoblasts were significantly higher than in the peripheral blood lymphocytes of the control animals. 5'-nucleotidase, however, was significantly less in the leukemic lymphoblasts. No significant change in acid DNase was observed in the leukemic lymphoblasts and lymphocytes from the control animals.

Acid and alkaline phosphatases in the spleen and bone marrow of the leukemic mice were significantly higher than in the corresponding controls. Acid DNase activity (per mg protein) in the spleen and bone marrow remained
unchanged. However, activities of acid DNase (per 100 mg tissue) and SDH were significantly lower in the leukemic spleen than in the control tissue. 5'-nucleotidase activity was markedly low in the leukemic spleen and bone marrow in comparison to the corresponding tissue in the control whereas reverse was true for ATPase and LDH. Identical pattern of the enzyme activities in the spleen and bone marrow on one hand, and leukemic cells on the other is suggestive of higher concentration of the leukemic cells in these organs.

Initial changes in the ICRC mice after multiple treatments with DMBA and MCA, and radiation exposures at various post-treatment intervals were also studied. Hematological alterations and organ weight changes were observed after successive treatments. Progressive decrease in the body weight, weight of the thymus and spleen, erythrocyte/leucocyte counts in the peripheral blood and hematocrit were observed eight days after each of the four successive treatments with DMBA and MCA, and radiation exposures. Similarly, changes in the weight and the nucleic acids contents of the spleen were studied at 1, 4 and 8 days after each of the four successive treatments with DMBA and MCA, and radiation exposures. Initial decrease was followed by fast recovery in the post-treatment period. Differential leucocyte count of the peripheral blood also reveals higher concentration of the neutrophils at the initial periods.
indicating enhanced phagocytic activity following damage to the hemopoietic tissues by the carcinogens. Decrease in the erythrocytic elements in the bone marrow smears indicates setting in of anemia as a consequence of DMBA and MCA treatments, and radiation exposures.

Enzyme analysis of the bone marrow and spleen following multiple treatments with DMBA and MCA, and also after radiation exposures was carried out at various post-treatment intervals and the results discussed in the light of the concomitant histological disruption.

Higher level of phosphatases and nucleotidases in the regenerating bone marrow and spleen after multiple treatments and also in leukemic cells/tissues are considered helpful in providing high energy phosphorous, nucleotides and nucleosides which in turn are utilized in the varied synthetic machinery of cells. However, lower levels of 5'-nucleotidase activity in leukemic cells and tissues is attributable to the detachment of 5'-nucleotidase-rich vesicles from the leukemic cell surfaces. Increase in the activity of LDH in bone marrow and spleen with the number of treatments of DMBA and MCA, has also been related to the enhanced synthetic activity in regenerating tissues. Similarly, corresponding decrease in SDH activity in the spleen after multiple treatments may be due to accumulation of pyrophosphate as a result
of high phosphatase activity, which in turn is a SDH inhibitor. Higher level of LDH in leukemic cells/tissues and, lower level of SDH in leukemic spleen has been related with the enhanced anaerobic oxidation in the neoplastic tissues. Similarly higher level of ADA activity in the leukemic cells is attributed to the overall higher metabolic activity characteristic of the malignant cells. Higher level of acid phosphatase and DNase specially at initial intervals of the multiple treatments with DMBA and MCA, and radiation exposures is suggestive of enhanced lysosomal activity. However, no significant change in activity of acid DNase was observed in leukemic cells and bone marrow to their corresponding controls. Depletion of acid DNase in leukemic spleen (activity per 100 mg tissue weight) may be due to the fall in number of lysosomes per gm of tissue or depletion of the lysosome rich cell population per gm of tissue.