Influence of Therapeutic Radiation on Serum Enzyme Levels in Cancer Patients

BY

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I INTRODUCTION

Cancer - a mysterious plague and the greatest bugaboo of man, is itself plagued by scientific double think. On the one hand, it has been speculatively described as the most fascinating, formidable, and wonderful problem almost beyond comprehension by human intellect. On the other hand, mankind has been repeatedly promised a victory against cancer (Manu and Lopa, 1978).

There are about 1.5 million cancer patients in India out of 6.9 million suffering from the dreaded ailment in the world. There had been an addition of 5 lakh people, who fall a prey to the fatal ailment of cancer in the country every year. Most of the male patients were the victims of the oral cancer and the women suffer from cancer of the cervixuterus (Gupta, 1987).

In U.S.A. one in four suffers and one in six dies of cancer. Lung cancer is common in Western Countries due to smoking. Cancer in stomach is the main cause of death in Japan and Poland probably due to their habits of using fried sea fish and purified rice. Liver cancer and jaw tumour known as Burkitt's lymphoma is more common in African countries. A common example of occupational cancer is 'Kangri' cancer seen in Kashmir, produced by Chronic irritation of hot earthern pots around umbilicus (Panda, 1982).

Cancer is a disease that will touch most of us directly or indirectly (Cairns, 1985).

Cancer tissue competes with normal tissues for nutrients. Cancer cells continue to proliferate indefinitely, their number multiplying day by day. One can readily understand that the cancer cells will soon demand essentially all nutrients available to the body. As a result the normal tissues normally suffer nutritive death (Guyton, 1981).

Cancer cells have a distinctive type of metabolism. The rate of oxygen consumption of cancer cells is somewhat below the values given by normal cells, but the utilisation of glucose is 5 to 10 times as much for normal tissue, and convert most of them into lactate. The lactate is converted back to blood glucose, in the liver at a large net cost in ATP. Cancer cells are thus 'metabolic parasites' (Lehninger, 1980).

Four modes of cancer therapy applied at present are surgery, radiotherapy, chemotherapy and immunotherapy. Radio therapy destroys microscopic cancer cells which may remain in the surrounding tissues following surgery or a tumour. In many instances, the advantage of radiotherapy is its ability to preserve the function of an organ as well as to minimise the mutilating effect of surgery. Gamma rays from

naturally occurring radioactive isotopes and X-rays are used most often in radiotherapy against cancer (Bahadur, 1983).

Tumour markers are required for the diagnosis of malignant tumours and to monitor the progress of tumour therapy in man. Most of the hitherto investigated tumour markers are proteins. Enzymes are good tumour markers for diagnosis and therapy control in man, because enzymes reflect ideally the changes in metabolism of tumours (Schmidth et al., 1979).

Vitamin A taken as carotene is believed to inhibit certain cancers (Seshadri, 1986).

There is an universe relationship between the risk of cancer and consumption of foods that contain vitamin A or its precursors. Vitamin A deficiency generally increases susceptibility to chemically induced neoplasia and that an increased intake of the vitamin appears to protect carcinogenesis in most but not all cases (Krishnamoorthy, 1986).

In the present study on attempt has been made to find out the 'influence of therapeutic radiation on the activities of selected serum enzymes' vitamin A and protein.

Review of Literature

II REVIEW OF LITERATURE

The review of literature pertaining to the study "Influence of therapeutic radiation on serum enzyme level in cancer patients" is discussed under the following headings:

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1. Introduction

A group of cells in the body suddenly start multiplying irregularly and form a lump or a tumour. Tumours are of two types: benign, and malignant. Abnormal and persistent cell division that remains localised at the spot of origin results in the so called benign tumours. Tumour cells may be carried by the blood stream or the lymphatic system or direct penetration, to other parts of the body, where they may induce secondary tumours; which ultimately result in the death of the organism and are said to be malignant. The growth of malignant tumours is known as cancer (Panda, 1982).

2. History

Cancer was known in antiquity, being described in the early writings of Greek and Romans. Tumours in Egyptian mummies dating back 5000 years represnet the first known human malignant growths, although there is pathalogic evidence of bone tumours occuring in dinosaurs and other prehistoric animals (John Lasala, 1985).

3. Incidence

Cancer death rates very enormously between countries. As an example the highest male cancer death rate among 46 countries reporting such information is in Scotland, followed by Czechoslovakia, Uruguay, and Australia. For women the highest cancer death rates are in the Netherlands, followed by Chile, Denmark, Uruguay and Scotland. The lowest cancer death rate for both sexes is in Thailand. Japan leads the world in both male and female stomach cancer, but is 41st in breast cancer. Scotland has the highest lung cancer death rate for both sexes but is 31st in uterine cancer (International Union against cancer, 1980). Oral cancers are among the most frequently encountered malignant diseases in India (Lara, 1980). The incidence of cancer is more in men than in women in India (Canda, 1983). The number of cancer cases has increased by virtue of the increase in the relatively aged population (Weinberg, 1985). The

highest incidence of cancer was noted at the age of 40-45 years (WHO, 1980).

4. Types of Cancer

Cancer is not one single disease but a complex of many diseases. About 200 distinct types of cancer have been recognised. These can be grouped into four main types: Carcinoma, Sarcoma, Lymphoma and Leukaemia.

Carcinomas are tumours made up principally of epithelial cells of endodermal or ectodermal origin. These include cervical, breast, skin and brain carcinomas. About 85 per cent of cancers are carcinomas.

Sarcomas are tumours made up principally of connective tissue cells, which are of mesodermal origin. They constitute only about 2 per cent of human cancers.

Lymphomas are cancers in which there is excess production of lymphocytes by the lymph nodes and spleen. Lymphomas constitute about 5 per cent of human cancers.

Leukaemias are neoplastic growths of leucocytes (W.B.C) and are characterised by excessive production of the cells. They constitute about 4 per cent of human cancers.

In addition the types of cancers mentioned above there may be mixed malignant tumours (Powar, 1983).

5. Distribution of Cancer

Neoplasms of many different sites and tissues occur in all species of animals that have been studied. They occur in lower forms such as amphibia and fish and many plants develop cellular reactions similar to cancer (Juan, 1985).

6. Theories of Cancer formation

The principal theories of cancer formation are generally genetic or nongenetic. The predominant evidence points towards the genetic theory. This augues that tumour development begins with changes in genetic information encoded in the cell either by addition, alteration or subtraction. Cancer causing viruses add genetic information and chemicals or radiation can alter or subtract genetic information (Bahadur, 1983).

7. Predisposing factors

The basic cause (or causes) of tumour formation and human carcinogenesis are unknown. There are however, a number of types and sites of tumours, where contributory or predisposing causes are known and which threw some light on the possible factors involved in neoplasia in general.

a. Race

Whilst all races are susceptible to malignant disease, there is considerable geographical variation in organ incidence. For instance liver tumours are uncommon in Europe, but are extremely common in Bantus and carcinoma of the breast occurs more frequently in limited states of Europe than in Japan (Thompson and Cotton, 1983).

b. Heredity

The fact that not all humans exposed to the same dose of carcinogens develop cancer, indicates that host factors play an important role in the cancer inducing process. Certain familial and genetic disorders may increase the risk of cancer (Petersdorf et al., 1985).

c. Sex

There is considerable variation in sex incidence of different tumours. Carcinomas of the bronchus, tongue, and lip are predominantly diseases of males.

d. Age

In general the prevalence of tumours increases with age. Carcinomas are commonest over 50 years. Some carcinomas occur in young persons, while rare embryonal tumours occur only in childhood (Davis, 1985).

e. Occupation and environment

Bladder cancer in males is predominantly related to industrial contaminants, found in areas of high industrialization and around oil-processing areas. Skin cancer is associated with residence in the sunbelt. The hepatomas of Africa are related to exposure to aflatoxins. Japanese migrating to Hawaii or to the continental United States have a decreasing risk of cancer of the stomach, emphasizing the importance of environmental factors. An excess of both lung cancer and cancer of the nasal sinuses have been found among nickel refinery workers (Ultmann and Golomb, 1985).

f. Physical factors

Repetitive burns on the lip of clay pipe smokers predispose to carcinoma at this site. Chronic irritation by calculi in the biliary and urinary tract may result in squamous metaplasia and leads to the development of carcinoma.

Various types of radiation may lead to malignancy. Excessive exposure to sunlight (ultraviolet rays) especially in fair-skinned persons may result in basal cell or squamous carcinoma. An increased incidence of skin cancer was noted in the pioneers of radiology. Survivors of the Japanese atombomb explosions have an increased incidence of leukemia (Watson, 1977).

g. Chemical factors

More than 15 carcinogens including hydrocarbons and aromatic amines have been isolated from tobacco smoke. Asbestos and uranium exposure act synergistically to increase the risk. Exposure to heavy metals like arsenic, cadmium, chromium and nickel has been associated with an increased incidence of certain cancers (Robert et al., 1985).

Radioactive substances like uranium, radium and thorium are carcinogenic. Tobacco smoke contains dimethyl nitrosamine benzo (α) pyrene hydrazine, vinyl chloride and several other toxic materials which are found to be carcinogenic. Various dye stuffs, amino and nitroso compounds and alkylating agents are known to be carcinogenic (Jussen, 1985).

h. Hormones

Cancers in certain 'target organs' particularly carcinomas of the breast and prostate are profoundly influenced in growth and behaviour by hormones. So they are some times classified as 'hormone dependent'. Some cancers may arise following hormonal stimulation. For instance carcinoma of endometrium in menopausal women receiving hormone replacement therapy and carcinoma of the breast associated with mammary displasia (Reus, 1985).

i. Nutrition

High dietary fat may affect the risk of colonic cancer and endometrial cancer. The risk of colonic cancer has been inversely related to the consumption of fibre. Cancers of the lung and other sites have been associated with a low intake of vitamin A, carotene and selenium. Cruciferous vegetables containing indole compounds can inhibit carcinogenesis (Boyd, 1985). Aflatoxins produced by fungi growing on the food crops are carcinogenic (Bahadur et al., 1983).

j. Infective agents

Viral DNA is present in all the tumour cells and determine the expression in them of virus-coded neoantigens. Of the other cancers of which there is evidence of viral infection, the most important is cancer of the cervix uterii (Hiatt et al., 1977). The tumourogenic viruses may be of two types: those that induce tumours within a few weeks, or the chronically transforming viruses, which require months (Jerry, 1985).

k. Drugs

Various drugs have been shown to be associated with the induction of cancer. Drugs are capable of interacting with DNA (alkylating agents) as well as immuno suppressive

agents and hormones. All have the potential for causing neoplasms in humans (Peulev, 1975).

1. Alcohol

Consumption of alcoholic beverages has been shown to multiply the effects of tobacco smoking on cancers of the mouth, pharynx, oesophagus, and larynx. Heavy drinking also causes the risk of liver cancer, particularly among cirrhotic patients (Weatherall, 1984).

m. Immunologic factors

Cancers may be associated with or lead to an altered host immune response. Malignant tumours can develop in otherwise healthy hosts only if there has been a breakdown of the host's surviellance mechanism. An increased incidence of malignancy associated with long-term administration of immunosuppressive drugs has been noted in patients with renal transplants (Robert et al., 1985).

8. Physiological properties of cancer cells

Cancer cells are space occupying lesions and produce some distortion of surrounding tissues. Obstruction may be caused by tumours in the lumen, arising from the wall or pressing on the wall from outside. Deposits of tumour results in the formation of an inflammatory exudate of high protein

content which is frequently blood stained as in malignant ascites. There is frequent progressive weakness and loss of weight in malignant disease. Pyrexia is a frequent accompaniment of malignant disease and is usually due to infection. Progressive anemia is frequently found (Thomson and Cotton, 1983).

i. Ultrastructural variations of cancer cells

The nucleus is usually enlarged and is irregular in outline. Deep invaginations or infoldings of the nuclear membrane give an irregular shape to the cancer cell/nuclei. The nucleolus is often hypertrophied, and also the shape is irregular. Most often the mitochondria of cancer cells are swollen and have structural differences. Polysomes are more abundant in cancer cells. In adenovirus - induced tumour cells the endoplasmic reticulum is very little but ribosomes are more. Cancer cells have a reduced or under developed golgiapparatus. Annulate lamellae are most frequent in tumour cells than in normal cells. Some pathologic inclusions are seen in the cytoplasm of the malignant cell (Agarwal and Sastry, 1981).

The process of cell change in which a cell loses its ability to control its rate of cell division, and thus become a tumour cell, is called cell transformation.

ii. Immortalization

Normal cell cultures do not survive indefinitely. Human cell cultures die after about 50 generations. On the other hand transformed cell cultures are immortal and can grow indefinitely.

iii. Loss of contact inhibition

Normal cells in a culture stop growing when their plasma membrane come into contact with one another which is called as contact inhibition. But transformed cells lack this property (Power and Daginawala, 1982).

iv. Reduced cellular adhesion

Normal cell membranes show stickiness or adhesiveness. Cancer cells do not show this property.

v. Lower requirement of serum

Normal cells, when grown in tissue culture *invitro*, generally show a requirment for a certain amount of serum or any other specific growth factor found in serum. In the case of malignant cells, this requirement if at all, is much less stringnent (Vollemers *et al*., 1984).

vi. Lack of Anchorage dependence

Normal cells require to be anchored to a proper substrate before going into the cell division cycle. Malignant cells however, do not show this property because of their ability to metastasize (Roberts and Robertis, 1981).

9. Biochemical changes in cancer

Cancer cells have a dingtinctive type of metabolism. Although they posess all the enzymes required for most of the central pathways of metabolism cancer cells of nearly all types show an anomally in the integration of glycolytic sequence and the T.C.A. cycle. The rate of oxygen consumption of cancer cells is shmewhat below the values given by normal cells. Malignant cells utilise 5 to 10 times as much glucose as normal tissues and convert most of them to lactate. The formation of a molecule of glucose from lactate requires input of six molecules of high-energy phosphate in the liver, whereas the cancer cells require only two molecules of ATP. Hence the cancer cells may be looked upon as a metabolic parasite, dependent on the liver for the substantial part of its energy (Lehninger, 1983).

The cancer tissue competes with normal tissues for nutrients. Because cancer cells continue to proliferate indefinitely their number multiplying day by day the cancer cells will demand essentially all the nutrition available

to the body. As a result the normal tissues gradually suffer nutritive death (Guyton, 1981).

i. Carbohydrate metabolism

A decrease in overall carbohydrate content as well as structural changes in saccharide chains of membrane glycoproteins are seen in malignant cells. The decreased content of sialic acid and galactose, which are constituents of the outer branches of saccharide chains, has been attributed to low activities of the corresponding glycosyl transferase (Okamoto et al.,1983). Tumour associated hypoglycemia occur most frequently with neoplasms that can be loosely termed as mesotheliomas. A hypoglycemic factor which mimics insulin activity is often demonstrable in cancers. Insulin itself is usually not demonstrable by insulin radioimmuno assay (Plevink et al., 1979). Serum sialic acid is elevated in cancer of the cervix, ovary, vagina and endometrium (Maity et al., 1983). The concentration of serum protein bound hexoses may be markedly increased in patients suffering from physiological conditions like malignancy (Lodha et al., 1981).

An absolute increase in the amounts of hyaluronic acid, chondroitin-6-sulfate, and dermatan sulfate fraction of the oral cancer tissues, were observed in cancer patients (Pillai et al., 1981).

ii. Lipid metabolism

In cancer patients there is higher concentration of total lipids, phospholipids and cholesterol than in normal cases (Gupta Harman, 1981). In tumour tissues there is a specific blockade of the enzyme 3-hydroxy, 3 methyl glutaryl CoA reductase which is responsible for the various pathways that produce, among other lipid components, cholesterol, dolichol, ubiquinone, and the isopentenyl moiety of isopentenyl t-RNA. As a result of the blockade, uncontrolled cholesterol synthesis may occur. Prostaglandins, prostacyclins, and leukotrienes, phosphatidyl inositols and gangliosides display association with rapid cell proliferation. The cellular membranes do as their lipids tell them. Some studies have shown a significant inverse correlation between the level of plasma cholesterol and the overall incidence of cancer (Sabine, 1983). Breast cancer patients have a high serum cholesterol and they have poor prognosis (Sarin et al., 1983).

Unequal changes in the contents of total phospholipids, lecithin, lysolecithins, linoleic acid and arachidonic acid in blood were revealed in patients with pulmonary cancer (Norman et al., 1982).

iii. Protein metabolism

According to Schersten et al., (1982) muscle wasting in patients with cancer is a clear evidence of altered protein metabolism. In most of the cancer patients the plasma albumin concentration is low (Moore et al., 1982 and Raines et al., 1982).

Benign tumour patients have a high mean serum total protein levels i.e. about 7.7g/dl (Normal 6.4g/dl) (Kapoor et al., 1983).

Increased concentrations of serum glycoproteins have been noted in malignancy. Levels of α1-acid glycoprotein, ceruloplasmin, and α1-antitrypsin are increased and α2-HS-glycoprotein, β2 glycoprotein I and prealbumin levels are decreased and haptoglobulin and haemopexin levels are elevated in cancer patients. Serum group specific protein concentration was found to be increased in male patients with cancers of lymphatic tissue. A female with a liposarcoma has been reported with nerve growth factor in serum and tumour cells produced this factor in culture. Increased β-lipoprotein values have been reported in breast carcinoma. The values for high density lipoprotein in cancer were significantly below those in normal subjects (Bagshawe, 1975).

iv. Enzymes in serum

There are a number of possible mechanisms for the appearance of abnormal activities of enzymes in the serum. The include: over production of the enzymes by the tumour, as in the case with Alkaline phosphatase in osteogenic sarcoma, tumour blockage of the duct system through which enzymes pass, induction of enzyme by the presence of tumour. There may be induction of both alkaline phosphatase and 5'Nucleotidase in normal liver cells when metastatic tumour is present, and change in permeability of the cell allowing leakage of soluble enzymes into the circulation.

(a) Acid phosphatase (ACP)

Serum acid phosphatase is a variable biological marker in prostate cancer (Crawfold and Dawkins, 1986). The value of elevated serum acid phosphatase is used in the detection of bone metastases from prostatic carcinoma. Acid phosphatase activity in non prostatic tissue is low but prostatic enzyme is distinguished from the contribution of erythrocytes by being formol stable. Prostatic ACP tends to be confined within the gland unless there is secondary spread or manipulation. High serum ACP has been reported in patients with myeloproliferative disease and lympoblastic leukemia (Sobin et al., 1986).

The mean serum acid phosphatase activity is increased in all types of cancer compared to the normal. The highest serum acid phosphatase activity is found in liver in cancer and the lowest in Oesophageal cancer (Giri, et al., 1986).

b. Alkaline phosphatase (ALP)

The activity of this enzyme is elevated in cases of cancers involving G.I.tract or liver. The extent of elevation is a function of the type of bone lesion (Yeshowardhan et al., 1985). ALP values are low in chronic myelogenous leukemia and less regularly in acute myeloblastic leukemia. Normal or high scores are found in other leukemias. Placental alkaline phosphatase like activity was found in serum of bronchial carcinoma patients. Placental ALP like activity was found in the sera of upto one in 7 cancer patients. HeLa cells are known to produce a placental ALP like enzyme. A number of studies of ALP present in tumours or the sera of cancer patients have shown substantial variations in their properties (Harkal, 1978).

c. Amylase

More than 85 per cent of patients with acute pancreatitis have abnormally high serum amylase activity. Only 14% of the patients with cancer of pancreas had such high values. Serum amylase may be lower than normal in cancers

with liver diseases (Jungles et al., 1986). Amylase level is increased in all types of cancers, the maximum increase seen in pancreatic cancer (Giri, et al., 1984).

d. Leucine aminopeptidase

In a report of 51 cases of neoplasm of the liver, activity was within normal limits in 23% of the patients, elevated to twice the normal range in 31% of the patients, and greater than 3 times the normal range in 28% of cases. Leucine aminopeptidase activity has been reported to be higher in pleural fluid of patients with cancer (Rao et al., 1978).

e. 5' Nucleotidase (5'NT)

5'NT activity which is present in lymphocytes isolated from the blood of normal subjects is markedly diminished or not detectable in patients with chronic lymphocytic leukemia (CLL). In certain patients with CLL the decreased enzyme level has remained constant over more than 24 months of observation (Quagliata et al., 1974).

Abnormally high activities of 5'NT has been reported for patients with liver carcinoma (Schwartz, 1978). The mean serum 5' nucleotidase activity is increased in all types of cancer (Giri et al., 1986).

f. Transaminases (SGOT and SGPT)

In hepatocellular carcinoma it has been noted that SGOT levels are consistently higher than SGPT. SGOT level was the highest in patients with Hepatocellular carcinoma associated with cirrhosis. SGOT/SGPT ratio was also the highest being above 3 (Yutakashimokawa et al., 1978).

g. Lysozyme (Muraminidase)

Increased concentrations of lysozyme are found in patients with acute and chronic monocytic leukemia and those with acute myelomonocytic leukemia. High urinary and serum muraminidase consentrations have been reported in patients with carcinomas of the prostate, bladder, kidney, urether, but not with most bladder papillomas (Nagahawe, 1978).

h. Lactate dehydrogenase

The serum levels of total LDH as well as LDH 4 and LDH 5 were increased in most of the malignancies except in case of thyroid in which there was increase of total LDH and LDH_3. Total LDH is increased in breast carcinoma in advanced stages as compared to early lesions (Saravanan et al., 1977). LDH plays a key role in the normal cell metabolism and is known to be elevated in human neoplastic disease. Total LDH and its isoenzymes could serve as a biochemical parameter for the detection of recurrence and metastatis (Bhatnagar et al., 1983). Serum LDH levels were

markedly raised in acute as compared to chronic leukemias (Tyagi et al., 1986).

v. Enzymes in urine

Urinary Amylase activity was found to be greater than in serum in patients with pancreatic carcinoma. The activity of muraminidase was reported to be elevated in the urine of patients with leukemia. β-glucuronidase in urine plays a role in the carcinogenesis of bladder carcinoma.

vi. Enzymes in other biological fluids

In studies with 6-phosphogluconate dehydrogenase or β-glucuronidase, elevations were observed in their activity in vaginal fluid in a majority of women, with uterine malignancy. Phosphohexose isomerase was markedly elevated in vaginal fluid in women with gynecological cancers (Schwartz, 1978).

vii. Enzymes in the diagnosis of cancer

a. Enzymes in the diagnosis of solid tumours

Acid phosphatase activity is increased in the serum of patients with carcinoma of the prostate. Several other enzymes like amylase, lipase, chymotrypsin, and alanine aminopeptidase in pancreatic carcinoma, glucose 6-phosphatase

dehydrogenase and β-glucuronidase in cervix and corpus cancer α 1-4 glucosidase and sialyl transferase in breast carcinoma or aryl sulfatase B in colon cancer have been proposed in cancer diagnosis (Bosman and Hall, 1974).

b. Enzymes in the diagnosis of metastatic cancer

In contrast to solid tumours, the unspecific increase of glycolytic enzymes may be of diagnositic help in metastatic cancer. An increase of cholestatic enzymes is characteristic for liver metastases and an increase of alkaline and acid phosphatases may be indicative of bone metastases (Lehmann, 1975).

c. Enzymes in the diagnosis of exudates

The determination of enzymes in exudates of unknown origin of little help for the differential diagnosis of exudates. Glycolytic enzymes like lactate or malate dehydrogenases, hexokinases, and others are only indicative for a malignant effusion in a non-hemorrhagic malignant effusion (Schmidt et al., 1979).

d. Other enzymes

The levels of acetyl choline, cortisol histamine and dopamine hydroxylase were found to be significantly raised in all cancer patients, whereas the levels of acetylcholine

esterase and monoamine oxidase were found to be significantly decreased. The increased levels of cortisol catecholamine, acetylcholine and histamine observed in the study may be responsible for the abnormal cell growth in cancer. Stress may be one of the important factors involved in the pathogenesis of malignant transformations (Dohadwala and Chawdhan, 1980).

Glutamine was shown to be essential for the growth of tumours. Injection of purified glutaminase brought about a complete regression of a number of tumours in animals. Uraemia results from bilateral ureteric obstruction due to a pelvic tumour.

Putrescine, spermidin and spermine are found in highest concentrations in rapidly proliferating tissues. Increased t RNA-methylase activity is a common characteristic of malignancy. A glycoprotein RDCI is isolated in the urine of patients with acute myelogenous leukaemia. A distinct form of serum glycosyl transferase has been reported in patients with neoplastic disease (Slungaard et al., 1983).

viii. Isoenzymes in cancer

The serum isoenzyme patterns may reflect the primary tumours and the release of isoenzymes from destroyed normal

tissue adjacent to the tumour. LDH isoenzymes have been studied in cancer patients.

Serum total LDH enzyme assay revealed higher activity in cancer patients as compared to controls. This increase was due to increased activity of isoenzymes (LDH 1,2,3 and 5) in patients. The most significant finding of LDH isoenzyme assay was the detection of absent LDH 4 isoenzyme activity in 77% of patients which could be due to either defective association of subunits (H and M) of LDH_4 or alteration in the template resulting in defective LDH isoenzyme formation and loss of its activity (Tuteja et al., 1980). An extra band existed in LDH and MDH (Malate dehydrogenase) in a few tumours at the position of most anodic side of regular isoenzymes. Glucose-6-phosphate dehydrogenase isoenzymes were resolved into 3 components and various forms were shown according to the activity of each compartment (Yoshimura et al., 1973) whereas a significant increase of LDH4 and LDH5 except in case of carcinoma of thyroid was reported (Markel, 1978).

Three isoenzymes corresponding to this enzyme have been identified. A fourth isoenzyme (Regan), identical to placental ALP also has been described. The bone isoenzymes is elevated in 90% prostate cancers that have metastasized to bone. The Regan isoenzyme will be high in approximately 20% of patients with advanced stage disease. Serial monitoring of ALP correlates with tumour progression as well

as response to therapeutic intervention (Crawford and Dawkins, 1986). The mean values of the isoenzymes A P-1, ACP-2 and ACP-3 of serum acid phosphatase increase in all types of cancer compared to the normal (Giri et al.,1986). The isoenzymes of aldolase have been shown to differ in human cancers. Primary tumours of the liver have been observed to contain aldolase-A and not the B-form found in normal liver (Schwartz, 1978).

Ix. Minerals

Malignant disease is one the commonest causes of hypercalcemia (Stevenson and John 1985). Experimental tumours indicate that there is a relationship between calcium incorporation and phospholipid concentration (Anghilari et al., 1973). Hyper-calcemia may also result from the release of hormone like substances from extraskeletal tumours in the absence of skeletal metastases (Weatherall et al., 1984). In a large series of patients with bronchogenic carcinoma 12.5 per cent were hypercalcemic (Boyd, 1985). The mean serum iron level is decreased in all types of cancer compared to the normal (Giri, et al., 1986).

Increase in nickel, copper, zinc, and manganese occurs in peripheral blood cells in leukemia. Copper and manganese levels are increased in leukocytic leukaemia, especially in chronic myelocytic leukaemia where decreased concentration of selenium and low rhubidium concentrations

in leukemic blood are also observed (Mangal, 1981). Hodgkin's disease is associated with excessive uptake of iron by liver and spleen and results in hypoferremia (Beeson, 1978). The mean serum zinc and selenium levels is decreased in all types of cancer compared to normal (Giri et al., 1986).

A rare syndrome of profound hypophosphatemia with normal serum calcium is seen in a variety of prostatic carcinoma. Patients with prostatic carcinoma are found to have hypophosphatemia (Daniels et al., 1979).

Selenium has been found to reduce the incidence and progression of cancer in animals exposed to carcinogens. There is a strong association between a low serum selenium concentration and an increased risk of death from cancer (Solomon and solomon, 1985).

Hyponatremia syndrome may result from hypocorticalism due to adrenal replacement by metastatic tumour (Weatherall et al., 1984).

x. Vitamins

A high dosage of vitamin A decreases the incidence and severity of tumour development in mice inoculated with murine sarcoma virus (Seifter and Martin, 1973). Vitamin A

taken as carotene is believed to inhibit certain cancers. Vitamin C is a cancer inhibitor as well as an effective therapeutic agent. According to other studies, so does vitamin A (Seshadri, 1986). Several studies indicate that riboflavind deficiency inhibits tumour growth in experimental animals and possibly in man. Riboflavin influences uptake of chemotherapeutic druggs, in atleast one instance into neoplastic cells (Richards, 1981). Serum α-tocopherol alone showed no consistent independent relation to the risk of cancer. Vitamin E did not show an association with any specific cancer site. Vitamin E showed strong synergism with selenium on the risk of fatal cancer. The impact of selenium deficiency in cancer risk seemed to be noticeably greater at low serum vitamin E concentrations (Soleman et al., 1985).

Serum vitamin A level is significantly decreased in all types of cancer compared to normal (Giri et al., 1986). Nicotinamide deficiency occurs in a small proportion of patients with carcinoid syndrome, and affected patients develop pellegra like skin lesions (Chowdhari, 1980).

xi. Hormones

Hypoadrenalism results from replacement of both adrenals by metastatic bronchial carcinoma. In some cases there is evidence of the production by the tumour of a

substance with thyroid stimulating properties (Weatherall et al., 1964). Elevated levels of calcitonins have been described in patients with carcinomas of the lung, colon, breast, and pancreas. Ectopic production of vasopressin is common in patients with carcinoma of the lung. Elevation of plasma neurophysin has been found in patients with lung cancer (Slungaard et al., 1983).

xii. Cyclic AMP and catecholamines in cancer

The circulating levels of CAMP were found to be decreased, whereas the CAMP phosphodiesterase activity was increased in all cases of cancer. The urinary excretion of adrenaline and noradrenaline were increased in these patients. After surgery the levels of CAMP increased towards normal and levels of CAMP - phosphodiesterase, urinary adrenaline and noradrenaline declined towards normal. Decreased levels of CAMP may be responsible for the loss of contact inhibition of growth in malignant tissue. Thus CAMP and catecholamines have an important role in the pathogenesis of cancer (Patel et al., 1980).

xiii. Tumour markers

a. Oncofetal antigens: Oncofetal antigents which are partially or completely repressed in adult life is activated, and appear in cancer patients and thus can act as an index of neoplastic transformation.

b. Carcinoembryonic antigen: Abnormal serum carcinoembryonic antigen levels occur in a wide variety of malignancies, particularly in endodermally derived tumours of the G.I. tract and lung.

c. Alfa-fetoprotein: IS synthesised by the liver, yolksac, and G.I.tract of the human fetus. Abnormal serum levels of AFP have been noticed in serum of patients with hepatocellular carcinoma. Elevated levels of AFP are also found in association with teratocarcinomas and embryonal cell carcinoma of testes, ovary and extragonadal sites (Boyd, 1985).

d. Ectopic polypeptides: Many tomours produce augmented quantities of hormones or proteins that are normally secreted by the cell origin. They include the hypersecretions of insulin and gastrin by islet cell tumours, the M-spike of multiple myeloma, and the elevated serum ACP activity associated with metastatic cancer of prostate (Hammond and Winnicker, 1974).

e. Eosinophile protein: An undifferentiated lung carcinoma was found to contain large amounts of an eosinophil protein like material (Slu ggard et al., 1983).

10. Prognosis of cancer

The warning signals of cancer are:

a. Change in bowel or bladder habbits

b. A sore that does not heal

c. Unusual bleeding or discharge

d. Thickening or lump in breast or elsewhere

e. Indigestion or difficulty in Swallowing

f. Obvious change in wart or mole and

g. Nagging cough or hoarseness

Laboratory diagnosis

a. Histological examinations

The tumour cells are removed and fixed, embedded in paraffin, sectioned and stained. The sections are studied paying attention to (1) the cytological appearances of the tumour cells (2) the relationship of these cells to the surrounding tissues, especially as regards the invasion of this tissue by the tumour cells.

b. Cytological diagnosis: Cancer cells lose the stickiness or adhesiveness characteristic of normal cells so that they tend to be cast off from the surface easily in the disease, even in the preinvasive state. The cells are found in exudates.

c. Fluorescent microscopy: Fluorescent microscopy with acridine orange is a rapid and easy procedure. The technique gives a highly polychromatic picture, that serves to differentiate the DNA of the nucleus from the RNA of the cytoplasm - the

RNA staining a flemming red or orange compared with DNA which gives a green to yellow fluorescence. The malignant cells stain mainly red or orange and the normal cells yellow or green, a distinction which is readily recognisable with low power of the microscope.

d. Chemical tests: Epithelium of the prostate normally elaborates ACP. When the epithelium is greatly increased in amount as in the frequent massive metastatic carcinoma, the serum ACP is likely to be raised to a significant degree Cancers of placental tissue may be detected by the presence of chorionic gonadotropic hormone in urine (Boyd, 1985).

11. Treatment of cancer

The five modes of cancer therapy applied at present are: (i) Radiotherapy (ii) Surgery (iii) Chemotherapy (iv) Immunotherapy and (v) Diet therapy.

i. Radiotherapy

Radiotherapy is the application of ionising radiation to the treatment of malignant disease. Ionising radiations deliver energy in photons sufficiently high to destroy the cancer cells by impairing their reproductive capacity (Joslin, 1986).

a. History

In February 1896 Veigt in Hamburg treated by radiation nasopharengeal carcinoma by radiation. Freund in November 1896, treated a hairy mole and carcinoma of cervix uteri was first treated in 1902 by Radiation. Fibrosis of the lung as a complication of deep radio therapy was recognised as early as 1922 (Gupta et al., 1985).

b. Applications of Radiotherapy

Radiology concerns one of the most exciting aspects of therapeutic achievements in clinical oncology (Duncan, 1985). Oesophageal cancer receive radiation therapy for cure or palliation (ACTA Radiologica, 1986).

Upper and lower half body irradiation have been utilised to treat patients with advanced and widely metastatic lung cancer (Salazar and Scarantio, 1980). Irradiation is most useful in lesions of the tongue, lip and the cheek sufficiently away from the bones. The commonest indication for radiotherapy is in the management of patients with metastatic breast cancer for the relief of bone pain (Jarnod, 1985). Radiotherapy is largely concerned with the cure of carcinomata arising from the skin and from within the head and neck and pelvis as well as within certain unifocal lymphoreticular and embryonal tumours (Sutton and Ronald, 1985).

c. Types of ionising radiations

X-rays, Roentogen rays or Gamma rays from naturally occurring or artificially produced radioactive elements such as radium or cobalt-60, are used in radiotherapy. The ionising effects following irradiation lead to the production of free radicals in the water molecules of the cell microenvironment. Such free radicals and oxidising agents interact with DNA molecules and produce a large number and variety of DNA breaks and damage. Thus a change in transcription or defective repair takes place leading to cell death (Ultsmann and Golomb, 1985). The chief methods used are: external radiation (roentgent rays and radium) (2) Surface application (radium) (3) intestinal radiation (radium) (Boyd, 1985).

d. Dose and risk defined

The physical unit of absorbed dose is the rad (100 ergs per gram of tissue) equal to 0.01 gray. Different kinds of radiation are differently effective. Gamma rays may be 1/3 as effective as neutron rays at a dose of 25 rads, but only 1/25 as effective at 1 rad (Kohn et al., 1984).

Radiation therapy may also induce malignant changes at other sites. Cancer has occurred as a secondary effect

of radiation therapy both for benign and malignant diseases (Smith et al., 1982). The higher the dose the more likely oncogenesis (Hommed et al., 1984).

e. Effects of Radiation therapy

The effect is two fold (1) Arrest of mitosis and (2) degradation and destruction of cells (Boyd, 1985).

Weight loss was observed in patients on radiation treatment for localised cancers (Woodard, 1985). Radiation beams, during treatment almost always traverse the lung parenchymal cells and cause structural and functional damages (Jain et al., 1982).

Older persons may have diminished tolerance to radiation and recover more slowly from adverse effects on normal tissue (Jain et al., 1982). Ionising radiation can cause cell death by a variety of pathways and would more likely alter membrane permeability. High levels of serum enzyme activity usually observed before therapy suggested increased leakage of cancer cell enzymes into the circulatory system. The initial elevation was followed by a decrease in enzyme activity after therapy (Nyandieka, 1984).

Vitamin A and Vitamin D levels are reduced during radiotherapy. Decreases of vitamin E, vitamin C, vitamin B_1 B_{12} and folic acid levels were observed. Thiamine and vitamin B_{12} deficiencies are rare in cancer patients but

serum folate, ascorbic acid and vitamin A levels were low in 20-45% of cases. Malabsorption of vitamin B_{12} and other nutrients are seen after radiotherapy. Vitamin B_{12} deficiency was found in patients who underwent radiotherapy. Radiotherapy thus has adverse effects on nutritional status of cancer patients (Bruning et al., 1985).

ii. Surgery

Surgery is used to treat localised tumours but this method required complete removal of the tumoured organ. The effectiveness of surgery is not only limited by the size of the tumour but also by its distribution. Even a very large tumour can be removed completely by surgery if it has not spread to other parts of the body (Bahadur, 1983).

iii. Chemotherapy

Chemotherapeutic agents with cytotoxic effects on the most rapidly dividing cells are used for cancer treatment. Methotrexate and some related compounds are used for curing certain tumours. Antifolate drug and fluorinated pyrimidines are effective as anticancer drugs. Cyclophosphamide is used in treating lymphosarcomas, Hodgkin's disease as well as breast, ovarian and lung cancers. Antibiotics like daunomycin and adiramycin are also used in treating leukemias and lymphomas. Natural alkaloids like vinblastine and vincristine are used in treating some types of cancers (Todd, et al., 1983).

iv. Immunotherapy

This method directs body's natural defense system to combat cancer by destroying invading organism and other foreign particles. Scientists are trying a variety of approaches including purified tumour antigents, substances isolated from active W.B.C. that produce general immune system. Boosting the body's own defense mechanism by gene-cloning is also done to destroy the cancerous cells (Mahajan, 1986).

v. Diet in cancer therapy

It has been estimated that the portion of total cancer incidence directly related to diet and nutrition is 60 per cent for women and greater than 40 per cent for men. Experiments have shown an increasing number of linkages between vitamin levels in the diet and prevalence of certain cancers. Vitamin A taken as carotene is believed to inhibit certain cancers. Vitamin C is a cancer inhibitor as well as an effective therapeutic agent. Intakes of minerals toomuch or too little may favour the formation of tumours. Dietary fibre or roughage is beneficial in avoiding cancers of gastro-intestinal tract. Buttermilk is found to be excellent for cancers of the lower intestinal tract. Thus diet is at best an adjacent to treatment and not a therapy itself. It is tailored to weaken the enemy (Seshadri, 1986).

Experimental Procedure

III EXPERIMENTAL PROCEDURE

The experimental procedure pertaining to the study "the influence of therapeutic radiation on serum enzyme levels in cancer patients is presented in the following sequence:

1. Selection of subjects
2. Collection of Blood
3. Separation of serum
4. Estimation of serum total protein
5. Estimation of serum vitamin A
6. Estimation of creatine kinase
7. Estimation of total alkaline phosphatase
8. Estimation of amylase
9. Estimation of total lactate dehydrogenase
10. Estimation of seru-glutamate oxaloacetate transaminase
11. Estimation of serum glutamate pyruvate transaminase

1. Selection of subjects

Eighteen patients with histologically or cytologically proven cancer, receiving therapeutic radiation at Sri Kuppuswamy Naidu Hospital, Coimbatore, were chosen for the present study. They were all inpatients and were treated with the cobalt-60 moving strip technique. The radiation was given daily for five days a week and the treatment was

aimed to deliver a total tumour dose of 3000 to 6000 rads. The patients were of both sexes (suffering from different types of cancer) and belonged to the age group of 35-55. Ten healthy individuals of both sexes and of the same age group were also selected as controls for comparison.

2. Collection of blood

The blood was collected as follows (Omer, 1976):

Tied a tourniquet (of soft rubber tubing or a strip of bandage) tightly around the arm of the patient, a couple of inches above the elbow. Had the subject clench his fist firmly, washed the skin surface about the prominent vein on the inner surface of the elbow (usually the median basilic) with 70% alcohol, allowed to dry, held the vein immobile by pressing on it with the thumb below the elbow and into the vein inserted a sharp, sterile hypodermic needle (No.20) an inch and a half long which was attached to a dry sterile syringe of suitable capacity. The needle should penetrate the vein from the side and at an angle of 30° with the surface of the arm, the level of opening of the needle being kept upward to the side. As soon as the blood was seen to enter the syringe, retracted the plunger slowly until the desired amount of blood had entered the syringe. Before removing the needle from the vein, loosened the tourniquet had the patient unclench his fist and on the

skin, at the point of entrance of the needle held in place a small pad of folded gauze moistened with 70% alcohol, withdrew the needle, detached it from the syringe (not too vigorously which might cause hemolysis), and then transferred the blood to a centrifuge tube. Pressure on the gauze pad will effectively prevent bleeding from the skin puncture. It is important that the pressure be maintained for a minimum of five minutes, to prevent the formation of a painful hematoma at the site of puncture.

3. Separation of serum

The blood after being transferred to a centrifuge tube, was allowed to clot. The clot was carefully removed and centrufuged after which the supernatant was separated. The separated serum was frozen till used for analyses.

4. Estimation of serum total protein

Serum total protein was estimated by Biuret method (Teitz, 1976). The details are presented in Appendix I.

5. Estimation of serum vitamin A

The vitamin A was estimated by the trifluroacetic acid method of Gyorgy and Pearson (1967). The details are given in Appendix II.

6. Estimation of serum creatinine kinase

The level of serum total creatine kinase was determined by the Sigma-kit procedure, the details of which are given in Appendix III.

7. Estimation of serum total alkaline phosphatase

Total level of Alkaline phosphatase in serum was estimated by the method of King-Adul-Fadle and Walker (Varley, 1985). The procedure is given in Appendix IV.

8. Estimation of serum Amylase

Serum amylase was estimated by the method of Somogyi (Wootton, 1965). The details are presented in Appendix V.

9. Estimation of serum total Lactate dehydrogenase

Serum total lactate dehydrogenase was estimated by the method of King (Varley, 1975). The details are presented in Appendix VI.

10. Estimation of serum Glutamate-Oxaloacetate transaminase

Serum Glutamate-oxaloacetate transaminase was estimated by Reitman and Frankel (1957) method. The details are presented in Appendix VII.

11. Estimation of Serum Glutamate Pyruvate transaminase

Serum glutamate-pyruvate transaminase was estimated by the Reitman and Frenkel (1957) method. The details are presented in Appendix VIII.

Results and Discussion

IV RESULTS AND DISCUSSION

The results and discussion of the study 'Influence of therapeutic radiation on serum enzyme levels in cancer patients' are described under the following headings:

1. Distribution of patients
2. Alterations in selected serum constituents due to radiotherapy
3. a. Total proteins
   b. Alkaline phosphatase
   c. Amylase
   d. Creatine Kinase
   e. Lactate dehydrogenase
   f. Glutamate Oxaloacetate transaminase
   g. Glutamate pyruvate transaminase and
   h. Vitamin A

1. Distribution of patients

As described earlier in Chapter III patients of both sexes suffering from five different types of cancers (Bone, breast, cervix, lung and oesophagus cancers) were selected for the study. They belonged to the age group 35-55. Table I presents the distribution of patients.

TABLE I

DISTRIBUTION OF PATIENTS

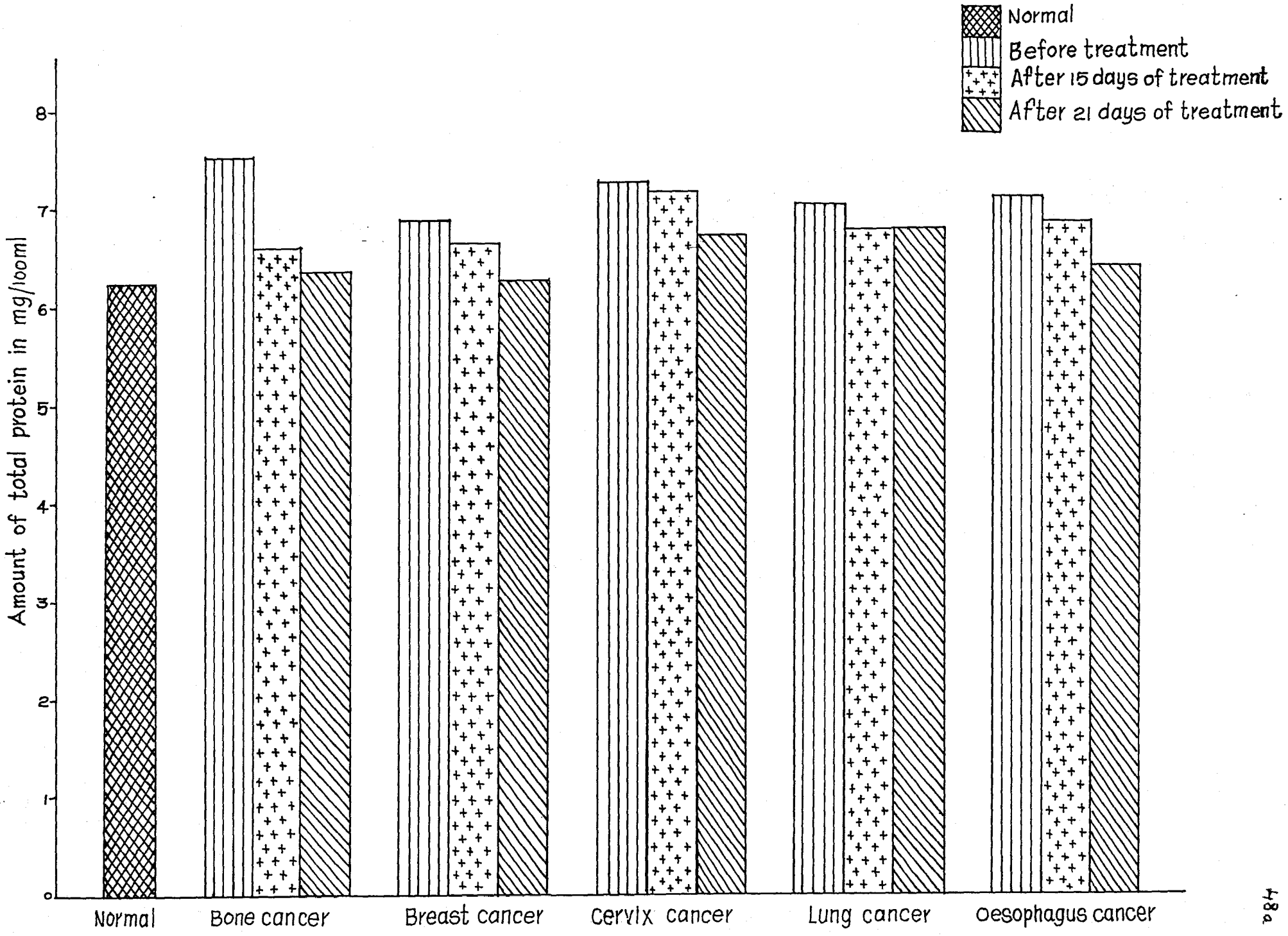
Types of cancer	Bone	Breast	Cervix	Lung	Oesophagus	Total
Number of patients	5	4	4	3	2	18

2. Alterations in selected serum constituents due to radiotherapy

The levels of selected serum constituents of cancer patients are compared with the normal levels at three different stages of treatment (Before treatment, after 15 days of treatment and after 21 days of treatment).

Fig-1

ALTERATIONS IN TOTAL PROTEIN LEVELS DUE TO RADIOTHERAPY



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a. Alterations in serum total protein level

Table II gives the alterations in serum total protein level due to radiotherapy in different types of cancer.

From the table it is clear that the serum total protein levels are significantly increased in all types of cancers except breast cancer, before giving radiotherapy.

The serum total protein level in different types of cancers in g/dl in increasing order is:

Normal (6.23 ± 0.25) < Breast (6.88 ± 0.80) < Lung (7.07 ± 0.08) < Oesophagus (7.10 ± 0.14) < Cervix (7.25 ± 0.77) < Bone cancer (8.08 ± 0.41)

In bone, cervix and oesophagus cancer a significant increase (at 1 per cent level) in serum protein level is seen before treatment. In lung cancer the increase is significant only at 5 per cent level.

In breast cancer there is no significant change in protein level throughout the treatment period. In bone cancer the protein level returns to normal by 15 days of treatment itself, whereas in cervical, oesophagus and lung cancers, the levels come down to normal only after 21 days of treatment.

The increase in total proteins in bone cancer may be due to the presence of markedly elevated levels of myeloma

proteins (monoclonal immunoglobulins or paraproteins). Changes in the hormone levels like steroids, androgens, corticosteroids, corticotrophin or other hormones which may be decreased or elevated in cancer may increase protein synthesis and the serum protein level (Tietz, 1978). The reason for the unchanged concentration of total proteins in serum of breast cancer patients may be due to the non disturbance of the constant equilibrium between the plasma amino acids and most of the proteins in the cells of the body. Generally if any particular tissue losses proteins, it can synthesize new protein from the amino acids of the blood. In turn, these are replaced by degradation of proteins from other tissues which become markedly depleted (Guyton, 1981).

b. Serum total alkaline phosphatase

Table II depicts the alterations of serum total alkaline phosphatase level due to radiotherapy.

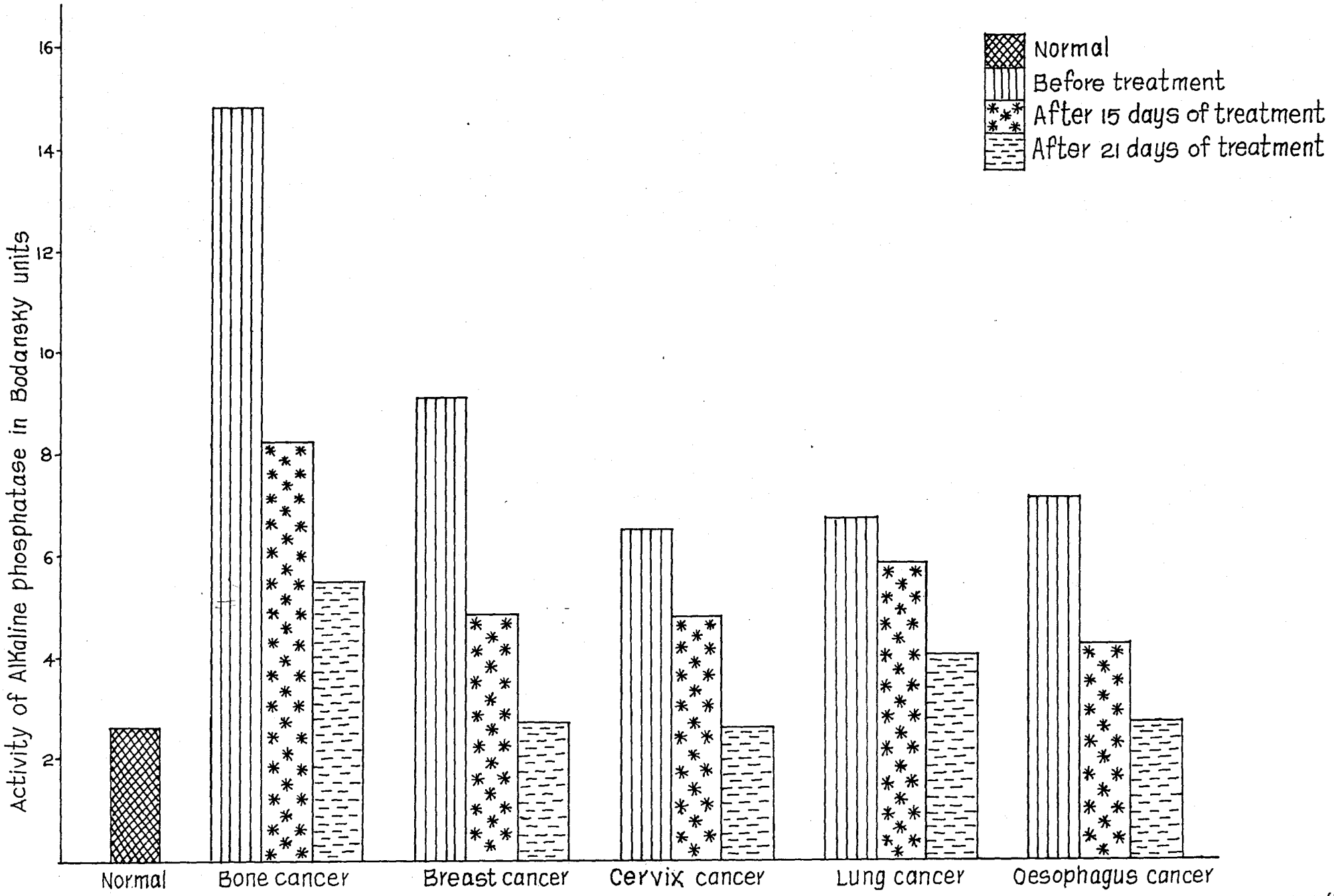
A significant increase in serum total ALP activity at 1 per cent level is seen in all types of cancer before treatment.

Following is the pattern in total ALP activity (Bodansky units) in the decreasing order.

Normal (2.62 ± 0.73) < Cervix (6.45 ± 0.57) <
Lung (6.77 ± 0.21) < Oesophagus (7.10 ± 0.41) <
Breast (9.15 ± 1.86) < Bone cancer (14.86 ± 4.25)

In bone cancer the total ALP level is increased about five fold before therapy. The ALP values remain high after 15 days treatment in all cases of cancer. In bone and cervix cancer the increase is significant at 1 per cent level, whereas, in breast, lung and oesophagus cancer the increase is significant only at 5 per cent level. Except in bone cancer, the ALP activity falls down to normal in all other types of cancer after 21 days of treatment. The present study shows maximum elevation in ALP activity in bone cancer. This may be due to the secretion of large quantities of ALP by the osteoblasts, when they are actively depositing bone matrix, which may diffuse into the blood and increase the ALP level (Guyton, 1981). Placental ALP like activity was found in the sera of upto one in 7 cancer patients, which may be the reason for increased mean ALP activity found in cancer. HeLa cells are especially known to produce a placental ALP like enzyme (Markel, 1978).

FIG-2


ALTERATIONS IN SERUM ALKALINE PHOSPHATASE DUE TO RADIOTHERAPY
Normal
Before treatment
After 15 days of treatment
After 21 days of treatment
Activity of Alkaline phosphatase in Bodansky units
16
14
12
10
8
6
4
2
Normal
Bone cancer
Breast cancer
Cervix cancer
Lung cancer
Oesophagus cancer

Serum amylase

Table IV reveals the alterations in serum amylase activities due to radiotherapy. There is significant increase in amylase activity in all types of cancers except oesophagus.

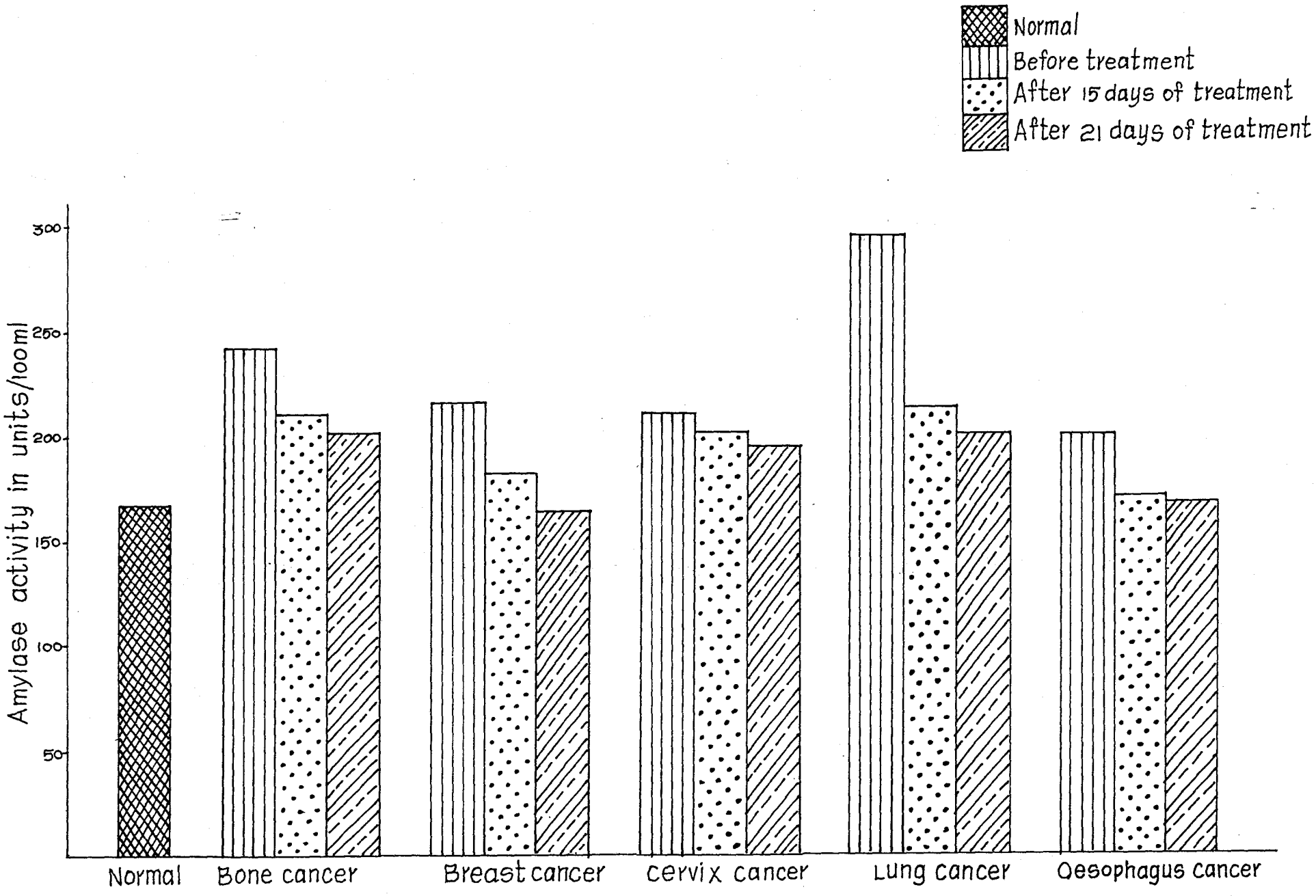
The activity of serum amylase (Unit/dl) in the increasing order is as follows:

Normal (168.00 ± 23.40) < Oesophagus (200.00±12.50) <
Cervix (215.66± 25.68) < Breast (217.92 ± 43.50) <
Bone (246.13 ± 31.29) < Lung cancer(294.01±11.84)

In bone, cervix, and lung cancer there is significant increase in amylase activity at 1 per cent level, whereas in the case of breast cancer the increase in activity is significant only at 5 per cent level.

The amylase activity returns to normal after 15 days of treatment in breast and lung cancer whereas normal activity is restored only after 21 days of treatment in bone, and cervix cancer. This increase in activity may be due to the increased leakage of amylase enzyme produced by cancer cells into the circulation.

FIG-3 ALTERATIONS IN SERUM AMYLASE ACTIVITY DUE TO RADIOTHERAPY



Serum creatine kinase

Table V indicates the changes in serum creatine kinase activity due to radiotherapy in cancer patients.

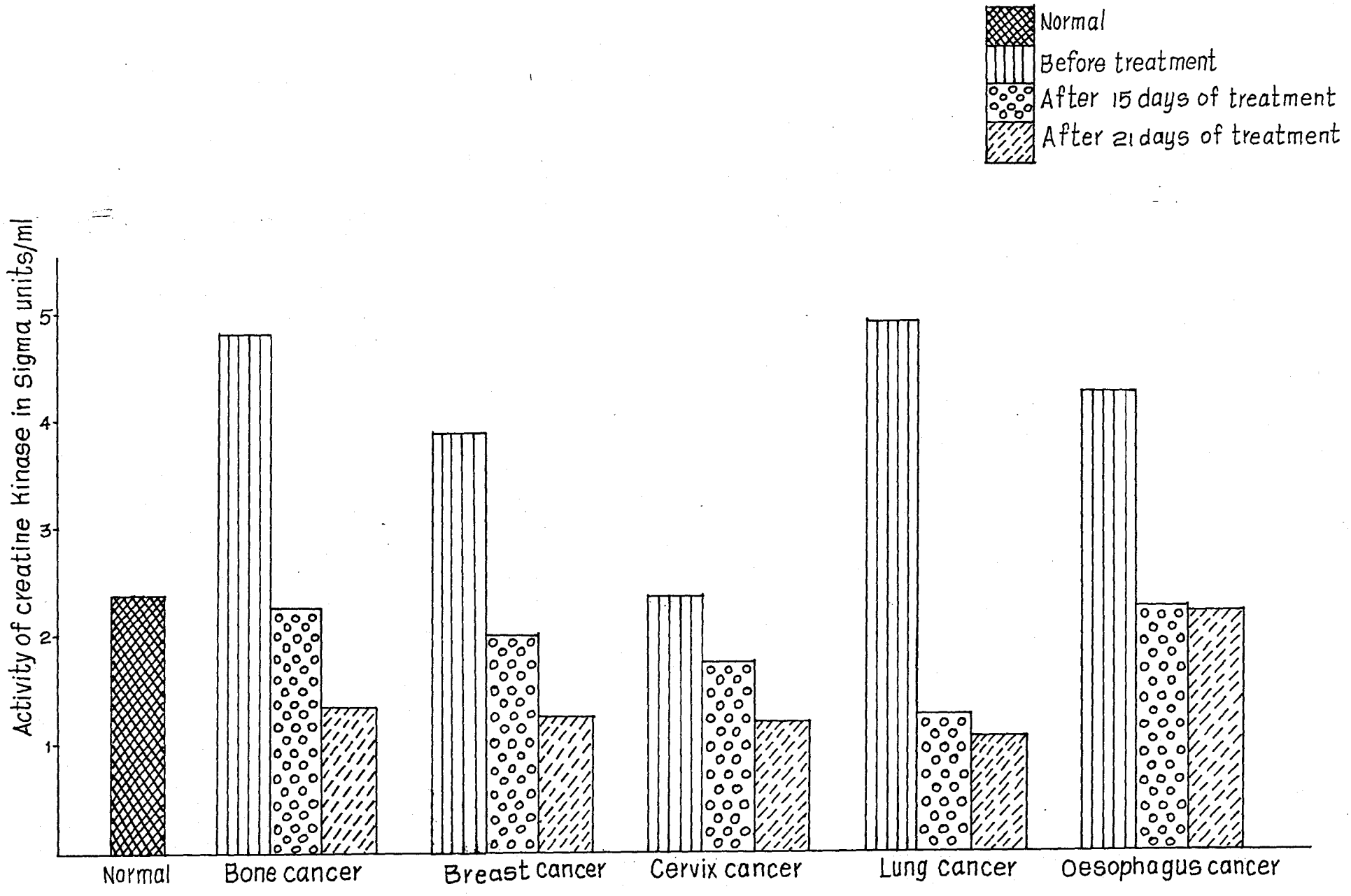
All types of cancers show a significant increase in total creatine kinase level before treatment. Maximal increase in creatine kinase is observed in lung cancer followed by bone cancer, oesophagus cancer, breast cancer, and cervical cancer compared to normal. The activity of creatine kinase (in sigma units/ml) in the increasing order is:

Normal (4.82 ± 0.53) < Cervix (2.35 ± 0.78) <
Breast (3.90 ± 0.61) < Oesophagus (4.25 ± 0.64) <
Bone (4.82 ± 0.53) < Lung cancer (4.90 ± 0.29)

The total creatine kinase activity reverts to normal level only after 21 days of treatment in bone and breast cancers. In cervix and lung cancers the level comes back to normal by 15 days of treatment itself. In oesophagus cancer even after 21 days of treatment the creatine kinase level does not reach the normal.

FIG-4

ALTRATIONS IN SERUM CREATINE KINASE ACTIVITY DUE TO RADIOTHERAPY



Serum lactate dehydrogenase activity

Table VI records the change in serum lactate dehydrogenase activity in cancer before and after radiotherapy. The enzyme activity increases significantly at 1 per cent level in all types of cancer before treatment when compared with normal. The activity pattern of lactate dehydrogenase (units/l) is as follows:

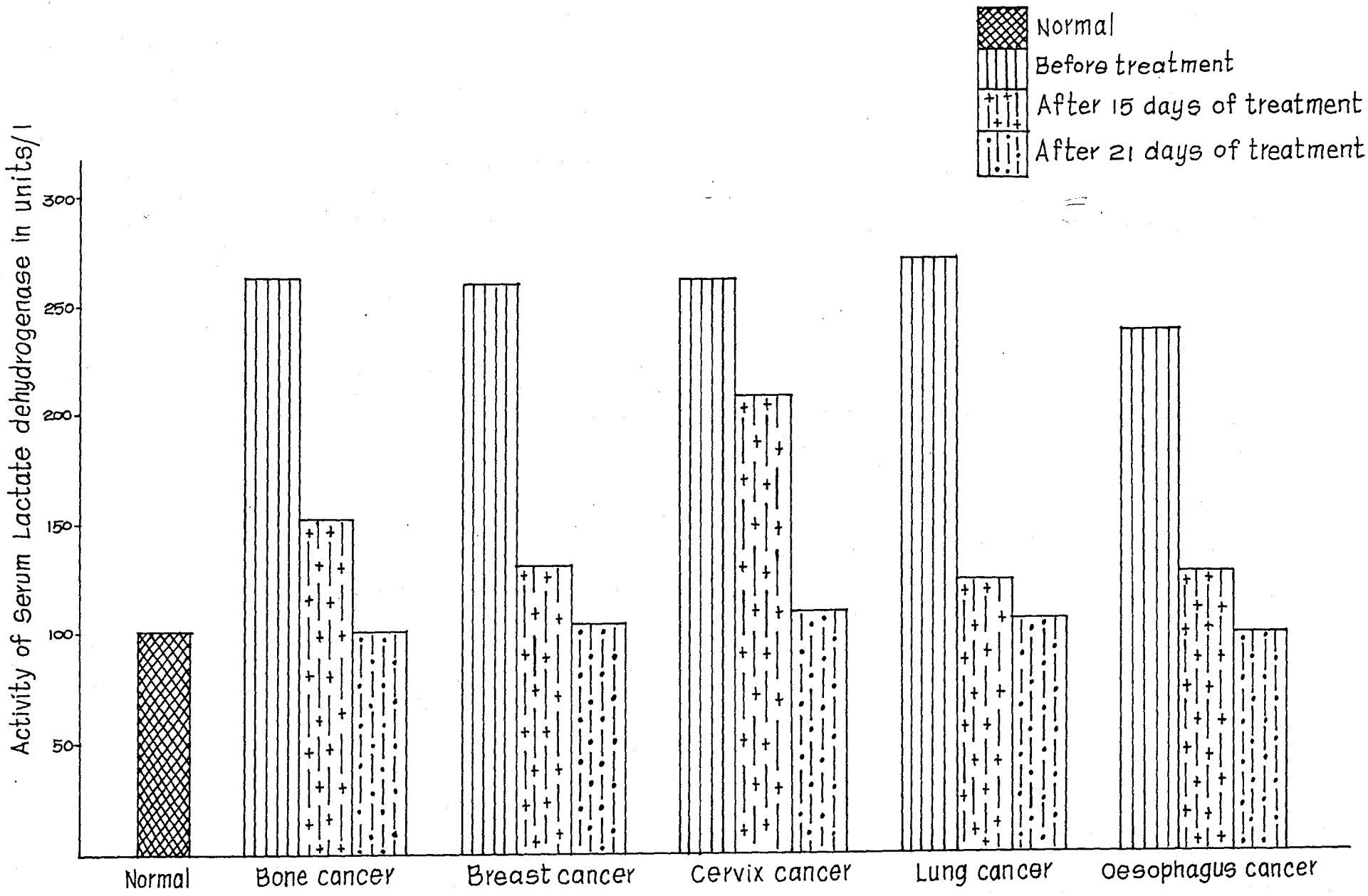
Lung (272.50 ± 22.90) > Breast (268.87 ± 53.29) > Cervix (265.00 ± 10.00) > Bone (259.50 ± 18.06) > Oesophagus (101.00±22.40) > Normal (100.00±30.35)

In all types of cancer, after 21 days of treatment the enzyme levels come down to normal (No significant difference). Lung, breast and Oesophagus cancer, need only 15 days treatment to come to normal.

LDH plays a key role in the normal cell metabolism and is elevated in human neoplastic disease (Bhatnagar et al., 1983). The results of the present study agree with the above report.

ALTERATIONS IN SERUM LACTATE DEHYDROGENASE ACTIVITY DUE TO RADIOTHERAPY

FIG-5



Serum Glutamate Oxaloacetate transaminase

Table VII presents the influence of radiotherapy on SGOT activity. SGOT values are increased several fold in all types of cancer. Though the level decreases on radiotherapy it does not reach the normal even after 21 days treatment. Since there is a downward trend on radiotherapy, it is likely that SGOT will take a much longer time to attain the normal level.

SGOT values in IU/l of different types of cancer in the increasing order are:

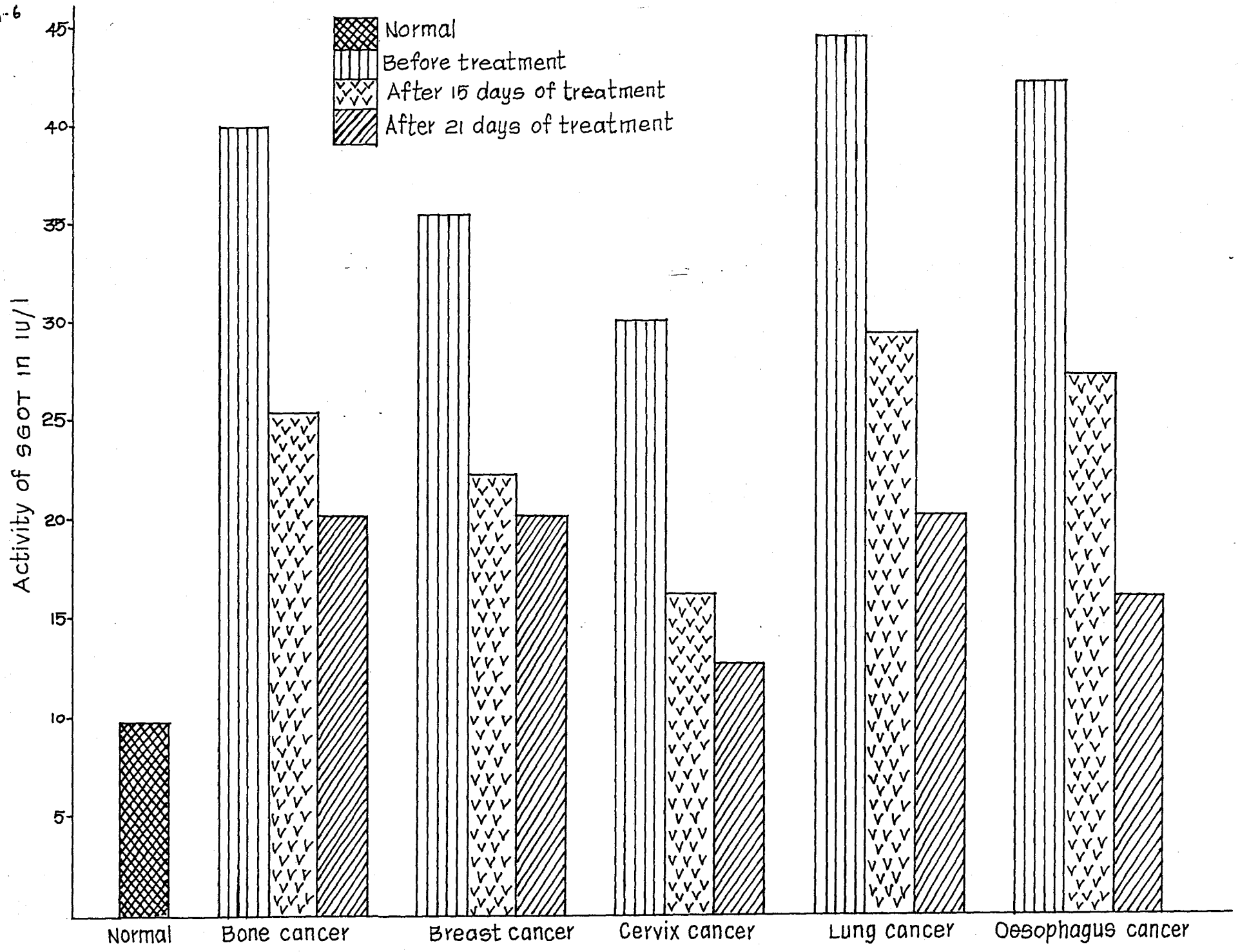
Normal (8.60 ± 3.35) < Cervix (32.00 ± 3.04) < Breast (36.00 ± 1.22) < Bone (39.30 ± 3.80) < Oesophagus (42.25 ± 1.06) < Lung 44.50 ± 1.50)

The maximum increase in SGOT activity is seen in lung cancer and the minimum increase in cervix cancer. The increase is significant at 1 per cent level in all types of cancer before treatment.

This increase in SGOT activity is seen in cancer agrees with the observations of Greengard et al. (1983).

ALTERATIONS IN SERUM GLUTAMATE OXALOACETATE TRANSAMINASE DUE TO RADIOTHERAPY

FIG-6



g. Alterations in serum glutamate pyruvate transaminase activity due to radiotherapy

Table VIII gives the changes in SGPT activity due to radiotherapy.

In all the types of cancer studied, the SGPT activity is increased tremendously (Significant at 1 per cent level) before treatment. The pattern of SGPT activity in the decreasing order is as follows:

Lung (39.50 ± 3.12) > Oesophagus (39.00±2.12) > Bone (33.90 ± 3.11) > Breast (26.37 ± 1.75) > Cervix (20.37 ± 2.56) > Normal (8.15 ± 2.60)

The activity pattern is similar to SGOT, the maximum being in lung cancer and minimum in cervix cancer.

After 15 days of treatment SGPT values fall. However they are still high wen compared to the normal. On 21 days treatment there is only a slight decrease, in all types of cancer the values being still higher than the normals.

The increased activities of transaminase even after the completion of treatment may be due the presence of liver metastases or other pathological diseases, along with the primary cancer (Nyandieka, 1984).

ALTERATIONS IN SERUM GLUTAMATE PYRUVATE TRANSAMINASE DUE TO RADIOTHERAPY

FIG-7

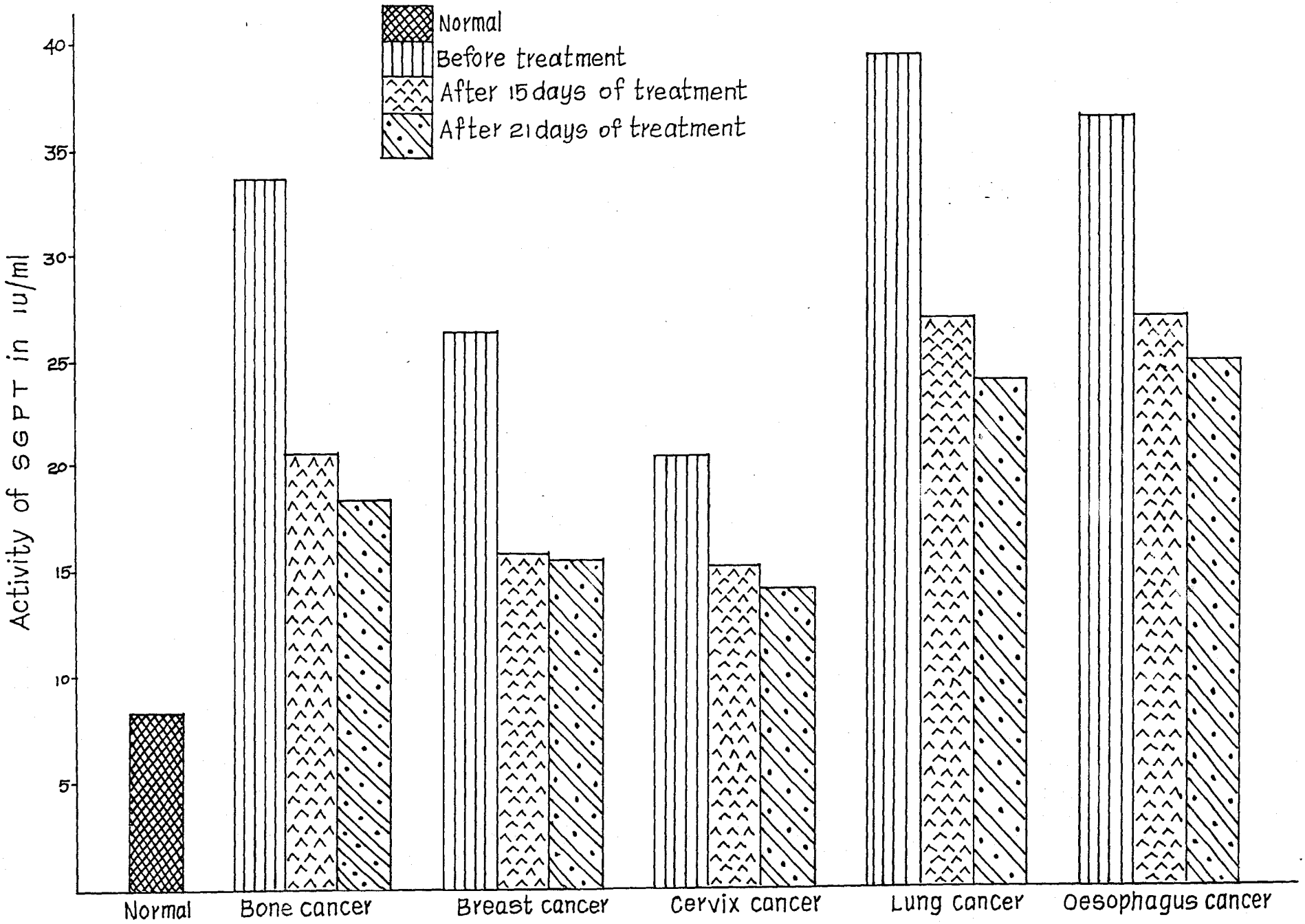
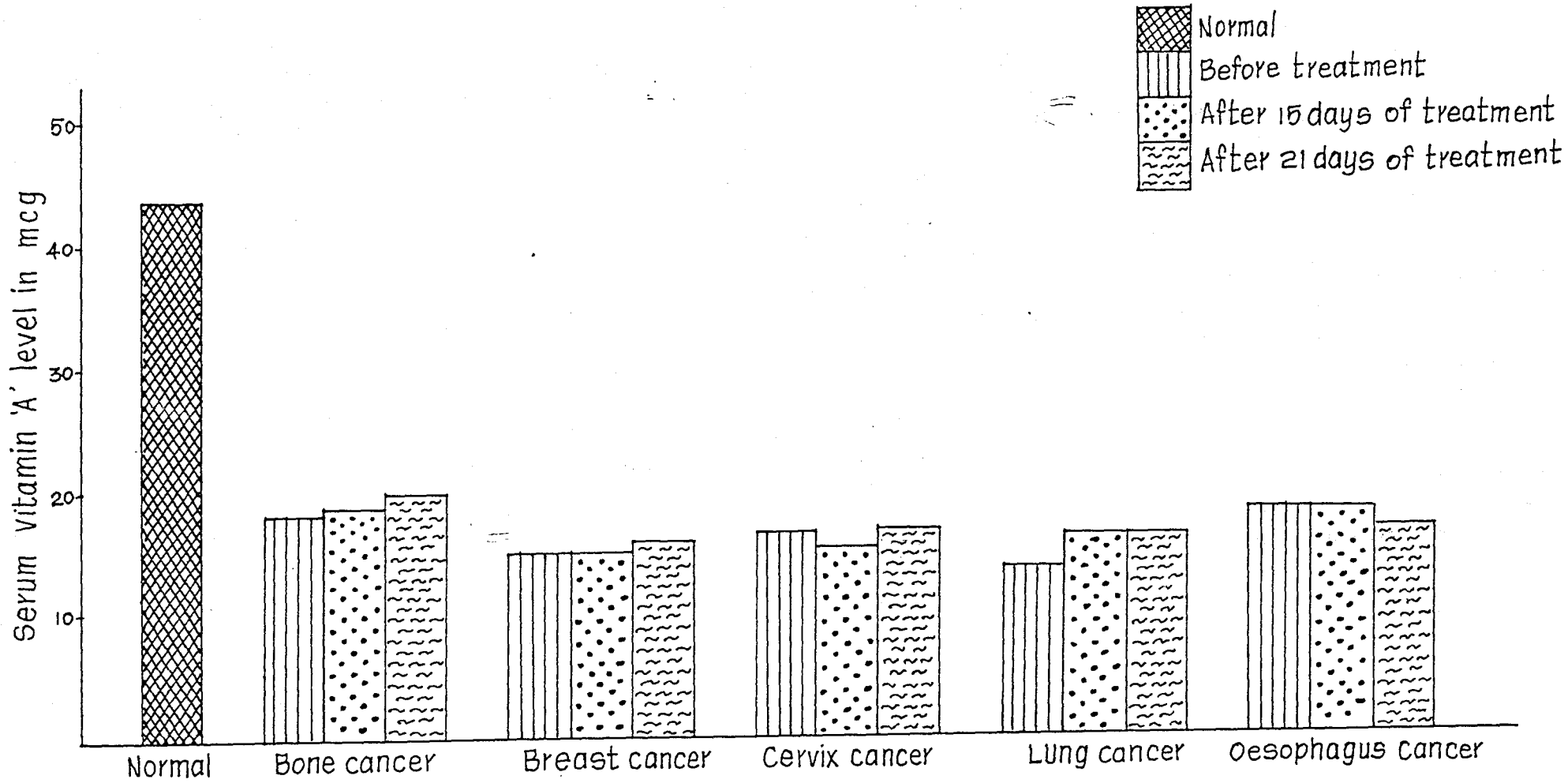


Figure. 8

ALTERATION IN SERAM VITAMIN 'A' LEVELS DUE TO RADIOTHERAPY



h. Serum vitamin A

Table IX depicts changes in serum vitamin A content in different types of cancer. The vitamin A content in mcg/dl in different types of cancer in the increasing order is:

Lung (14.50 ± 2.30) < Breast (15.00± 2.70) < Cervix (16.50 ± 2.70) < Oesophagus (18.00±2.12) < Bone cancer (18.00± 1.84)

A significant decrease of vitamin A level at 1 per cent level is seen in all types of cancer, the levels remain significantly decreased even after the treatment for 21 days.

A high dosage of vitamin A decreases the incidence and severity of tumour development in mice inoculated with murine sarcoma virus (Seifter and Martin, 1973). The results obtained in the present study agree with the observations of Giri et al. (1986).

Radiotherapy does not improve vitamin A status in cancer patients.

Summary and Conclusion

V. SUMMARY AND CONCLUSION

The study 'Influence of therapeutic radiation on serum enzyme levels in cancer patients' was carried out with the aim, of evaluating some of the biochemical parameters in malignancy of various types, changes in these levels after 15 and 21 days of radiotherapy, and comparing the levels at each stage of treatment with the normal.

Eighteen patients of the age group 35-55 suffering from different types of cancer such as bone, breast, cervix, lung and oesophagus were selected for the study from Sri G.Kuppuswamy Naidu Hospital, Coimbatore. The patients were all inpatients and were receiving radiotherapy (3000 rads). Ten normal healthy individuals were chosen as controls and they belonged to the same age group.

Estimations in serum of total protein, alkaline phosphatase, amylase, creatine kinase, lactate dehydrogenase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and vitamin A were carried out. The results obtained are summarised below:

(a) <u>Total protein:</u>

Serum total protein levels were significantly elevated in all types of cancers except breast cancer, before giving radiotherapy.

The serum total protein level in different types of cancer in g/dl in increasing order was:

Normal (6.23 ± 0.25) < Breast (6.98 ± 0.8) <
Lung (7.07 ± 0.08) < Oesophagus (7.10 ± 0.14) <
Cervix (7.25 ± 0.77) < Bone cancer (8.08 ± 0.41)

In bone, cervix, and oesophagus cancer a significant increase at 1 per cent level in serum protein level was seen before treatment. In lung cancer the increase was significant only at 5 per cent level. In breast cancer there was no significant change in protein level on radiotherapy even after 21 days.

In bone cancer the protein level returned to normal on 15 days of treatment itself, whereas in cervical, oesophagus and lung cancers the levels came down to normal only after 21 days of treatment.

The increase in total proteins in bone cancer may be due to the presence of markedly elevated levels of myeloma proteins (monoclonal immunoglobulins or para proteins). Changes in the hormone levels like steroids, androgens, corticosteroids, corticotrophin or other hormones which may be decreased or elevated in cancer may increase protein synthesis and the serum protein level (Tietz, 1976). The reason for the unchanged concentration of total proteins in

serum of breast cancer patients may be due to the non disturbance of the constant equilibrium between the plasma amino-acids and most of the proteins in the cells of the body.

(b) Serum total alkaline phosphatase

A tremendous increase in serum total ALP activity (significant at 1 per cent level) was seen in all types of cancer before treatment. Following is the pattern in total ALP activity (Bodansky units) in the decreasing order:

Normal (2.62 ± 0.73) < Cervix (6.45 ± 0.57) < Lung (6.77 ± 0.21) < Oesophagus (7.10 ± 0.41) < Breast (9.15 ± 1.96) < Bone cancer (14.86 ± 4.25)

In bone cancer the total ALP level was increased about five fold before therapy. The ALP values continued to remain high even after 15 days of treatment in all cases of cancer. In bone and cervix cancer the increase was significant at 1 per cent level, whereas in breast, lung, and oesophagus cancer the increase was significant only at 5 per cent level. Except in bone cancer, the ALP activity came down to normal in all other types of cancers after 21 days of treatment.

The present study shows maximum elevation in ALP activity in bone cancer. This may be due to the secretion of large quantities of ALP by the osteoblasts, when they are

actively depositing bone matrix, which may diffuse into the blood and increase the ALP level (Guyton 1981). Placental ALP like activity was found in the sera of upto one in 7 cancer patients, which may be the reason for increased mean ALP activity in cancer (Marhal, 1978).

(c) Serum amylase

There was significant increase in amylase activity in all types of cancer except oesophagus:

The activity of serum amylase (units/dl) in the increasing order was as follows:

Normal (168.00 ± 23.40) < Oesophagus (200.00±12.50) < Cervix (215.66 ± 25.68) < Breast (217.92 ± 43.50) < Bone (246.13 ± 31.29) < Lung cancer (294.01 ± 11.84)

In bone, cervix and lung cancer there was significant increase in amylase activity at 1 per cent lvel, whereas in the case of breast cancer, the increase in activity was significant only at 5 per cent level. In oesophagus cancer there was no significant change in serum amylase activity both before and after treatment.

The amylase activity returned to normal after 15 days of treatment in breast and lung cancer, whereas normal activity was restored only after 21 days of treatment in bone and cervix cancer. This increase in activity may be

due to the increased leakage of amylase enzyme produced by cancer cells into the circulation.

(d) Serum Creatine Kinase

All types of cancer except cervix cancer showed a significant increase at 1 per cent level in creatine kinase level before treatment while in cervix cancer the increase was significant at 5 per cent level. The activity of creatine kinase (in sigma units/ml) in the increasing order was:

Normal (1.24 ± 0.47) < Cervix (2.35 ± 0.78) <
Breast (3.90 ± 0.61) < Oesophagus (4.25 ± 0.64) <
Bone (4.82 ± 0.53) < Lung cancer (4.90 ± 0.29)

The total creatine kinase activity was reverted to the normal level only after 21 days of treatment in bone and breast cancer.

In cervix and lung cancer the level came back to normal by 15 days of treatment itself. In oesophagus cancer even after 21 days of treatment the creatine kinase level did not reach the normal.

(e) Lactate dehydrogenase

There was significant increase in enzyme activity at 1 per cent level in all types of cancers before treatment compared with normal. The activity pattern of lactate dehydrogenase (units/l) was as follows:

Lung (272.50 ± 22.90) > Breast (268.87 ± 53.29) > Cervix (265.00 ± 10.00) > Bone (259.50 ± 18.06) > Oesophagus (101.00 ± 22.40) > Normal (100.00 ± 30.35)

In all the types of cancers after 21 days of treatment, the enzyme levels came down to normal (No significant difference). Lung, breast and oesophagus cancers needed only 15 days treatment to come to normal.

LDH plays a key role in the normal cell metabolism and is elevated in human neoplastic disease (Bhatnagar et al. 1983). The results of the present study agree with the above report.

(f) Serum glutamate oxalo acetate transaminase

SGOT levels (IU/l) were increased several fold in all types of cancer and eventhough the level decreased on radiotherapy it did not reach the normal even after 21 days of treatment. Since a downward trend is noticed on radiotherapy it is likely that SGOT will take a much longer time to attain the normal level.

SGOT values in IU/l of different types of cancers in the increasing order were:

Normal (8.60 ± 3.35) < Cervix (32.00 ± 3.24) < Breast (36.00 ± 1.22) < Bone (39.30 ± 3.8) < Oesophagus (42.25 ± 1.06) < Lung (44.50 ± 1.5)

The maximum increase in SGOT activity was seen in lung cancer and the minimum increase in cervix cancer. The increase was significant at 1 per cent level in all types of cancer before treatment.

The increase in SGOT activity seen in cancer agrees with the observations of Greengard et al. (1963).

(g) Serum glutamate pyruvate transaminase

In all the types of cancers studied, the SGPT activity was increased tremendously (significant at 1 per cent level) before treatment.

The pattern of SGPT activity in the decreasing order was as follows:

Lung (39.50 ± 3.12) > Oesophagus (39.00 ± 2.12) > Bone (33.90 ± 3.11) > Breast (26.37 ± 1.75) > Cervix (20.37 ± 2.56) > Normal (8.13 ± 2.6)

The activity pattern of SGPT was similar to SGOT, the maximum being in lung cancer and minimum in cervix cancer.

After 15 days of treatment SGPT values declined. However, they were still high when compared to the normal. On 21 days treatment there was only a slight decrease, in all types of cancer, the values being still higher than the normal.

The increased activity of transaminases even after the completion of treatment may be due to the presence of liver metastases or other pathalogical diseases, along with the primary cancer (Nyandieka, 1984).

(h) Serum Vitamin A

The vitamin A content in µg/dl in different types of cancer in the increasing order was:

Lung (14.50 ± 2.30) < Breast (15.00 ± 2.7) < Cervix (16.50 ± 2.7) < Oesophagus (18.00 ± 2.12) and Bone cancer (18.00 ± 1.84)

A significant decrease of vitamin A level at 1 per cent level was seen in all types of cancers. The levels remained significantly decreased even after the treatment for 21 days.

Deficiency of vitamin A increases susceptibility to neoplasia low levels of serum vitamin A in cancer indicate deficiency of the vitamin.

A high dosage of vitamin A decreases the incidence and severity of tumour development in mice inoculated with murine sarcoma virus (Seifter and Martin, 1973). The results obtained in the present study agree wih the observationos of Giri et al. (1986)

Deficiency of vitamin A probably promotes cancer. Radiotherapy does not improve vitamin A status in cancer patients.

The abnormal increase in serum enzymes in cancer may be due to the increased leakage of cancer-cell enzymes into the circulation because of necrosis. Increased membrane permeability caused by the altered metabolism of malignant cell, may be another reason for increased serum enzyme levels.

It is evident that the initial elevation of serum enzyme activity is followed by marked decrease in activity during the course of treatment. This change may be due to the cancer cell death caused by radiotherapy subsequent to tumour regression.

To summarise, it may be said that radiotherapy though effective, does not often cause a 100% regression of tumour cells.

Determination of the enzyme activities at different stages of treatment is of prognostic importance, as it gives a clinical evidence of the extent of tumour regression. Especially LD activity in the serum can be used as an effective diagnostic tool and as a good monitor of radiotherapy, as it falls back to the normal level after the completion of treatment in all types of cancer.

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Appendices

APPENDIX I

ESTIMATION OF TOTAL PROTEIN, ALBUMIN, GLOBULIN RATIO BIURET METHOD (Tietz, 1976)

Principle

The colorimetric method for protein estimation makes use of Biuret reaction. Substances which contain CO-NH2 group joined directly or through a single carbon or nitrogen atom give a blue purple colour with alkaline copper sulfate solution. Proteins thus give a purple colour which is different for different proteins. The reaction takes its name from the complex form that is Biuret.

Reagents

(1) Stock biuret reagent: Dissolved 45g of Rochelle salt in about 400ml of 0.2N sodiumhydroxide and 15g of copper sulfate 5 H20, stirring continuously until the solution was complete. Added 5.0g of potassium iodide and made upto a litre with 0.2N sodium hydroxide.

(2) Biuret reagent (Dilute); Diluted 200ml of stock reagent to a litre with 0.2N sodium hydroxide which contains 5.0g of potassium iodide per litre.

(3) Standard protein solution: Weighed 400mg of albumin and dissolved in 0.9% sodium chloride solution so that 1.0ml of this solution contains 4.0$\frac{m}{\lambda}$g of protein.

(4) 0.9% Saline

(5) 22.5% sodium sulfate solution

Procedure

Into a series of test tubes pipetted out 0.5-2.5ml of standard protein solution. The volume was then made upto 2.5ml with water. Into another test tube added 0.4ml of serum and diluted with 0.9% saline to 10.0ml. From this 2.5ml of the solution was taken and treated as unknown. Now added 3.0ml of dilute Biuret reagent to all the tubes. Along with this blank was also taken. The colour developed was read at 500mμ colorimetrically after 30 minutes.

A standard graph was drawn by plotting concentration on X-axis and colorimeter reading on the Y-axis. The amount of protein present was calculated. This gives the total protein value for the serum.

APPENDIX II

ESTIMATION OF SERUM VITAMIN A (RETINOL)

The serum vitamin A was estimated by the trifluoroacetic acid method of Neeld and Pearson as modified and suggested by Roles et al. quoted by Gyorgy and Pearson (1967).

Procedure

Reagents:

1. Absolute ethanol purified for spectrophotometry
2. n Hexane. Fischer certified reagent special for spectrophotometry
3. Chloroform: March reagent special for spectrophotometry
4. Trif-luoroacetic acid: reagent grade (Sigma)
5. IN alcoholic KOH
6. (A) Stock vitamin A solution: 344mg of vitamin A was dissolved in chloroform and made upto 100ml 1.0ml of stock contains 3000mcg of retinol

B. Intermediate standard (1) 0.1ml of stock diluted to 100ml with chloroform

2. 0.1ml of stock diluted to 50ml with chloroform

3. 0.15ml of stock diluted to 50ml with chloroform

4. 0.1ml of stock diluted to 25ml with chloroform

C. Working standard: Each intermediate standard was again diluted in the ratio 1:10 and from each standard finally 1.0ml was taken

Method

The serum (0.5ml) was saponified with an equal volume of 1N ethanolic KOH in a water bath at 60°C for 20 minutes. The mixture was cooled and vigorously shaken in a glass, stoppered tube with an equal volume of (1ml) of n-hexane for 10 minutes. The tube was centrifuged for 1 minute at 100g to separate the layers. An aliquot (0.8ml) of the n-Hexane layer was pipetted off for determination of retinol. The n-Hexane was evaporated from this aliquot in a water bath at 60°C in a stream of oxygen free nitrogen. The last traces of n-Hexane were removed by nitrogen blowing at room temperature. The residue was taken upin (0.5ml) chloroform. 1.0 drop of acetic anhydride was added followed (0.1ml) trifluroacetic acid. The mixture was shaken vigorously and the optical density at 600nm was determined exactly 30 seconds after addition of the trifluroacetic acid.

APPENDIX III

ESTIMATION OF SERUM CREATINE KINASE

Creatine kinase in serum was estimated by the Sigma-kit procedure.

Principle

Creatine kinase catalyses the following reaction:

ATP + creatine $\rightleftharpoons$ ADP + Creatine phosphate. In the colorimetric procedure for creatine kinase estimation, the phosphocreatine formed is hydrolysed to release inorganic phosphate which is then determined colorimetrically.

Creatine phosphate $\xrightarrow{H_2SO_4}$ creatine + pi

Reagents

(1) Tris Magnesium sulfate buffer, pH $\overset{9.0}{9.0}$ at 37°C - Dissolved 0.2550g Magnesium sulfate 7H20 (0.0140M) and 2.7853g of Tris (0.23M) in about 75ml of distilled water. Adjusted the pH to 9.0, and made upto 100ml.

(2) Creatine solution: Dissolved 0.85824 g (0.057M of creatine monohydrate in 100ml of buffer (molecular weight of creatine monohydrate is 149).

(3) TCA - 20%. Dissolved 20g in 100ml of water.

(4) ATP disodium (0.005) stable while frozen - Reconstituted just before use with 2ml of water (sufficient for four tests). Both tests and blank should be from the same solution. The solution is sufficiently stable for several weeks while frozen.

(5) Molybdate solution - 2.5% - Ammonium molybdate - $(NH_4)_6 MO_7 O_{24} .4H20$ in distilled water.

(6) Fiske and Subbarow solution (for phosphate estimation)

Added 0.5g of 1,2,4, ANSA to 195ml of 15% sodium bisulphite and 5.0ml of the 20% sodium sulphite. Stoppered and shaken until it dissolved. (If the bisulphite solution is old, more than 5ml of sulphite may be needed, in which case, more sulphite may be added, 1.0ml at a time shaking well after each addition until complete solution is obtained).

(7) Phosphorus standard solution - 2mg of phosphorus/ml

(8) 5N sulphuric acid - 25ml of concentrated sulphuric acid was poured into 155ml of water with stirring.

Procedure: Into 15ml test tubes or centrifuge tubes labelled Test-1 and Blank-2, carefully added.

Reagent	Test-1(1)	Blank-2(2)
Magnesium tris buffer	—	1.0ml
Creatine solution	1.0ml	-
Serum	0.4ml	0.4ml
Water	0.8ml	0.8ml

Both the tubes were placed in a water bath at 37°C, Added 0.2ml ATP solution to each tube to start the reaction. Noted the exact time. The tubes were gently shaken and replaced into the bath and incubated for 60 minutes. While the above tubes were incubating, the following 3 test tubes were prepared.

Reagent	Reference(3)	Test 4(4)	Blank 5(5)
Molybdate solution	0.5ml	0.5ml	0.5ml
5N sulphuric acid	0.5ml	0.5ml	0.5ml
Water	5.0ml	3.0ml	3.0ml

At the end of 60 minutes incubation added to tubes (1) and (2) 1.6ml of cold TCA to stop the reaction. Mixed by inversion and centrifuged. Transferred 2.0ml each from Test-1 into Test-4 and from Blank-2 into Blank-5. Allowed to stand for 30 minutes to hydrolyse. Added to each of the tubes (No.3,4,5) 0.25ml of Fiske and Subbarow reducer solution and mixed. After 10 minutes the colour was read at 660mμ. Tube No.3 reference. Determined Creatine phosphokinase activity for tubes 4 and 5 from the calibration curve (for inorganic phosphorus). Substracted activity of blank 5 from that of Test 4. This is corrected creatine phosphokainase activites in Sigma (Units /ml).

APPENDIX IV

ESTIMATION OF ALKALINE PHOSPHATASE (Varley, 1975) (KIND ADUL FALL AND WALKER METHOD)

Principle

This method is that of King and Amstrong in which disodium phenyl phosphate is hydrolysed with the liberation of phenol and disodium phosphate. The amount of inorganic phosphate so formed is estimated colorimetrically.

Reagents

(1) Buffer substrate: 2.0g of disodium phenyl phosphate per litre of alkaline buffer. Filtered and checked the pH.

(2) Alkaline buffer (pH 10 ± 0.5)

Dissolved 1.5g anhydrous sodium carbonate in about 500ml of water and then added 0.84g of sodium bicarbonate. Shook well and made upto a litre. Filtered if necessary and checked the pH.

(3) 20% Trichloroacetic acid.

(4) Acid molybdate solution: Dissolved 5.0g of ammonium molybdate in 100ml of 2N sulfuric acid (14ml of sulfuric acid to 86ml of water).

(5) Aminonaphthol sulfonic acid reagent

(6) Stock phosphorus solution: Monopotassium dihydrogen

phosphate solution. Dissolved 35.1mg in 1ml of 10N sulfuric acid and made upto 100ml with water.

(7) Working standard: 10ml of stock standard was diluted to 200ml with water. 1.0ml of this solution contains 8.0mmg of phosphorus.

Procedure

Measured 6.0ml of buffered substrate into a test tube and placed in water bath at 37°C for few minutes. Added 0.2ml of the serum, stoppered the tube and incubated at 37°c for 15 minutes. Removed the tubes and added 1.2ml of 20%. Trichloroacetic acid. Shook well and filtered, at the same time, set up a control and a blank. For the blank used 0.2ml of water and 6.0ml of buffer substrate, for the control added 0.2ml of serum, and 6.0ml of water. To each tube added 1.2ml of 20% trichloroacetic acid. Mixed filtered and pipetted out 5.0ml of each of the supernatant.

Prepared a standard ranging from 1.0-5.0ml and made up the volume in all the tubes to 5.0ml with water. To all the tubes then added 0.6ml of acid molybdate followed by the Aminonaphthol sulfonic acid reagent, mixed and allowed to stand for 10 minutes. Read the colour developed in a colorimeter against a reagent blank at 660mμ.

APPENDIX V

ESTIMATION OF AMYLASE

Serum Amylase was assayed by the method of Somogyi (Wootton, 1964)

Principle

The Amylase of serum has a pH optimum in the region of 6.4 to 7.0. It is activated by chloride ions, so that dilutions of serum must be made in physiological saline.

Reagents

1. Buffered starch substrate (pH 7.0)

Dissolved 13.3g of dry anhydrous disodium hydrogen phosphate and 4.3g of benzoic acid in 250ml water. Boiled and then mixed 0.2g of soluble starch in 5-10ml cold water in a beaker and added it, all to the boiling mixture, rinsing the beaker out with additional cold water. Continued boiling for one minutes, then cooled to room temperature and diluted to 500ml. Kept the solution at 4°C in a brown bottle and prepared freshly each month.

2. Stock iodine solution (0.1N)

Dissolved 13.2g of pure sublimed iodine in a stock solution of 24g of potassium iodide in about 100ml of water and made to one litre with water.

3. Working iodine solution (0.01N).

Dissolved 50g of potassium fluride in a little water and added 100ml of stock iodine solution and made upto one litre with water. Stored at 4°C in a brown bottle.

Test (T)

Diluted the serum 1 in 10 with 0.9% saline, pipetted out 1.0ml of buffered starch substrate into a test tube and placed in water bath at 37°C. After 3 minutes, added 0.1ml of diluted serum mixed gently and incubated for exactly 15 minutes. Removed the tubes from the bath and added 0.4ml of working iodine solution, mixed well and then added 8.5ml of water and mixed again.

Control (C)

Mixed together in order - 1.0ml of buffered substrate 8.6ml of water and 0.4ml of iodine. Compared the colour immediately at 660mμ using water as blank (B).

The control tube contained 0.4mg of starch. The amount of starch which has been digested is therefore,

$$\frac{C - T}{C - B} \times 0.4mg$$

The amylase unit is the amount of enzyme digesting 5.0mg of starch in these conditions. The amount of enzyme in 0.01ml of serum is

$$\frac{C - T}{C - B} \times \frac{0.4}{5} \text{ Units}$$

$$\text{Thus serum amylase (Units/100ml)} = \frac{C-T}{C-B} \times \frac{0.4}{5} \times \frac{100}{0.01} \frac{C-T}{C-B} \times 800$$

APPENDIX VI

ESTIMATION OF TOTAL LDH
(Varley, 1975)

(Method of King, using dinitrophenyl hydrazine with lactate as substrate)

Reagents

1. Glycine buffer: 100mmol/litre (Sorensen), 7.505g glycine and 5.85g sodium chloride/l water.

2. Buffered substrate: pH 10.0: Add 125 ml glycine buffer and 75ml of 100mmol/l sodium hydroxide to 5ml sodium/lactate solution. Alternatively 4g lithium lactate may be used. This is more stable than the sodium salt.

3. NAD^+ solution 10mg/2ml in water. Keep at 4°C.

   NAD^+ is table longer if dissolved in nicotin amide solution. (200mmol/l 24.4mg/ml). It can then be kept upto 6 months at 0-4°C.

4. 2,4 - Dinitrophenyl hydrazine (DNPH) reagent. Dissolve 200mg in hot 1 mol/l hydrochloric acid and make upto a litre with acid.

5. Sodium hydroxide 400mmol/l (16g/l).

6. Standard pyruvate solution 1 micromole/ml. 11mg sodium pyruvate in 100ml buffered substrate.

7. NADH solution - 1micromole/l in buffer substrate

(8.5mg/100ml assuming a purity of 85%). Usually supplied as the disodium salt, molecular weight 710 of stated purity, from which the amount can be calculated.

Technique

Placed 1.0ml buffered substrate and 0.1ml serum. Diluted 1 to 5 with water into each of two tubes. Added 0.1ml water to one, the blank, placed both in a bath at 37°C and allowed them to reach this temperature. Then to the other, the test, added 0.2ml NAD^+ solution and shook to mix. Incubated for 15 minutes, then to each added 1.0ml DNPH reagent, mixed, left in the bath for a further 15 minutes, removed and added 10ml sodium hydroxide and read at 440nm 1 to 5 minutes later. For the standard curve set up the following tubes and developed as for the test.

Serum LD Activity (U/l)	0	167	333	500	667	833	1000
NADH solution (ml)	0	0.05	0.10	0.15	0.20	0.25	0.3
Pyruvate standard (ml)	0	0.05	0.10	0.15	0.20	0.25	0.3
Buffered substrate (ml)	1.0	0.9	0.8	0.7	0.6	0.5	0.4
NAD^+ solution	0	0.2	0.2	0.2	0.2	0.2	0.2
Water (ml)	0.3	0.2	0.1	0.1	0.1	0.1	0.1

For specimens with activity above 1000 U/l dilute the serum appropriately.

Note:

NAEH is added in preparing the standard curve since it gives a colour with DNPH at 400nm. NAD^+ also dies but in lactate substrate the extinction of NAD-DNPH is small (0.015). So King did not think it necessary to vary the amount of NAD^+.

APPENDIX VII

ESTIMATION OF SERUM GLUTAMIC OXALOACETIC TRANSMINASE (Method of Reitman and Frankel, 1957)

Principle

The serum is treated with the substrate and the liberated oxaloacetic acid is allowed to react with 2, 4 dinitrophenyl Hydrazine and then with sodium hydroxide. The colour developed is read at 540millimicron and compared with pyruvate readings, which is also treated similarly at 37°C.

Reagents

1. Stock pyruvic acid: 0.8ml pyruvic acid was diluted to 100ml. The pyruvic acid should be previously warmed (1.0mg per cent of pyruvic acid.) 1.0ml therefore contains 1.0mg of pyruvic acid.

2. Working standard: 1.0ml of the stock was diluted to 100ml with water. 1.0ml contains 10mg of pyruvic acid.

3. Phosphate buffer of pH 7.5: Mixed 840ml of M/15 disodium hydrogen phosphate (11.876g of disodium hydrogen phosphate 2^{H20}/litre) and 160ml of M/15 potassium dihydrogen phosphate/litre.

4. Substrate: Dissolved 0.3g of L.Aspartic acid and 50mg of L-Oxo lutaric acid. In 20 to 30ml of phosphate buffer added 10% sodium hydroxide to bring pH to 7.5. Made

upto 100ml with phosphate buffer. Stored the substrate in refrigerator.

5. Aniline citrate reagent: Dissolved 50g of citric acid in 50ml of water to this added equal volume of redistilled aniline.

6. Dinitro phenyl hydrazeane reagent: Dissolved 200mg of 2,4 dinitrophenyl hydrazeane reagent in 85ml of concentrated Hydrochloric acid and made upto a litre with water.

7. 0.4N sodium hydroxide - 16g of sodium hydroxide per litre of water.

Procedure

Pipetted out 1.0ml of the substrate into two tubes and placed in a water bath at 37°C, one (the test) added 0.2ml of the serum and shook gently to mix. Exactly an hour later, with tubes still in the water bath added 2 drops (0.07) ml of aniline citrate reagent to both and 0.2ml of serum to the other (blank). Left for 20 minutes. Into a series of clean dry test tubes pipetted out the working standard 3.5, 4.0, 4.5, 5.5, 6.5 and 7.5ml corresponding to 35, 40, 45, 55, 65 and 75mcg pyruvic acid. Added distilled water to make upto 10ml. Kept in a water bath at 37°C. After 20 minutes added 10.0ml of 2,4, dinitro phenylhydrazine to the tubes (test and standard and kept it at 37°C for 20 minutes in the water bath.

Removed from the water bath and added 10ml of 0.4N sodium hydroxide and read at 540millimicron after ten minutes. A standard graph was drawn and from the values got for the test, the serum glutamic oxaloacetic transaminase activity of serum can be calculated (Varley, 1980).

APPENDIX VIII

ESTIMATION OF SERUM GLUTAMATE PYRUVATE TRANSAMINASE (Method of Reitman and Frankel, 1957)

Principle

The serum is treated with the substrate and the liberated pyruvic acid is allowed to react with 2,4 dinitrophenyl hydrazine and then with sodium hydroxide. The colour developed is read at 520nm and compared with pyruvate standard readings which is also treated similarly at 37°C.

Reagents

(1) Stock pyruvic acid - 1.0mg/ml of pyruvic acid

(2) Working standard: 1.0ml stock was diluted to 100ml with water. 1.0ml contains 10mcg of pyruvic acid.

(3) Phosphate buffer of pH 7.5

(4) Substrate: Dissolved 5.0g of L.Alanine and 30mg of L.Oxoglutaric acid in 20-30ml of phosphate buffer and added 10% sodium hydroxide to bring pH to 7.5. Made upto 100ml with phosphate buffer.

(5) Aniline citrate reagent

(6) Dinitrophenyl hydrazine reagent

(7) 0.4N sodium hydroxide

Procedure

Pipetted out 1.0ml of substrate in two tubes and placed in an incubator at 37°C for few minutes to reach the temperature. To one added 0.2ml of serum and shook gently to mix. Exactly half an hour later with the tube still in the incubator added 2 drops of (0.07 M) of aniline citrate reagent to both and added 0.2ml of serum to the other (control). Kept at 37°C for twenty minutes. Then the tubes were removed from the incubator to which 10ml of 0.4N sodium hydroxide was added and the colour developed was read at 520millimicron after 10 minutes.

Into a series of dry test tubes pipetted out the working standard 3.5, 4.0, 4.5, 5.5, 6.5 and 7.5 ml correspending to 35, 40, 45, 55, 65 and 75mcg of pyruvic acid respectively. Added distilled water to make upto 10ml.

Incubated at 37°C for 20 minutes and then added one ml of 2-4-g dinitrophenyl hydrazine reagent to all the tubes (test and standard) and removed from the water bath and added 10ml of 0.4N sodium hydroxide and read at 540mμ using green filter after 10minutes.

A graph was drawn taking colorimeter reading on X-axis and concentration of pyruvic acid on Y axis. From the value got for the test, the serum glutamic pyruvic transaminase activity of the serum can be calculated (Varley, 1980).

TABLE II

ALTERATIONS IN SERUM TOTAL PROTEIN LEVEL DUE TO RADIOTHERAPY IN CANCER PATIENTS

Group	Types	Protein content in g/dl	Groups compared	't' value
A	Normal control	6.23 ± 0.25		
	Bone cancer			
B1	Before treatment	8.08 ± 0.41	A vs B1	10.07**
B2	After 15 days of treatment	6.60 ± 0.40	A vs B2	2.05 NS
B3	After 21 days of treatment	6.38 ± 0.39	A vs B3	0.84 NS
	Breast cancer			
C1	Before treatment	6.88 ± 0.80	A vs C1	2.37 NS
C2	After 15 days of treatment	6.65 ± 0.34	A vs C2	2.11 NS
C3	After 21 days of treatment	6.27 ± 0.20	A vs C3	0.30 NS
	Cervix cancer			
D1	Before treatment	7.25 ± 0.77	A vs D1	3.45**
D2	After 15 days of treatment	7.17 ± 0.34	A vs D2	5.36**
D3	After 21 days of treatment	6.73 ± 0.59	A vs D3	2.05 NS
	Lung cancer			
E1	Before treatment	7.07 ± 0.80	A vs E1	2.63*
E2	After 15 days of treatment	6.76 ± 0.20	A vs E2	3.16**
E3	After 21 days of treatment	6.76 ± 0.73	A vs E3	1.81 NS
	Oesophagus cancer			
F1	Before treatment	7.10 ± 0.14	A vs F1	3.78**
F2	After 15 days of treatment	6.80 ± 0.21	A vs F2	3.04*
F3	After 21 days of treatment	6.40 ± 0.28	A vs F3	0.64 NS

**Significant at 1 per cent level
*Significant at 5 per cent level
NS - Not significant

TABLE III

ALTERATIONS IN TOTAL ALKALINE PHOSPHATASE ACTIVITY DUE TO RADIOTHERAPY

Group	Types	Activity of serum Alkaline phosphatase in Bodansky units (B.U)	Groups compared	't' value
A	Normal control	2.63 ± 0.73		
	Bone cancer			
B1	Before treatment	14.86 ± 4.25	A vs B1	8.25**
B2	After 15 days of treatment	8.16 ± 2.14	A vs B2	6.84**
B3	After 21 days of treatment	5.49 ± 2.29	A vs B3	3.35**
	Breast cancer			
C1	Before treatment	9.15 ± 1.86	A vs C1	8.74**
C2	After 15 days of treatment	4.80 ± 2.19	A vs C2	2.57*
C3	After 21 days of treatment	2.70 ± 0.53	A vs C3	0.71NS
	Cervix cancer			
D1	Before treatment	6.45 ± 0.57	A vs D1	6.69**
D2	After 15 days of treatment	4.80 ± 0.98	A vs D2	4.95**
D3	After 21 days of treatment	2.64 ± 0.98	A vs D3	0.029NS
	Lung cancer			
E1	Before treatment	6.77 ± 0.21	A vs E1	8.95**
E2	After 15 days of treatment	5.80 ± 3.08	A vs E2	2.75*
E3	After 21 days of treatment	4.00 ± 3.02	A vs E3	1.21NS
	Oesophagus cancer			
F1	Before treatment	7.10 ± 0.41	A vs F1	7.897**
F2	After 15 days of treatment	4.20 ± 0.85	A vs F2	2.47*
F3	After 21 days of treatment	2.70 ± 0.10	A vs F3	0.39NS

**Significant at 1 per cent level
*Significant at 5 per cent level
NS - Not significant

TABLE IV

ALTERATIONS IN SERUM AMYLASE ACTIVITY DUE TO RADIOTHERAPY IN CANCER PATIENTS

Group	Types	Activity of serum amylase in units/dl	Groups compared	't' value
A	Normal control	168.00 ± 23.48		
	Bone cancer			
B1	Before treatment	246.13±31.29	A vs B1	5.04**
B2	After 15 days of treatment	210.29±33.91	A vs B2	2.62*
B3	After 21 days of treatment	208.00±36.33	A vs B3	2.39*
	Breast cancer			
C1	Before treatment	217.92±43.46	A vs C1	2.59*
C2	After 15 days of treatment	182.86±18.66	A vs C2	0.76NS
C3	After 21 days of treatment	169.00±10.00	A vs C3	0.01NS
	Cervix cancer			
D1	Before treatment	215.66±25.68	A vs D1	3.09**
D2	After 15 days of treatment	205.71±18.66	A vs D2	3.36**
D3	After 21 days of treatment	195.00±19.14	A vs D3	1.89NS
	Lung cancer			
E1	Before treatment	294.01±11.84	A vs E1	8.25**
E2	After 15 days of treatment	213.33±47.60	A vs E2	2.06NS
E3	After 21 days of treatment	206.66±1.63	A vs E3	1.88NS
	Oesophagus cancer			
F1	Before treatment	200 ± 12.50	A vs F1	1.76 NS
F2	After 15 days of treatment	171.45±14.00	A vs F2	1.79NS
F3	After 21 days of treatment	170.00±14.14	A vs F3	0.11NS

**Significant at 1 per cent level
*Significant at 5 per cent level
NS - Not significant

TABLE VI

ALTERATIONS IN SERUM LACTATE DEHYDROGENASE ACTIVITY DUE TO RADIOTHERAPY

Group	Types	Activity of Serum LD activity Units/l	Groups compared	't' value
A	Normal control	100.00± 30.55		
	Bone cancer			
B1	Before treatment	259.50±18.06	A vs B1	9.99**
B2	After 15 days of treatment	152.00± 31.62	A vs B2	2.85*
B3	After 21 days of treatment	108.00± 15.00	A vs B3	0.51NS
	Breast cancer			
C1	Before treatment	268.87± 53.30	A vs C1	6.87**
C2	After 15 days of treatment	138.50± 53.15	A vs C2	1.57NS
C3	After 21 days of treatment	105.30± 7.50	A vs C3	0.32NS
	Cervix cancer			
D1	Before treatment	265.00±10.00	A vs D1	9.79**
D2	After 15 days of treatment	209.25 ± 44.14	A vs D2	4.88**
D3	After 21 days of treatment	110.20±8.50	A vs D3	0.6 NS
	Lung cancer			
E1	Before treatment	272.50±22.90	A vs E1	4.05**
E2	After 15 days of treatment	125.20 ± 20.00	A vs E2	1.24NS
E3	After 21 days of treatment	107.00 ± 15.20	A vs E3	0.35NS
	Oesophagus cancer			
F1	Before treatment	290.00±10.6	A vs F1	5.84**
F2	After 15 days of treatment	132.00±14.14	A vs F2	1.34NS
F3	After 21 days of treatment	101.00±22.40	A vs F3	0.04 NS

**Significant at 1 per cent level
*Significant at 5 per cent level
NS - Not significant

TABLE V

ALTERATIONS IN SERUM CREATINE KINASE ACTIVITY DUE TO RADIOTHERAPHY IN CANCER PATIENTS

Group	Types	Activity of creatine kinase in sigma Units/ml	Groups compared	't' value
A	Normal control	1.24 ± 0.47		
	Bone cancer			
B1	Before treatment	4.82 ± 0.56	A vs B1	12.43**
B2	After 15 days of treatment	2.23 ± 0.61	A vs B2	2.30*
B3	After 21 days of treatment	1.33 ± 0.57	A vs B3	0.30NS
	Breast cancer			
C1	Before treatment	3.90 ± 0.60	A vs C1	8.13**
C2	After 15 days of treatment	2.00 ± 0.58	A vs C2	2.36*
C3	After 21 days of treatment	1.25 ± 0.46	A vs C3	0.03NS
	Cervix cancer			
D1	Before treatment	2.35 ± 0.78	A vs D1	3.02*
D2	After 15 days of treatment	1.75 ± 0.96	A vs D2	0.21NS
D3	After 21 days of treatment	1.20 ± 0.57	A vs D3	0.13NS
	Lung cancer			
E1	Before treatment	4.93 ± 0.29	A vs E1	11.87**
E2	After 15 days of treatment	1.25 ± 0.35	A vs E2	0.02NS
E3	After 21 days of treatment	1.06 ± 0.23	A vs E3	0.57NS
	Oesophagus cancer			
F1	Before treatment	4.25 ± 0.64	A vs F1	7.08**
F2	After 15 days of treatment	2.25 ± 0.35	A vs F2	2.63*
F3	After 21 days of treatment	2.20 ± 0.28	A vs F3	2.55*

**Significant at 1 per cent level
*Significant at 5 per cent level
NS - Not significant

TABLE VII

ALTERATION IN SERUM GLUTAMATE OXALOACETATE TRANSAMINASE ACTIVITY DUE TO RADIOTHERAPY

Group	Type	Activity of SGOT in IU/l	Groups compared	't' value
A	Normal control	8.60 ± 3.34		
	Bone cancer			
B1	Before treatment	39.30 ± 3.88	A vs B1	14.75**
B2	After 15 days of treatment	25.50 ± 3.62	A vs B2	8.34**
B3	After 21 days of treatment	20.30 ± 1.20	A vs B3	7.03**
	Breast cancer			
C1	Before treatment	36.00 ± 1.22	A vs C1	14.76**
C2	After 15 days of treatment	22.75 ± 4.13	A vs C2	6.17**
C3	After 21 days of treatment	15.13 ± 3.07	A vs C3	3.12**
	Cervix cancer			
D1	Before treatment	32.00 ± 3.24	A vs D1	11.04**
D2	After 15 days of treatment	16.25 ± 0.86	A vs D2	4.18**
D3	After 21 days of treatment	12.87 ± 1.43	A vs D3	2.29*
	Lung cancer			
E1	Before treatment	44.50 ± 1.50	A vs E1	16.59**
E2	After 15 days of treatment	29.67 ± 3.51	A vs E2	8.66**
E3	After 21 days of treatment	20.33 ± 5.90	A vs E3	4.02**
	Oesophagus cancer			
F1	Before treatment	42.25 ± 1.00	A vs F1	12.85**
F2	After 15 days of treatment	27.75 ± 1.06	A vs F2	7.31**
F3	After 21 days of treatment	16.25 ± 1.06	A vs F3	2.90*

**Significant at 1 per cent level
*Significant at 5 per cent level
NS - Not significant

TABLE VIII

ALTERATIONS IN SERUM GLUTAMATE PYRUVATE TRANSAMINASE DUE TO RADIOTHERAPY

Group	Types	Activity of SGPT in IU/ml	Groups compard	't' value
A	Normal control	8.15 ± 2.60		
	Bone cancer			
B1	Before treatment	33.90 ± 3.11	A vs B1	15.54**
B2	After 15 days of treatment	20.60 ± 2.51	A vs B2	8.11**
B3	After 21 days of treatment	19.40 ± 3.11	A vs B3	6.79**
	Breast cancer			
C1	Before treatment	26.38 ± 1.75	A vs C1	11.73**
C2	After 15 days of treatment	15.80 ± 1.20	A vs C2	5.13**
C3	After 21 days of treatment	15.50 ± 2.10	A vs C3	6.99**
	Cervix cancer			
D1	Before treatment	20.38 ± 2.56	A vs D1	7.44**
D2	After 15 days of treatment	15.23 ± 0.95	A vs D2	4.81**
D3	After 21 days of treatment	14.375 ± 1.80	A vs D3	3.96**
	Lung cancer			
E1	Before treatment	39.50 ± 3.12	A vs E1	15.54**
E2	After 15 days of treatment	27.00 ± 1.50	A vs E2	10.79**
E3	After 21 days of treatment	24.00 ± 0.42	A vs E3	9;47**
	Oesophagus cancer			
F1	Before treatment	39.00 ± 2.12	A vs F1	14.18**
F2	After 15 days of treatment	27.00 ± 1.10	A vs F2	9.01**
F3	After 21 days of treatment	25.10 ± 0.85	A vs F3	8.15**

**Significant at 1 per cent level

TABLE VII

ALTERATION IN SERUM GLUTAMATE OXALOACETATE TRANSAMINASE ACTIVITY DUE TO RADIOTHERAPY

Group	Type	Activity of SGOT in IU/l	Groups compared	't' value
A	Normal control	8.60 ± 3.34		
	Bone cancer			
B1	Before treatment	39.30 ± 3.88	A vs B1	14.75**
B2	After 15 days of treatment	25.50 ± 3.62	A vs B2	8.34**
B3	After 21 days of treatment	20.30 ± 1.20	A vs B3	7.03**
	Breast cancer			
C1	Before treatment	36.00 ± 1.22	A vs C1	14.76**
C2	After 15 days of treatment	22.75 ± 4.13	A vs C2	6.17**
C3	After 21 days of treatment	15.13 ± 3.07	A vs C3	3.12**
	Cervix cancer			
D1	Before treatment	32.00 ± 3.24	A vs D1	11.04**
D2	After 15 days of treatment	16.25 ± 0.86	A vs D2	4.18**
D3	After 21 days of treatment	12.87 ± 1.43	A vs D3	2.29*
	Lung cancer			
E1	Before treatment	44.50 ± 1.50	A vs E1	16.59**
E2	After 15 days of treatment	29.67 ± 3.51	A vs E2	8.66**
E3	After 21 days of treatment	20.33 ± 5.90	A vs E3	4.02**
	Oesophagus cancer			
F1	Before treatment	42.25 ± 1.00	A vs F1	12.85**
F2	After 15 days of treatment	27.75 ± 1.06	A vs F2	7.31**
F3	After 21 days of treatment	16.25 ± 1.06	A vs F3	2.90*

**Significant at 1 per cent level
*Significant at 5 per cent level
NS - Not significant