Table 2. Inhibitory effect of sugars on the haemagglutinating activity of modeccin*

Modeccin (μg/ml)	62.5	31.2
Sugars	Sugars concentration (mM) inhibiting agglutination	
D-galactose	12.5	3.1
D-fucose	50	12.5
Lactose**	not tested	12.5
Melibiose	25	3.1
Raffinose	50	12.5

*Assayed on rabbit erythrocytes. **Lactose at 25 mM and higher concentrations causes by itself some haemagglutination.

and serial dilutions (1:1, starting from 500 μg/ml of final volume) of the lectins. Sugars were tested for inhibitory effect at serial dilutions (1:1, starting from 50 mM final) with 100 μl of erythrocyte suspension in a final volume of 200 μl.

Results and discussion. The haemagglutinating activities of modeccin and ricin are reported in table 1. Modeccin agglutinates erythrocytes from several mammalian species with no specificity for human blood groups. Maximal agglutination was observed with rabbit erythrocytes, whereas sheep and ox erythrocytes were not agglutinated by modeccin at the highest concentration tested. In all cases except rabbit erythrocytes, agglutination was enhanced by neuraminidase, to an extent variable from species to species. In all cases the agglutinating activity of modeccin was lower than that of ricin, which also was enhanced by neuraminidase, except in the case of rabbit and pig erythrocytes.

Several sugars were tested for inhibitory effect on haemagglutination of rabbit erythrocytes by modeccin. The following sugars had no effect at concentrations up to 50 mM: D-ribose, D-xylose, L-arabinose, D-fructose, D-glucose, 1-O-methyl-α-D-glucopyranoside, 1-O-methyl-β-D-glucopyranoside, D-mannose, D-glucosamine, N-acetyl-D-glucosamine, D-galactosamine, N-acetyl-D-galactosamine, maltose, sucrose, cellobiose, trehalose, melezitose. Only fucose, galactose and galactose-containing di- and trisaccharides were inhibitory (table 2).

Our results demonstrate that modeccin has an haemagglutinating activity, which is different on erythrocytes from different species. Previous apparent inconsistencies[2,5] are thus accounted for by the fact that Refsnes et al.[2] used human erythrocytes, whereas we used rabbit erythrocytes[5], which are more sensitive to the agglutinating lectin. The inhibitory effect of galactose and of galactose containing sugars indicates that modeccin, like ricin, binds to galactosyl residues on the red cell membrane, which is consistent with previous findings with Ehrlich[5] and HeLa[2] cells. The lower agglutinating activity of modeccin as compared with ricin may indicate that, on the membrane of erythrocytes, there are fewer receptors for modeccin than for ricin, as is the case in HeLa cells[9]. The enhancement of agglutination by neuraminidase is consistent with results obtained with modeccin on human erythrocytes and HeLa cells[2], and with ricin on Novikoff tumour cells[10], and may be due to unmasking of galactosyl receptors on the cell membrane[9].

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The effect of dietary fat on the anticoagulant activity of aflatoxin B_1

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Summary. A single i.p. dose of aflatoxin B_1 had no significant effect on the thrombotest clotting times of monkeys subsisting on low-fat and high-fat dietary regimens, respectively. There was a significant interaction between aflatoxin and dietary fat level.

Haemorrhage has been a common clinical and pathological sympton associated with aflatoxicosis in many animal species[2-6]. This bleeding often precedes death[5,7,8]. Related to these findings are reports that aflatoxin lengthened blood clotting time in the dog, rat, chicken, guinea-pig and monkey[5,9-12]. In a review by Schoental[13], emphasis was placed on the need for studies on the anticoagulant property (amongst other pharmacological properties) of aflatoxin. It has been proposed that the aflotoxins as coumarin compounds, having a central 5-methoxy moiety, act as anticoagulants[9,14]. However, the aflatoxins have been reported to be much more effective than coumarin in prolonging the time necessary for blood clotting to occur in the rat[9,15-16]. In this paper, we report our observations on the influence of dietary fat on the anticoagulant activity of aflatoxin B_1 in the Nigerian monkey (*Cercopithecus aethiops*, Tantalus).

Materials and methods. The experimental animals were obtained from the stock quartered at the Primate Colony of the Department of Biochemistry of Ibadan University. They had weights between 2.8–3.5 kg. Their ages were not known because none of them was born in captivity. All monkeys were in apparent good health and were individually housed in cages which were supplied with food trays. Tap water was available ad libitum. Monkeys were alloted into 6 treatment groups of 6 animals in each. Groups I, II and III were maintained on a stock, low-fat (2.2%) diet, while groups IV, V and VI received a high-fat (18.1%) diet.

Table 1. Composition of stock, low-fat (2.2%) and high-fat (18.1%) diets for experimental monkeys

Ingredients	Low-fat diet (stock) (%)	High-fat diet (%)
Yellow maize, ground	76.6	60.3
Corn starch	7.0	7.0
Brown fish meal	3.0	3.0
Casein	4.0	5.3
Brewer's dried yeast	1.4	1.4
Rice bran	5.0	5.0
Sodium chloride	0.3	0.3
Dicalcium phosphate	2.2	2.2
Vitamin mixture*	0.5	0.5
Lard	-	15.0

* Pfizer Products (Ikeja, Nigeria) comprizing Vitamin A (10 mill. units), vitamin D_3 (2 mill. units), vitamin E (7000 mill. units), vitamin B_{12} (10 µg), vitamin K (2000 mg), riboflavin (4200 mg), nicotinic acid (25,000 mg), pantothenic acid (10,000 mg), folic acid (1000 mg), iodine (2016 mg), manganese (34,720 mg), zinc (100,800 mg) and iron (100,800 mg).

The composition of each diet is shown in table 1. Vitamin C was added (0.1%) as extra supplement to each diet[17]. No other food supplements were offered. The experimental design was completely randomized. After 15 days, groups II and V were given a single i.p. dose (60 µg/kg of b.wt) of aflatoxin B_1 while groups III and VI received 100 µg/kg of b.wt. Aflatoxin was dissolved in 0.5 ml of dimethylsulphoxide. Groups I and IV were kept as control and received 0.5 ml of solvent. Aflatoxin B_1 was produced by the method of Shotwell et al.[18] as modified by Llewellyn et al.[19] and quantitatively estimated using the method of Rodricks and Stoloff[20]. Blood was taken from the femoral vein of unanesthetized monkeys 1 h after aflatoxin administration. The maximum anticoagulant activity of the toxin has been found to occur at this time in the Nigerian monkey[12]. Blood from each animal was collected in a different plastic tube (11 × 1.5 cm) and clotting prevented by mixing in a 1:9 (v/v) ratio of sodium citrate (3.13%, w/v) and blood. The thrombotest clotting time was determined by the method of Owren[21]. All reactions were carried out at 37±1 °C. A factorial analysis of variance was used to assess the statistical significance of differences between groups of animals.

Results and discussion. The thrombotest clotting times of the control and aflatoxin-treated monkeys are shown in table 2. The results indicate that at the 60 µg/kg level, aflatoxin B_1 had no significant ($p > 0.05$) effect on the thrombotest clotting times of monkeys on the low-fat and high-fat dietary regimens, respectively. However, when given at a dose of 100 µg/kg, the thrombotest clotting time was significantly ($p < 0.01$) prolonged in animals subsisting on the low-fat diet, but not in those maintained on the high-fat dietary regimen. A factorial analysis of variance revealed that there was a significant ($p < 0.01$) interaction between aflatoxin and dietary fat concentration. As a result of this interaction, the anticoagulant activity of aflatoxin was decreased when the toxin was administered i.p. to monkeys subsisting on the high-fat diet. The results of this experiment suggest that the level of fat in the diet has a significant effect, on the anticoagulant activity of aflatoxin. Similarly, high-lipid diets lead to a decreased mortality by aflatoxicosis in turkeys[22] and chickens[23]. Rats on high-protein diets have been reported to be less susceptible to aflatoxin poisoning than low-protein diet rats[24]. High-protein diets have been similarly reported to protect the liver from necrosis by aflatoxin in rats[25]. These findings suggest that the composition of diets affects the metabolism and toxicity of aflatoxin in these animals. In the thrombotest technique, all coagulation factors are held constant except the vitamin K-dependent clotting factors II, VII, IX and X. The thrombotest clotting time is therefore dependent exclusively upon the concentration of the factor which is rate limiting. Prolongation of clotting time in monkeys on the low-fat dietary regimen therefore suggests clotting factor deficiencies not present in monkeys on the high-fat dietary regimen.

Table 2. Effect of dietary fat on the anticoagulant action of aflatoxin B_1

Treatment	Clotting Time (sec) mean ± SD
2.2% fat diet:	
Control	34.49 ± 0.30
Aflatoxin B_1 (60)[a]	35.58 ± 0.49[d] (3.1)
Aflatoxin B_1 (100)[b]	41.27 ± 0.23[c] (19.4)
18.1% fat diet:	
Control	31.01 ± 0.84
Aflatoxin B_1 (60)[a]	31.18 ± 0.44[d] (0.6)
Aflatoxin B_1 (100)[b]	32.58 ± 0.56[d] (5.1)

Figures in parentheses indicate percentage increases in thrombotest clotting time. Results are expressed as mean values for 6 determinations. [a] Aflatoxin B_1 at 60 µg/kg of b.wt. [b] Aflatoxin B_1 at 100 µg/kg of b.wt. [c] Significantly different from the control monkeys given a low-fat diet ($p < 0.01$). [d] Not significantly different from the control monkeys given a low-fat or high-fat diet ($p > 0.05$).

Table 3. Effect of diet on the anticoagulant action of aflatoxin B_1 (µg/kg)

Fat level	Time (sec) 0	60	100
2.2%			
	34.25	35.0	41.0
	34.25	36.0	41.4
	34.90	35.5	41.3
	34.20	35.0	41.0
	34.55	36.0	41.6
	34.80	36.0	41.3
18.1%			
	32.0	31.75	33.0
	30.0	31.0	32.25
	30.5	31.3	32.5
	32.0	31.5	33.5
	30.5	30.5	32.25
	31.0	31.0	32.0

Comments. Our experience is that unlike measurements of serum lipids which varied considerably in blood samples from different animals and even in separate measurements of the same probe, measurements of thrombotest clotting time appeared to be more consistent in different animals in the same treatment group. These observations have been confirmed with repeated measurements.

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Activation by S-adenosylhomocysteine of norepinephrine and serotonin in vitro uptake in synaptosomal preparations from rat brain[1]

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Summary. S-Adenosylhomocysteine (10^{-7}–10^{-5} M) activated norepinephrine (NE) and serotonin (5HT) in vitro uptake in synaptosomal preparations from rat brain, but did not affect dopamine (DA) uptake. When administered to rats (7 mg/kg i.p.), it had the same effect on in vitro NE and 5HT uptake. It did not affect NE and 5HT release.

S-Adenosylhomocysteine (SAH) is an inhibitor in numerous methylations[2], particularly methylations of nucleic acids and neurotransmitters[3]. Some studies have been performed relating to a possible antitumoral activity[4]. Recently we have discovered that SAH is sleep-inductive and anticonvulsant (electric shock) with rabbit, rat and cat (administered i.v., i.p. or p.o. in 1–10 mg/kg-doses)[5]. This result led us to consider the action of SAH on neurotransmitters uptake and release in synaptosomal preparations from rat brain.

Methods. The method of Snyder and Coyle[6] was used to measure dopamine (DA), norepinephrine (NE) and 5 hydroxytryptamine (5HT) uptake on a crude synaptosomal fraction, obtained from homogenates of part of male Wistar rat (150–200 g) brain. The brain was dissected out according to the method prescribed by Glowinski and Iversen[7]. After homogenization in a 0.32-M sucrose solution and centrifugation (1000× g), 0.3 ml of the synaptosomal suspension were incubated in 3 ml of Krebs Henseleit medium ($CaCl_2$ concentration 1.13 mM) containing ascorbic acid 1 mM, glucose 10 mM and nialamide $1.25 \cdot 10^{-5}$ M. The suspension was incubated for 10 min at 37 °C, before adding 0.2 ml of Krebs solution containing the tritiated neurotransmitters (0.2 µM), and for 5 min after addition; then it was chilled and centrifuged for 20 min (20,000× g at 4 °C). After rinsing twice the pellet with 3 ml of NaCl 9‰, and homogenizing in absolute alcohol, the radioactivity taken in synaptosomes was measured with liquid scintillation counting.

Release evaluation was achieved according to the method of Mulder et al.[8]. Synaptosomes (corresponding to about 50 mg fresh tissue), charged for 20 min with tritiated neurotransmitter, were laid on a Sephadex G 25 bed contained in a syringe (diameter 0.6 cm× 10 cm). After 35 min perfusion (0.25 ml/min), Krebs medium was replaced by identical medium containing SAH, then, 20 min later, for NE and 5HT tests, KCl concentration was

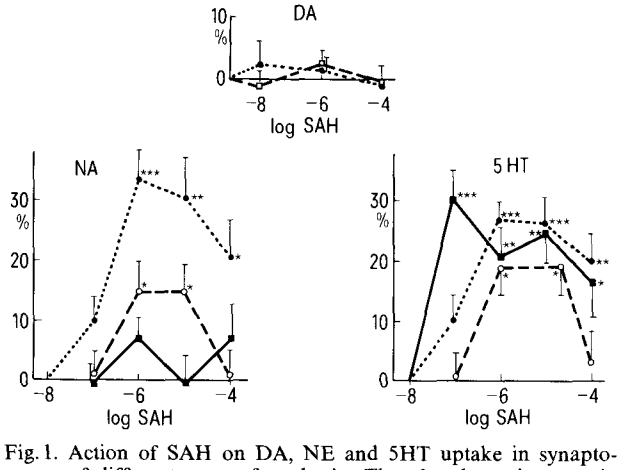

Fig. 1. Action of SAH on DA, NE and 5HT uptake in synaptosomes of different parts of rat brain. The plot shows increase in DA, NA, 5HT uptake in the presence of SAH, in percent of control values. Each point is the average of 8–12 determinations; statistical meaning of the difference between test and control sample is determined with t-test (*$p<0.05$; **$p<0.01$; ***$p<0.001$). ●···●, cortex; ○---○, midbrain + hypothalamus; ■——■, brainstem; □---□, corpora striata.

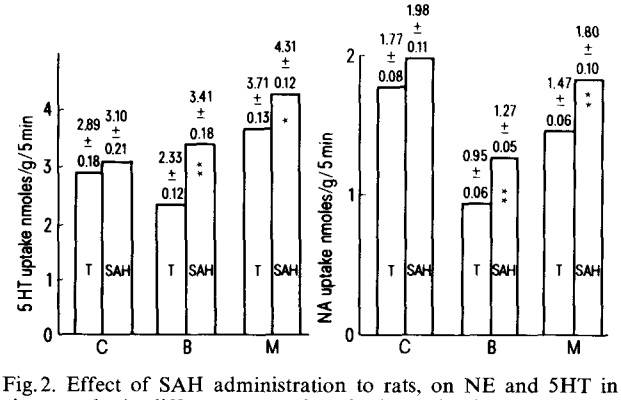

Fig. 2. Effect of SAH administration to rats, on NE and 5HT in vitro uptake in different parts of rat brain. Animals receive either SAH (7 mg/kg i.p.), or saline solution 1 h prior to sacrifice. The rest of the procedure is as before. Each value is the average of 7 determinations±SEM. Statistical meaning is determined with t-test (*$p < 0.05$; **$p < 0.01$). C: cortex + corpora striata; B: brainstem; M: midbrain + hypothalamus.