# THE EFFECT OF DIETARY CONSTITUENTS ON TOXICITY AND CARCINOGENICITY OF AFLATOXIN $B_1$ IN RATS

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# D E C L A R A T I O N

THIS THESIS IS MY ORIGINAL WORK AND HAS NOT BEEN PRESENTED FOR A DEGREE IN ANY OTHER UNIVERSITY

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THIS THESIS HAS BEEN SUBMITTED FOR EXAMINATION WITH OUR APPROVAL AS UNIVERSITY SUPERVISORS

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## KEY TO ABBREVIATION

| | | |
|---|---|---|
| GOT | - | Glutamate oxaloacetate transaminase |
| GPT | - | Glutamate pyruvate transaminase |
| AP | - | Alkaline phosphatase |
| CD | - | Chlorpromazine demethylase |
| $AFB_1$ | - | Aflatoxin $B_1$ |
| DMSO | - | Dimethyl sulphoxide |
| IU/L | - | International Units per litre |
| kg | - | Kilogrammes |
| mg | - | Milligrames |
| NADH | - | Nicotinamide adenine dinucleotide (reduced). |
| NADPH | - | Nicotinamide adenine dinucleotide phosphate (reduced form) |
| PPM | - | Parts per million |
| DMBA | - | Dimethyl Benz (a) Anthracine. |
| GSH | - | Reduced glutathione |

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# THE EFFECT OF DIETARY CONSTITUENTS ON TOXICITY AND CARCINOGENICITY OF AFLATOXIN$_1$. THE HEPATIC MIXED FUNCTION OXIDASE ACTIVITY

## ABSTRACT

A long-term study, using male Wistar rats, was initiated to determine whether the effects of dietary constituents on $AFB_1$ -induced liver cancer could be associated with altered microsomal enzyme activity. They were maintained on mice pellets mixed with specific dietary constituents for 7 days and then given a single carcinogenic dose of $AFB_1$ (500ug/rat). After three months, the dietary constituents were discontinued and the animals were left on mice pellets and drinking water only for a period of about 20 months. At the end of the trial period, it was observed that dietary mixtures containing small quantities of either B-carotene, ascorbic acid, GSH, vitamin E, selenium salt, or uric acid inhibited the development of $AFB_1$ - induced liver cancer and induced increased microsomal enzyme activity at least by 50%. Where B-carotene and uric acid were the most effective inhibitors, vitamin E as was the least, yet a significant inhibitor of liver cancer. Hepatic levels of cytochrome P-450, aniline hydroxylase and chlorpromazine demethylase were significantly induced

in rats fed fortified food followed by $AFB_1$ treatment than in control animals. The inhibition of liver cancer by dietary factors was probably due to their ability to induce the activity of hepatic microsomal enzymes. Increased enzyme activity could lead to rapid activation of $AFB_1$ metabolites that attack cell components. Inhbition of liver cancer is therefore associated with induction of increased microsomal enzyme activity.

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# CHAPTER I

# I N T R O D U C T I O N

## THE BIOLOGICAL POTENCY OF AFLATOXINS

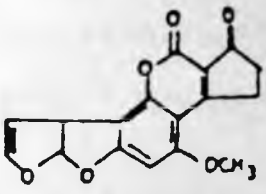
Aflatoxins were discovered as a consequence of an outbreak of disease that resulted in the deaths of many thousands of young turkeys and ducks in England in 1960 and isolated from peanut meal in 1961 (Schoental, 1961). This disease was characterized by lethargy and loss of appetite resulting in death within a week.

A similar outbreak of the disease occurred simultaneously in ducklings in Kenya and Uganda (Allcroft, 1969). The source of the toxin that caused the disease in animals in England was traced to a shipment of Brazilian peanut meal used as a protein supplement in poultry feeds. The meal proved to be highly toxic for many species of animals and from it the common fungus known as Aspergillus flavus was isolated.

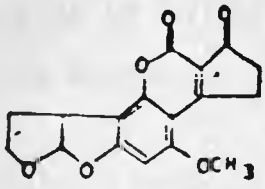
Subsequent to the observations in England and Africa, cancers of the liver in trout associated with these toxins were observed in broad stock fish from hatcheries throughout the United States. These tumours in fish were eventually tranced to rations that contained

either aflatoxin - contaminated peanut meals or cotton seed meal (Newberne, 1984). In the intervening years since the early reports became available, it has been revealed that virtually every domestic and laboratory animal species is sensitive to either the toxic or carcinogenic effects of aflatoxins. Studies in Kenya and elsewhere have, therefore, been mounted to assess whether these toxins, which are available on many cereals in rural areas, play a role in the genesis of liver tumours.

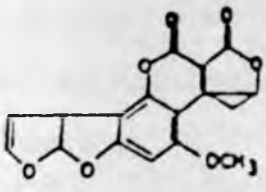
Structure of the four naturally occuring Aflatoxins.



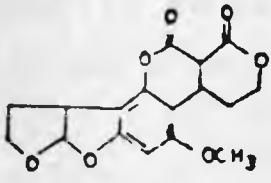
Aflatoxin $B_1$



Aflatoxin $B_2$



Aflatoxin $G_1$



Aflatoxin $G_2$

## Occurrence Aflatoxins

Chemically, the aflatoxins are a family of Coumarins that have a fused dihydrofurofuran configuration peculiar to a limited number of compounds of natural origin. The compunds occur in two series, aflatoxin $B_1$ and derivatives and aflatoxin $G_1$ and derivatives. Their structures are shown in Fig. 1.

The aflatoxins are produced by only a few strains of Aspergillus flavus and Aspergillus parasiticus, whose spores are widely distributed, especially in soil, microflora, air and found living on or in living or dead plants and animals throughout the world.

With respect to substrate, requirements for toxin production are relatively non-specific, and the mold can produce the compounds on virtually any food (or indeed on simple synthetic media) that will support growth. Thus any food material can be subjected to aflatoxin contamination if it becomes moldy, due to humidity.

## Aflatoxicosis

The toxicity and biochemical effects induced in various biological test systems by aflatoxins have been

extensively studied and only a brief summary is possible. Acute or sub-acute poisoning can be produced in animals by feeding aflatoxin contaminated diets or by dosing with purified compounds (Wogan, 1973). Symptoms of poisoning are produced in most domestic animals by aflatoxin levels in the feed of 10 - 100 mg/kg body weight or less (Newberne, Buttler, 1969). As regards lethal potency to experimental animals, the oral or parenteral $LD_{50}$ values are generally in the range 5-15 mg/kg body weight for aflatoxin $B_1$. The value for trout, for the most sensitive species is less than 0.5 mg/kg. In both acute and subacute poisoning, the liver is the main target organ for aflatoxin $B_1$. The chief pathologic lession in liver associated with acute or chronic toxicity are periportal or centrilobular necrosis, bile duct proliferation, and in some species, cirrhosis (Wogan, 1973).

Many of the biochemical changes induced in various biological systems by aflatoxin $B_1$ follow a consistent pattern. Administration of the toxin to rats is quickly followed by pronounced inhibition of DNA and RNA polymerases in liver and similar responses have been observed in human and animal cell cultures. Protein synthesis is also impaired, particularly under conditions

where synthesis is strongly influenced by alterations in de novo RNA synthesis (Wogan, 1973). Available evidence indicates that polymerase inhibition is an indirect sequence of impared template activity of chromatin subsequent to toxin chromatin interaction. Consequently, interaction between aflatoxin or some derivative of it with DNA or another component of chromatin is viewed as the intiating event in the observed series of reactions (Wogan, 1973). Another line of evidence that may be related to the mode of action of the toxins deals with their ability to interact with membrane of endoplasmic reticulum and thereby alter polysomal binding to those membranes. However, the available evidence is as yet inadequate to explain in detail, the biochemical basis of the cytotoxicity effects of these toxins.

Here in Kenya, Ngindu and coworkders (1982) reported an outbreak of aflatoxin poisoning in Makueni Division of Machakos District. In this incidence, 20 cases of aflatoxicosis occured after consumption with aflatoxin. 12 of these cases were fatal. A more serious incidence of aflatoxin poisoning from the ingestion of heavily contaminated maize meal was reported from India in which 100 fatalities were recorded by Krishnamachari

(1975). There were similarities in these two incidences in that death in dogs preceded human illness and the unweaned infants were not affected by the poison.

Depending on harvesting practices and processing techniques, the aflatoxins can be sporadic, frequent or almost constant contaminants of oil seeds (such as peanuts and cottonseed) and grains (such as corn). Sorghum and Cassava are less frequent sources of the aflatoxins. Less obvious sources have been garlic, dried chilli peppers and dried fish (Shank et al., 1972).

AFLATOXIN CARCINOGENESIS

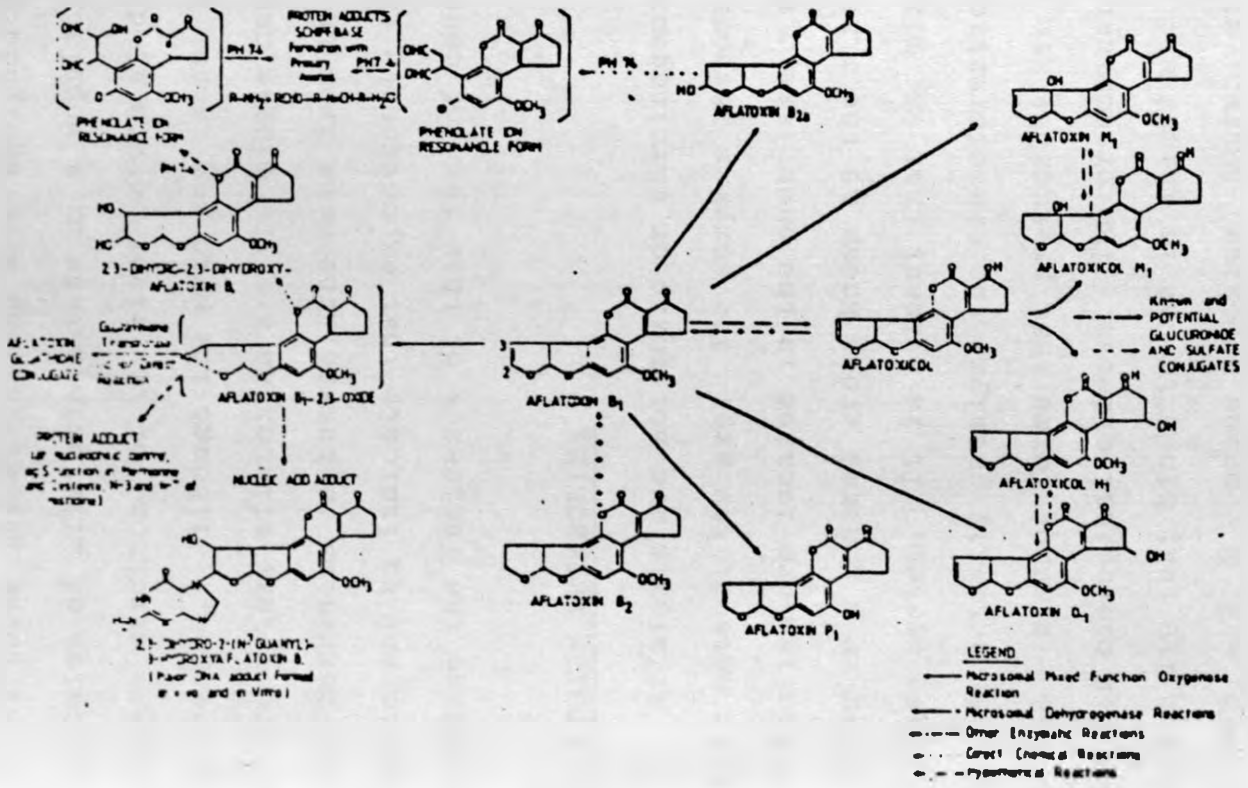
Aflatoxins have been shown to have carcinogenic activity in many species of animals including rodents, non human primates, birds and fish. The primary organ for aflatoxin $B_1$ in most rat strains is the liver in which it induces hepatocellular carcinomas and cholangio-carcinomas among other lesions. However, under some circumstances, significant incidence of tumours at sites other than liver has been recorded (Newberne & Rogers, 1973). Tumours of other tissues have been observed in aflatoxin $B_1$ treated rats include carcinomas of the glandular stomach and adenocarcinoma of the colon.

The majority of published information of aflatoxin carcinogenicity deals with experiments in rats because they are more susceptible to the toxin. Many early experiments involved the feeding of diets containing aflatoxin contaminated foodstaffs. Levels of the toxin were determined by chemical assays and manipulated by dilution with uncontaminated material. As a group, these experiments established the fact that aflatoxin levels of 0.1 mg/kg diet and higher consistently induced liver carcinogenesis in rats Wogan (1973) Buttler (1964).

Liver carcinomas have been induced in subhuman primates and althogh the total number of positive response is very small, it seems clear that aflatoxin $B_1$ is an effective carcinogen for the livers of Monkey, Madhavan, et al. (1972), Tree Shrew and Duck; Carnagha (1965) and Marmoset; Groopman J.D., et al. (1986).

A positive correlation has also been demonstrated between aflatoxin $B_1$. Information is available from studies carried out by several groups of investigators which demonstrated a positive correlation between aflatoxin ingestion and liver cancer incidence in human, Alpart and Davidson (1969), Peers and Linsel (1973).

# METABOLIC TRANSFORMARTIONS OF AFLATOXIN $B_1$



All of these investigations were designed to obtain estimates of aflatoxin intake of a population in which primary carcinoma of the liver occurs at different incidences. Although the evidence cannot be regarded as proof that aflatoxins are the single cause of liver cell carcinoma in humans, the data from the studies is sufficient to indicate that exposures to the carcinogen increase the incidence of this form of cancer.

## AFLATOXIN METABOLISM

Aflatoxin are not toxic or carcinogenic per se, but their metabolites are. The enzymes responsible for their metabolism are located in the endoplasmic reticulum and belong to a general group known as the mixed function oxidase system. It is evident that the mixed function oxidase activity catalyze the transformation of $AFB_1$ to DNA - binding forms that presumably are responsible for its biological effects. The predominant $AFB_1$ metabolite that binds to DNA in animal and human tissues is $AFB_1$ - 2, 3 - oxide (epoxide) Autrup, et al. (1978). All of the metabolic transformations of aflatoxin $B_1$ known to take place in animals are indicated by the pathways outlined in Fig. 2. $AFB_1$ and to a lesser extent, $AFG_1$ are responsible for the biological potency of aflatoxin - contaminated foodstuffs. These two

toxins posses an unsaturated bond at position 2 and 3 on the terminal ring whereas $AFB_2$ and $AFG_2$ are saturated at these positions.

Besides their activation, aflatoxins can be dextoxified by the same enzyme system to soluble compounds that are excreted in urine. The detoxification of $AFB_1$ is accomplished by enzymatic conjugation of the hydroxylated metabolites with sulfate or glucuronide esters taht are excreted in urine. Another route for removal of $AFB_1$ from the body involves the enzyme catalyzed reaction of the epoxide metabolite with glutathione and its subsequent excretion in the bile.

Interest in cancer chemotherapy using anti-oxidants has recently produced evidence indicating that protective effects of these compounds (anti oxidants) arise from increased carcinogen inactivation by enzymatic activity. Since many carcinogens are metabolized by the hydroxylating enzymes of the liver microsomes, it seems possible that induction of microsomal enzyme activity would also alter hepato-toxicity and carcinogenesis of $AFB_1$. This alteration may either be the activation of the carcinogen compound into its reactive form that attack cells components or its inactivation into a harmless metabolite Miller (1970). Experiments were therefore carried out to determine if there were any changes in

aflatoxin carcinogenesis in animals given accessory foodstaffs such as vitamins.

INITIATION OF LIVER CANCER.

Initiation is the induction of an essential irreversible genetic changes that results in increased cell volume of malignant transformation. In liver initiation results from exposure to either a high or a low dose given once or severally. This process is accomplished rapidly and is irreversible. The initiated cell will develop into a tumour if the tissue is exposed repeatedly to a tumour promoter.

Cellular metabolism plays an important role in initiation and promotion of tumour growth. This is because most chemical carcinogens are inactive and therefore require metabolic activation to exert their biological effects. They are metabolised mainly by the mixed function oxidase activity into highly reactive derivatives that react with cell components such as DNA, RNA and protein Miller (1970). The activation of a carcinogenic compound into its reactive form is probably accompanied by several competing pathways resulting in production of both carcinogenic and non-carcinogenic metabolites, Miller (1970). Cellular metabolism may

therefore be an important step for both activation and inactivation of many chemical carcinogens. The ultimate carcinogenic form will bind to DNA to form carcinogen - DNA adduct. The identification and the metabolism of these DNA - adducts has been demonstrated in animals Lin, et al (1971); Austrup, et al. (1975).

## INHIBITION OF CARCINOGENESIS BY DIETARY FACTORS

Specific environmental chemicals can promote or inhibit the process of chemical carcinogenesis. Most of the inhibitors presently known are synthetic compounds and natural food products of vegetable origin such as vitamins and mineral salts. It has been suggested that good nutrition protects against the carcinogenic effects of $AFB_1$ Newberne and Rogers (1973). There is epidemiological evidence that consumption of vegetables in the diet can reduce the incidence of certain tissue specific cancers Graham, et al (1978). There is also evidence that certain naturally occurring constituents of common vegetables can inhibit the induction by $AFB_1$ of liver tumours Wattenberg and Loub (1978).

Food high in fibre, or rich in vitamins A, C and E or in the mineral selenium and the indole compond (food in cabbage family), are linked to prevention of cancer Chen and Menguia (1986).

It has been suggested that upto 90 per cent of human cancers are associated with environmental factors Higginson(1968). Fifty per cent of deaths are from cancers possibly related to dietary factors Wynder and Gori (1977). Several dietary factors, such as retinoids, carotenoids, selenium, vitamin A, different growth factors (protease inhibitors) and certain nutrients factors required by many cell lines, are reported to inhibit tumour growth in experimental animals. In fully formed tumours, dietary lipids, proteins and hormones may affect growth by influencing tissue growth factors and hormones Chen and Menguia (1986).

Restriction of total diet or the number of calories fed to rats and mice inhibits the formation of tumours in several tissues. Vitamins have specific effects on the activity of certain carcinogens. There is adequate information available on vitamin A which has been shown to inhibit or enhance carcinogenesis and vitamin C which by reducing sodium nitrite prevents nitrosation of secondary and tertiary amines. Shamberger (1976) showed that topically applied carotene reduced the incidence of papillomas induced by DMBA. The inhibitory effect of vitamin A on carcinogenesis was suggested to be due to distabilization of the lysosomes of premalignant cell Shamberger (1969).

## OBJECTVES

The nutritional state of an animal may influence greately its response to toxic and carcinogenic compounds that are handled by the liver. Many of these compounds are metabolized by the hydroxylating enzyme system of the liver microsomes. Hydroxylation and dimethylation may be an essential reaction in the activation of the carcinogenic compounds into the reactive forms that attack cell components. These reactions may also result in inactivation of these compounds into harmless metabolites that are excreted out of the body.

Since hydroxylating and dimethylating enzyme systems of the liver are highly responsive to changes in the diet, it is important to evaluate the relationship between the drug-metabolizing enzymes and the plasma level of marker enzymes of liver disease. Aflatoxin $B_1$ ($AFB_1$) was chosen as an example of hepatotoxic and hepatocarcinogenic compound and investigated its action on enzyme activities in rats in response to the presence and absence of dietary protein and accessory food factors such as vitamins and mineral rats.

In order to evaluate the mechanism in action of the toxin on the liver, the effects of aflatoxin $B_1$ on the enzymes involved in its transformation, such as aniline hydroxylase, chlorpromazine demethylase and cytochrome P-450 were studied. At the same time, liver damage was determined by measuring the activities of plasma alkaline phosphatase, glutamate pyruvate transaminase (GPT), and glutamate oxaloacetate transaminase (GOT).

## EFFECTS OF $AFB_1$ AND ETHANOL ON DEFICIENT RATS

### EXPERIMENTAL ANIMALS.

Male albino wistar rats bred in our animal house were used. They were housed in plastic cages and fed on mice pellets from Unga Feeds Limited. Aflatoxin analysis carried out on the Mice pellets revealed no contaminations. $AFB_1$ was administered to experimental growth by gastric intubation and was dissolved in 0.2 ml of dimethyl sulphoxide (DMSO).

### CHEMICALS

Crystaline $AFB_1$ was obtained from sigma chemica GMBH Munchem FRG. NADP-NA, NADPH, NADH and Isocitrate dehydrogenase were obtained from Boehringer Manhein FRG. Isocitrate sodium salt was from Serva Frehbichemica Heidelberg. All other chemicals including carbon monoxide were of laboratory reagent grade.

### TREATMENT OF ANIMALS

Male wistar rats weighing 100-150g were randomly divided into seven groups, each consisting of 4 rats and treated as follows:-

GROUP 1: Rats received normal diet and drinking water alone throughout the experimental period.

GROUP 2: Rats received normal diet and 20% v/v ethanol as the only source of drinking water daily.

GROUP 3: Rats received normal diet and daily dose of 25ug $AFB_1$ for the first week, 50ug $AFB_1$ for the second week and 100ug $AFB_1$ for the third week. Treatment was done for five days a week.

GROUP 4: Rats received normal diet plus ethanol and $AFB_1$ and treated as in group 3.

GROUP 5: Rats received a protein deficient diet and drinking water throughout the experimental period.

GROUP 6: Rats received protein deficient diet and 20% v/v ethanol as the only source of drinking water.

GROUP 7: Rats received protein deficient diet and ethanol with a daily dose of $AFB_1$ as in group 3..

COMPOSITION OF ANIMAL DEITS

The normal diet was Commercial Pellets of rat (oxoid modified diet), with distribution of protein, fat and carbohydrate. The percentage of protein was altered (lowered) to give a very low percentage of the protein in the diet thus qualifying it as the protein deficient diet. These dietary components were supplied by Unga Limited, Nairobi. The anti-oxidants were commercially supplied high grade chemicals.

PREPARATION OF ENZYME FRACTIONS.

At the end of the trial period, rats were sacrificed by decapitation, the livers were quickly removed and washed in ice-cold 0.9% Nacl solution and homogenised with six volumes of a buffer containing o.25M sucrose, o.03M Tris-HC1, 5.5mM EDTA, pH 7.5M. The homogenate was centrifuged for twenty minutes at 12,000g in a Beckman refrigerated centrifuge (at Kenya Medical Research Institute). The supernatant was pelleted by the same Beckman refrigerated Ultra-centrifuge at 105,000g for sixty minutes. The microsomal pellets were washed once with a buffer containing 0.12M KC1 and 0.05M Tris-HC1 PH 7.5. The pellets were resedimented again by the Beckman refrigerated Ultra-centrifuge at 105,000g for sixty minutes resuspended in the same buffer to give a final protein concentration of 2mg.

Protein Estimation

Protein estimation was based on the colorimetric determination of the Lowry method, Lowry, et al (1957). The blue colour formed is said to be due to a complex between the alkaline copper phenol reagent and the tyrosine and trytophan residue of the protein.

Preparation of Carbon Monoxide

Carbon monoxide was prepared from the reaction of

concentrated sulphuric acid on formic acid. The released gas was passed through three washings, water sodium hydroxide and water again before being passed through cotton gauze and used directly in bubbling into the cuvette containing microsomal fraction for thirty seconds.

Determination of Cytochrome P-450 and b5.

Cytochrome P-450 was estimated as described by Omura and Sato (1964) from the carbon monoxide differences spectra. The measurements were made in a Perkin Elmer 550s recording Spectrophotometer. Immediately before measurement, a few grams of sodium dithionate were added to both sample and reference cuvettes. After base line were recorded, carbon monoxide was carefully bubbled through the sample cuvette for 20 seconds and differences in absorbance between 600nm and 380nm recorded.

Microsomal Incubation

The incubation medium for in vitro tests was prepared in 25ml Erlenmeyer Flask with a final volume of 5.0ml. The incubations were carried out in a shaking water bath at 30° C. The incubation medium contained 50mM buffer (containing 0.12M KCI and 0.05M Tris-HCI $P^H$ 7.5). 5 µmoles $MgCl_2$ 1 µmole NADP - Na, 8 µmole isocitrate - Na and 16 µmole of aniline or Chlorpromazine substrate.

After preincubation for 5 minutes, the reaction

was started by the addition of 0.5ml aniline or chlorpromazine substrate.

Immediately after the addition of the substrate, 1.5ml of the incubation medium was taken off and pipetted into a test tube containing 7.5% TCA for (Chlorpromazine) or 15% TCA (for aniline). This was for zero (0) time determination. Further reaction was followed by taking off a sample after 2.5min and 5min (Chlorpromazine) or 10 and 20 minutes (aniline). The protein was completely precipitated by TCA by storing the samples at 4°C for at least 30 minutes. The samples were then centrifuged for 10 minutes at 5000rpm and the supernatant was used to determine the reaction products.

Chlorpromazine demethylase activity

Chlorpromazine demethylase activity was determined by measuring formaldehyde produced using the pH6.0 Nash reagent (Nash,1953) as follows: 1.5ml TCA supernatant plus 1.5m, Nash reagent was mixed and incubated for 12 minutes in a water bath at 58°C. The samples were cooled and extinction was read at 412nm.

Aniline hydroxylase activity

Aniline hydroxylase was determined by the method of Imai and Sato (1966) as follow:- 1.5ml TCA supernatant

plus 1.5ml phenol reagent plus 1.5ml 1M $Na_2CO_3$ solution was mixed and left at room temperature for 30 minutes before taking readings at 630nm ($500_s$ Perkin Elmer Spectrophotometer).

Plasma Enzyme Determination

The activity of plasma alkaline phosphatase was determined according to the method described by Berger and Rudoph(1965) and was expressed as International Units per litre of plasma (IU/L).

Plasma levels of glutamate oxaloacetate transaminase (GOT) was assayed in a Spectrophotometer ($55_s$) as described by Karmen (19559 and the activity was expressed as International Units per litre of plasma. The activity of glutamate pyruvate transaminase (GPT) in serum was assayed by Spectrophotometric techniques as described by Wroblewski and La Due (1956) and expressed as International Units per litre of serum.

## EXPERIMENTAL RESULTS

### a) The effect of $AFB_1$ and Ethanol

In table 1, body weight of rats fed with Aflatoxin $B_1$ and at different diet status, were compared. The average weights were between 100-250gms at the start

# CHAPTER TWO

## R E S U L T S

Table 1

The effects of $AFB_1$ dosage and alcohol on the growth of dietary protein deficient rats

| | Nutrition status | $AFB_1$ Given 500ug | 250ug | 125ug |
|---|---|---|---|---|
| 1. | Normal diet (No $AFB_1$) | 231 ± 8.54 | 240.13 ± 3.66 | 207.50 ± 2.01 |
| 2. | Normal diet + Ethanol (No. $AFB_1$). | 103.75 ± 8.54 | 66 ± 1.83 | 166.50 ± 3.11 |
| 3. | Normal diet + $AFB_1$ | 204.25 ± 3.97 | 101.25 ± 17.02 | 167.75 ± 5.32 |
| 4. | Normal diet + $AFB_1$ + Ethanol | 136.5 ± 2.45 | 55.00 ± 12.91 | 190.25 ± 4.11 |
| 5. | Deficient diet (No $AFB_1$) | 94.75 ± 9.54 | 109.00 ± 7.87 | 124.5 ± 9.20 |
| 6. | Deficient diet + Ethanol (No $AFB_1$). | 82.5 ± 13.02 | 66.75 ± 0.50 | 66.25 ± 4.72 |
| 7. | Deficient diet + $AFB_1$ + Ethanol | 56.5 ± 1.85 | 71.50 ± 1.91 | 117.25 ± 6.08 |

Values are mean ± S.D. They are averages of 4 animals. (n=4).
Weight was given in grammes. Animals in groups with both normal diet and protein deficient diet were not given AFB, but were sacrificed at the same time with the other animals fed with different amounts of aflatoxin.

of experiments. The weight differences were not very significant over the range of diets. As can be seen, there was a small difference between the weights before and after feeding with aflatoxin $B_1$. Also, there was no difference in weight increase as the amount of aflatoxin given was increased.

The drop in weight seen in animals given protein deficient diet and alcohol and also $AFB_1$ was quite low. It was also low in animals given alcohol and protein deficient diet alone. The above low levels in protein deficient rats could not be explained on the basis of Aflatoxin doses alone. It is known that chronic alcohol intake retards the body growth rate and, because of protein deficiency, no increase in body weight could be expected.

b) Plasma Enzyme levels in Deficient Rats

Table 2 shows the levels of plasma enzymes as affected in each dietary status in response to different $AFB_1$ dosage given. There was a general increase in levels of plasma enzyme as the level of aflatoxin dose was increased from 125ug to 500ug, but the increases were more distinct in protein deficient animals fed alcohol and aflatoxin. These levels are indication to the status of the liver and the increases are indication of the damage on the liver as the levels of aflatoxin was increased.

Table 2

Plasma enzyme levels in dietary protein deficient rats in response to different AFB dosage.

| | Nutrition status | 500ug | | | 250ug | | | 125ug | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | GOT | GPT | AP | GOT | GPT | AP | GOT | GPT | AP |
| 1. | Normal diet (No $AFB_1$) | 56.00 ±0.82 | 102.8 12.22 | 259.20 ±1.29 | 49.50 ±1.30 | 100.75 ±2.30 | 261.25 ±2.05 | 49.75 ±1.29 | 100.50 ±2.33 | 266. 75 ±2.01 |
| 2. | Normal diet + $AFB_1$ | 166.30 ±0.29 | 160.32 ±0.70 | 360.50 ±1.29 | 52.00 ±1.87 | 146.00 ±1.83 | 316.73 ±4.20 | 49.50 ±4.20 | 112.00 ±5.72 | 219.50 ±0.58 |
| 3. | Normal diet AFB + Ethanol | 157 ±1.29 | 190.15 ±0.24 | 406.0 ±2.56 | 66.0 ±4.32 | 121.50 ±2.65 | 337.25 ±4.86 | 52.50 ±2.08 | 73.25 ±4.65 | 269.75 ±3.30 |
| 4. | Deficient Diet.(No $AFB_1$) | 113.25 ±5.56 | 173.25 ±5.56 | 352.25 ±0.96 | 71.75 ±6.24 | 120.0 ±3.74 | 372.5 ±4.80 | 56.00 ±2.16 | 71.00 ±0.638 | 263.75 ±10.66 |
| 5. | Deficient Diet + Ethanol (No $AFB_1$) | 126.00 ±1.83 | 201 ±1.41 | 466.75 ±2.63 | 64.75 ±3.77 | 134.5 ±3.42 | 400 ±9.97 | 64.50 ±3.42 | 60.75 ±0.65 | 302.50 016.58 |
| 6. | Deficient Diet AFB and Ethanol | 99.00 ±1.30 | 150.75 ±0.79 | 540.50 ±0.79 | 89.50 ±13.32 | 142.25 ±2.22 | 441.50 ±6.70 | 68.00 ±4.32 | 92.50 ±3.51 | 346.00 ±2.16 |

Values are mean ± S.D. in IU/litre n = 4 animals.
GOT - Glutamate Oxaloacetate Transaminase, GPT - Glutamate Pyruvate Transaminase and AP - Alkaline Phosphatase.

Animals on normal diet and deficient diet given Aflatoxin but treated as for group I. were no

c) Changes in Microsomal Enzymes.

Table 3 and 4 show the changes in cytochrome P-450 hydroxylation of aniline and demethylation of chlorpromazine and cytochrome $b_5$ in response to varying dosages of aflatoxin $B_1$ administered by gastric intubation. There was significant decrease in all the four parameters, as the dose of aflatoxin was increased.

The decreases were more clearly defined for aniline hydroxylase, cytochrome P-450 and cytochrome $b_5$. For the levels of chlorpromazine, the decrease was not so clearly defined but it was still quite low in animals deficient in diet.

Table 3

Effects of chronic administration of Aflatoxin $B_1$ with or without alcohol on microsomal cytochrome P-450 and $b_5$.

| Nutrition status | 500ug | | 250ug | | 125ug | |
|---|---|---|---|---|---|---|
| | cyt P-450 | cyt $b_5$ | cyt P-450 | cyt $b_5$ | cyt P-450 | cyt $b_5$ |
| Normal diet (No $AFB_1$) | 0.78 ±0.03 | 0.70 ±0.01 | 0.60 ±0.01 | 0.60 ±0.15 | 0.77 ±0.02 | 0.54 ±0.02 |
| Normal diet $AFB_1$ | 0.49 ±0.01 | 0.41 ±0.01 | 0.5 ±0.01 | 0.54 ±0.01 | 0.66 ±0.02 | 0.55 ±0.01 |
| Normal diet + $AFB_1$ Ethanol | 0.30 ±0.01 | 0.39 ±0.01 | 0.49 ±0.01 | 0.51 ±0.01 | 0.49 ±0.01 | 0.52 ±0.01 |
| Deficient diet (No $AFB_1$) | 0.30 ±0.01 | 0.32 ±0.02 | 0.39 ±0.02 | 0.41 ±0.01 | 0.43 ±0.02 | 0.42 ±0.01 |
| Deficient diet + $AFB_1$ + Ethanol | 0.26 ±0.18 | 0.23 ±0.16 | 0.37 ±0.02 | 0.43 ±0.01 | 0.46 ±0.01 | 0.41 ±0.02 |

The activities of cytochrome P-450 and $b_5$ were given in nmoles/mg protein/ millitre.

Values are mean ± S.D. from 4 animals. (n=4 ).

Animals on normal diet & deficient diet treated as for table 1

Table 4

Changes of Chlorpromazine-N-demethylase activity and aniline hydroxylase activity after Chronic Aflatoxin administration.

| Nutrition | Quantity of $AFB_1$ given | | | | | |
|---|---|---|---|---|---|---|
| | 500ug | | 250ug | | 125ug | |
| | AH | CD | AH | CD | AH | CD |
| Normal diet given (No $AFB_1$) | 1.06 ±0.09 | 15.53 ±0.46 | 1.06 ±0.06 | 10.15 ±0.13 | 1.04 ±0.06 | 11 ±0.45 |
| Dietary protein + $AFB_1$ | 0.29 ±0.01 | 19.51 ±0.38 | 0.34 ±0.03 | 19.4 ±0.29 | 0.42 ±0.01 | 17.0 ±0.06 |
| Dietary protein + $AFB_1$ + Ethanol | 0.33 ±0.01 | 17.2 ±0.29 | 0.42 ±0.01 | 16.8 ±0.22 | 0.62 ±0.01 | 16.15 ±0.13 |
| Protein deficient (No $AFB_1$) | 0.26 ±0.01 | 10.01 ±0.17 | 0.45 ±0.01 | 12.3 ±0.050 | 0.76 ±0.01 | 14.53 ±0.41 |
| Protein deficient diet + Ethanol (No $AFB_1$) | 0.33 ±0.02 | 16.1 ±0.11 | 0.43 ±0.02 | 16.1 ±0.65 | 0.57 ±0.03 | 19.6 ±0.63 |
| Protein deficient diet + $AFB_1$ + Ethanol | 0.26 ±0.18 | 12.1 ±0.89 | 0.44 ±0.03 | 15.3 ±0.57 | 0.52 ±0.01 | 19.3 ±0.49 |

AH - Aniline hydroxylase given nmoles of P-aminophenol formed/mg protein/minute

CD - Chlorpromazine Demethylase (nmoles of Formaldehyde/mg protein/minute

Values are mean ± S.D.

# CHAPTER THREE

## INHIBITION OF AFLATOXIN CARCINOGENESIS

### 1. Treatment of Rats

48 albino wister male rats aged 7 and 8 weeks and weighing 120 to 130 grams were started on an experiment designed to test for the inhibitory effects of a variety of dietary factors on $AFB_1$ - induced tumours of the liver. The rats were divided into 8 groups of six rats each and housed in different plastic cages. Each group was treated with $AFB_1$ administered by gastric intubation and was dissolved in 0.2ml DMSO.

GROUP 1:- The rats in this group were fed on crushed mice pellets and received water ad libitum for 7 days. On the 8th day, they were given 500ug/kg body weight of DMSO each using stomach tube.

GROUP 2:- Rats in this group were fed on crushed mice pellets and received water ad libitum for 7 days. On the 8th day, they were given a carcinogenic dose of 500ug/kg body weight of $AFB_1$ each using stomach tube.

GROUP 3:- Rats in this group were fed on crushed mice pellets mixed with 1mg/kg body weight of β-carotene and water for 7 days. On the 8th

day, each rat was given by stomach tube a carcinogenic dose of $AFB_1$ (500ug/kg body weight)

GROUP 4:- Rats in this group were fed on mice pellets mixed with 10mg/kg body weight of ascorbic acid for 7 days. Water was given ad libitum daily. On the 8th day, a carcinogenic dose of 500ug/kg body weight of $AFB_1$ was administered to each rat.

GROUP 5:- Rats in this group were fed on mice pellets mixed with reduced glutathione (1mg/kg body weight) and water ad libitum. On the 8th day, each rat received a carcinogenic dose of $AFB_1$ (500ug/kg body weight).

GROUP 6:- Rats were fed on crushed mice pellets mixed with 1mg/kg body weight of vitamin E and water for 7 days. On the 8th day, each rat was given $AFB_1$ dose (500ug/kg body weight).

GROUP 7:- Rats were fed on mice pellets mixed with 1ug/kg body weight of selenium and water for 7 days. On the 8th day, each rat received a carcinogenic dose of 500ug/kg body weight of $AFB_1$.

Group 8:- Rats received crushed mice pellets mixed with uric acid (1mg/kg body weight) and water for

7 days. On the 8th day, each animal was given a carcinogenic dose of $AFB_1$ (500ug/kg body weight).

All rats were left to continue on their respective dietary conditions for a further three weeks after which they were left on mice pellets and drinking water only until they were sacrificed, that is, 22 months later.

2. Enzyme Assays.

At the end of the trial period, the surviving rats were killed by decapitation and the blood was collected for plasma enzyme determinations. The livers were homogenized for microsomal preparations for enzyme assays as described in Chapter two.

3. Histological examination

At autopsy, small pieces of fresh liver were fixed in 10% neutral buffered formalin, processed for routine histological methods, and examined in sections stained by hematoxylin and eosin for tumour incidence. The livers of aflatoxin fed group were grossly abnormal with greenish or whitish brown tumours (Fig. 3).

Table 5

Inhibition of Liver tumour growth by anticarcinogenic agents

| . | Dietary mixtures | Initial No. | Mortality | Tumour | Non Tumours | % age tumours |
|---|---|---|---|---|---|---|
| 1. | Control diet + DMSO | 6 | 0 | 0 | 6 | 100 |
| 2. | Control diet + $AFB_1$ | 6 | 1 | 4 | 1 | 80 |
| 3. | $AFB_1$ + 1mg b-carotene | 6 | 0 | 0 | 6 | 0 |
| 4. | $AFB_1$ + 10mg ascorbic acid | 6 | 1 | 1 | 4 | 20 |
| 5. | $AFB_1$ + 1mg GSH | 6 | 2 | 1 | 3 | 25 |
| 6. | $AFB_1$ + 1mg vitamin E | 6 | 2 | 2 | 2 | 50 |
| 7. | $AFB_1$ + 1mg Selenium | 6 | 1 | 1 | 4 | 20 |
| 8. | $AFB_1$ + 1mg Uric acid | 6 | 4 | 0 | 2 | 0 |

Rats were fed on mice pellets mixed with various dietary constituents for 7 days and then given a carcinogenic dose of $AFB_1$ (500ug/rat) by stomach tube. They were left to continue with their respective dietary mixtures and develop tumours. They were sacrificed after 22 months on these dietary constituents. The percentage tumour production was based on animals that survived for the entire experimental period.

Table 6

Effects of anti-circinogenic agents on Rat liver microsomal enzymes

| | Dietary mixtures | Cytochrome P-450 | Aniline | Chlorpromazine demethylase |
|---|---|---|---|---|
| 1. | Control + DMSO | 0.65 ± 0.01 | 0.85 ± 0.05 | 10 ± 0.65 |
| 2. | Control + $AFB_1$ | 0.75 ± 0.03 | 1.09 ± 0.09 | 12 ± 0.90 |
| 3. | $AFB_1$ + Ascorbit Acid | 0.90 ± 0.03 | 1.55 ± 0.14 | 18 ± 2.00 |
| 4. | $AFB_1$ + B-carotene | 1.81 ± 0.08 | 2.11 ± 0.15 | 21 ± 3.21 |
| 5. | $AFB_1$ + GSH | 0.90 ± 0.02 | 1.50 ± 0.08 | 15 ± 0.95 |
| 6. | $AFB_1$ + Vitamin E | 0.83 ± 0.04 | 1.29 ± 0.07 | 14 ± 0.87 |
| 7. | $AFB_1$ + Selenium | 0.88 ± 0.03 | 1.44 ± 0.09 | 17 ± 3.11 |
| 8. | $AFB_1$ + Uric acid | 1.79 ± 0.09 | 2.14 ± 0.10 | 23 ± 2.11 |

The quantity of $AFB_1$ and of each dietary constituents are given in Table I.
Cytochrome P-450 (nmoles/mg protein) aniline hydroxylase nmoles (amino phenol/mg protein)
Chlorpromazine demethylase (nmoles formaldehyde/mg protein
Values are ± mean S.D. of 6 rats. (n=6).

## 4. EXPERIMENTAL RESULTS

### A) Inhibition of Tumours by Dietary Factors

Table 5 describes the diet fed to each group of animals and lists the mortality rate and tumours incidence in each group.

Whereas no rat died or developed liver tumours in rats fed control diet, four rats out of six in $AFB_1$ - fed group had developed liver tumours while one had died early probably due to $AFB_1$ poisoning. No single rate died with mice pellets fortified with $\beta$-carotene and given $AFB_1$.

In another group of rats fed with mice pellets mixed with Ascorbic acid, only one rat out of five rats that survived the entire experimental period developed liver tumours. One rat had died early within the first week of feeding. The same results were obtained when selenium salt was used as a dietary mixture for another group of rats.

In another group of animals fed with mice pellets mixed with reduced glutathione (GSH) and $AFB_1$, two animals died early during the trial period and one of the remaining four rats which survived the experimental period had developed liver tumours. When vitamin E was substituted

for (GSH), the mortality rate was the same as in reduced glutathione (GSH) fed group. Of the 4 rats that survived, 2 of them had liver tumours representing 50% of the animals that survived the entire experimental period.

In the last group where uric acid was mixed with the mice pellets, four animals died in the first week of the experimental period. The two surviving rats had no liver tumours of lessions.

b) Effects of fortified food on microsomal enzymes

Table 6 shows the activities of hepatic microsomal cytochrome P-450, aniline hydroxylase and chlorpromazine demethylase. There was a general induction in enzyme activities in groups fed fortifeid food. In the group fed mice pellets mixed with β-carotene and uric acid, the inductive effect was the greatest. The inductive effect on the activity of these enzyme systems remained moderate in food factors such as ascorbit acid, reduced glutathione (GSH) and selemium salt.

The protein content of microsomes was not statistically different in experimental and control gropps. For the control diet and aflatoxin $B_1$ the protein content of the microsomes was quite low.

Table 7

Plasma enzyme levels in rats given anticarcinogenic agents combined with $AFB_1$

| | Dietary mixture | Liver protein content | GOT | GPT | Alkaline Phosphatase |
|---|---|---|---|---|---|
| 1. | Control diet + $DMSO_4$ | 7.13 ±0.31 | 59.56 ±0.83 | 85.20 ±2.33 | 82 ±13.82 |
| 2. | Control diet + $AFB_1$ | 4.0 ±0.15 | 132.65 ±1.83 | 140.75 ±1.83 | 150.00 ±0.70 |
| 3. | $AFB_1$ + 1mg B-carotene | 6.90 ±0.22 | 104.72 ±5.56 | 103.75 ±3.74 | 121.0 ±2.65 |
| 4. | $AFB_1$ + 10mg Ascorbic acid | 6.80 ±0.16 | 103.86 ±1.30 | 126.60 ±3.03 | 130.2 ±3.42 |
| 5. | $AFB_1$ + 1mg GSM (reduced glutathione) | 7.44 ±0.22 | 169.20 0.28 | 167.84 ±4.32 | 132.00 ±3.74 |
| 6. | $AFB_1$ + 1mg vitamin E | 7.50 ±0.22 | 100.84 ±1.30 | 90.20 ±3.51 | 139.00 ±1.83 |
| 7. | $AFB_1$ + 1mg Selenium ± 2.01. | 6.80 ±1.50 | 121.22 ±0.63 | 79.86 ±2,23 | 125.00 ±1.15 |
| 8. | $AFB_1$ + 1mg Uric acid | 6.88 ±2.01 | 119.73 ±1.30 | 133.43 ±3.42 | 132.0 ±0.79 |

Rats were fed as in table IV. Plasma enzyme activity, GOT, GPT and Alkaline Phosphatase was given in International units per litre. Values are mean ± S.D. in 1u/lite. (n = 6.)

c) Effect of fortified food on plasma enzymes

Table 7 summarises the effects of fortified food on the levels of Glutamate Oxaloacetate transaminase (GOT Glutamate Pyruvate Transaminase (GPT) and Alkaline Phosphatase in plasma. The levels of the three enzymes were highest in rats fed aflatoxin $B_1$ alone in the group fed fortified food, the levels were moderate, except the vitamin E fed group where the levels were unchanged.

# CHAPTER FOUR

## GENERAL DISCUSSION

The effects of $AFB_1$ on plasma enzyme levels and liver microsomal demethylase and aniline hydroxylase were determined in rats restricted to protein deficient diets. The levels of cytochrome P-450 and $b_5$ were also measured in the livers of these animals.

There was a general decrease in activities of P-hydroxylation of aniline, N-demethylation of chlorpromazine and also a decrease in levels of microsomal cytochrome $b_5$ and cytochrome P-450 with the increase of $AFB_1$ doses. This is in agreement with the observations of Mclean and Maclean (1960), who showed that the activities of the microsomal enzymes namely aniline hydroxylase and chlorpromazine demethylase along with cytochrome P-450 were lowered in protein deficient rats.

The effects of aflatoxin $B_1$ on the mixed function oxidase induction are rather complex, and may not be dose dependent. They are probably influenced by the ability of $AFB_1$ to inhibit proteinsynthesis. Also it might invoke its capabilities to activate existing enzyme activities and also induce the mixed function oxidase synthesis, Campbell and Hayes (1974).

The enzymes involved in activation of most known hepatocarcinogens are located predominantly in the microsomal endoplasmic reticulum. Their activities are subject to major positive and negative influences of diet and drugs. Some of these modulations can prevent cancer induction by potent carcinogens presumably by interfering with their activation to form reactive derivatives that attack cell components, Garner and Martin (1979).

The ability of aflatoxin $B_1$ to act as an inhibitor of microsomal enzyme activity should be related to its reported inhibitory effect on protein biosynthesis, Smith and Mckerman (1962). The decrease in hepatic microsomal drug-metabolising enzymes during the present study may have been due to an inhibition of protein synthesis by $AFB_1$. This observation is in agreement with data reported by other workers who have demonstrated that $AFB_1$ has a direct action on RNA and polysome synthesis, Rao (1971); Sarasin and Moule (1973).

From the results, it is demonstrated that chronic alcohol intake increases the level of cytochrome P-450 slightly. Rogers and Newberne, (1965) demonstrated that a diet relatively low in first class protein reduces the acute toxicity but may increase in the chronic

toxicity of $B_1$ in rats. In contrast, Madhavan and Gopalan (1986) showed that when rats are fed with low protein diet, they become more sensitive to the acute effects of aflatoxin toxicity. Similar work by Anukarahanota, et al (1978) showed that in animals fed with a low protein diet, aflatoxin induced extensive toxic and carcinogenic effects.

From the results, it is demonstrated that protein deficient diet substantially decreased aminopyrine demethylase and P-nitro phenol hydroxylase. These effects were further aggravated by the administration of $AFB_1$. Furthermore it has been shown that protein deficiency caused significant decrease in cytochrome P-450 content. Therefore, increased mixed function oxidase actually is associated with a low tumour yield and a high toxic effect by chemical carcinogens. Dietary reduction depress either quantity and quality of microsomal mixed function oxidase activities and this has a direct application to the toxicity of drugs and chemical agents taken when dietary protein is low. The result thus demonstrated that the depression of the mixed function oxidase by $AFB_1$ is associated with liver tumour inhibition. This can be explained in that the metabolite $AFB_1$ slow protein synthesis and consequently its formation.

Plate 3a. Shows gross appearance of liver tumour from $AFB_1$ - treated rats.

Plate 3b. Shows gross appearance of rat liver from normal control group.

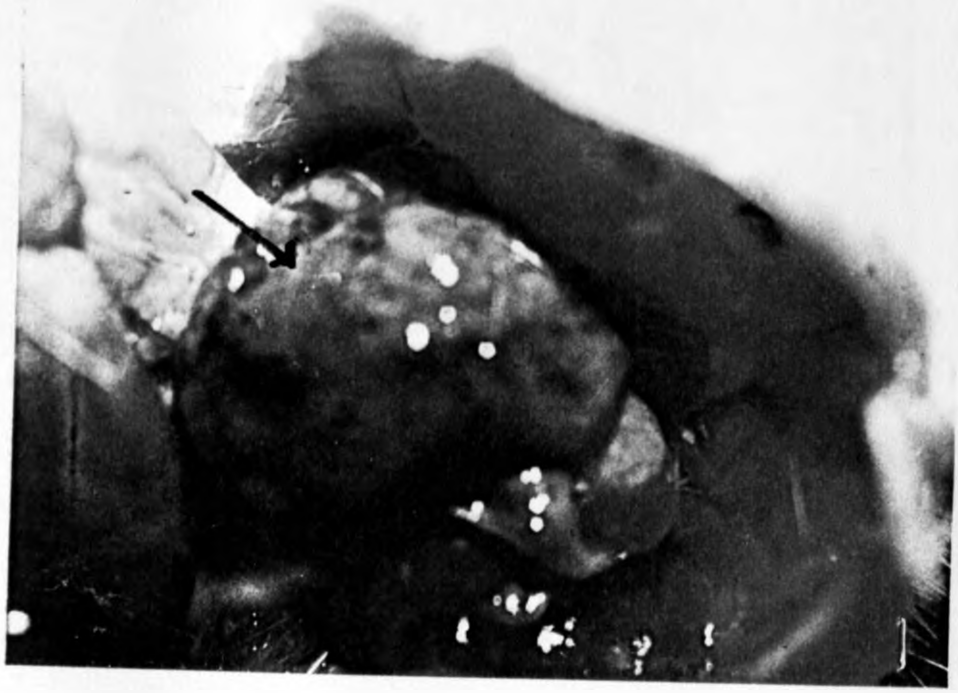


PLATE 3A

PLATE 3B

Plate 3c. Histological appearance of moderately differentiated hepatocellular carcinoma from $AFB_1$ - treated animals.

Plate 3d. Histological appearance of rat liver from normal control group.

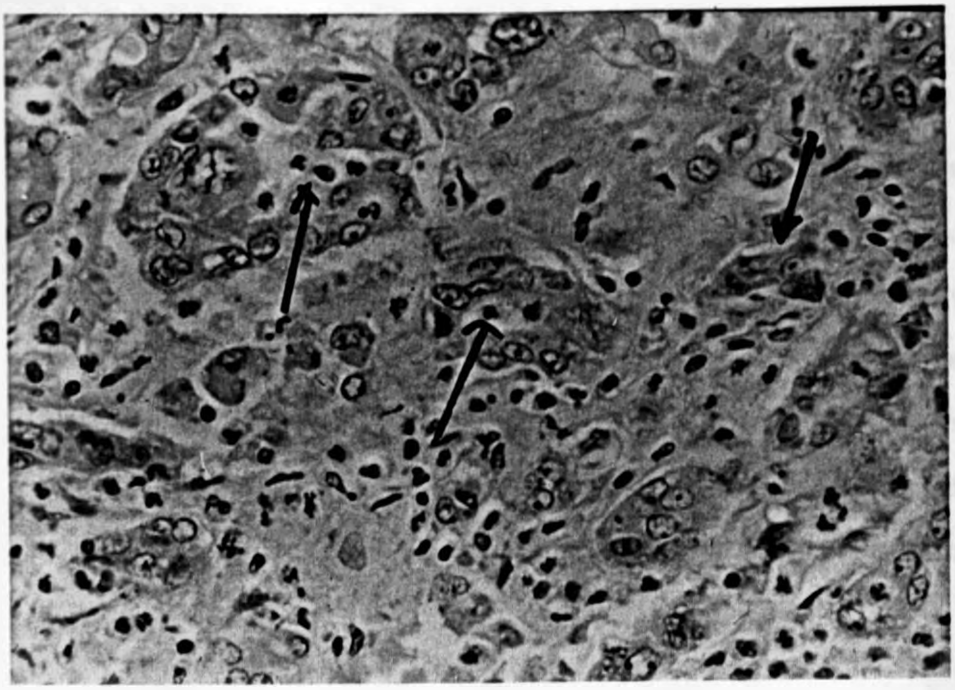


PLATE 3C

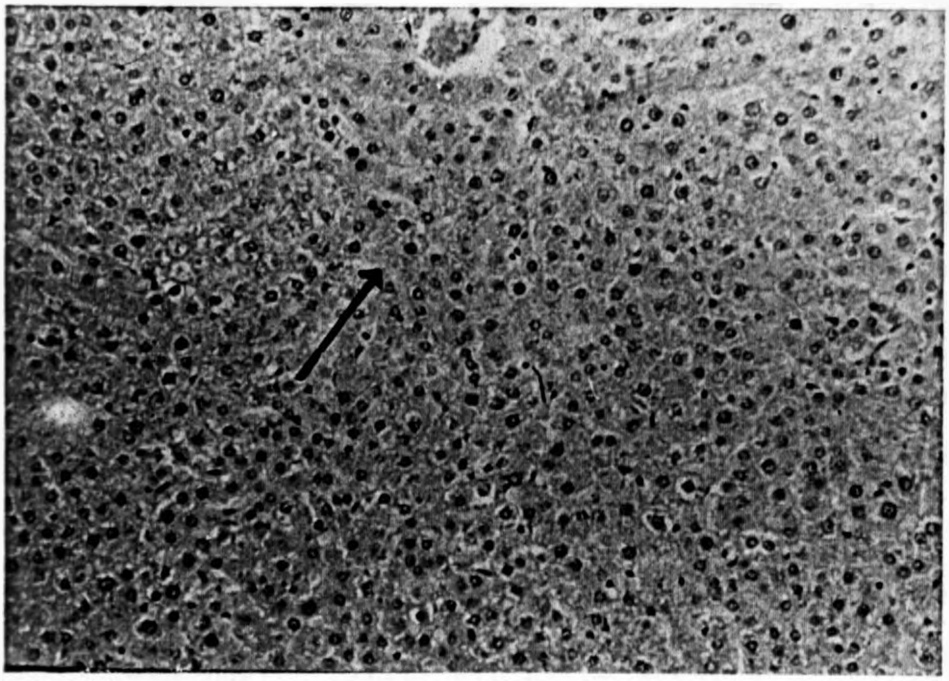


PLATE 3D

Current observations show that there was an increase in plasma enzyme levels as the dosage of aflatoxin $B_1$ was increased, indicating the status of the liver. Such high levels will indicate that the liver was extensively damaged and histological slides showed large areas covered with tumours. Increased plasma parameters during the present study reflected the extent of liver damage by $AFB_1$.

THE INHIBITION OF LIVER TUMOUR GROWTH BY ANTICARCINOGENIC AGENTS.

Enzymes activities were induced although there were significant differences between the rats fed mice pellets with $AFB_1$ and those given $AFB_1$ and accessory food factors or vitamins. 80% of the rats on $AFB_1$ alone developed massive liver tumours than in other groups of animals as shown in Table 1. Naked eye appearance of the liver of the animals fed $AFB_1$ alone showed that the livers were usually either grossly abnormal, blotched, grey or green with multiple tumour nodules; in some cases, with essentially no normal liver remaining (Figure 3). In contrst, animals that had either selenium, ascorbic acid, -carotene no tumours developed even after sacrifice and all survived the trial period.

The complexity of dietary interaction with chemical carcinogenes in animal models are evident from many studies. The studies of Basu and coworkers (1982) and Wattenberg (1975) have shown an inhibitory effect on tumour formation. These authors showed that there was a decrease of various drug-metabolising enzymes and that cytochrome P-450 and cytochrome $b_5$ were major components. Zannoni and coworkers (1972) observed a significant decrease in overall drug oxidation typified by aniline hydroxylation, aminopyrine N-demethylation and [illegible]-Nitroanisole. He also observed a decrease in demethylation and cytochrome P-450 in microsomes isolated from vitamine E deficient guinea pigs.

Current results (Table 6) show that there was no tumour formation in the group of animals fed vitamin A followed by $AFB_1$ treatment. All the six animals used in this study survived the entire experimental period of over 22 months and none of them had any tumours. This means that vitamin A had protected the rats from both toxic and carcinogenic effects of aflatoxin $B_1$. This is in line with the work of Reddy, B.D. et al (1977) who whowed the preventive role of vitamine A in colon carcinogenesis in rats. Newbern and Rogers (1973) on the other hand noted an increase in the incidence of colonic carcinoma when aflatoxin $B_1$ was administered

to rats which had a low dietary intake flow of stored vitamin A.

In a group of rats given vitamin E followed by aflatoxin $B_1$, two animals died early although this was suspected to be due to the toxicity since this happened within two weeks after administration of aflatoxin $B_1$. Among the remaining 4 animals, 2 had tumours, representing 50% of the animals that survived the entire experimental period. This indicates that vitamine E has some anti-carcinogenic effect. Studies by Rowel and Wills (1975), showed that vitamin E could act in several ways. It could play a role as an antioxidant preventing lipid peroxidase formation in the diet before feeding or in the endoplasmic reticulum protecting the unsaturated fatty acids of the membrane. Alternatively, it may play a specific role as part of a structure of the membranes of the endoplasmic reticulum and thus be essential for maximum activity of oxidative demethylation.

In the selenium fed group, one rat died early and histological tests showed no tumour growth, thus toxic effect might have been the cause. The five animals that survived the entire experimental period, one had visible tumours of the liver and the other four had no tumours. The tumour incidence represented only 20% of the animals that survived. Thus 80% of the animals

that survived were protected by selenium against aflatoxin carcinogenesis. High dietary selenium has been reported to inhibit or delay carcinogeneis, Le Boeuf, et al., (1985). Selenium has also been reported to inhibit the induction of tumours of skin colon, liver and mammay glands in experimental animals, Ames (1983). As a component of the enzyme glutathione peroxidase, selenium probably acts to detoxify hydrogen peroxide radicals generated by $AFB_1$, Slaga and Digiovanini (1984).

Although 4 rats out of 6 in the uric acid group with aflatoxin $B_1$ died early in the trial period, the liver histological studies showed no tumours. Out of the remaining two animals, no tumours were observed. The large number of early deaths indicates toxic effect meaning that uric acid had no preventive effect against toxicity. The remaining 2 rats had no tumours. That shows that uric acid had more preventive effect on cancer formation caused by aflatoxin $B_1$. It must be noted, however, that uric acid is a strong anti-oxidant that apears to inhibit carcinogenesis by inducing the activity of drug metabolizing enzymes, Ames (1983).

In the group given ascorbic acid, one rat died

early with no tumours formation, one had tumours and 4 of the animals were protected against $AFB_1$ carcinogenesis. That means that ascorbic acid has high preventive role for toxic and carcinogenic effect of $AFB_1$, Zannoni, V.G., et al. (1972); Slaga and Digiovanoni (1984). Although large doses of ascorbic acid have been found to lower the level of various drug - metabolising enzymes including cytochrome P-450 and cytochrome $b_5$, Basu, et al. (1982), low levels of ascorbic acid were also shown to interfere with the construction of the cytochrome P-450 molecule. Hence it appears that there may be a biphasic effect of ascorbic acid on drug metabolising enzymes with an inhibitory effect on both high and low concentrations.

Ascorbic acid deficiency in guinea pigs was shown to result in marked reduction in the activity of the hepatic microsomal drug metabolising activity, Zannoni, V.G., et al. (1972). In vitro studies with liver microsomes isolated from vitamin C deficient guinea pigs have indicated a significant decrease in overall drug oxidation typified by aniline hydroxylation, aminopyrine N-demethylation and -Nitroanisole-O-demethylation, Zannoni, V.G., et al. (1972). In the group of rats that received high amounts of reduced glutathione, a 75%

protection effect against aflatoxin carcinogenesis was observed. Only 25% of the surviving animals had tumours. This inhibiting effect of reduced glutathione against carcinogenesis observed in this study is in agreement with the observations of Nove (1981) who observed that reduced glutathione produced effective protection against aflatoxin tumourigenesis. Reduced glutathione can form AFB-SG with 2, 3 dehydrodiol of $AFB_1$ hydrolysis which is considered to be a detoxication product of liver metabolism. Moreover epidemiological evidence suggests that consumption of vegetables rich in glutathione is associated with reduction in the incidence of cancer in humans, Kensler, T.W. et al. (1987).

The inhibition of liver tumours during the present study was probably due to rapid activation of $AFB_1$ metabolism by microsomal enzyme activity. Nutritional factors such as vitamins could inhibit chemical carcinogenesis through their ability to induce the activity of hepatic microsomal enzymes. Inhibition of liver cancer during the present study was therefore associated with induction of increased microsomal enzyme activity and the plasma level of marker enzymes of liver disease.

## C O N C L U S I O N

1. This study demonstrates that changes observed in both drug-metabolising enzymes and plasma enzyme activities are related to the degree of liver poisoning caused by $AFB_1$. Whereas increased plasma parameters reflected the extent of liver injury produced by $AFB_1$, decreased activities of hepatic drug-metabolising enzymes reflected inhibition of protein synthesis by the direct action of $AFB_1$. Inhibition of protein synthesis could increase in the toxicity of repeated $AFB_1$ doses.

2. The inhibition of $AFB_1$ - induced liver tumours observed during the present study was probably due to increased induction of hepatic microsomal enzymes by dietary factors. Increased enzyme activity could lead to rapid activation of $AFB_1$ metabolism to non-carcinogenic metabolites. Inhibition of liver tumours is therefore associated with induction of increased hepatic microsomal enzymes in response to the presence of accessory food factors.

This study clearly demonstrates that compounds such as B-carotene, ascorbic acid GSH, vitamin E and selenium salts have strong inhibitory effect

against aflatoxin carcinogenesis. Since these compounds are present in fresh vegetables such as cabbages and in fresh fruits such as oranges and lemons, people should be encouraged to consume much more frequently in order to reduce cancer incidence.

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