EFFECT OF ASCORBIC ACID AND DIMETHYLBENZ(a)ANTHRACENE ON CERTAIN LYSOSOMAL ENZYMES IN NORMAL AND PARTIALLY HEPATECTOMIZED MOUSE LIVER

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M Y B E L O V E D

(LATE) B I T I A J I J J I

PREFACE

The research work embodied in this thesis has been carried out in the School of Life Sciences, Jawaharlal Nehru University, New Delhi. The work is original and has not been submitted so far, in part or full, for any other degree and diploma of any University.

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CONTENTS

INTRODUCTION

It is well established now that several natural and synthetic chemicals are capable of inducing cancer in man as well as in experimental animals. Those carcinogenic chemicals which trigger neoplastic transformation in liver cells are either reactive electrophils per se or get converted metabolically into electrophils which interact with and become covalently bound to important macromolecules. Carcinogenic Polycyclic Aromatic Hydrocarbons (PAH) first undergo metabolic activation to epoxides in a reaction catalysed by the microsomal mixed function oxidases. These epoxides may produce phenols nonenzymatically or be hydrated by epoxide hydrase to transdihydrodiols enzymatically. They may also be conjugated to reduced glutathione (GSH) enzymatically as catalysed by GSHS-epoxide transferase or be complexed to macromolecules, such as DNA, RNA and proteins. These carcinogens thereby alter the genetic and homeostatic characteristics of the cell and bring about changes in normal metabolism.

7,12-Dimethylbenz(a)anthracene (DMBA), one of the first pure substances to be shown to possess carcinogenic activity (Kennaway, 1930; Shear, 1938), is a potent skin carcinogen and is capable of producing tumor after single intravenous or intragastric dose in mammary glands (Huggins

et al., 1961; Shimkin et al., 1967), lungs (Waters, 1966; Waters and Roe, 1966), ovary (Jull et al., 1968; Uematsu and Higgins, 1968). It has also produced leukemias (Uematsu and Huggins, 1968a,b) and tumors of zymbals gland (Heimann et al., 1968; Shimkin et al., 1967). DMBA reacts, in vivo, with nucleic acids and proteins (Brookes and Heidelberger, 1969; Brookes and Lawley, 1964; Heidelberger, 1964) and inhibits the synthesis of DNA both in vitro (Alfred, 1965; Alfred and DiPaolo, 1968) and in vivo (Jenson et al., 1963; Juhn and Prodi, 1965; McCarter and Quastler, 1962; Philips et al., 1969; Shimkin et al., 1967; Uematsu and Huggins, 1968). General body growth is also inhibited by potently carcinogenic polycyclic aromatic hydrocarbons (Haddow et al., 1937), but of even greater significance may be the observations that the action of polycyclic aromatic hydrocarbons like DMBA are particularly directed, in vivo, against cell renewal systems with high proliferative activity such as bone marrow, lymphoid tissue and intestinal epithelium (Cawein and Sydnor, 1968; Philips et al., 1969; Shubik and Porta, 1957).

The carcinogenic activity of DMBA is not prominent in resting liver in which proliferative activity and DNA synthesis are minimal. However, Pound (1968) has shown that hepatomas are induced in significant numbers if the agent is given to mice with regenerating livers. Such a result is consistent with the possibility that there is a casual

relationship between susceptibility to tumor induction and interference with nucleic acid synthesis in cell populations with high proliferative activity (Jenson et al., 1963; Philips et al., 1969).

Regenerating liver provides an opportunity to study the control of cellular proliferation and has been proposed as an experimental model for hepatoma (Bresnick, 1971). On the other hand, regenerating liver continues to carry out hepatic function, often at rates equivalent to or higher than normal liver (Mutschler and Gorden, 1966; Henderson and Kersten, 1970).

In regenerating liver, DNA synthesis is increased but when DMBA is administered it reduced the DNA content (Marquardt et al., 1970). It may bring some changes in the catabolic enzymes e.g. acid ribonuclease (EC.3.1.4.22), deoxyribonuclease I (EC.3.1.4.5) and acid phosphatase (EC.3.1.3.2). Though most of the enzymes of regenerating liver have been studied, but the lysosomal enzymes have not yet been extensively studied. With the expectation that it might provide a relationship in liver, the present work was undertaken to study the effect of DMBA on certain lysosomal enzymes in rapidly dividing hepatic cells.

Effect of ascorbic acid on DNA, RNA and protein synthesis have already been observed. Ascorbic acid helps

in collagen synthesis and enhances total protein content of the tissue. But *in vitro* studies have shown that it has a breaking action on DNA strand (Omura *et al.*, 1975). Ascorbic acid might have an effect on these catabolic enzymes, i.e. nucleases and phosphatases and thus might help in preventing the tissue from the effects of any carcinogen. Ascorbic acid, since breaks DNA molecule, is considered to be a preventor of severel infectious diseases, but its effects on these lysosomal enzymes are not known. In the present study an attempt was made to examine the changes in specific activities of the above mentioned lysosomal enzymes in partially hepatectomized mouse liver treated with DMBA and ascorbic acid.

REVIEW OF LITERATURE

Carcinogenesis involves so many changes in the physiological behaviour as well as in the physical appearance of the tissue. It is now known that during carcinogenesis, changes in the genetic material i.e. DNA occur. But the exact nature of carcinogenic tissue is still a disputed question.

It has been shown that initiation of chemical carcinogenesis requires cell proliferation (Cayama et al., 1978; Warwick, 1971; Borek and Sachs, 1968). The carcinogenecity of a chemical is determined by the proliferative activity of a tissue or organ at the time of treatment (Warwick, 1971). Cell replication has a drametic effect on the response of cells to toxic compounds. Thus while a compound may have no apparent effect on a non-dividing cell, it may kill cell which are passing through the cell cycle (Farber, 1972).

Evidence for the relevance of cell replication in carcinogenesis came initially from experiments involving skin cancer. Berenblum (1954) suggested that carcinogens may induce dormant tumor cells which remain in a latent state until an additional stimulus causes them to grow to form tumors.

Partial hepatectomy of rats or mice is followed by an initial burst of rapid compensatory growth of the remaining liver lobes. After the first two or three rounds of cell division, the frequency of mitosis decreases and returns to normal by two weeks after surgery. At this time, the restoration of liver mass is essentially complete (Bresnick, 1971). Regenerating liver provides an opportunity to study the control of celluler proliferation and has been proposed as an experimental model for neoplasia (Bresnick, 1971).

Several chemical carcinogens have been tried for their carcinogenecity by administering them in regenerating liver. Cayama et al., (1978) has given the supportive evidence that several chemicals that normally do not cause liver cancer in intact adult animals especially with a single dose, become carcinogenic if administered as a single injection after partiel hepatectomy. In these conditions, it is thought that partial hepatectomy may act during initiation, presumably by fixation of some carcinogen-induced DNA damage through replication of altered DNA.

It is reported that carcinogenic activity of 7,12-Dimethylbenz(a)anthracene (DMBA) is not prominent in resting liver in which proliferative activity and DNA synthesis are minimal. However, Pound (1968) has shown

that hepatomas are induced in significant numbers if the agent is given to mice with regenerating liver. A number of compounds which are non-carcinogenic for various tissues when given to the adult animal, were found to induce hepatomas in mice when given by a single injection soon after birth (Pietra et al., 1961) as young animals are always with a higher mitotic index than adult animals. Diethylnitrosamine was one of the first carcinogens to be studied in this way. This compound was known to induce liver cell cancer in rats when fed in the diet, but a single treatment of adult rats induced only kidney tumors. However, one injection given to newborn rats caused a low incidence of liver cell cancer (Terracini and Magee, 1964). Similar results have been obtained in experiments with mice, although mice are less suitable than rats for the study of liver cancer, owing to the occurance of spontaneous liver cell tumors. It was found that a single treatment of newborn mice with dimethylnitrosamine (DMN) and diethylnitrosamine (DEN) induced haeangioma, haemangiosarcoma and liver cell tumors (Toth et al., 1964; Toth and Shubik, 1963; Gargus et al., 1969). Dibutylnitrosamine, which induces bladder tumors in adult mice, induces liver tumors but not bladder when given to newborn mice (Wood et al., 1970). Nitrosomethylurea induced liver

tumors in newborn mice but, surprisingly, not when given to newborn rats (Terracini and Testa, 1970). Cycasin is another carcinogen which probably acts in a similar manner to DEN, as a similar alkylating intermediate is probably formed by the two compounds (Kobayashi and Matsumoto, 1965; Shank and Magee, 1967; Negata and Matsumoto, 1969). Cycasin induces liver cell cancer when fed in the diet (Laqueur *et al.*, 1963; Laqueur and Spatz, 1968) but a single dose given to adult animals causes an enlargement of liver cell tumors (Zedeck *et al.*, 1970). In addition to the alkylating agents, discussed above, many other compounds induce liver cell cancer after a single treatment of neonatal animals.

Some chemicals like Maleic Hydrazide, was not shown to be carcinogenic in adult animals (Barnes *et al.*, 1957) but does induce liver cell cancer in suckling mice (Epstein *et al.*, 1967). β -propiolactone is a well known skin carcinogen, but when given to suckling mice, it induces liver cell cancer (Chernogemski and Warwick, 1970). This difference in organotropy between adult and neonatal animal is also seen with polycyclic aromatic hydrocarbons, PAH (Roe and Waters, 1967) and with certain aromatic amines

(Gorrod *et al.*, 1968; Roe *et al.*, 1969). Recently Pound and McGuire (1977a,b) have shown that repeated partial hepatectomy acts as a promoting stimulus for carcinogenic response of liver to nitrosamines in rats and DEN in mice.

Thus, from all the above reports, it is evident that the regenerating liver and the tissue, which are with a high proliferative activity as in newborn or suckling mice, are more sensitive to carcinogens than resting cells.

If liver cells while replicating are found to be more sensitive to carcinogens, then what is the explanation of this effect? Very probably a number of different factor(s) are involved in different situations. In the case of hepatocarcinogenic alkylating agents, it is possible that a base change in DNA is involved in the initiation of the carcinogenic process. In this case, the effect of cell replication may be due to the occurance of DNA synthesis while abnormal bases capable of mispairing are present in the template molecules or to aberrant repair of DNA if this takes place during S phase. An effect of cell replication on viral induced tumors might be explained if enzymes which incorporate the genetic material of the virus into the host genome are also those responsible for DNA replication. Thus, the sensitivity of a regenerating

tissue is affected by the effects of carcinogen on DNA synthesis as well as on the enzymes of nucleic acid metabolism. Under certain conditions, carcinogens themselves stimulate cell replication, so that proliferating cells are being affected even although the carcinogen was not administered to a system of replicating cells. Alternatively, under other circumstances, when replicating cells are treated with a carcinogen, the cell cycle may be rapidly inhibited, so that proliferating cells are not in fact being acted on by the carcinogen for as long as would otherwise have been so. These effects of carcinogens on cell replication have been studied by making estimations of mitotic rate, but more often by measurement of DNA synthesis, assayed by incorporation of (^{3}H) thymidine into acid insoluble material.

Several investigations have been made to examine the effect of different carcinogens on DNA synthesis as well as on RNA. Rate of DNA synthesis has also been investigated in tumors of different organs and it has been found that there are several chemical carcinogens which react with DNA *in vivo*, and inhibit *de novo* DNA synthesis. In many cases the abnormal product formed in the DNA is excised, together with a section of DNA (Regan and Setlow, 1973). Resynthesis of this 'eroded' DNA, using the complementary DNA strand as a template is thought to be responsible for

the unscheduled DNA replication which often occurs after treatment of cells with carcinogens. One or more days after administration of a carcinogen, there is often an increase in the rate of DNA synthesis. In the case of liver, this is usually considered to be due to regeneration following necrosis. Different carcinogens have affected DNA synthesis variously. Gronow (1971) has shown that the specific activity of DNA decreases significantly in the rat liver during neonatal carcinogenesis induced by DMN in 3 and 5 days old animals. But the liver DNA of these animals exhibited a 3-fold increase in the specific activity over the controls at the post-weanling stage. Changes in composition of rat liver chromatin fraction during nitrosamine carcinogenesis have also been studied (Gronow and Theckrab, 1974). These workers observed that in the carcinogen treated animals given tritiated thymidine, there was again a 3-fold increase in the specific activity of DNA. Jackson and Irving (1973) have studied the effects of 2-Acetylaminofluorene (AAF) on liver cell proliferation after partial hepatectomy of female rats and they found that livers of normal female rats were highly resistant to the carcinogen while partial hepatectomy of female animals maintained on diet containing 0.04% AAF disclosed a marked hepatotoxicity which resulted in abnormal nodular regeneration. They have shown that the incorporation of Thymidine-^{3}H into DNA and the mitotic index were markedly

inhibited in liver of AAF treated animals following partial hepatectomy. Similarly, the work with nitrosamine has shown that hyperplasia occurs during carcinogenesis. Rubin et al. (1964) were among the first to consider the role of cell proliferation in hepatocarcinogenesis. They found that administration of DEN led to the formation of hyperplastic nodules in liver and autoradiography after the injection of tritiated thymidine showed an increase in DNA synthesis in the nodules and in the carcinomas, but not in the surrounding normal liver. However, Guzzo and Glazer (1976) have found no effect of N-Acetoxy-2-AAF and N-methyl-N-nitroso-nitroguanidine on nuclear DNA synthesis in the nuclei prepared from normal or regenerating liver. But they did observe exogenous activated DNA preferentially stimulated 5-methyl-^{3}H thymidine triphosphate incorporation in nuclei from regenerating liver.

Along with these chemicals, effect of Urethane on DNA synthesis in regenerating rat liver has been studied. Hweng et al. (1973) have shown that when Urethane was given after 12 hr after partial hepatectomy the first wave of DNA was suppressed by 50-60%, while the second cycle of DNA replication was essentially uneffected.

In addition, PAH have also been studied in this aspect. Tominaga et al. (1970) have shown that the

incorporation of tritieted thymidine into female mammary gland was severely inhibited at 6 hr and 1, 2 and 4 days after a single oral dose of 20 mg DMBA. A similar inhibitory effect was observed in male rats although the level of inhibition was less than in the female rats.

Studies of Marquardt and Philips (1970) also indicate that DMBA inhibits the incorporation of DNA precursors into DNA of rapidly dividing liver cell. The selective and transient effect of DMBA is closely similar to that of irradiation (Lehnert and Okada, 1966a,b; Van Lancker, 1960). Both agents delay liver regeneration in rats in so far as DNA synthesis and mitotic activity are concerned for about 24 hr without interfering with RNA synthesis or total protein content. The interference of DMBA with DNA synthesis is not restricted to dividing liver cells; the inhibition has been demonstrated in a wide variety of proliferating tissue *in vitro* (Alfred 1965, 1968) as well as *in vivo* (Jensen, 1963; Juhn, 1965; McCarter and Quastler, 1962; Philips *et al.*, 1969; Shimkin *et al.*, 1967; Uematsu and Huggins, 1968).

Reports on the effect of carcinogens on ribonucleic acid in regenerating liver as well as in normal tissue

are also there. Glazer (1973a,b) has shown that partial hepatectomy results in a five to nine fold elevation on the incorporation of Orotic acid - 5-^{3}H into free and membrane bound ribosomal RNA 18 hr after operation. Treatment of animals with Urethane 6 hr after operation resulted in 50-55% inhibition of the incorporation of labelled orotic acid into nuclear ribosomal RNA and free RNA in partially hepatectomized rats but not in sham-operated animals. Witschi (1973) have studied the effect of DEN on RNA and Protein synthesis in the liver and the lung of the Syrian golden Hamster. He found that DEN inhibited the incorporation of labelled orotic acid into RNA in both the organs at early treatment and increased 2-3 days later. Markov et al. (1975) have shown that partial hepatectomy caused a sharp transient increase in the specific radioactivity of the endogenous low molecular weight precursors of RNA in the livers of both normal and irradiated rats (Studies on the incorporation of 6-^{14}C orotic acid).

Mechanism of the inhibition of nucleic acid synthesis by carcinogens is not yet known. Several investigations have been made and it is proposed that increase in the nucleic acid content during liver regeneration and the process of carcinogenesis is because of the decrease in catabolic enzymes, ribonuclease and deoxyribonuclease

(Deoust and Amano, 1963, 1968; Farber, 1973) and vice versa. At present, authors are not aware of any such report which can provide a clear-cut relationship between the nucleic acids and the enzymes of nucleic acid metabolism in carcinogenic tissue.

Weber (1961) has given a general interpretation about the enzyme levels in cancerous tissue. According to him, increased enzymatic activity, as assayed under optimum *in vitro* conditions, is likely to reflect the presence of an increased enzyme population of tissue. Since enzymes are usually present in an excess in the tissue, an increased enzyme concentration may be a valuable indication of an increased stimulus or elevation in metabolic activity.

Both nucleases and polymerases have been studied in regenerating liver and tumors induced by several chemical carcinogens. Taper *et al.* (1971) have shown that the histochemical activities of alkaline and acid nucleases decrease considerably in focal areas and later in the hyperplastic nodules, whereas the surrounding liver parenchyma demonstrated normal activity of these enzymes in the rat liver parenchyma during N-nitrosomorpholine carcinogenesis. In the necrobiotic cells of those malignant tumors a reappearance of nuclease activity was

observed. He suggested that nuclease might be involved in the protection of genetic stability of normal cells against transforming nucleic acids. Daoust and Amano(1963) have studied RNase and DNase in experimental and humen tumors by histochemical substrate film method and reported that the connective tissue stroma and the necrotic regions of the tumor masses showed various levels of nuclease activity, whereas the neoplastic cells showed no demonstrable activity. It appears that deficiencies in RNase and DNase activities represent general properties of cancer cells. These workers reported that losses of RNase and DNase activities take place in rat liver during azo-dye carcinogenesis. The loss of RNase activity is progressive and occurs before parenchymal cells become cancerous, whereas the loss of DNase activity is abrupt and closely associated with the neoplastic transformation of parenchymal cells. DNA polymerase has also been extensively studied in relation to cancer. Ove et al. (1969) reported that a correlation exists between DNA synthesis and DNA polymerase activity partially purified from normal liver and from twelve types of hepatomas. In all these cases DNase activity with denatured DNA as substrate was lower in tumors than in the livers of normal rats. He, therefore, suggested that increased DNA synthesis by these tumors is supported by a rise in

key anabolic enzyme and a fall in catabolic enzyme. DMBA, in mammary gland, however, is found to enrich the activity of DNA polymerase within 24 hr after the administration, but thereafter it decreased to a low level between 2 and 6 days (Tominage et al., 1970). But DNA polymerase was found to be insensitive to Urethane treatment in normal and regenerating liver (Hwang et al., 1973). Much reports are there on RNase. Roth (1957) has shown that alkaline RNase activity of mitochondria was depressed by an average of 50% during the entire course of experiment. Serum alkaline RNase in the AAF-fed animals was depressed to a lesser extent and there was a significant change in acid RNase activity. However, both enzymes increase in the primary tumor. Allard et al. (1957) have reported that RNase activity was similar in Dimethylaminoazobenzene (DAB)-fed animals, liver tumors and regenerating liver. But in contrast, Mayer and Greco (1956) observed that acid as well as alkaline RNase were higher in hepatoma transplants. It was observed that relative concentration of acid ribonuclease (RNase) was greater in the cytoplasmic particulates of hepatoma as well as in DAB-induced tumor than in normal liver. These authors have also found that when the administration of 3-methyl-DAB was followed by partial hepatectomy, acid ribonuclease was increased, while alkaline RNase activity was unaffected. In the earlier work on RNase in tumor tissue, Greenstein (1943)

studied RNase in normal and neoplastic tissues compared with controls, little change of activity was reported in rat hepatoma, but higher activities were observed in mouse hepatoma than in normal mouse liver (Greenstein and Thompson, 1944). Cantero et al. (1950) fed DAB in 0.06% concentration to rats and found a considerable variation in RNase activity. There was an increase to maximum at 90 days followed by a progressive decrease to control levels at 150 days. Later, Lamirande et al. (1953) indicated that the above change is probably accounted for by an increase in the number of cells, the activity per cell remaining constant. Again, Lamirande and his coworkers (1954a,b) observed that two RNase(s) as well as an inhibitor for one of them (Lamirande et al., 1956) are present in rat liver and these observations make a re-examination of RNase activity in precancerous and cancerous state of interest. It is possible that considerable changes may occur in individual components of the RNase system during the feeding of carcinogen, if these changes are in different directions, they might not be detected by determining only the over all activity of the cell.

Acid and alkaline phosphatases have also been affected by tumor initiation by carcinogens. Not much literature is available on this. DeDuve et al. (1955)

and Lamirande *et al.* (1957) have reported that the specific activity of acid phosphatase was unchanged in whole liver homogenate following partial hepatectomy. But in the liver of animals fed DAB, however, the acid phosphatase activity was decreased. When animals were fed the stronger azodye carcinogen 3'-methyl-DAB, acid phosphatase was lower than that in the controls. In mice bearing Ehrlich ascites tumors, alkaline phosphatase activity was increased by five fold in the liver and by 50% in the kidney (Kojima and Sakurada, 1976). In mice bearing solid tumors, caused by inoculation of tumor cells into the axillary region, the activity of this enzyme in the liver was increased by 11-fold, whereas the activity in the kidney was unaltered. Serum phosphatase activity was increased in bone tumors as alkaline phosphatase and acid phosphatase in carcinoma of the prostate (Woodward, 1942, 1952). Similar results were obtained by Gutman and Gutman (1938) that serum acid phosphatase activity is increased in patients with carcinoma of the prostate with metastasis.

Effect of polycyclic aromatic hydrocarbons on these enzyme are not well studied.

On the other hand, several investigations are in the process to look for a remedy for cancer and several

chemicals have been tried as an anticancer agent. Ascorbic acid which has been used in the treatment of cold, cough and several other infectious diseases, has also been found to prevent tumor growth (Chan and Fong, 1977) by aminopyrene and nitrite in rats. These workers have shown that massive doses of vitamin C protected rats against liver tumor production by aminopyrene and Sodium Nitrite; but the protection against lung and kidney tumor production was incomplete. They proposed that the mechanism of protection is in part due to blockage of *in vivo* nitrosation. Greenblett (1973) has shown that ascorbic acid blocks aminopyrene nitrosation in NZO/Bl mice.

Mirvish *et al.* (1972) reported that the nitrosation of several secondary and tirtiary amines is blocked *in vitro* by the addition of ascorbic acid. They suggested that this effect may be useful in preventing the production of carcinogenic N-nitroso compounds when foods containing a high nitrite content are eaten with secondary or tirtiary amines. Kamm *et al.* (1973) have observed that the oral treatment of rats with ascorbic acid (sodium salt) in combination with Sodium Nitrite and aminopyrene prevents the rise in serum alanine amino-transferase (EC.2.6.1.2) observed when nitrite and aminopyrene are

given alone. Ascorbic acid has protective effects against tumor growth, whereas dehydroascorbic acid exerts no protective effect. It is reported that ascorbic acid inhibits the formation of carcinogenic and hepatotoxic N-nitroso compounds from nitrite and nitrogen bases (Kamm *et al.*, 1975; Mirvish *et al.*, 1972). This inhibition that occurs *in vivo* and *in vitro* is believed to involve the reductive deactivation of nitrite by ascorbate (Guttenplan, 1977; Mirvish *et al.*, 1972). Ascorbic acid is also reported to protect against the induction of bladder tumors in mice by 3-hydroxyanthranilic acid (Schlegal *et al.*, 1970) and against skin tumor induction in mice by DMBA plus croton oil (Shamburger, 1972; Slaga and Bracken, 1977). Because these carcinogens are believed to require oxidative metabolism before exerting carcinogenic effects, the mechanism of protection by ascorbic acid was suggested to involve the antioxidant property of ascorbic acid. It was suggested by Guttenplan (1977) that the mechanism of inhibition of microbial mutagenesis by two profound N-nitrosocompounds - DMN and N-methyl-N'-nitroso-N-nitroguanidine, involves the nucleophilic deactivation of electrophilic alkylating metabolites of N-nitroso compounds by ascorbates, but reductive deactivation of the parent N-nitroso compounds was not excluded. Magee and Barnes (1962) noted that the

site of tumor production following DMN administration to the rats shifted from the livers to the kidney as the concentration of carcinogen increases in the diet. Therefore, the complete absence of liver tumor in the presence of kidney tumor production in the group animals means that there must be some factor(s) operating other than the reduction or complete blockage of nitrosation of aminopyrene by vitamin C. One other factor may be altered liver metabolism following massive doses of ascorbic acid. McLean and McLean (1969) cited example in which altered liver function affected chemical hepatic toxicity and carcinogenesis. They found that protein deficiency protects the rat liver against hepatic toxicity and carcinogenic influence of DMN. One other reason of this protection of tumor growth by ascorbic acid may be its effects on nucleic acids and nucleic acid metabolism. Omura et al. (1975) has shown that ascorbic acid has a breaking action on nucleic acids, both DNA and RNA. It was observed that ascorbic acid and its oxidised derivatives caused some retardation of the growth of Sarcoma-180 implanted in mice and the lowering of the viscosity of DNA solution.

On the other hand, Conney and Burns (1959) have observed that the carcinogenic hydrocarbons are potent

stimulators of L-ascorbic acid biosynthesis. These compounds are also known to be extremely potent in inducing the synthesis of several liver microsomal enzymes which metabolize foreign compounds. But how does ascorbic acid affect the enzymes of nucleic and metabolism in regenerating liver and in precancerous and cancerous tissue is still to be known.

MATERIALS AND METHODS

ANIMALS:

All the experiments were conducted on random-bred Swiss albino mice, about 20±2 gm in weight and on average of 10-12 weeks of age. Animals were obtained from Disease Free Small Animal House, Haryana Agricultural University, Hissar, India. They were housed in aluminium cages with a bedding of Paddy-husk. They were grouped in tins of six and provided with Rat food (Hindusten Lever's Limited, India) and water *ad libitum*.

CHEMICALS:

7,12-Dimethylbenz(a)anthracene (DMBA), Triton-x-100, Sucrose, Deoxyribonucleic Acid (DNA), Ribonucleic Acid (RNA), p-Nitrophenyl Phosphate (p-NPP), Bovine Serum Albumin (BSA) were obtained from Sigma Chemical Company, U.S.A.

Tris-Salt Sodium Acetate, Acetic Acid, Sodium Citrate, Citric Acid, Sodium Hydroxide, Sodium Carbonate, Magnesium Sulfate and Oxalic Acid were obtained from B.D.H., India.

Folin-Ciocalteau Reagent and Ascorbic Acid were obtained from V.P. Chest Institute, Delhi, India.

Anaesthatic Ether I.P. was obtained from Alembic Chemical Works Co. Ltd., Baroda, India.

Autoclips (No. B-2355-100 Wound Clips), used for stitching the wound were obtained from Cley-Adams, U.S.A.

EXPERIMENTS:

Mice were selected at random to form 9 groups of 20 animals each as follows:

Group 1: Normal mice without any operation and treatment.

Group 2: Sham-Operated animals.

Group 3: Partially hepatectomized animals.

Group 4: Sham-Operated animals treated with ascorbic acid (dose: 50 mg per 0.3 ml in 1.0% oxalic acid to each individual) after twenty four hours of surgery.

Group 5: Sham-Operated animals treated with DMBA (dose: 5 mg/0.1 ml olive oil to each individual) after twenty four hours of surgery.

Group 6: Sham-Operated animals treated with DMBA and ascorbic acid (doses as in group 5 and group 4 respectively) in combination after twenty four hours of surgery.

Group 7: Partially hepatectomized animals treated with ascorbic acid (same dose as in group 4).

Group 8: Partially hepatectomized animals treated with DMBA (same dose as in group 5).

Group 9: Partially hepatectomized animals treated with ascorbic acid (same dose as in group 4) and DMBA (same dose as in group 5) in combination after twenty four hours of surgery.

Ascorbic acid was given by intramuscular route and DMBA was administered by intragastric intubation.

All Sham-Operations and partial hepatectomies were performed between 10 a.m. to 11 a.m. Surgery was carried out under antiseptic conditions.

For Sham-Operation, median and left lateral liver lobes were delivered to the outside and allowed to slip back into the body cavity.

Partial hepatectomy was performed by using the procedure of Higgins and Anderson (1931), but only a single ligature was used to avoid local tissue reaction. Ligation and excision of the median and left lateral lobes of liver constituted about 50% partial hepatectomy. The incision was about 1.0 - 1.5 cm in length. After excision of the liver lobes, the muscular layer was stitched with surgical thread and skin wound with autoclips.

The operated animals were given post-operative care. The animals of each group were sacrified by cervical decapitation and subjected to liver enzyme analysis at the post-operation intervals of 5, 10 and 15 days.

HOMOGENISATION AND FRACTIONATION:

Homogenisation and fractionation were carried out inside a cold room (temp. $3\pm2^{o}C$). Ten per cent liver homogenates (W/v) were prepared in a Potler-Elvehjem homogeniser for about 1 minute in 0.25 M sucrose solution prepared in 0.1 M Tris-buffer (pH 7.4). The homogenate, thus prepared, was subjected to density gradient centrifugation at 2500-3000 rpm in refrigeretor - centrifuge for 10 minutes. The pellet obtained was suspended in the required volume of 0.25 M sucrose in Tris-buffer (0.1 M; pH 7.4) to obtain the final concentration of 10%. Deoxyribonuclease I (DNase) assay was done in this fraction. The supernatant was recentrifuged at 15000 rpm for 10 minutes. The pellet obtained was discarded and the supernatant obtained as a result of second centrifugation was recentrifuged at 15000 rpm for 10 minutes. The supernatant was discarded and pellet was suspended in the required volume of 0.25 M sucrose in 0.1 M Tris-buffer (pH 7.4) to obtain the final concentration of 10%. In this fraction assay for acid phosphatase and acid ribonuclease (RNase) was carried out. For all the three enzymes, assay was done after Triton-x-100 treatment (0.05 ml of 10% Triton-x-100/ml of extract).

PROCEDURE FOR ENZYME ASSAY:

The assay for each enzyme was done by spectrophotometric method using Carl-Zeiss Spectrophotometer.

a) Acid ribonucleese assay was done by using the method of Bergmeyer (1974). Assay mixture contained 1.5 ml of RNA (substrate)/acetate buffer (0.1M, pH 5.0; 1 mg RNA/ml of buffer), 1.4 ml of doubly distilled water and 0.1 ml of enzyme extract. The cuvette containing the assay mixture was allowed to run at scale expansion twice to obtain a base line and the increase in the optical density was followed until there was no further increase in optical density (O.D.). The O.D. was measured at 300 nm at room temperature (25°C). The activity was expressed as units per gm of tissue. One unit of activity being defined as an increase in extinction of 0.001/minute under the assay condition.

b) Assay for Deoxyribonucleese I (DNase) was done using the method of Bergmeyer (1974). The assay mixture contained 2.5 ml substrate (DNA) in acetate buffer (0.1 M, pH 5.0; 40 µg DNA/ml, 5 mM $MgSO_4$) and 0.1 ml of enzyme extract. The cuvette containing assay mixture was allowed to run at scale expansion twice to obtain a base line and an increase in the optical density was followed until there was no further increase in O.D. The O.D. was measured at 260 nm at room temperature (25°C).

A unit of the enzyme activity was defined which causes an increase in extinction of 0.001/minute under the assay conditions.

c) Acid phosphatase (AcP) activity was measured by the method of Bergmeyer (1974). The assay mixture contained 1.0 ml of 0.1 M citrate buffer (pH 5.6); 0.03 ml of 0.6 M p-Nitrophenyl Phosphate-Sodium Salt (Substrate) and 0.02 ml of the enzyme extract. This was incubated for 5 minutes at room temperature and 2.0 ml of 0.5 N NaOH was added. The O.D. was measured against blank at 405 nm. The activity was expressed as μ moles of p-Nitrophenol released per minute and was measured by using the following formula:

$$\text{Activity per gm tissue} = \frac{\Delta E \times \text{volume of assay mixture} \times \text{dilution}}{\varepsilon \times \text{Vol. of enzyme sample} \times \text{incubation time} \times \text{light path}}$$

or $$\frac{\Delta E \times V \times d}{\varepsilon \times v \times t \times l}$$

where ε = Extinction coefficient

ΔE = Change in Extinction.

Specific activity of each enzyme was expressed as units per mg of protein.

PROTEIN ESTIMATION:

Protein was estimated by the method of Lowry *et al.* (1951).

Reagents:

i) 2% Sodium Carbonate in 0.1 N NaOH.

ii) 1% Copper Sulfate $.5H_2O$.

iii) 2% Sodium Tartarate.

Alkaline reagent was prepared by mixing 25 ml of soln.(i); 0.5 ml of Soln. (ii) and 0.5 ml of soln.(iii).

Folin-Ciocalteau reagent was diluted with equal amount of doubly distilled water immediately before use.

Bovine Serum Albumin (BSA) was used as a standard for protein estimation.

To 0.05 ml of sample, 0.45 ml of doubly distilled water was added to make the volume 0.5 ml. This was followed by the addition of 5.0 ml of Alkaline reagent. The solution was allowed to stand for 5 minutes at room temperature. Finally 0.5 ml of diluted Folin-Ciocalteau reagent was added to the reaction mixture. The optical density was measured against blank (without protein sample) at 660 nm after 30 minutes of Folin-Ciocalteau reagent addition.

P.Hy. = Partial Hepatectomy.

RESULTS

The observations from various treatments are as given below:

1. Effect of Sham-Operation on Acid phosphatase, Acid ribonuclease and Deoxyribonuclease I activity in mouse liver:

The effect of Sham-Operations on enzyme activities was studied on 5th, 10th and 15th day after surgery. Data presented (Table I) show that acid phosphatase activity is significantly ($P \leq 0.01$) lower at all the three post-operation intervals as compared to that of normal. It was also observed that there was a significant ($P \leq 0.01$ on 5th day and 10th day; $P \leq 0.05$ on 15th day) increase in acid ribonuclease (RNase) activity as compared to normal control values (Table II). The deoxyribonuclease I (DNase) activity remained unaffected on 5th day (Table III). However, it showed a significant ($P \leq 0.01$) increase on 10th and 15th day after surgery.

2. Changes in Acid phosphatase, RNase and DNase after Partial hepatectomy:

The effect of P.Hy. on Acid phosphatase, RNase and DNase was studied on 5th, 10th and 15th day after surgery.

The specific activity of acid phosphatase showed a markedly significant ($P \leq 0.01$) decrease on the 5th day, whereas significant ($P \leq 0.01$) increase was observed on 10th day as compared to Sham-Operated controls. However, no change in

the specific activity of the enzyme was observed on the 15th day of operation (Tables IV & VII).

The specific activity of RNase was not affected on 5th and 10th day of operation, but showed a significent ($P \leqslant 0.01$) increase on the 15th day (Tables V & VIII).

A significent increase ($P \leqslant 0.01$) in the specific activity of deoxyribonuclease I (DNase) was observed on 5th and 10th day. However, there was a decrease ($P \leqslant 0.01$) on 15th day after partial hepatectomy as compared to Sham-Operated controls (Tables VI & IX).

3. Effect of Ascorbic Acid on Acid phosphatase, RNase and DNase in Sham-Operated mouse liver:

Specific activity of acid phosphatase was not effected in ascorbic acid treated Sham-Operated mice liver at any of the three (5th, 10th and 15th day of operation) intervals as compared to that of control i.e. Sham-Operated controls (Table IV)

The specific activity of RNase in the animals of this group remained uneffected on 5th and 10th day after surgery. It, however, registered a significent ($P \leqslant 0.01$; $\backsim$ 2 fold) decrease on the 15th day of operation (Table V).

Specific activity of DNase enriched very significently ($P \leqslant 0.01$; $\backsim$ 5-10 fold) by ascorbic acid treatment in Sham-Operated animals on 5th day after surgery. It, however,

decreased significantly ($P \leq 0.01$) on 10th day and remained unaffected on the 15th day after operation (Table VI).

4. Effect of Ascorbic acid on Acid phosphatase, RNase and DNase in Partially hepatectomized mouse liver:

The effect of ascorbic acid on specific activities of acid phosphatase, RNase and DNase was investigated in partially hepatectomized animals on 5th, 10th and 15th day of operation.

Specific activity of acid phosphatase registered a significant ($P \leq 0.01$; $\backsim 5$ fold) increase on 5th day in ascorbic acid treated animals. It, however, decreased significantly ($P \leq 0.01$) on 10th day after operation. Ascorbic acid did not affect the specific activity of acid phosphatase after 15th day of operation (Table VII).

In the animals of this group, there was a significant increase ($P \leq 0.05$ on 5th day and $P \leq 0.01$ on 10th and 15th day) in the specific activity of RNase at all the three intervals. The effect of ascorbic acid on the specific activity of RNase increased with the increase in the time after surgery. The specific activity increased by 1.3 fold; 2.6 fold and 5.8 fold on 5th, 10th and 15th days respectively (Table VIII).

Ascorbic acid did not affect the specific activity of DNase in partially hepatectomized animals on the 5th day

after operation. It, however, decreased the specific activity of the enzyme on 10th and 15th day after operation ($P \leq 0.05$) (Table IX).

5. Effect of DMBA on the specific activities of the enzymes, studied in Sham-Operated mouse liver:

Specific activity of acid phosphatase was not effected by DMBA treatment on 5th day after operation but it registered a significant ($P \leq 0.01$) increase over that of control (Sham-Operated) on 10th and 15th days of operation (Table IV).

The DMBA treated animals, the specific activity of RNase was inhibited till the 10th day of operation. DMBA did not affect the RNase activity on 15th day of operation (Table V).

The effect of DMBA on the specific activity of DNase was observed only on the 5th day of operation. It induced $\backsim$3 fold increase ($P \leq 0.01$) in the specific activity of the enzyme (Table VI).

6. Effect of DMBA on Acid phosphatase, RNase and DNase in Partially hepatectomized mouse liver:

The effect of DMBA on the specific activity of acid phosphatase was observed only on the 5th day of operation. DMBA induced $\backsim$5 fold ($P \leq 0.01$) increase in the specific activity of the enzyme over that of control (Sham-Operated) (Table VII).

DMBA was not found to effect the specific activity of RNase on the 5th day and 10th day of surgery but it induced ∽1.5 fold ($P \leqslant 0.01$) increase over that of control (Sham-Operated) on 15th day of operation (Table VIII).

It did not affect the specific activity of DNase at any of the three assay intervals (Table IX).

7. Combined Effects of DMBA and Ascorbic Acid on Acid phosphatase, RNase and DNase in Sham-Operated mouse liver:

There was no effect of DMBA and ascorbic acid on the specific activity of acid phosphatase when used in combination on the 5th day of operation. However, there was a significant increase in the specific activity of the acid phosphatase on 10th ($P \leq 0.01$) and 15th ($P \leqslant 0.05$) days after surgery, when the two test chemicals were administered in combination. Under the comparable conditions ascorbic acid had no effect whereas DMBA induced a significant ($P \leqslant 0.01$) increase on the specific activity of acid phosphatase (Table IV).

The specific activity of RNase registered a significant ($P \leqslant 0.01$) decrease over that of control on 5th, 10th and 15th day of operation, when the two test chemicals were administered in combination (Table V).

The specific activity of DNase registered ∽4.5 fold increase over that of sham control on 5th day of operation

in animals treated with DMBA and ascorbic acid in combination. There was, however, no effect on the specific activity of DNase on the 10th and 15th days of operation when these two were administered in combination (Table VI).

8. Effect of DMBA and Ascorbic Acid in combination on Acid phosphatase, RNase and DNase in Partially hepatectomized mouse liver:

When DMBA was administered in combination with ascorbic acid, the specific activity of acid phosphatase increased significantly ($P \leq 0.01$; $P \leq 0.05$ and $P \leq 0.01$ on 5th, 10th and 15th day of operation respectively) in partially hepatectomized animals (Table VII).

There was a significant ($P \leq 0.01$) increase in the specific activity of RNase at all the three intervals when DMBA and ascorbic acid were administered in combination (Table VIII).

In partially hepatectomized animals, given a combined treatment of DMBA and ascorbic acid, the specific activity of DNase registered a significant decrease on 5th day ($P \leq 0.01$) and 10th day ($P \leq 0.05$) of operation. Surprisingly, on 15th day the specific activity increased significantly ($P \leq 0.01$) by $\backsim 6$ fold (Table IX).

TABLE I: Effect of Sham-Operation on specific activity of Acid Phosphatase(AcP) in mouse liver. Specific activity is expressed as Mean ± S.E. for livers from six mice.

Time(in days) after operation	5		10		15	
Group	Units of Activity per gm tissue	Specific Activity	Units of Activity per gm tissue	Specific Activity	Units of Activity per gm tissue	Specific Activity
1. NORMAL	2.91±0.384	0.043±0.0069				
2. SHAM-OPERATION	0.219±0.0356	0.016±0.003*	0.37±0.07	0.0064±.0009*	0.65±0.076	0.0102±0.0014

* Significantly different from unoperated group at $P \leq 0.01$.

TABLE II: Effect of Sham-Operation on specific activity of Acid Ribonuclease (RNase) in mouse liver. Numbers represent Mean ± S.E. for livers from 6 mice in each case.

Time(in days) after operation / Group	5		10		15	
	Units of Activity per gm tissue	Specific Activity	Units of Activity per gm tissue	Specific Activity	Units of Activity per gm tissue	Specific Activity
1. NORMAL	6.50±101.03	9.6±1.43				
2. SHAM-OPERATION	3035±463.35	45.72±8.13*	1622±296.4	27.78±3.23*	818.33±31.83	14.63±1.36**

* Significantly different from unoperated group at $P \leq 0.01$.

** Significantly different from unoperated group at $P \leq 0.05$.

TABLE III: Effect of Sham-Operation on specific activity of Deoxyribonuclease I (DNase) in mouse liver. Specific activity is given as Mean ± S.E. for livers from 6 mice in each case.

Time(in days) after operation	5		10		15	
Group	Units of Activity per gm tissue	Specific Activity	Units of Activity per gm tissue	Specific Activity	Units of Activity per gm tissue	Specific Activity
1. NORMAL	4033.3±546.7	88.19±10.7				
2. SHAM-OPERATION	3313±243.13	99.08±4.86	20150±1023	257.66±12.44*	16379±1279	257.22±47.76

* Significantly different from normal control at $P \leq 0.01$.

TABLE IV: Effect of DMBA and Ascorbic acid treatment on specific activity of Acid Phosphatase in Sham-Operated mouse liver. The numbers represent Mean ± S.E. for livers from 6 mice in each case.

Time(in days) after operation	5		10		15	
Group	Units of Activity per gm tissue	Specific Activity	Units of Activity per gm tissue	Specific Activity	Units of Activity per gm tissue	Specific Activity
1. SHAM-OPERATION	0.219±0.036	0.016±0.003	0.37±0.07	0.0064±0.0009	0.65±0.076	0.0102±.001
2. SHAM-OPERATION AND ASCORBIC ACID	0.365±0.054	0.0108±0.003	0.51±0.002	0.008±0.002	0.44±0.082	0.0076±.0016
3. SHAM-OPERATION AND DMBA	1.013±0.035	0.023±0.005	1.87±0.26	0.028±0.0028*	1.24±0.123	0.03 ±0.002*
4. SHAM-OPERATION, ASCORBIC ACID AND DMBA	0.635±0.031	0.0124±0.002	1.49±0.0023	0.0255±0.0023*	0.8595±0.13	0.0156±0.002**

* Significantly different from Sham-operated control at $P \leq 0.01$.

** Significantly different from Sham-operated control at $P \leq 0.05$.

TABLE V: Effect of DMBA and Ascorbic Acid Treatment on Specific Activity of Acid Ribonuclease in Sham-Operated mouse liver. Specific activity is expressed as Mean ± S.E. for livers from 6 mice in each case.

Time(in days) after operation	5		10		15	
Group	Units of Activity per gm tissue	Specific Activity	Units of Activity per gm tissue	Specific Activity	Units of Activity per gm tissue	Specific Activity
1. SHAM-OPERATION	3035±463.35	45.72±8.13	1622±296.45	27.78±3.23	818.33±31.83	14.63±1.36
2. SHAM-OPERATION AND ASCORBIC ACID	2451.66±377.77	37.76±5.38	1005±152.29	21.42±2.6	515±26.29	7.63±0.79*
3. SHAM-OPERATION AND DMBA	1345±149.64	25.82±1.67**	858.3±108.99	16.26±1.66*	1455±183.37	20.42±2.62
4. SHAM-OPERATION, ASCORBIC ACID AND DMBA	858.3±113	25.21±4.17*	1443.33±253.4	17.14±1.41*	443.3±59.04	6.56±0.845*

* Significantly different from Sham-operated control at $P \leq 0.01$.

** Significantly different from Sham-operated control at $P \leq 0.05$.

TABLE VI: Effect of DMBA and Ascorbic Acid on Deoxyribenuclease I specific activity in Sham-Operated mouse liver. The numbers represent Mean ± S.E. for livers from 6 mice in each case.

Time(in days) after operation	5		10		15	
Group	Units of Activity per gm tissue	Specific Activity	Units of Activity per gm tissue	Specific Activity	Units of Activity per gm tissue	Specific Activity
1. SHAM-OPERATION	3313±243.13	79.08±4.86	20150±1023	257.66±12.44	16379.16±1279	257.2±47.76
2. SHAM-OPERATION AND ASCORBIC ACID	14508±1722	371.74±43.74*	13108.3±1668	192.06±13.86*	13245±1224.7	174.78±13.8
3. SHAM-OPERATION AND DMBA	13441.6±922	261.39±19.35*	18285±2430	247.0±24.47	12750±1814.5	234.5±29.96
4. SHAM-OPERATION, ASCORBIC ACID AND DMBA	15125±1586	354±29.41*	13958±916.7	229.16±21.89	10603±941	199.7±17.51

* Significantly different from Sham-operated control at $P \leq 0.01$.

TABLE VII: Effect of Ascorbic Acid and DMBA on specific activity of Acid Phosphatase in Partially Hepatectomized mouse liver. Specific Activity is expressed as Mean ± S.E. for livers from 6 mice in each case.

Time(in days) after operation	5		10		15	
Group	Units of Activity per gm tissue	Specific Activity	Units of Activity per gm tissue	Specific Activity	Units of Activity per gm tissue	Specific Activity
1. PARTIAL HEPATECTOMY	0.91±0.22	0.007±0.001	1.112±0.139	0.023±0.0027	1.46±0.243	0.0156±.003
2. PARTIAL HEPATECTOMY AND ASCORBIC ACID	2.84±0.289	0.0352±0.004*	0.63±0.065	0.0085±0.002*	1.113±0.142	0.0143±.0025
3. PARTIAL HEPATECTOMY AND DMBA	0.675±0.059	0.0356±0.0056*	1.066±0.126	0.028±0.0028	0.744±0.081	0.012±0.002
4. PARTIAL HEPATECTOMY, ASCORBIC ACID AND DMBA	1.05±0.114	0.027±0.005*	1.02±0.084	0.044±0.0087**	1.67±0.244	0.0616±.008

* Significantly different from Partially Hepatectomized group at $P \leq 0.01$.

** Significantly different from Partially Hepatectomized group at $P \leq 0.05$.

TABLE VIII: Effect of Ascorbic acid and DMBA on specific activity of Acid Ribonuclease in Partially Hepatectomized mouse liver. The Numbers represent Mean ± S.E. for livers from 6 mice in each case.

Time(in days) after operation	5		10		15	
Group	Units of Activity per gm tissue	Specific Activity	Units of Activity per gm tissue	Specific Activity	Units of Activity per gm tissue	Specific Activity
1. PARTIAL HEPATECTOMY	2055±103.8	32.03±1.64	1000±125.8	28.24±2.15	1366.6±136.8	21.26±1.689
2. PARTIAL HEPATECTOMY AND ASCORBIC ACID	2513.3±247	42.23±4.25**	3650±359.39	72.24±5.11*	4750.8±376.5	122.3±5.75*
3. PARTIAL HEPATECTOMY AND DMBA	2762.5±359.2	30.94±5.11	1341.6±212.89	25.84±3.23	2233.3±55.27	32.68±1.93*
4. PARTIAL HEPATECTOMY, ASCORBIC ACID AND DMBA	3388.3±370.97	151.6±14.22*	2250±381.8	104.78±6.67*	4950±234.5	136.8±7.11*

* Significantly different from Partially Hepatectomized group at $P \leq 0.01$.

** Significantly different from Partially Hepatectomized group at $P \leq 0.05$.

TABLE IX: Effect of DMBA and Ascorbic Acid on specific activity of Deoxyribonuclease I in partially Hepatectomized mouse liver. Numbers represent Mean ± S.E. for livers from 6 mice in each case.

Time(in days) after operation	5		10		15	
Group	Units of Activity per gm tissue	Specific Activity	Units of Activity per gm tissue	Specific Activity	Units of Activity per gm tissue	Specific Activity
1. PARTIAL HEPATECTOMY	1738.3±1083	270.34±21.40	22580±2908	480.79±29.39	4838±337.8	76.42±9.06
2. PARTIAL HEPATECTOMY & ASCORBIC ACID	13911.6±1671.2	257.7±22.4	16367±1444.4	391±30.75**	5225±678.0	42.0±9.98**
3. PARTIAL HEPATECTOMY AND DMBA	19083.3±1008	291.26±14.38	21650±1407.5	431±29.66	3908.3±2805	95.49±9.79
4. PARTIAL HEPATECTOMY, ASCORBIC ACID AND DMBA	3392±491.83	104±15.42*	12800±1261.28	393±24.15**	19183±2096.8	453±30.47*

* Significantly different from Partially Hepatectomized group at $P \leq 0.01$.

** Significantly different from Partially Hepatectomized group at $P \leq 0.05$.

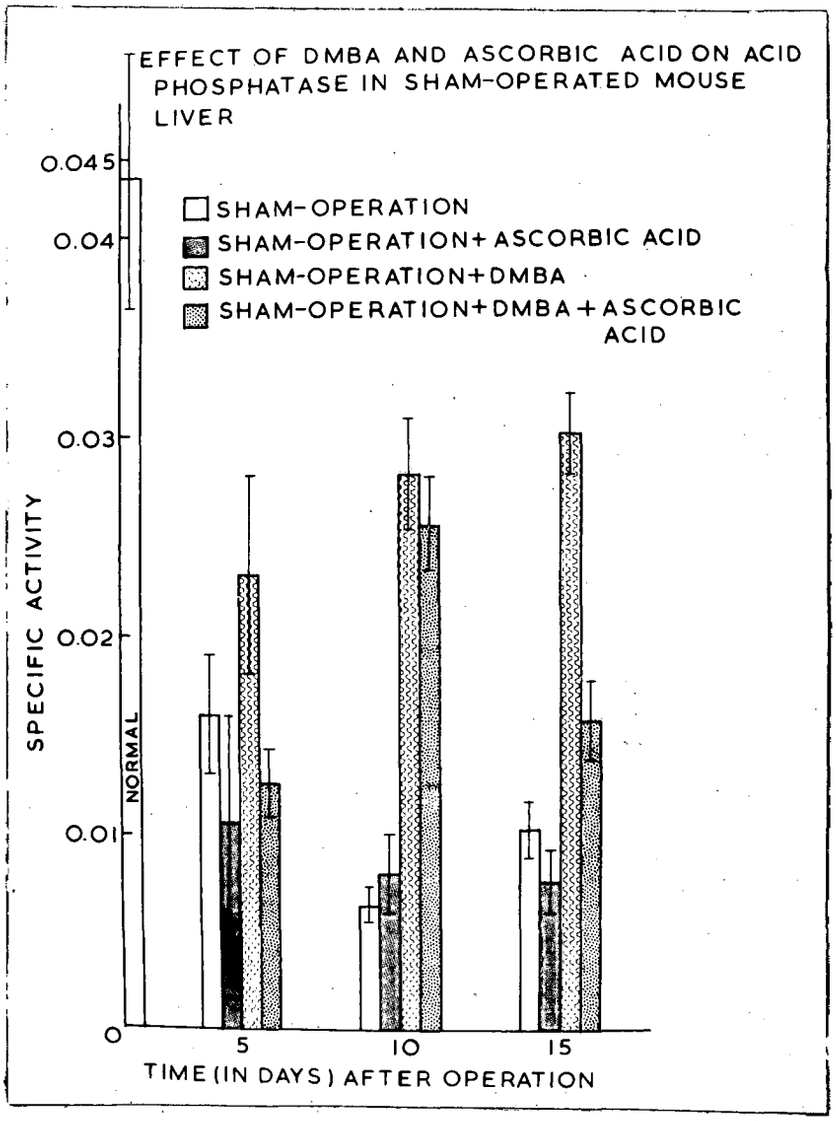
EFFECT OF DMBA AND ASCORBIC ACID ON ACID PHOSPHATASE IN SHAM-OPERATED MOUSE LIVER
SHAM-OPERATION
SHAM-OPERATION+ASCORBIC ACID
SHAM-OPERATION+DMBA
SHAM-OPERATION+DMBA+ASCORBIC ACID
SPECIFIC ACTIVITY
0.045
0.04
0.03
0.02
0.01
0
NORMAL
5
10
15
TIME (IN DAYS) AFTER OPERATION

Fig. 1

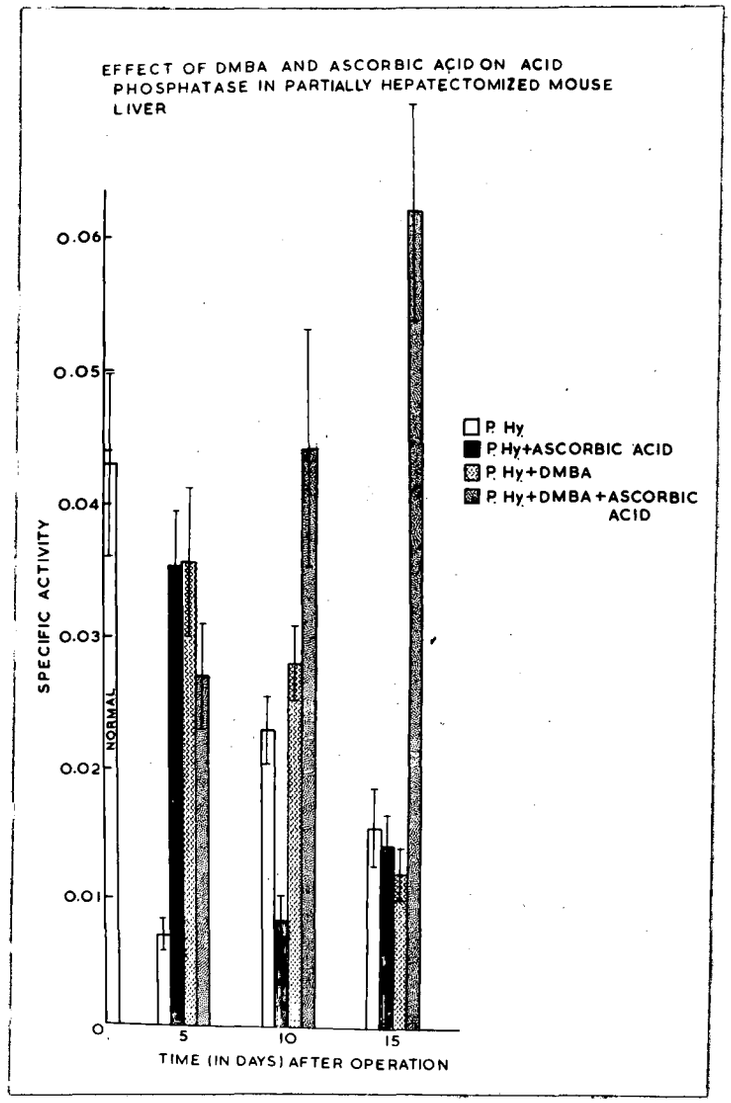
EFFECT OF DMBA AND ASCORBIC ACID ON ACID PHOSPHATASE IN PARTIALLY HEPATECTOMIZED MOUSE LIVER
SPECIFIC ACTIVITY
0.06
0.05
0.04
0.03
0.02
0.01
0
NORMAL
5
10
15
TIME (IN DAYS) AFTER OPERATION
P. Hy
P. Hy+ASCORBIC ACID
P. Hy+DMBA
P. Hy+DMBA+ASCORBIC ACID

Fig. 2

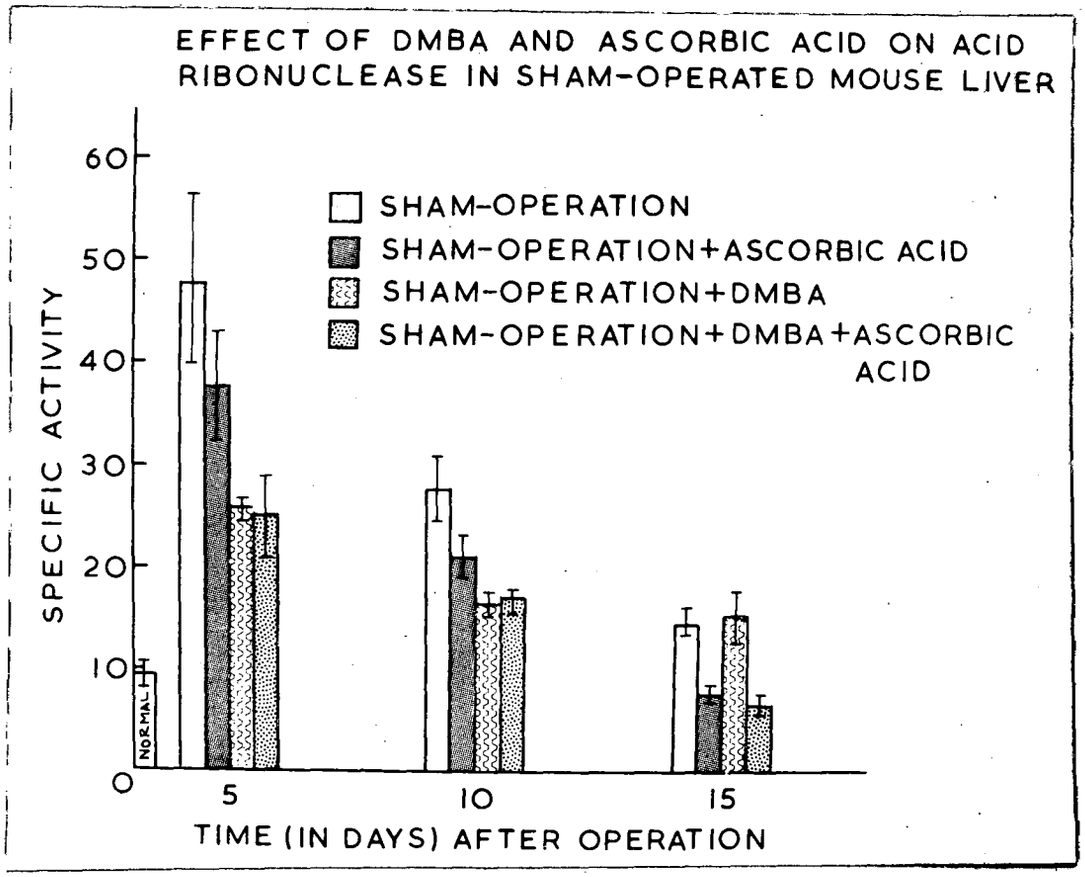
EFFECT OF DMBA AND ASCORBIC ACID ON ACID RIBONUCLEASE IN SHAM-OPERATED MOUSE LIVER
SPECIFIC ACTIVITY
60
50
40
30
20
10
0
NORMAL
SHAM-OPERATION
SHAM-OPERATION+ASCORBIC ACID
SHAM-OPERATION+DMBA
SHAM-OPERATION+DMBA+ASCORBIC ACID
5
10
15
TIME (IN DAYS) AFTER OPERATION

Fig. 3

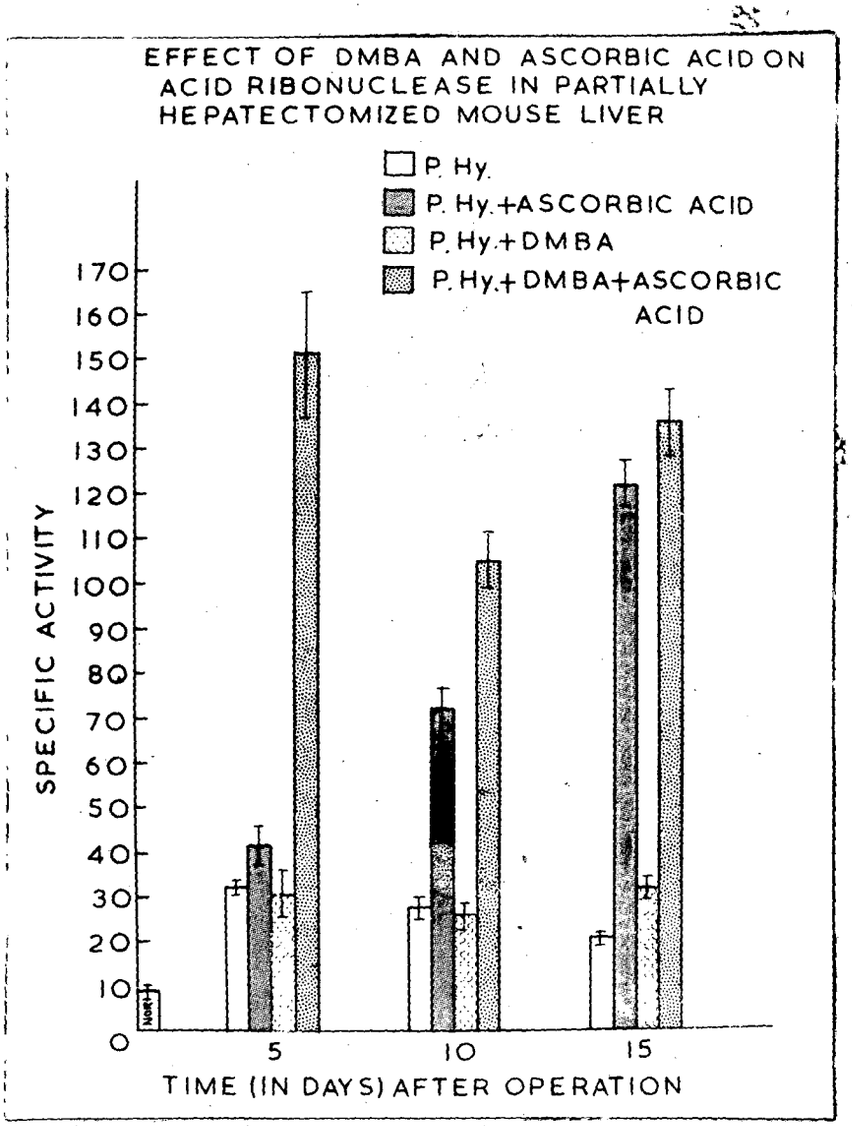
EFFECT OF DMBA AND ASCORBIC ACID ON ACID RIBONUCLEASE IN PARTIALLY HEPATECTOMIZED MOUSE LIVER
P. Hy.
P. Hy.+ASCORBIC ACID
P. Hy.+DMBA
P. Hy.+DMBA+ASCORBIC ACID
SPECIFIC ACTIVITY
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
Norm
5
10
15
TIME (IN DAYS) AFTER OPERATION

Fig. 4

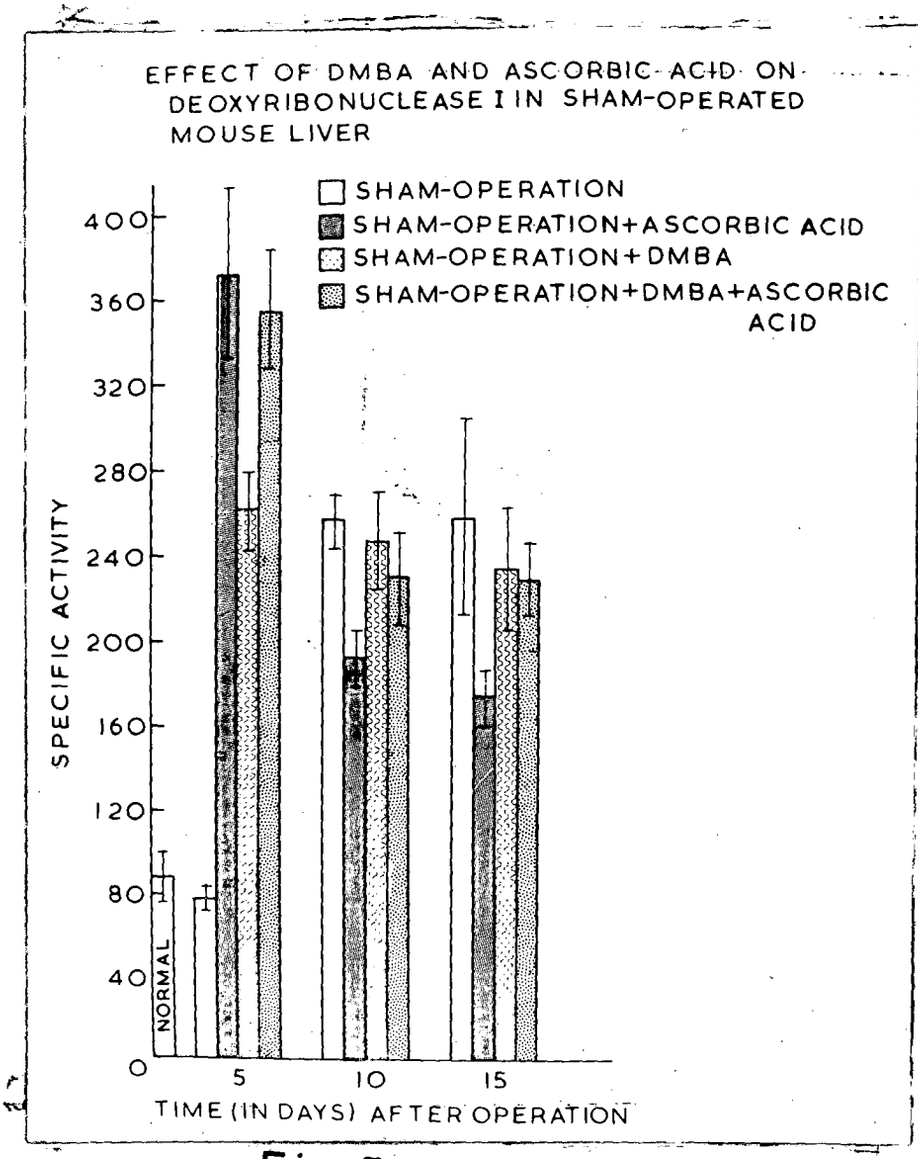
EFFECT OF DMBA AND ASCORBIC ACID ON DEOXYRIBONUCLEASE I IN SHAM-OPERATED MOUSE LIVER
SHAM-OPERATION
SHAM-OPERATION+ASCORBIC ACID
SHAM-OPERATION+DMBA
SHAM-OPERATION+DMBA+ASCORBIC ACID
SPECIFIC ACTIVITY
400
360
320
280
240
200
160
120
80
40
0
NORMAL
5
10
15
TIME (IN DAYS) AFTER OPERATION

Fig. 5

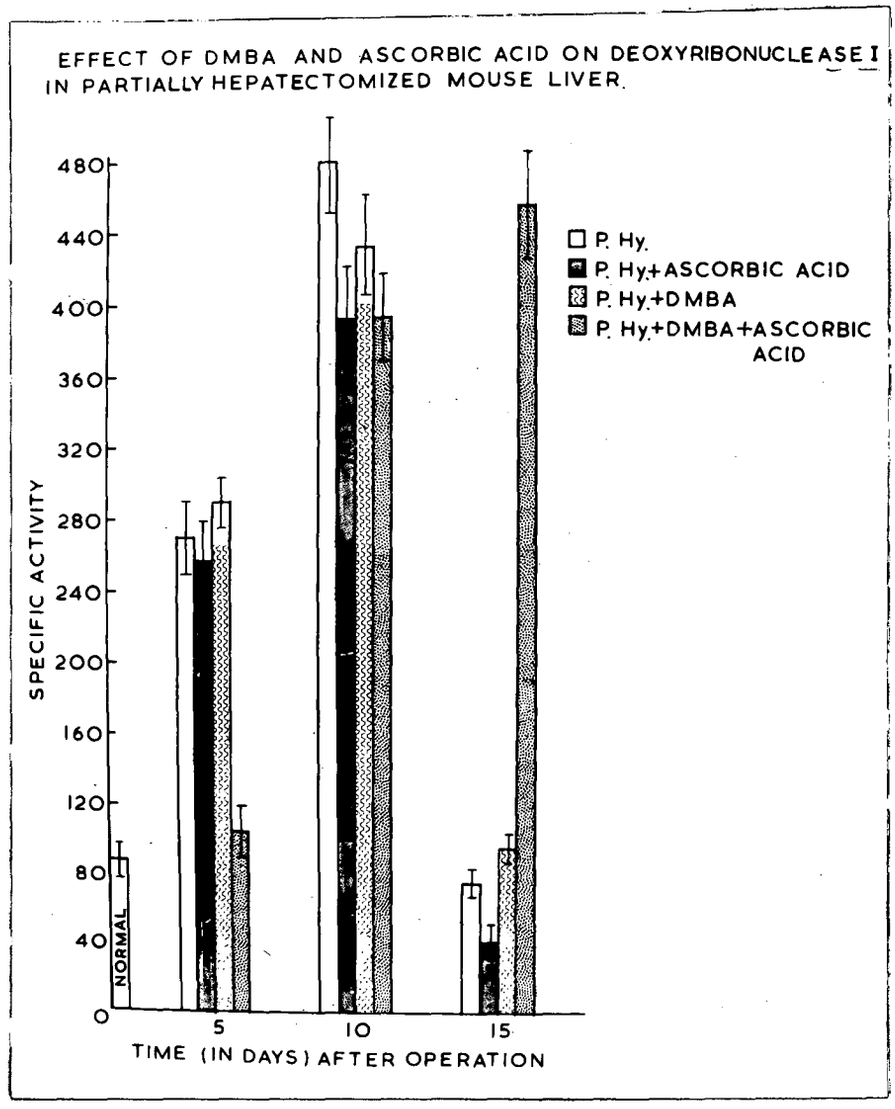
EFFECT OF DMBA AND ASCORBIC ACID ON DEOXYRIBONUCLEASE I IN PARTIALLY HEPATECTOMIZED MOUSE LIVER.
SPECIFIC ACTIVITY
480
440
400
360
320
280
240
200
160
120
80
40
0
NORMAL
5
10
15
TIME (IN DAYS) AFTER OPERATION
P. Hy.
P. Hy.+ASCORBIC ACID
P. Hy.+DMBA
P. Hy.+DMBA+ASCORBIC ACID

Fig. 6

DISCUSSION

Several studies have already been done on enzyme profiles in regenerating, precancerous and cancerous liver. Acid phosphatase, RNase and DNase I, in spite of being important enzymes of nucleic acid metabolism, have not been extensively studied.

The present study reveals that in partially hepatectomized mouse liver, specific activity of acid phosphatase is decreased on 5th day but an increase is observed on 10th day. Later on the activity is unaffected as compared to Sham-controls (Tables IV and VII). Similar results were reported in regenerating rat liver (Fiszer-Szafarz and Nadal, 1977) and in actively growing hepatoma (Fiszer-Szafarz and Szafarz, 1973). However, there are some reports that there is no change in the specific activity of acid phosphatase in whole liver homogenates (DeDuve *et al.*, 1955; Lamirande *et al.*, 1957). The changes in the specific activity of the enzyme can be explained through the changes in the organelles of the cell during subsequent stages. In regenerating liver, the dividing cells, probably, reorganize their structure, one aspect of which is an increase in the activity of the lysosomal enzymes responsible for organelle degradation and of constituents not involved in division.

These enzymes are then gradually used up and are not renewed. This phenomenon would then be direct cause of the loss of lysosomes before mitosis reverted to a low level by 5th day. Later on reconstitution of cell organelle takes place and it is reflected in the increased lysosomal activity measured between 5th and 10th day after partial hepetectomy. An increase in the lysosomal enzyme activities in cultured cells, reaching the stage of contact inhibition, was also observed by Horvat and Acs (1974).

Data presented in Tables VI and IX show an increase in the specific activity of DNase in partially hepatectomized mouse liver. This increase might be due to an increase in the cell population and showing a higher rate of metabolism. Whereas the decrease at the later intervals might be because of feed back inhibition.

The specific activity of ribonuclease remains unaffected at earlier intervals, but an increase is observed on later interval in partially hepatectomized mouse liver (TablesV and VIII). Glazer (1973a,b) has shown that there is an increase in the RNA synthesis in partially hepatectomized rat liver. The increase in RNA synthesis might be due to an increase in the activity of RNA polymerase and not because of change in the levels of RNase.

Table IV and Fig. 1 show the effect of ascorbic acid and DMBA on acid phosphatase activity in sham-operated control mouse liver. It reveals that ascorbic acid is not affecting the enzyme activity as compared to sham-control. But DMBA causes an increase in the specific activity at later intervals. These two test chemicals, in combination, show similar effect on specific activity of acid phosphatase as in DMBA treated sham-control. Here we see that ascorbic acid is not having any effect on the specific activity of acid phosphatase. Ascorbic acid, thus, is not able to inhibit the action of DMBA on acid phosphatase specific activity.

In partially hepatectomized animals these two chemicals have entirely different effects on the specific activity of acid phosphatase as compared to sham-operated control treated with DMBA and ascorbic acid (Table V; Fig.2). Here ascorbic acid is found to increase the specific activity of acid phosphatase. This indicates that an increase in the catabolic enzyme might help in the prevention of neoplastic transformation. Our data show that DMBA has also increased the specific activity of the enzyme at earlier intervals. Similar results were obtained in the solid tumor developed by inoculated tumor cells in axillary region (Kojima and Sakurada, 1976) and in the

carcinoma of the prostate (Woodward, 1942, 1952; Gutman and Gutman, 1938). However, when these two test chemicals were given in combination, they have increased the specific activity of acid phosphatase as compared to that of partially hepatectomized mouse liver, at all the three intervals. The increase in the specific activity on 15th day appears to be a synergistic effect of these two chemicals but at other two intervals again DMBA and ascorbic acid are showing independent effects on the specific activity of acid phosphatase in partially hepatectomized mouse liver also.

Specific activity of acid ribonuclease in sham-operated mouse liver has shown various levels of activity when treated with ascorbic acid and DMBA (Table V and Fig.3). Ascorbic acid does not affect the specific activity of RNase at earlier intervals but a decrease is observed on 15th day of surgery. However, DMBA has shown a decline in the specific activity at earlier intervals but the specific activity of enzyme shows no variation on 15th day. This decline is consistent with the increased RNA synthesis (Daoust and Amano, 1963, 1968 and Farber, 1973) in animals treated with different chemicals. Taper *et al.* (1971) have also observed a decline in the level of acid nuclease in rat liver during N-Nitrosomorpholine carcinogenesis. Similar results were obtained in animals fed Acetylamino-fluorene in rat liver during azodye carcinogenesis (Roth, 1957)

in experimental and human tumors (Daoust and Amano, 1963). On 15th day, it is possible that considerable changes might occur in individual components of RNase system during the feeding of carcinogen. If these changes are in different directions, they might not be detected by determining only the overall activity of the enzyme as has been done in the present study.

A combined treatment of the two test chemicals shows a fall in the specific activity of ribonuclease in sham-operated animals, again showing that ascorbic acid and DMBA are acting independently on acid ribonuclease. The decrease on 5th and 10th day is similar to DMBA effects when given alone while on 15th day the fall in the specific activity is similar to the ascorbic acid effect when administered alone.

In partially hepatectomized animals, ascorbic acid induces an increase in the specific activity of RNase at all the three intervals (Table VIII, Fig. 4). This induction of enzyme by ascorbic acid can be correlated with the reports of Omura *et al.* (1975) that ascorbic acid decomposes nucleic acids. This breaking of nucleic acid molecule i.e. RNA might be because of the induction of catabolic enzyme-ribonuclease by ascorbic acid. On the other hand DMBA has not affected the levels of ribonuclease at earlier intervals but an increase is observed on 15th

day after partial hepatectomy (Table VIII, Fig. 4). Similar results were obtained in the experiments with cytoplasmic particulates of hepatoma transplants (Mayer and Greco, 1956) and with neoplastic tissue (Greenstein, 1943; Greenstein and Thompson, 1944). Our data is also consistent with the reports of Cantero et al. (1950), Daoust and Cantero (1950), and Lamirande et al. (1953). According to these workers, the increase in the specific activity of ribonuclease in the animals at later intervals may probably be accounted for by an increase in the number of cells, the activity per cell remaining constant.

A combined treatment of these two test chemicals when given to partially hepatectomized animals, the specific activity of RNase is increased during the entire course of experiment. This result again confirms that ascorbic acid and DMBA are acting independently on the enzyme level. The increase in the specific activity of RNase on 15th day is almost equal to the total increase in the specific activity caused by these two test chemicals when given separately.

The data in Table VI (Fig. 5) show the effects of these two test chemicals on the levels of DNase I in sham-operated mouse liver. It is observed that ascorbic acid enriches the specific activity of the enzyme at earlier intervals. Omura et al. (1975) have reported that ascorbic

acid breaks DNA molecule. This breaking action of ascorbic acid, thus, can be explained by the observations in the present study that it increases DNase I level and thus decreases the DNA content. However, later on, a decline in the specific activity of DNase I is observed.

DMBA, in sham-controls, has increased the activity only at earlier intervals (5th day). Marquardt and Philips (1970) have reported that DMBA decreases the total DNA content. Similar reports have been given using other carcinogens like DMN (Gronow, 1971); Nitrosamine (Gronow, and Thakrab, 1974); and 1,2-Dimethylhydrazine (Brambilla et al., 1978). This decrease in DNA content is proposed to be due to interaction of carcinogen with DNA molecule. It may also be due to the increase in the specific activity of DNase I by the carcinogen as observed in the present studies.

However, these two test chemicals when administered in combination to sham controls, a rise in the specific activity of DNase I is observed only on 5th day. In this group, we see that decrease in the specific activity of the enzyme, caused by ascorbic acid on later intervals, has been nullified by DMBA. Here, the effects of DMBA are more prominent.

In partially hepatectomised animals, DMBA and ascorbic acid effect the levels of DNase I in an entirely different manner from sham-controls (Table IX, Fig. 6). Ascorbic acid is not affecting DNase at earlier interval but a fall in the specific activity of the enzyme is observed on 10th and 15th day of operation. This might be due to a decreese in the cell population because of local tissue reaction which has occured already and also might be due to the induction of some inhibitory factors in the tissue. However, it is observed that DMBA does not alter the specific activity of DNase I at any of the three intervals. It is reported that by 5th day of operation, the mitotic activity almost comes to normal level and hence afterwards there would not be any change in the genetic material DNA and concerned enzyme system. Similar reports have come using Urethane in rat liver (Hwang *et al.*, 1973). These investigators have shown that Urethane suppressed the first wave of DNA replication while later DNA replication was essentially unaffected.

A combined treatment of the two test chemicals to partially hepatectomized animals causes a decline in the specific activity of DNase I at earlier intervals but surprisingly it is raised significantly on 15th day. The fall in the specific activity on 5th day appears to be a

synergistic effect of the two test chemicals. On 10th day, the decrease might be because of ascorbic acid which is found to decline the specific activity of DNase I when given alone.

On the basis of our results concerning the activities of the lysosomal enzymes in mouse liver treated with ascorbic acid and DMBA, it is very difficult to investigate the cause with respect to the degree of the effects brought about by the two chemicals and with the present literature available, any concluding remark cannot be given, yet there are certain possible mechanisms through which the changes of enzymatic activities in treated mouse liver can be explained:

- The increased enzymatic activity is likely to reflect the presence of an increased enzyme population of the tissue. Since enzymes are usually present in an excess in the tissue, an increased enzyme level may be a valuable indication of a stimulated or elevated metabolic activity by the treatment.

- The increase in the enzyme levels in partially hepatectomized animal may probably be due to an increase in the cell population.

- The changes in the enzyme activities may be because of the changes in the rate of synthesis or enzyme catabolism.

- The changes in the enzyme levels can also be explained through enzyme translocation from one organ to another.
- The changes in the enzymatic activity may also be due to the changes in the levels of inhibitors or activators because of the treatment.

SUMMARY

1. In sham-operated mouse liver, ascorbic acid has no effect on acid phosphatase. It shows a decrease in the specific activity of RNase at later intervals, whereas DNase specific activity is enriched at earlier intervals.

2. In partially hepatectomized mouse liver, ascorbic acid is found to increase the specific activity of acid phosphatase at earlier intervals. The specific activity of both the nucleases - RNase and DNase - is also enriched and thus, can be correlated with the breaking action of ascorbic acid on nucleic acids.

3. DMBA, in sham-operated mouse liver, increases the specific activity of acid phosphatase at later intervals only. It inhibits RNase. However, it has been found to increase the specific activity of DNase only on 5th day of operation.

4. In partially hepatectomized mouse liver, DMBA increases the specific activity of acid phosphatase at earlier interval (5th day) and of RNase at later intervals. However, it does not alter the specific activity of DNase at any of the three intervals.

5. Ascorbic acid and DMBA, when given in combination to both sham-operated as well as partially hepatectomized animals, show independent effects on the specific activities of both acid phosphatase and RNase. However, on the specific activity of DNase, the effects of DMBA are more prominent. It is, therefore, clear that ascorbic acid does not prevent tumor growth by affecting the lysosomal enzymes studied.

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