ther the dose–response curve nor the mean increase in reaction time was significantly altered by pretreatment with the cerebral homogenate from morphine-tolerant rats. The possible reasons for the lack of confirmation of results obtained by other workers were discussed.

We wish to thank Miss Patricia Thayer for her excellent technical assistance. We are also grateful to Dr. Jack W. Miller for his interest and aid.

1. Ungar, G. and Cohen, M., Intern. J. Neuropharmacol. **5**, 183 (1966).

2. Cohen, M., Keats, A. S., Krivoy, W., and Ungar, G., Proc. Soc. Exptl. Biol. Med. **119**, 381 (1965).

3. Smith, A. A., Karmin, M., and Gavitt, J., Biochem. Pharmacol. **15**, 1877 (1966).

4. Spoerlein, M. T. and Scrafani, J., Life Sci. **6**, 1549 (1967).

5. Tirri, R., Experientia **23**, 278 (1967).

6. Eddy, N. B. and Leimbach, D., J. Pharmacol. Exptl. Therap. **107**, 385 (1953).

7. Litchfield, J. T. and Wilcoxon, F., J. Pharmacol. Exptl. Therap. **98**, 305 (1949).

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Carcinogenicity of Long-Term Feeding of Cycad Husk to Rats* (32901)

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Cycads are used as food on Guam and other areas in the tropics and subtropics (1, 2). The cycad, *Cycas circinalis*, is native to Guam. Guamanians wash and grind the kernels of the seeds of the *C. circinalis* to produce a starchy flour resembling wheat flour in appearance and use (3). This cycad flour or processed kernel obtained from local markets on Guam was noncarcinogenic when fed to rats whereas the unprocessed (other than vacuum drying and grinding) kernels were toxic and carcinogenic (3–7).

The fleshy covering or husk of the kernel is used to relieve thirst or when dried is eaten like sweets (1). The use of the husk has special public health implication in that it is not processed. Children reportedly eat the husk since it is sweet and readily available. The acute toxicities of the husk have been demonstrated in rats.[1] These include anemic appearance, hemoconcentration, ascites, weight loss, and early mortality. The present experiments were designed to utilize cycad husk removed from cycad kernels obtained from Guam and the objective was to determine whether long-term ingestion of low levels of cycad husks would produce tumors.

Methods. A total of 120 Sprague-Dawley rats were used in 3 trials. Trials 1 and 2: 10 rats of each sex were fed a diet containing 2.0% or 1.0% husk[2] respectively. Five control rats of each sex were included. Trial 3: 10 rats of each sex were fed diets containing 0, 0.5, or 1.0% husk. Control rats were fed a basal diet[3] without husk.

All rats were housed in individual suspended wire cages and were fed the husk diets or the basal diet and water *ad libitum* starting at 26 days of age. Body weights were recorded weekly. A complete necropsy was performed on all rats which died or when they were killed. The surviving rats in trial 3 were killed and necropsied at the end of 253 days of feeding when the experiment was terminated. Gross lesions were recorded at the time of necropsy. Eighteen different tissues were

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[1] Yang, M. G. and Mickelsen, O., 1967, manuscript submitted for publication.

[2] We thank Drs. L. T. Kurland and M. G. Whiting of the Institute of Neurological Diseases and Blindness, Bethesda, Md. for the generous supply of processed *Cycas circinalis* kernals and husks.

[3] Composition of basal diet has been described in footnote 6 of ref. (3).

selected for histologic examination; however, only those with lesions were included in this report. Hematoxylin–eosin stain was used routinely. Special stains were applied where needed.

Results. Trial 1. There was pronounced growth inhibition of all rats fed 2.0% dried husk in the diet ($p<0.01$) (Fig. 1). The first

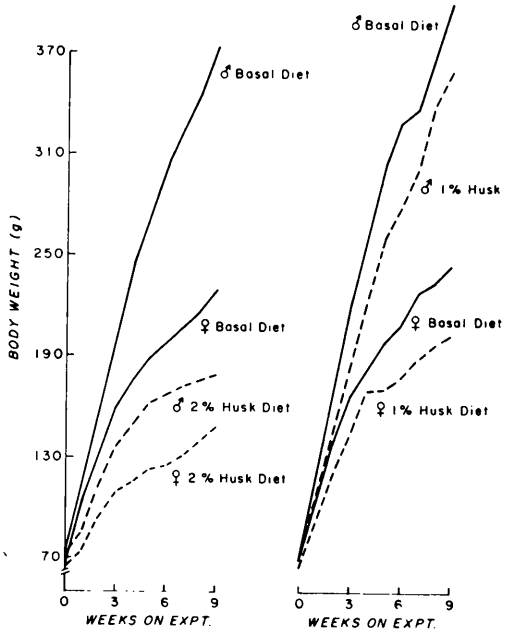

FIG. 1. Average body weight of rats fed different levels of cycad husk in their diets (left half from trial 1, right half, trial 2).

animals died after 33 days and by 83 days all but one female were dead or had been killed because they were moribund. At necropsy many of these rats had petechial hemorrhages in the livers and the pancreases were markedly edematous and appeared white rather than the usual brownish-pink (Fig. 2). All control rats were alive and in good health when the experiment was terminated. The microscopic findings were uniform throughout the group fed cycad husk and consisted of (i) nodular and early adenomatous hyperplasia of liver cells, (ii) multiple foci of bile duct proliferation in the liver, (iii) hemosiderin deposits in central or periportal zones indicating previous hemorrhage, (iv) marked erythrophagocytosis and hemosiderin deposits in peripancreatic lymph nodes, (v) interstitial pancreatic edema with variable cellular reaction suggesting some duration of the edema, (vi) variable degree of jaundice of organs, and (vii) hypoplasia of ovaries and testes. All control rats were normal.

Trial 2. Rats fed the 1.0% husk diet gained weight at a rate less than that of the controls ($p<0.01$) (Fig. 1). Mortality among the husk-fed rats did not begin until week 28 of the study. Except for one male rat all other rats, both males and females, fed the 1% husk diet had neoplastic growths in the livers. The tumors ranged in size from 1 to 5 cm in diameter and were frequently necrotic and hemorrhagic. Microscopic findings in these rats were similar to those found in rats fed 0.5 or 1.0% husk of trial 3 (Table I) and those fed low concentrations of unprocessed cycad kernel (5,6).

Trial 3. Body weight gains of these rats fed 1% husk were much less than those of control rats. The rats that were fed the 0.5% husk diet grew at about the same rate as the control rats (Fig. 3). The most significant lesions found in the rats of trial 3 were malignant neoplasms (Table I). Hepatic tumors developed in rats of both sexes; both levels of husk in the diet produced tumors. Hemorrhage and necrosis accompanied the neoplastic growth but also occurred independently. Necrosis of the pancreas and thyroid involved only small parts of the glands. In all instances, some functional tissues remained. In the thyroid gland, lining epithelial cells were gone from some follicles; the colloid was clumped and poorly stained and follicle walls were broken. No clinical signs of central nervous system involvement were noticed and no microscopic lesions of the central nervous system were found in husk fed rats in the 3 trials.

Discussion. The toxicity of cycad husks suggests a number of public health and research problems. These studies revealed that dried husk is as carcinogenic as unwashed cycad kernel in rats (6). Therefore, the possibility might be considered that consumption of husks by the people of Guam might produce injurious effects in man, particularly because the reports suggest that children from that island consume the husks

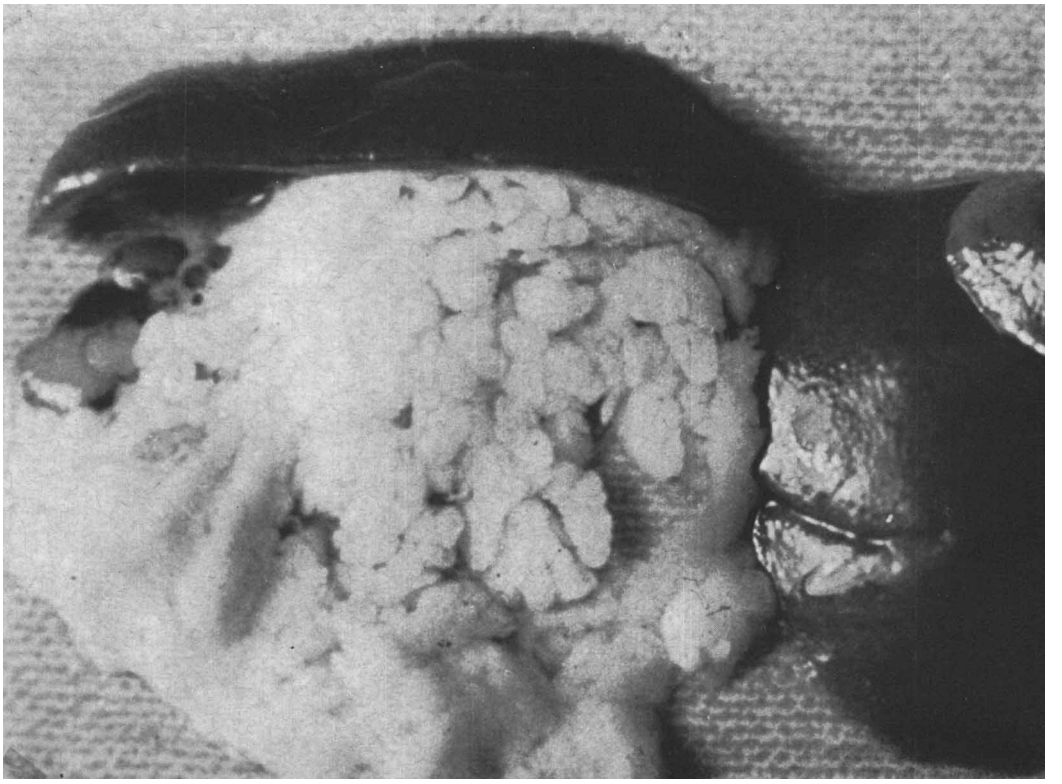

FIG. 2. Pancreas of a male rat fed a diet containing 2% husk for 11 weeks. Pancreas is white and edematous. Organs on top and right of picture are spleen and liver.

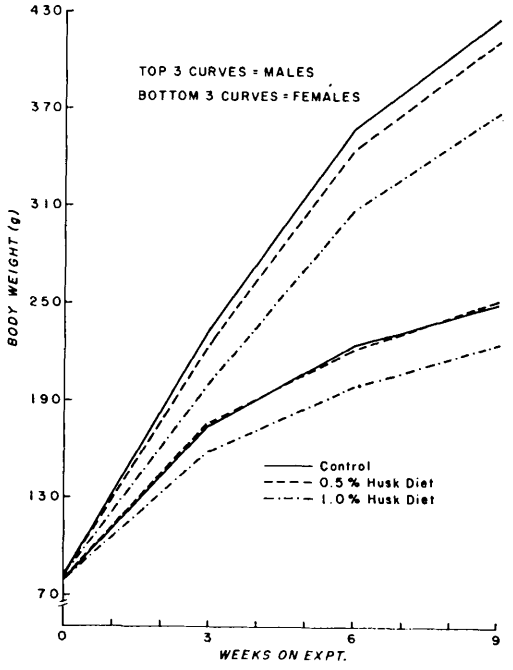

FIG. 3. Average body weight of rats fed diets containing cycad husk (trial 3).

as a confection (1). At present, there are no comprehensive statistics on the incidence of tumors among the Guamanians. Even if the incidence of hepatomas among the Guamanians were higher than among immigrants or foreigners, there would still remain the question whether ingestion of the husks was responsible for such an increase in incidence. Other work in this laboratory has shown that chickens and large animals such as pigs, cows and horses do not appear to be as susceptible to tumor formation as the rat when fed unwashed flour[4]. This observation suggests the possibility that variations in species susceptibility may extend to man who may not be adversely affected by long-term ingestion

[4] Sanger, V. L., Yang, M. G., and Mickelsen, O., Federation Proc. 26, 358. Abstract presented in FASEB Meetings, 1967.

TABLE I. Lesions in Male and Female Rats Fed Cycad Husks, Trials 2 and 3.[a]

	Cycad husk in diet (%)					
	0.5	1.0	1.0	0.5	1.0	1.0
	Males[b] (no. affected)			Females[b] (no. affected)		
Organ and lesion	Trial 3	Trial 2	Trial 3	Trial 3	Trial 2	Trial 3
Liver						
hepatoma	3	6	5	8	8	8
carcinoma	1	—	1	—	—	—
necrosis	2	1	3	3	2	—
hematocyst	1	—	—	3	—	—
bile duct hyperplasia	—	3	1	—	1	—
hemangioma	1	—	—	1	—	—
adenoma	—	1	—	—	—	—
sarcoma	—	1	—	—	—	—
Lung						
metastatic tumor	—	2	2	—	3	4
hemorrhage	—	—	—	—	3	1
Intestine	—	4	—	—	—	—
Spleen						
metastatic tumor	—	—	1	—	—	—
Kidney						
cellular degeneration	—	—	1	—	—	—
cystic tubules	4	—	—	2	—	—
interstitial fibrosis	—	—	—	—	1	1
adenoma	—	3	—	—	—	—
interstitial tumor	—	1	—	—	1	—
Pancreas						
necrosis	1	—	1	1	—	1
metastatic tumor	—	—	1	—	—	—
Miscellaneous[c]	—	1	—	—	—	—

[a] Controls: negative for lesions.
[b] Ten animals in each group.
[c] Intraperitoneal sarcoma, primarily site unknown.

of cycad husks. However, symptoms of acute toxicity such as violent illness, vomiting, loss of consciousness and death have been observed in people after consuming unwashed cycad (1). Although cycad products are ingested by Guamanians, efforts to secure information about the amounts consumed have not been successful. The ingestion of cycad is apparently a symbol of "low economic status" and for this reason Guamanians are reluctant to admit to its use despite the presence of cycad flour (made from the kernels) in a handy place in the kitchen of many homes on the island.[5]

Evidence is accumulating which suggests that different parts of the cycad plant produce different toxicological effects. The amount of cycasin, the known toxic glucoside of cycad products, in the batch of husks used in the present experiments suggests that the lesions produced by the husks may have a different etiological basis than that of the kernel. Assays revealed that this batch of husks contained only traces of cycasin. The concentration is so low that it could not have caused the damages observed. There is a

[5] Whiting, M. G., Inst. of Neurological Diseases and Blindness, NIH, Bethesda, Md. personal communication.

possibility that the toxins in this batch of husks are methylazoxymethanol, the aglycone of cycasin, and/or neocycasins. Neocycasins have the same aglycone as cycasin but differ in the glycoside residue.[6] The leaves of the plant may also differ in their toxicity at least in animals. All of the reports of pronounced neurological symptoms in animals feeding on cycad have involved the leaves of the plant (1). The ingestion of cycad kernel by cattle in amounts sufficient to produce a marked loss of appetite has only a minimal neurological effect and produces no histological lesions in either the brain or spinal cord.[7] The ingestion of cycad leaves by cattle produced locomotor disturbances of the hind limbs associated with lesions in the brain.[8]

These considerations suggest that the chronic type of cycad toxicity seem in rats with ultimate development of tumors may not apply to man—toxicity in the latter may be limited to the acute form which when severe enough may terminate fatally. As for animals, the kinds of toxicity which develop following the ingestion of cycad probably depends on the species of animals, amounts eaten, duration of ingestion, and the part of the plant consumed.

[6] Assays performed by Dr. Maria Spatz, Natl. Cancer Inst. NIH, Bethesda, Md.

[7] Mugera, M. G., thesis, Michigan State University, East Lansing, Mich., 1965.

[8] Hall, W. T. and McGavin, M. D., 1965 "Conference on the toxicity of cycads," (fourth), p. 165. Conference sponsored by Natl. Inst. of Neurological Diseases and Blindness, and Nat. Inst. of Arthritis and Metabolic Diseases, NIH, Bethesda, Md.

Summary. Guamanians eat the fleshy husk of the seeds of the cycad, *Cycas circinalis,* which is indigenous to Guam. The fresh husk is eaten to relieve thirst. The dried husk is eaten as candy. The kernel of this seed is soaked in water, sun dried, and ground for preparation as an ingredient in foods. This processed kernel was not toxic or carcinogenic in contrast to unprocessed kernels. Since the husk is consumed without cooking or processing other than drying, and since the husk is reported to be eaten primarily by children, it is imperative to evaluate the safety of this food. The acute toxic effects have already been studied whereas the carcinogenic properties of the husk have not. For this reason rats were fed diets containing 0.5–2.0% of the dry husk for prolonged periods. Malignant tumors were found in the liver and kidney of experimental animals. Metastatic tumors were found in the pancreas, spleen, and lung.

1. Whiting, M. G., Econ. Botany **17**, 271 (1963).
2. Thieret, J. W., Econ. Botany **12**, 3 (1958).
3. Campbell, M. E., Mickelsen, O., Yang, M. G., Laqueur, G. L., and Keresztesy, J. C., J. Nutr. **88**, 115 (1966).
4. Yang, M. G., Mickelsen, O., Campbell, M. E., Laqueur, G. L., and Keresztesy, J. C., J. Nutr. **90**, 153 (1966).
5. Laqueur, G. L., Mickelsen, O., Whiting, M. G., and Kurland, L. T., J. Natl. Cancer Inst. **31**, 919 (1963).
6. Laqueur, G. L., Federation Proc. **23**, 1386 (1964).
7. Spatz, M., Federation Proc. **23**, 1384 (1964).

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Response of Rat Kidney Na^+–K^+-Activated Adenosine Triphosphatase to Sodium Deprivation* (32902)

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There is abundant evidence indicating that sodium transport in isolated organ systems, (e.g., red blood cell, squid axon, toad bladder) as well as in the intact mammalian kidney, is an active process (1). The suggestion has been made that membrane Na^+–K^+-

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