STUDIES ON THE EFFECTS OF DIETARY MAGNESIUM AND MANGANESE ON EXPERIMENTAL TUMOUR CELL (in mice)

THESIS

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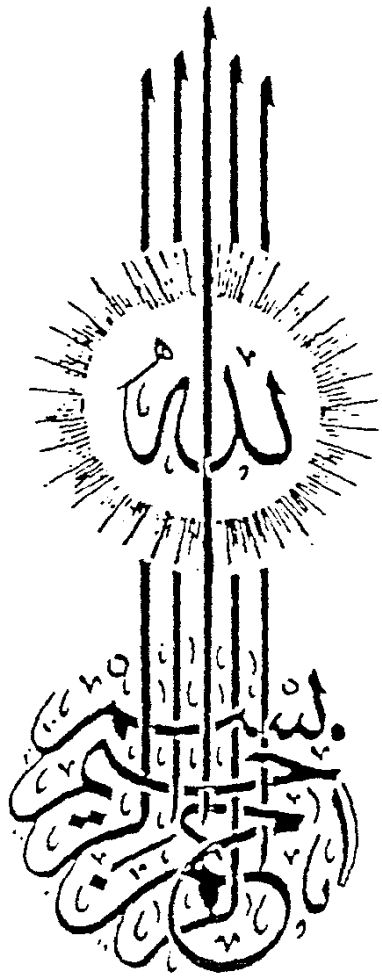

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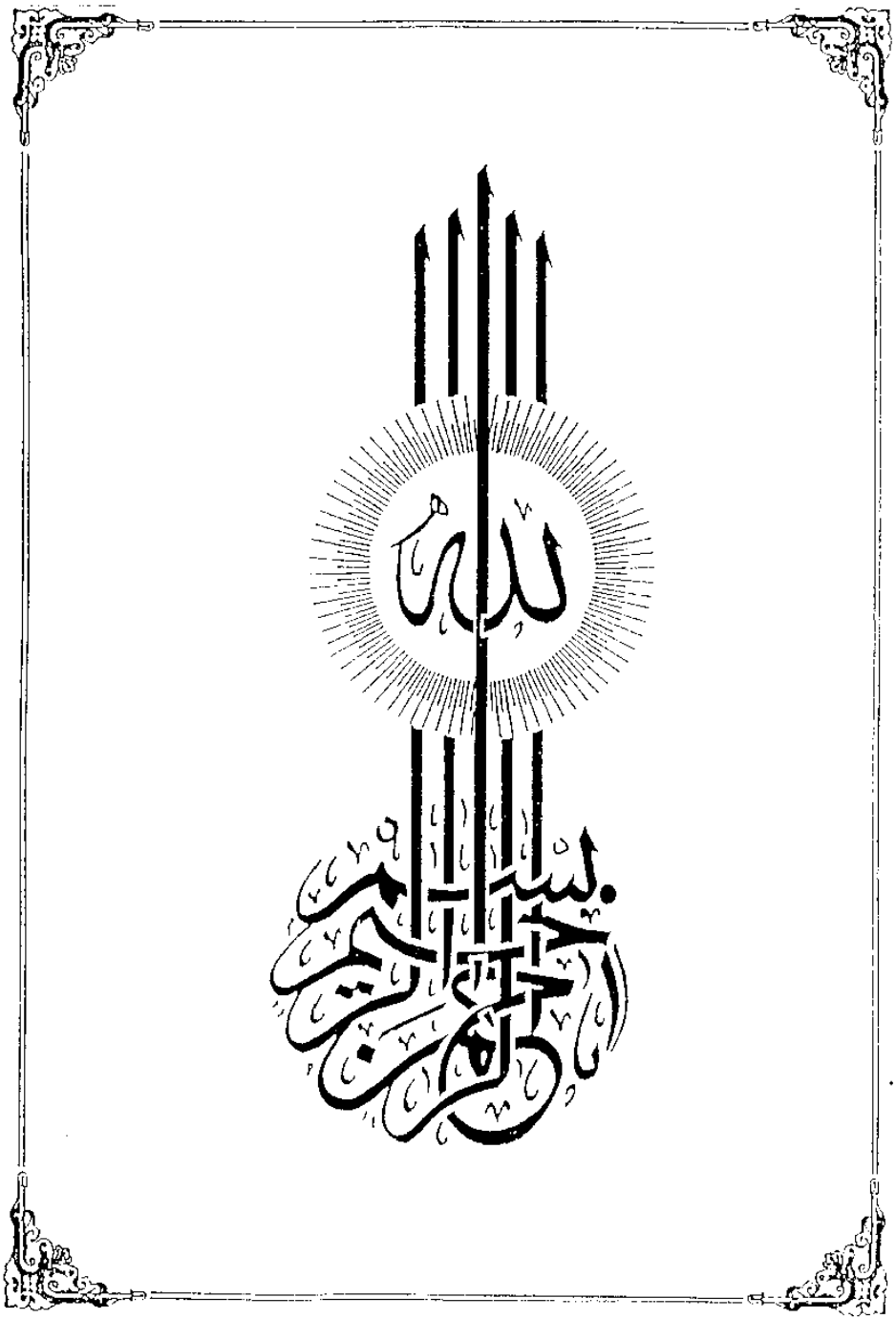

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To My Parents

THIS THESIS HAS NOT BEEN SUBMITTED FOR A DEGREE

AT THIS OR ANY OTHER UNIVERSITY

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ABBREVIATIONS USED

A	: Adenine or adenosine
ADP	: Adenosine diphosphate
AK-Pase	: Alkaline phosphatase
ATP	: Adenosine triphosphate
C	: Cytosine or cytidine
CDF	: Coupled deficient fed
CSF:	: Coupled supplemented fed
dATP	:2′-deoxyadenosine 5′-triphosphate
DNA	: Deoxyribonucleic acid
EAC	: Ehrlich ascites carcinoma
G-6-Pase	: Glucose-6-phosphatase
GTP	: Guanosine triphosphate
I	: Inosine
i.p.	: Intraperitoneal
LDH	: Lactate dehydrogenase
MgDF	: Magnesium deficient fed
MgSF	: Magnesium supplemented fed
MnDF	: Manganese deficient fed
MnSF:	: Manganese supplemented fed
N.S.	: Non Significant
p	: Probability
RNA:	: Ribonucleic acid
r.p.m.	: Revolutions per minute
s.c.	: Subcutaneous
S.E.	: Standard error
U	: Uracil or uridine
vs	: versus

CONTENTS

INTRODUCTION

INTRODUCTION

DIET AND CANCER

Cancer is a term used by both laymen and the medical profession to refer to malignant neoplasms or tumors. However, the term has no precise meaning and is not used by pathologists. Neoplasia or new growth is said to occur when cells in a tissue or organ proliferate without the normal controls on growth.

A malignant tumor arising in an epithelial tissue is known as a carcinoma and one arising in connective tissue as a sarcoma. Leukaemia is a malignant condition in which the abnormal cells are in the blood and bone marrow.

In malignant tumors the cells spread to neighbouring normal tissues by direct invasion or to distant organs by passage through blood or lymph vessels. Then a secondary tumor or metastasis forms. In benign neoplasms growth is limited to the organ of origin, and the tumor may have a well-defined capsule. Benign neoplasms usually cause symptoms only as a result of increasing size and the pressure which they may exert on other tissues.

It is commonly stated that most human cancer, perhaps as much as 80 to 90% is attributable to environmental influences (Doll, 1977). The assumption behind such an assertion is that the lowest rate for each type of malignancy identified in worldwide surveys is the natural basic incidence of the disease, and that higher rates found elsewhere are due to environmental factors specific to those areas.

There are many factors that can significantly modify apparent cancer death rates, including age and race structures of the respective populations, geographic location and its effects on, for example, the duration and intensity of sunlight and soil composition, morbidity and mortality from other diseases; levels of natural and occupational exposure

to carcinogens; features of the comparative lifestyles, including such things as diet, alcohol consumption and smoking. These factors may vary widely from one country to another, and perhaps also within one country, modifying the apparent incidence of cancer to a degree that can only be estimated roughly (Greasey, 1981).

When smoking and alcohol are discounted, nutrition remains as the single largest variable in the personal environment. The relationship between nutrition and cancer has been the focus of numerous investigations in recent years not only for possible therapeutic implications but also as prophylaxis.

Cancer in man is associated with dietary factors and falls into several categories. First, there are some carcinogens known to occur naturally in a number of foods (Miller & Miller, 1976). Because such foods are relatively few in number, and their consumption may be unique to specific regions or cultures, naturally occurring carcinogens are logical candidates to be causative agents for tumors of localized high rates of incidence. Second, there are substances that are added to foods deliberately, such as nitrites (Issenberg, 1976) or are present as contaminants (Harris *et al.*, 1977), that are known as suspected carcinogens. Third, there are variations in the consumption of normal dietary components such as fat, protein, fiber, vitamins and minerals, which may predispose toward, or help to prevent, some forms of cancer (Werther, 1980). Fourth, there are items suspected of being carcinogenic, which although they are not essential dietary components, are ingested or chewed. These items include betel nuts, tobacco and tea (Farnsworth *et al.*, 1976).

Turning from the role of diet in the development of cancer to the nutritional effects of the disease, profound changes may be noted. As cancer progresses, a complex anorexia-cachexia syndrome appears. Anorexia in the cancer patient is a loss of the desire to eat, leading often to cancer cachexia; a weight loss syndrome, which as it progresses leads to weakness, apathy, mental confusion, abnormalities of body composition and ultimate failure of vital functions.

Interestingly, some of the metabolic changes, particularly those involving breakdown of proteins and increased output of glucose by the liver, resemble those seen during some phases of starvation (Sandek & Felig, 1976). This finding is compatible with the long-held concept that the tumor is in competition with the host for nutrients. Competition for nutrients is only one element, and in most cases perhaps only a minor one, in the development of cachexia. Other factors that may play a role include toxic products and endocrine-like materials secreted by the tumor, mechanical interference with vital organ functions, side effects of therapy, deteriorating psychologic status (perhaps associated with pain and opportunistic infections).

Metals and Carcinogenesis:

It is impossible to assign specific roles to most trace elements in the process of carcinogenesis, as the available information deals mostly with data obtained by the administration of these elements to animals. Epidemiologic evidence from human studies is relatively sparse and is often concerned with environmental exposures that reflect the working situation rather than the diet. Only for arsenic, cadmium, iodine, lead and zinc can it be said that evidence is consistent enough to implicate these elements in the development or prevention of cancer.

Arsenic is widely distributed in nature and trace amounts occur in many foods. It has been claimed that a high intake of arsenic, as for example by vineyards workers who inhaled arsenical particles pesticides or drank contaminated wines, is associated with cancers of the skin and lung and with hemangioendotheliomas of the liver (Roth, 1957; Galy *et al.*, 1963 and Latarjet *et al.*, 1964). Furthermore, in Taiwan, the concentration of arsenic in well water could be correlated with the incidence of skin cancer (Tseng, 1977). Experimental studies in rats, mice and dogs which involved exposures of up to 2 years

duration, failed to disclose any increase in tumor incidence attributable to arsenic. The element did not appear to potentiate the action of known carcinogens (Furst, 1977).

Cadmium intake showed a positive relation with the incidence of renal (Kolonel, 1976) and prostate cancers (Adams *et al.*, 1969 and Lemen *et al.*, 1976). Such environmental exposure, notably in workers making batteries, bears little relationship to dietary exposure, however, indeed, when attempts have been made to distinguish the contribution of dietary cadmium, no association was evident (Kolonel & Winkelstein, 1977). Intramuscular or subcutaneous injections of cadmium powder or cadmium sulphide, oxide, sulphate or chloride in rodents cause sarcomas in the sites of injection.

Iodine deficiency experiments done by Eskin (1978) on rats led to epithelial dysplasia in mammary tissue, a change that could be exacerbated by estrogen treatment, leading in some cases to frank neoplasia. Supplementation of the deficient diets with iodide reversed the dysplasia, but supplemental thyroxine did not, suggesting that hypothyroidism itself was not the cause of the abnormal histology. The relevance of these data to human breast cancer is unclear (Edington, 1976 and Waterhouse, 1976).

Lead in drinking water showed a direct relation with the incidence of cancer, specifically stomach, intestinal, ovarian and renal cancers, myeloma, lymphomas and leukemias (Berg & Burbank, 1972). Other investigators (Nelson *et al.*, 1973; Cooper & Gaffey, 1975 and Robinson, 1976) found no significant correlations in surveys of people with major occupational lead exposure. In contrast to the generally negative epidemiologic findings, experimental evidence clearly suggests that lead has a carcinogenic potential. It appears that lead phosphate, lead acetate and basic lead acetate induces renal adenomas and renal adenocarcinoma in rodents following administration by parenteral and dietary routes. In addition, Zawiska & Medras (1968) have reported carcinomas of the testis and adenomas of the adrenal, thyroid, pituitary, prostate and lungs following chronic administration of lead acetate to rats.

Zinc is an essential element that is constituent of a large number of enzyme systems, including nucleic acid polymerases that are so vital in cellular proliferation. There is no evidence that zinc salts are carcinogenic after administration by any route except that of intratesticular injection (Riviere *et al.*, 1960). For example, Gunn *et al.*, (1964) failed to demonstrate any tumorigenicity of zinc acetate after subcutaneous injection, and Health *et al.*, (1962) failed to produce any tumors after intramuscular injection of zinc powder. Excess zinc has also been described as suppressing tumor induction by azo-dyes in rats (Duncan & Dresoti, 1975). In humans, similar conflict exists. It has been claimed that mortality rates from leukemia and cancers of the intestine, breast, prostate on skin correlate with higher zinc intake (Schrauzer *et al.*, 1977), whereas other investigators found lower levels in subjects with esophageal (Lin *et al.*, 1977), bronchogenic (Davies *et al.*, 1968) and prostate cancers (Gijorkey *et al.*, 1967).

Minerals as Therapy

The platinum salt, cis diamine dichloroplatinum was found to have broad anti-tumor activity in experimental tumors. Despite some troublesome side effects, especially nausea and vomiting, and potential renal impairment, this drug has been shown to be highly effective in patients with testicular, ovarian, head and neck malignancies (Gotthels & Drewinko, 1975).

Another heavy metal salt is gallium nitrate and in preliminary reports this drug has been noted to have anti-tumor effect against several human neoplasms (Brown *et al.*, 1978 and Valdivieso *et al.*, 1978).

MAGNESIUM

The presence of magnesium in living organism has been known for at least a century (Holmes, 1859). The adult human body contains 21-28 g of magnesium (Widdowson *et al.*, 1951). It is the fourth most abundant cation in the body and the second most plentiful intracellularly. Bone contains about 66% of the total body magnesium, the remainder being almost equally distributed between muscle and non muscular soft tissues (Forbes *et al.*, 1956). Of the nonosseous tissues, liver and striated muscle contain the largest quantities, 15-20 mEq/Kg. (Cotlove *et al.*, 1951, Widdowson *et al.*, 1951 and Baldwin *et al.*, 1952). The kidney and brain contain 17 and 13 mEq/Kg, respectively, while the content of red blood cells varies from 4.4 to 6 mEq/L (Valberg *et al.*, 1965 and Seller *et al.*, 1966). Atomic absorption methods show that mean values of magnesium in normal serum vary between 1.6 and 2.1 mEq/L (Alcock *et al.*, 1960; Wacker *et al.*, 1965 and Briscoe & Ragan, 1967). There is no difference between the concentration of the serum of infants and children and that of adults (Silverman & Gardner, 1954). About one-third of plasma magnesium is protein bound (Silverman & Gardner, 1954 and Massry, 1967); the major part of the remaining diffusible fraction is free ionized magnesium (Walser, 1967). Paradoxically, the concentration of magnesium in the cerebrospinal fluid is higher than in serum, it amounts to 2-2.4 mEq/L (Leusen, 1972).

Magnesium activates numerous important enzymes which split and transfer phosphate groups, among them the phosphatases and the many enzymes concerned in the reactions involving ATP. Since ATP is required for glucose utilization, fat, protein, nucleic acid and coenzyme synthesis, muscle contraction, methyl group transfer, sulfate, acetate and formate activation, by interference the activating effect of magnesium extends to all these functions. In addition to thiamine pyrophosphate, magnesium acts as a cofactor in decarboxylation. Certain peptidases are also said to require magnesium for their activity.