STUDIES ON THE IMMUNOLOGICAL SYSTEM OF RATS TREATED WITH CARCINOGENS AND BIOLOGICAL ANTICARCINOGENS

Thesis

Submitted By

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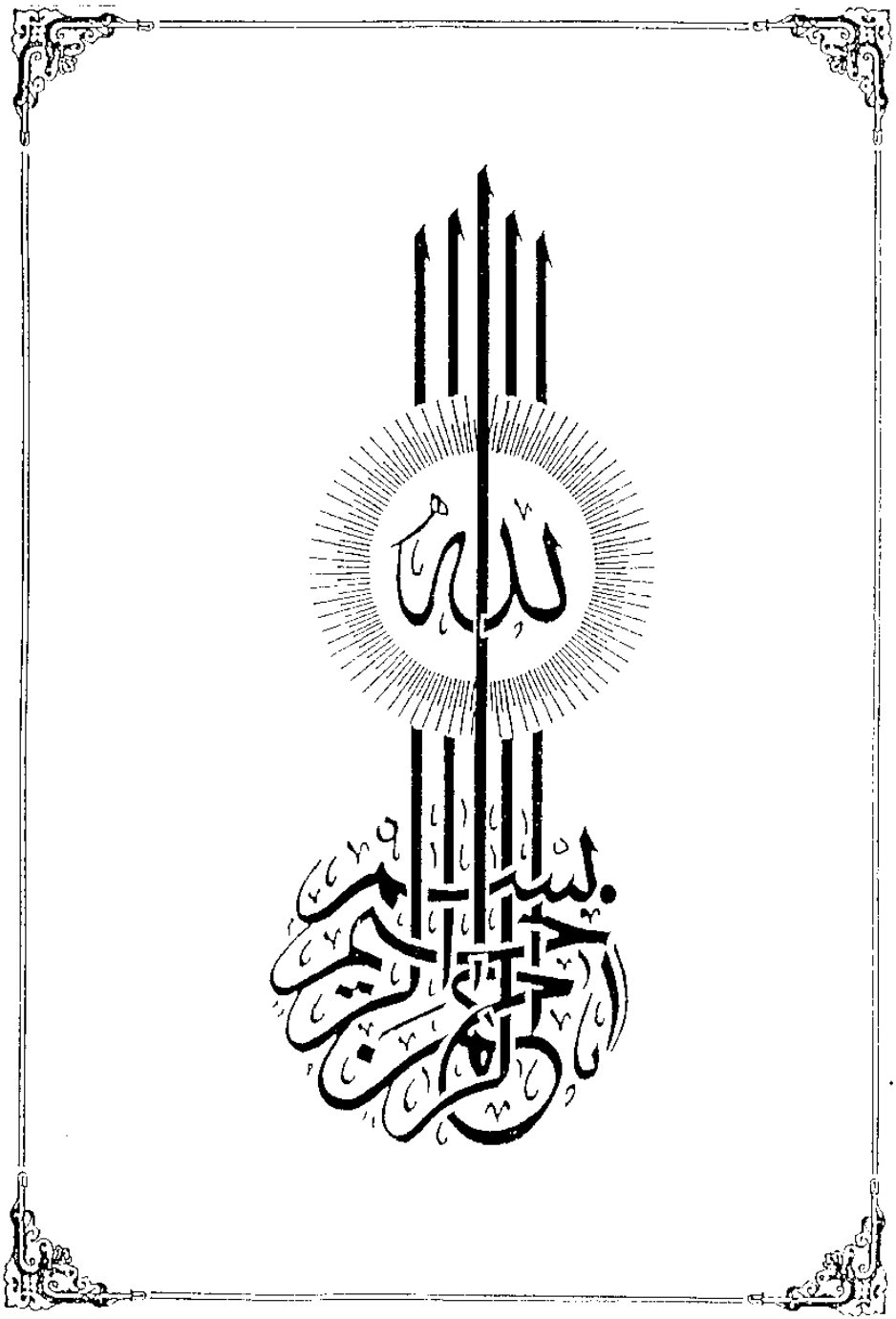
To My Children
Amr and Hebatallah



THIS THESIS HAS NOT BEEN SUBMITTED FOR
A DEGREE AT THIS OR ANY OTHER UNIVERSITY

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ABBREVIATIONS USED

BBN	:	N-butyl-N(4-hydroxybutyl)nitrosamine
C°	:	degree centigrade
cm*	:	centimetre
cpm	:	count per minute
DBA	:	N-dibutylamine
DBN	:	N-dibutylnitrosamine
dl*	:	decilitre
Fig.	:	figure
g*	:	gramme
^3H	:	tritium
HBSS	:	Hanks' balanced salt solution
kg*	:	kilogramme
L*	:	litre
M*	:	molar
mA	:	milliampere
mg*	:	milligramme
min.	:	minutes
ml*	:	millilitre
mm*	:	millimetre
ng*	:	nanogramme
NO_2^-	:	nitrite
N.S.	:	not significant
PHA	:	phytohemagglutinin
rpm	:	round per minute
S.E.	:	standard error
SRBCs	:	sheep red blood cells
μ*	:	micron
μCi*	:	microCurie
μg*	:	microgramme

* Official abbreviations cite in *"Current Therapy."* Edited by Howard H. Cohn. W.B. Saunders Company, Philadelphia, London, Toronto, p. 914 (1979).

PREFACE

Nitrosamines are one of the most potent chemical carcinogens known nowadays. They occur in the human environment and the ease of their formation from precursors abundant in our foodstuffs makes them and their precursors a potential health hazard to man.

More attention has been focused on nitrosamines after confirming that they could also be formed *in vivo* at any site where secondary amines, nitrite or nitrate reducing bacteria occur together. They could be formed *in vivo* in saliva, stomach and urine.

In patients with bacterial infection of the lower urinary tract, it is possible that small amounts of nitrosamines may be produced which could initiate neoplastic changes in the urothelium and furthermore, bladder cancer incidence should be higher in population subjected to chronic urinary tract infections.

The presence of nitrite and amines in human diet may also represent a health hazard. Cheese contains amines, and if consumed together with food rich in nitrite, toxic doses of N-nitroso compounds could be formed in the organism. White cheese, salted cheese and cottage cheese, which are consumed as main daily diets by the Egyptian population are contaminated with nitrosamines.

Linking vegetable consumption with negative risk for certain epithelial cancers, protease inhibitors have been shown to inhibit chemical carcinogenesis. One source of protease inhibitors is the soybean.

Soybean diets were found to protect experimental animals from tumour formation in the skin of mice, in X-ray induced breast cancer in rats and in

spontaneous liver cancer in mice. Soybean was also found to prevent nitrosamine formation from their precursors.

Vitamin C, present in many vegetables and fruits, was also found to inhibit the formation of carcinogenic N-nitroso compounds.

Thus, it is possible that ascorbate and soybean act as good prophylactic agents against cancer induction with chemical carcinogens.

On the other hand, it is known that cancer may result from the breakdown of the normal defense mechanism of the body and that cancer patients have diminished immunocompetence. Vitamin C is essential for the efficient working of the immune system and for the enhancement of the immunocompetence; however, little is known about the influence of soybean on the immune system.

Therefore, the present work was performed on experimental animals to study the possible *in vivo* formation of the nitrosamine from its precursors dibutylamine plus sodium nitrite and to investigate some of of its carcinogenic effects as well as its effects on the immune system. Also, the possible roles of sodium ascorbate and/or soybean in antagonizing the carcinogenic effect of the nitrosamine precursors (dibutylamine plus sodium nitrite) were studied, as well as their effects on the immune system.

The animals used in the present study were "Sprague Dawley" rats. They were divided into 7 groups, one group of rats received the nitrosamine precursors (dibutylamine plus sodium nitrite) dissolved in their drinking water, where the acidic pH of the stomach is suitable for the production of dibutylnitrosamine. Two groups of rats were given sodium ascorbate in the drinking water either alone or mixed with the nitrosamine precursors (dibutylamine plus sodium nitrite). Another two groups of rats were fed mildly autoclaved soybean, either alone or together with the nitrosamine

precursors (dibutylamine plus sodium nitrite). The study also included a group of rats receiving mildly autoclaved soybean together with sodium ascorbate and nitrosamine precursors (dibutylamine plus sodium nitrite).

Monthly samples of the rats from each experimental group as well as a group of normal untreated animals were subjected to the different analytical procedures performed in the present study that included the spleen lymphocyte transformation test, migration inhibition test, the demonstration of Fc receptors and Fc plus complement receptors on rat spleen lymphocytes by the rosette-forming test, the γ-globulin level and the level of the other electrophoretically separated protein fractions in the sera of the rats, the total serum protein level and the activity of the leucocyte alkaline phosphatase enzyme in the blood smears. Tissue sections from the bladder, the liver, the lung and the esophagus were prepared and histologically examined.

Body weight changes, survival rates and tumour incidence were also investigated in another set of animals.

All the results obtained were statistically analyzed and a thorough discussion is given to conclude this thesis and an extensive survey of the references concerning this subject is also included.

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I. INTRODUCTION

INTRODUCTION

There is a widespread concept that cancer results from a breakdown in the normal defense system of the body as a result of an improper diet combined with the effects of pollution and food additives, it is thus believed that this situation can be remedied by products of natural origin and diets that may have, at least in part, a rational basis (Creasey, 1981a). These dietary programs demand the avoidance of processed food, consumption of as many raw items as possible, large amounts of dietary fiber, vitamins and mineral supplements at levels greatly in excess of those officially regarded as adequate (Chope & Breslow, 1956).

Fiber has a major role in maintaining large bowel function. It increases stool out put, dilutes the contents of the colon, affects the absorption and metabolism of the intestinal contents, ensures a faster rate of passage through the gut and helps retain water in the stools (Stephen & Cummings, 1980). Colonic disease, including cancer, may be more common in those with small stool output, who are likely to suffer constipation and to have long gut transit times (Burkitt *et al.*, 1972), and the development of colon cancer might be prevented by increasing the fiber content in diets (Graham & Mettline, 1979). It has been suggested that retention in the gut allows greater metabolism of the contents, especially the bile pigments, to occur, and enhances the possibility that carcinogens are produced and concentrated in the reduced stool bulk (Hill *et al.*, 1971).

Vitamins are also known to inhibit the action of carcinogens. Vitamin A and other retinoids have proved to be capable of retarding or preventing the transformation of a normal to a neoplastic cell. Moreover, the reversion of transformed cells and the regression of certain tumours have even been observed. Based on this phenomenon, a new approach for the prevention of cancer by using

pharmacological doses, at non toxic levels of retinoids, had been developed (Sporn *et al.*, 1977 and Bollag, 1979).

13-cis-retinoic acid inhibited the incidence and extent of preneoplastic and neoplastic lesions of the urinary bladder (Squire *et al.*, 1977 and Becci *et al.*, 1978 & 1979b). Retinoids have preventive roles in the carcinogenesis of chemically induced colon carcinoma (Newberne & Suphakarn, 1977), carcinomas of the respiratory tract (Port *et al.*, 1975 and Cone & Nettesheim, 1976), mammary carcinomas (Grubbs *et al.*, 1977 ; Moon *et al.*, 1976; 1977 & 1979 and Bollag & Matter, 1981), and skin papillomas and carcinomas (Bollag, 1972a; 1972b and 1975).

Vitamin C has been also shown to exhibit inhibitory effects on carcinogenesis (Mirvish *et al.*, 1972; Cameron & Pauling, 1973 & 1974 and Cameron *et al.*, 1979).

Vitamin E was also found to be effective in preventing tumour development in mice suffering from tumours induced with 7,12-dimethylbenz(a)anthracene, and promoted with croton oil or resin (Shamberger, 1972).

Soybean has been also reported to exhibit inhibitory effects on carcinogenesis (Troll *et al.*, 1979a; 1979b & 1980; Kurechi *et al.*, 1981 and Hirayama, 1982).

Selenium has been described as a possible breast cancer-preventing micronutrient since its effectiveness in preventing vitally and chemically induced mammary tumorigenesis was demonstrated in animal experiments (Schrauzer & Ishmael, 1974; Medina & Shepherd, 1980; Clement, 1981; Clement & Sinha, 1981 and Clement & Daniel, 1985), and it was shown that the mortalities from female breast cancer in the U.S. population are lower in regions of the U.S.A naturally high in selenium (Schrauzer *et al.*, 1985).