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Production of reversible infertility in rats by feeding mimosine*

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MIMOSINE (Fig. 1) is a toxic amino acid occurring in *Leucaena leucocephala* and other leguminous plants. Feeding diets containing mimosine to rats causes loss of hair, decreased weight gain, and

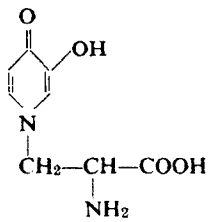

FIG. 1. Mimosine.

cataracts.[1] Several reports[2–8] have mentioned decreased fertility in animals fed rations containing *L. leucocephala* leaf meal. In the case of swine, this effect was ascribed to the presence of mimosine in the plant.[8] The fertility of animals fed experimental diets containing pure mimosine has not been determined. The purpose of our work was to determine conclusively whether mimosine has any effect on the fertility of non-ruminant animals, as exemplified by the rat.

EXPERIMENTAL RESULTS

Mimosine was isolated from *L. leucocephala* seed; it was recrystallized to constant purity and assayed at 99·9+ per cent mimosine.[1]

Six female rats of the Sprague-Dawley strain were determined to be fertile by the following criteria. Acceptance of a male on the evening of the first day of proestrus; sperm seen in the vaginal smear on the following morning; conception after one mating; delivery and nursing to weaning of at least 6 normal pups.

Three such animals, weighing 203, 207, and 213 g, were placed on an experimental diet† containing mimosine in the following amounts: first week, 0·5%; second week, 1%; third and subsequent weeks, 1·5%. Appreciable weight loss occurred on the 1·5% mimosine diet so that after four weeks the mimosine level was reduced to 1%. This level did not cause significant weight change during the experimental period. At this time an additional three females of proved fertility, weighing 198, 211, and 218 g, were added to the experiment after one week on 0·5% mimosine diet. Six weeks to two months after having been started on diets containing mimosine the animals were mated. All had been anestrous for two weeks and no cycling had been observed. Each animal was mated with three different males of proved fertility during a 14-day period. There was complete absence of sperm in the vaginal tracts of the animals and in no instance did conception occur.

TABLE 1. ORGAN WEIGHTS OF CONTROL AND EXPERIMENTAL ANIMALS FED 1% MIMOSINE

Group‡	Weights, uterus + tubes (mg/kg)	Ovaries
Control	936, 970, 1,104	333, 376, 386
Experimental	654, 671, 721	210, 235, 254

‡ Three animals in each group

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† Basal diet 15, components in g/kg: powdered skim milk, 212; whole wheat flour, 394; white flour, 91; cornmeal, 91; cornmeal, 91; brown rice meal, 91; soybean flour, 61; brewer's yeast, 18; cottonseed oil, 28; cod liver oil, 2; iodized salt, 6; calcium carbonate, 5·34; ferric citrate, 0·60; manganous sulfate, 0·06.

Three of the animals were killed and their organs weighed and subjected to gross and histological examination. No abnormalities were observed other than the differences in organ weights shown in Table 1.

The remaining three animals were returned to a mimosine-free diet and three days later had normal estrous cycles. They were mated after one week on this diet. All three conceived on the first mating. Two delivered and suckled normal litters and one resorbed.

In a subsequent experiment, four rats kept on diets containing 0·5% mimosine were examined daily for signs of estrus. After 30 days estrous cycling became irregular. Periods of atypical anestrus lasting as long as 23 days occurred, during which mucus was seen in the vaginal smears. After such periods of anestrus, however, apparently normal estrous cycles supervened in three of the four animals; thus estrous cycles, while irregular, were not completely abolished at this dosage of mimosine. All four animals proved infertile when mated with normal males.

The weight loss observed on the 1·5% diet may have been due to inanition. This is suggested by the data of Table 2 from an experiment with 1% mimosine diet in which food intake and weight gain were recorded every day.

TABLE 2. EFFECT OF MIMOSINE ON FOOD INTAKE AND WEIGHT GAIN OF TWO RATS*

Diet	Interval (days)	Average food consumed (g/day)	Average weight gain (g/day)
Control	0–5	11·1	2·5
Mimosine, 1%	6–18	8·7	1·6
Mimosine, 1%	19–35	5·3	−1·1

* Initial weights of animals: 195 and 203 g.

DISCUSSION

It is apparent from these results that feeding mimosine causes cessation of the estrous cycle and complete infertility. As little as 0·5% in the diet on prolonged feeding causes irregular and atypical estrous cycling. The mechanism of action of mimosine is not known, but the lower organ weight of infertile animals is suggestive of interference with gonadotrophin production or release. Srebnik[9] has reported anestrus and decreased weights of sex organs in female rats fed protein-free diets. Attempts were made to correlate the infertility effect of mimosine with a possible interference in protein synthesis. In three separate trials using the *in vitro* system of Moldave[10] mimosine did not affect the amount of incorporation of ^{14}C-leucine into newly synthesized protein.

Mimosine and the pyridoxine group of vitamins have certain structural similarities. Pyridoxine deficiency is known to cause appetite depression. The role of this vitamin in the biosynthesis of many amino acids is also well documented. These facts and the results presented in this paper suggest the possibility that an antagonism of vitamin B_6 may account for the observed effects of mimosine ingestion.

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Glycogen phosphorylase levels in the brain of rats treated with psychotomimetic drugs and with tranquilizers

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THE cause of psychosis is still obscure. It is the purpose of this communication to report a finding that appears promising as a possible lead in elucidating such a cause. It was found that a rather close and meaningful relationship exists between the level of glycogen phosphorylase *a* of rat brain and the type of drug that has been administered to the animal. The drugs that depressed the enzyme level belonged, in general, to psychotomimetic drugs, whereas those that enhanced it may be classified as therapeutic in mental illness. This investigation was prompted by a study by one of us* which pointed to a possible, although remote, involvement of the phosphorylase enzyme in psychosis.

Female albino rats of the Wistar strain were used. The animals were sacrificed after a definite period of time following s.c. administration of drugs, and the brains were assayed for phosphorylase activity. The phosphorylase activity determined represents the level found about 3 min after the death of the animal. At this time the activity of *a* is high and is declining very gradually.† Drummond *et al.* have reported that almost fully active *a* was found whether mice were dropped directly into liquid N_2 and the brain removed after freezing, whether anesthetized (pentobarbital) mice were decapitated and the heads frozen, or whether brains were removed from decapitated mice before freezing in liquid N_2.[1] Nevertheless, this high activity might be the result of effects on the "after-death" changes, in view of the report of Breckenridge and Norman that *a*, which is predominantly in an inactive form, is converted within seconds to an active form after death.[2] However, the experiments presented in this note were carried out with very carefully standardized conditions so that any difference observed would be due primarily to difference in treatment and may be significant. Moreover, substances absorbing at 260 mμ were always removed from the homogenate, as well as from glycogen, when deemed necessary, so that the values of *a* would not be influenced by AMP present in the homogenate or glycogen. This procedure was necessary because, without it, the detection of the effect of drugs was impossible.

Large variation in activity was not anticipated, because a slight deviation from homeostasis might be enough to cause mental aberrations. Care was taken, therefore, to enhance the sensitivity for detecting the effects of treatment by improving the technique of assay and by using appropriate experimental designs. The experimental designs were such that the effect of any factor, known or unknown, that might possibly influence the values of determinations would be removed from affecting the error variance by statistical treatment. Statistical analysis, including analysis of variance, was performed on each set of experiments.

The results of three sets of experiments are presented in Table 1. Effects of drugs should be compared with the control of that set of experiments only because conditions of standardization differ slightly from one set to another, resulting in different control values. Comparison of the effects of treatment was made by using the value of phosphorylase determinations without added AMP (active form *a*).

* T. T. Iriye, unpublished.
† Unpublished observation.