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.

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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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activated adenosine triphosphatase plays a critical role in the transport of both inorganic and organic ions (2). An adaptive increase in activity of this enzyme has been demonstrated in the avian salt gland in association with increased secretion of sodium (3).

The majority of tissues which demonstrate active transport of sodium have detectable levels of Na^+–K^+-activated adenosine triphosphatase and there appears to be a direct correlation between the level of this enzyme and the metabolic activity of the tissue studied (4). Ouabain, a specific inhibitor of Na^+–K^+-activated adenosine triphosphatase, has been shown to block sodium transport in the avian salt gland, red blood cell, and intestinal loop, as well as in bladder and kidney slices (2). To our knowledge, however, no previous studies have been made on the effect of salt deprivation (potentially a potent stimulus to aldosterone production, and, hence, increased active transport of sodium by the kidney) on the activity of Na^+–K^+-activated adenosine triphosphatase in the mammalian kidney *in vivo*.

Materials and Methods. Ten adult male Wistar rats, weighing from 388–488 gm, were maintained for a period of 8 days on 20 gm/day of an artificial neutral ash diet containing all essential nutrients and supplemented with 2.05 meq of sodium and 1.61 meq of potassium. The animals were then divided into two equal groups. The first group (control) was continued on the same diet for an additional 8 days. The second group was fed a diet containing the same constituents except that sodium was reduced to a level of 0.178 meq/day. All animals were maintained in metabolic cages and were trained to consume the entire diet. Daily urine collections were obtained for sodium determinations.

At the termination of the final urinary collection period, the animals were anesthetized with Dibutal and sacrificed by exsanguination. The kidneys were removed and weighed. The left kidney was grossly dissected into cortex, medulla, and papilla, and portions of each section were weighed and homogenized in ten volumes of sucrose. The activities of total Mg^{2+}-activated adenosine triphosphatase and Na^+–K^+-activated adenosine triphosphatase were measured in the various tissue fractions according to methods described previously by Bonting *et al.* (5). The activity of Na^+–K^+-activated adenosine triphosphatase was expressed both as an increase in total adenosine triphosphatase following Na^+ and K^+ stimulation and as reduction in total adenosine triphosphatase following ouabain inhibition. The activities of each enzyme were then related to protein content, using bovine serum albumin as a standard protein.

Results. Mean urinary sodium excretion on the day prior to sacrifice in the animals ingesting the normal diet was 0.45 meq/day, with a standard deviation of 0.20 meq/day and a range of 0.26–0.82 meq/day. In the animals on a low salt intake mean urinary sodium excretion was 0.04 meq/day, with a standard deviation of 0.01 meq/day and a range of 0.02–0.06 meq/day. Activities of

TABLE I. Total Adenosine Triphosphatase and Na^+–K^+-Activated Adenosine Triphosphatase Activity in Sections of Rat Kidney on Normal and Low Salt Intake.

Kidney fraction	Total adenosine triphosphatase activity[a]		Na^+–K^+ adenosine triphosphatase activity[a b]		Na^+–K^+ adenosine triphosphatase activity[a c]	
	Normal salt	Low salt	Normal salt	Low salt	Normal salt	Low salt
Cortex	3.072 ± 1.391	2.888 ± 1.021	0.330 ± 0.123	0.378 ± 0.175	0.405 ± 0.093	0.416 ± 0.204
Medulla	4.218 ± 2.162	3.928 ± 1.386	0.450 ± 0.496	0.664 ± 0.269	0.804 ± 0.513	0.766 ± 0.255
Papilla	0.974 ± 0.288	0.744 ± 0.197	0.172 ± 0.039	0.113 ± 0.051	0.118 ± 0.080	0.088 ± 0.084

[a] Mean and standard deviation. Results expressed as μmoles of PO_4 liberated/20 min per mg of protein at 37°C.

[b] Na^+–K^+-stimulated fraction of total adenosine triphosphatase.

[c] Ouabain-inhibited fraction of total adenosine triphosphatase.

Mg^{2+}-activated adenosine triphosphatase and Na^+–K^+-activated adenosine triphosphatase on both dietary regimens are presented in Table I. Examined either in terms of Na^+–K^+-stimulated adenosine triphosphatase activity or ouabain-inhibited activity, there was no significant difference in Na^+–K^+-activated adenosine triphosphatase in any portion of the kidney when the two dietary regimens were compared.

Discussion. The low urinary sodium values following 8 days of sodium deprivation indicated that a maximal stimulus for sodium reabsorption had been achieved. Although aldosterone excretion or secretion was not directly measured, it may be assumed that hormone production was increased by this physiological stimulus. Despite maximal sodium reabsorption, however, no increase in activity of either total Mg^{2+}-activated adenosine triphosphatase or Na^+–K^+-activated adenosine triphosphatase could be demonstrated in this experiment.

There is an accumulating body of evidence that aldosterone exerts its action on sodium transport via synthesis of new protein or enzyme (6). It could be speculated that aldosterone stimulates increased synthesis of Na^+–K^+-activated adenosine triphosphatase, an enzyme previously linked to sodium transport. The results of the present experiment, however, do not directly support this hypothesis. Chignell *et al.* (7) and Landon and Forte (8) have previously observed that adrenalectomy results in a marked diminution of Na^+–K^+-activated adenosine triphosphatase in rat kidney. The former workers also noted that acute administration of aldosterone and growth hormone to adrenalectomized rats did not increase the activity of the kidney enzyme to normal, although chronic administration of corticosterone was effective in restoring normal values. Incubation of nonadrenalectomized rat kidney homogenates with aldosterone failed to result in increased activity of Na^+–K^+-activated adenosine triphosphatase (9).

The negative results of the present experiment may therefore be accounted for in several possible ways. The sensitivity of the methodology for analysis of Na^+–K^+-activated adenosine triphosphatase may be insufficient to detect small increases in activity since the level of this enzyme is determined by difference (*vide supra*) and is not assayed directly. An alternative explanation, as suggested by the results of experiments quoted above, is that the Na^+–K^+-activated adenosine triphosphatase activity is nearly maximal under conditions of normal aldosterone production and excretion. Stimulation of aldosterone production by a low sodium intake might then result in a small increase in Na^+–K^+-activated adenosine triphosphatase which is physiologically important to the organism but not detectable by present methodology. Finally, an endogenous feedback control mechanism involving enzyme–inhibitor–activator complexes may be operative. Enzyme kinetic studies are currently underway to test the latter hypothesis.

Summary. Studies demonstrating the effect of low salt intake on the activity of Na^+–K^+-activated adenosine triphosphatase in the rat kidney have been described. The results indicated that no detectable changes in the level of this enzyme occurred.

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