EFFECT OF THE FOOD CONTAMINANT *ASPERGILLUS VERSICOLOR* TOXICITY ON AMINO ACID UPTAKE IN RATS

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STERIGMATOCYSTIN, a biogenetic precursor of aflatoxin B_1 is a carcinogenic major secondary metabolite of *Aspergillus versicolor* which had been reported to be toxic to various species of experimental animals[1, 2].

The main sites of action of most of the mycotoxins have been found to be liver, kidney, intestine and in some cases brain also. In the present investigation, to study the membrane transport, an attempt has been made to study the *in vivo* transport of two amino acids, ^{14}C methionine and ^{14}C alanine by intestinal perfusion experiment[3].

The removal of amino acids from the mucosal side of the gut to the serosal side of the tissue represents the amino acid uptake[4].

400 g of bread which contained 0.03% calcium propionate as preservative was mixed with 40% by weight of water, sterilized, cooled, inoculated with spores of *A. versicolor* (10^7 spores/ml) and allowed to incubate for 21 days at 29°C. After this period the contaminated bread was sterilized free of the fungus and dried. This contaminated diet was mixed with normal diet in the ratio of 1:2 and fed as the contaminated diet.

Weanling albino rats (40) of either sex were divided into two groups. The control group received normal diet, while the experimental group was fed with the contaminated diet. Water was given *ad libitum*. At the end of 60 days perfusion study was carried out by the method of Younoszai *et al*[3].

Perfusion fluid was 0.15 M sodium phosphate buffer (pH 7.5) containing sodium chloride 135 mM, potassium chloride 5 mM, the respective amino acids at 1 mM and C^{14} labelled amino acids.

The scintillating fluid was a mixture of dioxane and ethylene glycol (50:1 v/v) containing PPO (4 g/l), POPOP (200 mg/l) and naphthalene (60 g/l).

Rats were fasted for 24 hr before the experiment. The animal was anaesthetized by intraperitoneal injection of sodium phenobarbitone (50 mg/kg). The abdominal cavity was opened by a middle longitudinal incision, the duodenum was picked up and the common bile duct was ligated. The entire small intestine was used for absorption study as a single unit by inserting cannulas at the pylorus (inlet) and terminal ileum (outlet). The intestine was first washed with 159 mM sodium chloride solution. Then the perfusion solution was perfused at a constant rate (1.0 ml/min). After initial equilibration with buffer for 15 min, six "10 minutes-samples" were collected for analysis. The perfusate (0.1 ml) was taken immediately after perfusion and the radio activity was measured in a liquid scintillation counter with 10 ml of scintillating fluid. Radio activity of the perfusate was also measured separately.

Membrane-bound enzymes, namely total ATPase, Na^+K^+ dependent ATPase were studied by the method of Evans[5], alkaline phosphatase by the method described by King[6] and 5′ nucleotidase by the method of Camphell[7].

The results given in table 1 reveal that during *A. versicolor* toxicoses the levels of total ATPase, Na^+K^+ dependent ATPase and alkaline phosphatase are reduced while that of 5′-nucleotidase is increased.

Table 1 *Activities of total ATPase, Na^+K^+ dependent ATPase alkaline phosphatase and 5′ nucleotidase in intestinal tissue of control and experimental rats*

Enzyme	Control	Experimental
Total ATPase	3.79 ± 0.19	2.25 ± 0.24^a
Na^+K^+ dependent ATPase	2.01 ± 0.15	1.33 ± 0.09^a
Alkaline phosphatase	1.84 ± 0.11	1.52 ± 0.13^b
5′ nucleotidase	0.96 ± 0.08	1.86 ± 0.17^a

^{a} $p < 0.001$; ^{b} $p < 0.01$
Enzyme activities are expressed as μmol of product liberated per mg protein under incubation conditions (mean ± SD)

Na^+K^+ dependent ATPase is a membrane-bound enzyme and is important in maintaining intracellular sodium and potassium concentration. The decreased levels of the enzymes suggest that the *A. versicolor* toxins affect the cell membrane integrity and permeability. The decrease in alkaline phosphatase may be attributed to the fact that the membrane plasticity is being affected. 5′-nucleotidase being a marker enzyme for plasma membrane the increase in activity again reflects the membranal disturbances that are brought about by the *A. versicolor* toxicity.

Figures 1 and 2 give the uptake of ^{14}C-methionine and ^{14}C-alanine. The uptake of the two amino acids is significantly lowered in the intestinal tissue of the experimental group.

The decrease in the uptake of labelled amino acids caused by *A. versicolor* toxicoses could be discussed in terms of membrane damage, damage to the carrier

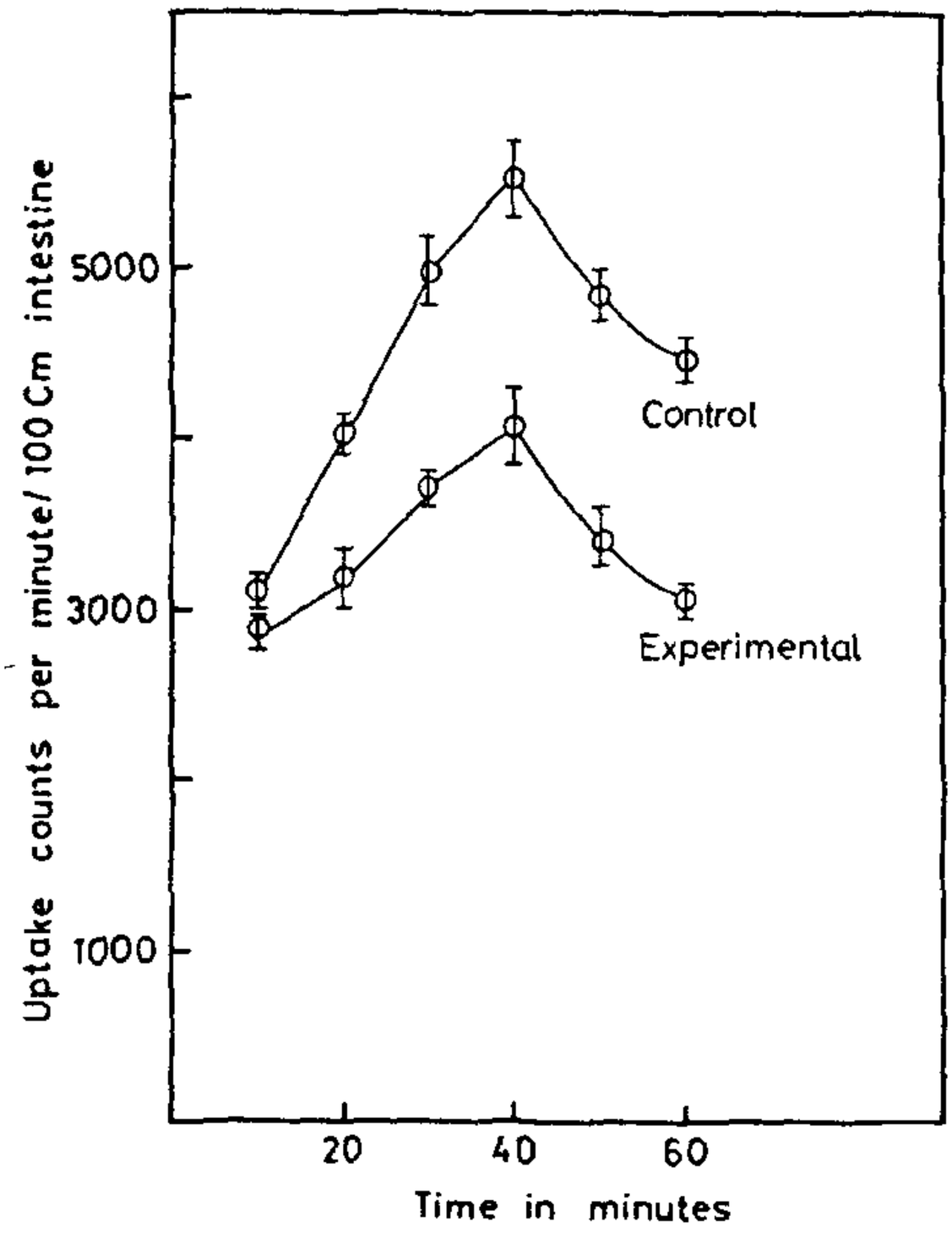

Figure 1. *In vivo* absorption of ^{14}C methionine by the intestine of control rats and *A. versicolor* infected diet fed rats expressed as counts per min per 100 cm intestine at various time intervals.

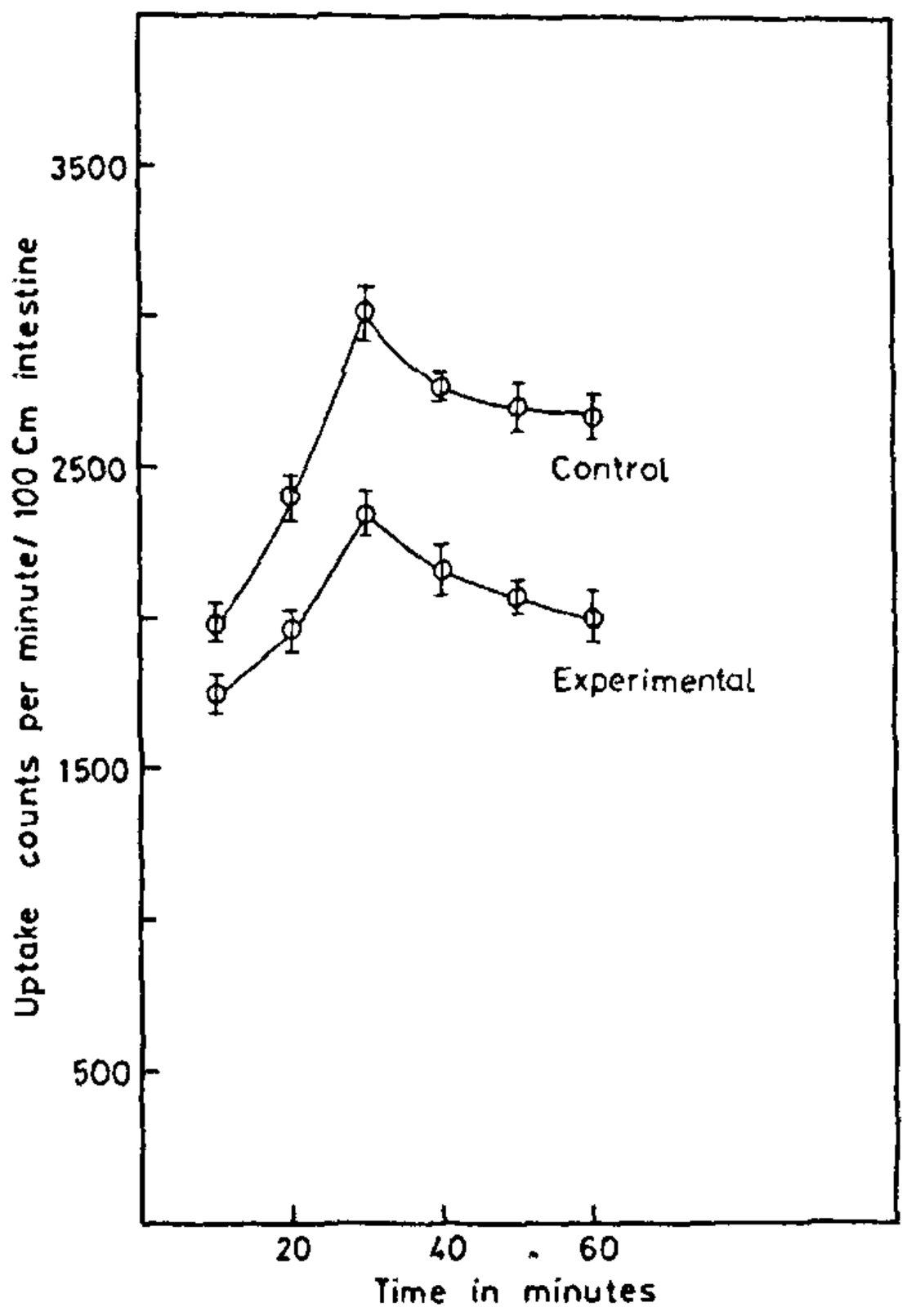

Figure 2. *In vivo* absorption of ^{14}C alanine by the intestine of control rats and *A. versicolor* infected diet fed rats expressed as counts per min per 100 cm intestine at variius time intervals.

systems or impairment of reactions which provide energy for active transport of amino acids.

The membrane damage is indicated by the variation in the alkaline phosphatase level and this membrane damage may affect the permeability of amino acids as observed by Ciegler *et al*[8] in their studies on patulin.

The uptake of amino acids requires sodium gradient [9, 10] and this concentration gradient is maintained by a membrane-localised sodium pump. The Na^+K^+ dependent ATPase which forms a part of the pump catalyses the outward flux of Na^+ ion across the membrane and inward flux of K^+ ion and this is responsible for the establishment of sodium gradient which drives amino acid transport. The inhibition of ATPases by the toxin may thus indirectly affect the amino acid uptake. It is pertinent to note that such a decrease in the aminoacid uptake during various mycotoxicoses has been established by Devaraj *et al*[11] and Saraswathi[12].

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PROBABILITIES OF DROUGHTS AND FLOODS OVER INDIA DURING THE SOUTHWEST MONSOON SEASON

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ABNORMALITIES in the performance of the southwest monsoon manifested as floods and droughts have disastrous effects on agriculture, industry and the generation of hydroelectric power, causing severe strain to the national economy. Almost every year some part or the other of the country suffers from such calamities. During the last two decades, the country experienced severe and widespread droughts during 1965–66, 1972 and 1979 which affected adversely the food production, power generation and industrial output. The drought of 1979 affected 240 districts covering 11 states and 240 million people; the Government had to spend Rs 1,600 crores on relief works[1]. Due to floods, on an average, during 1953–1978, an area of about 8.2 million hectares was affected, 9.2 million houses were damaged, 1240 human lives and 7700 cattle were lost per year[2]. In view of their great impact on the national economy a study of the probabilities of incidence of droughts and floods over different parts of the country has been made by utilising the southwest monsoon rainfall data for the 108 years, 1871–1978.

Three hundred and six stations, one from each of the districts in the plain regions of India were selected to form the network of raingauge stations. These stations are fairly uniformly distributed over the country and have rainfall data from 1871 onwards. The relevant rainfall data of southwest monsoon (June to September) were collected from the national data centre of the India Meteorological Department, Poona. Mean and standard deviations computed for all these stations for southwest monsoon have been utilised in this study.

Figure 1 shows the normal isohytal map of India for the southwest monsoon season drawn on the basis of the data of the 306 stations. Shaded hilly portions showed in the map have not been considered in the present study. High rainfall amounts over west coast and over Assam region and low rainfall over northwest India, central and eastern parts of southern peninsular India, are the salient features of the mean rainfall distribution. The central parts get moderate rainfall.

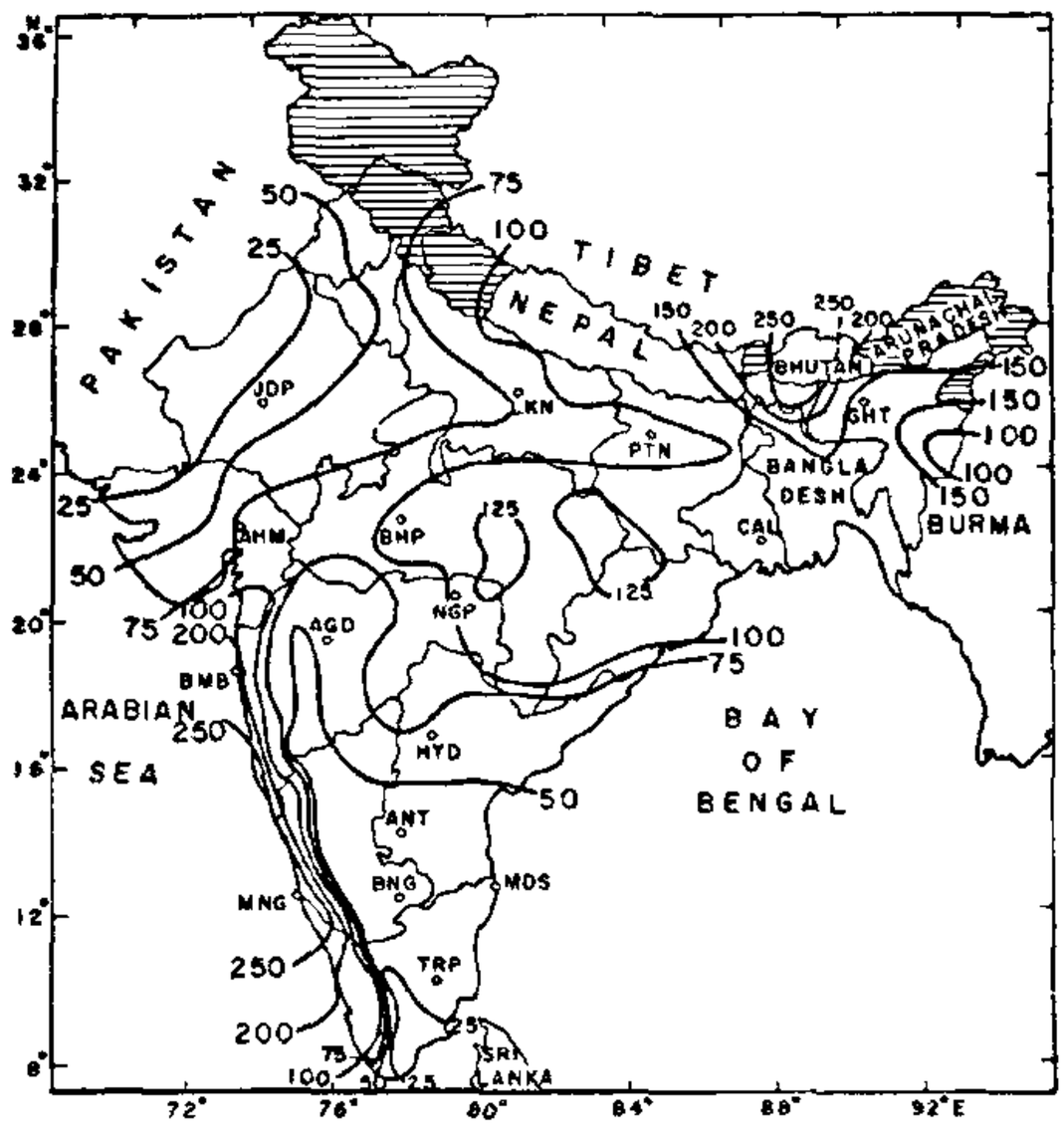

Figure 1. Normal southwest monsoon (June to September) rainfall (cm) of India: 1871–1978.

The inter-annual variation is a measure of the dependability of the rainfall from one year to another. The mean inter-annual variability (s) of rainfall at any station is defined as follows:

$$s = \frac{1}{N-1} \sum_{i=1}^{N-1} \left| R_{i+1} - R_i \right|$$

where R_i is monsoon rainfall for the ith year and N is the total number of years. The inter-annual variability was calculated for each of the 306 stations. A map showing the spatial variation of s is shown in figure 2. The highest values are noticed over the west coast and the lowest values over the west Rajasthan and the