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Response of Liver Nucleic Acids and Lipids in Rats Fed *Cycas circinalis* L. Endosperm or Cycasin. (29740)

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Cycad plants, which are found throughout the world in tropical and subtropical regions, provide both a staple and an emergency food for peoples where the plants are native. Recently interest in the biochemical and physiological effects of products from the cycads has arisen because of indications of toxicity when fed to animals(1). Of special interest is the fact that both liver and kidney tumors arise in rats(2) and liver tumors in guinea pigs (M. Spatz and G. L. Laqueur, personal communication) fed either the fresh ground endosperm of *Cycas circinalis* L. (cycad meal) or cycasin, a glycoside (methylazoxymethanol glycoside), isolated from it. From histological studies(2) it was observed that within 1-2 days after feeding either cycad meal or cycasin at appropriate levels, loss of cytoplasmic basophilia in liver cells begins to occur. The light microscopic observations correlate with an apparent loss of ribonucleoprotein particles (ribosomes) from the parallel arrays of the endoplasmic reticulum as observed in preliminary electron microscopic studies of the fine structures of these cells (B. Wetzel and G. L. Laqueur, personal communication).

Because of the association of ribosomes with the lipoprotein-rich endoplasmic reticular membrane, RNA and phospholipid concentrations in liver cells of rats after feeding cycad meal or cycasin were examined. A positive correlation of changes in concentrations of these substances with the histologically observed loss of cytoplasmic basophilia and probable disintegration of the endoplasmic reticulum would indicate that a primary effect of the cycad toxins might be at this locus of protein synthesis.

The guanine of both RNA and DNA is alkylated by dimethylnitrosamine(3), a compound structurally related to cycasin. Therefore, DNA was included in the present studies. In addition to phospholipids, neutral glycerides, cholesterol, and plasmalogens were studied to see if a general derangement of lipid metabolism occurs after cycad or cycasin feeding.

Experimental. Liver RNA and DNA were extracted by the procedure of Schneider(4). RNA was estimated by the orcinol reaction (4); and DNA, by the diphenylamine reaction(5). Phospholipid was measured by the method of Fiske and Subbarow(6), total li-

pid by the procedure of Bragdon(7), plasmalogens by the method of Williams *et al* (8), and total cholesterol by the method of Pearson *et al*(9), using washed liver lipid extracts prepared by the procedure of Folch *et al*(10). Neutral glycerides were calculated by difference between the total lipids and phospholipid plus cholesterol.

Two types of studies were carried out: (1) The response of the various liver components to the feeding of a single level of cycad meal or cycasin for various time intervals, and (2) dose-response experiments in which different levels of cycad meal or cycasin were fed for a single length of time. Cycad meal was prepared from vacuum-dried thin slices of fresh kernels of cycad nuts obtained by air shipment from Guam. Crystalline cycasin was prepared in the Dept. of Agricultural Chemistry, Kagoshima University, Kagoshima, Japan, and was made available to us through the courtesy of Dr. L. T. Kurland. The cycad meal or cycasin was added to ground stock ration* and fed to male albino rats (Osborne-Mendel strain) weighing 50-60 g. In all experiments pair-fed controls were included to obviate effects from lowering of food intake by the toxic substances. At termination of the experiment the rats were stunned and decapitated. The livers were removed and chilled in ice. Portions were frozen until analyzed for nucleic acids and lipids.

Results. Statistical analysis was made of results using Student's t test. If the mean of any group was significantly different from the mean of the corresponding control ($p < 0.005$), the point is encircled in the Figure.

RNA and DNA. In Fig. 1 are shown the results with RNA and DNA after feeding 0.2% cycasin for 0-8 days. The results are expressed as per cent of the values from control rats pair fed only the stock ration. After 1 day RNA values dropped to about two-thirds of normal and remained at that level for 5 days, followed by a tendency to return toward normal after 8 days. DNA concentrations were unaffected by feeding cycasin under these conditions.

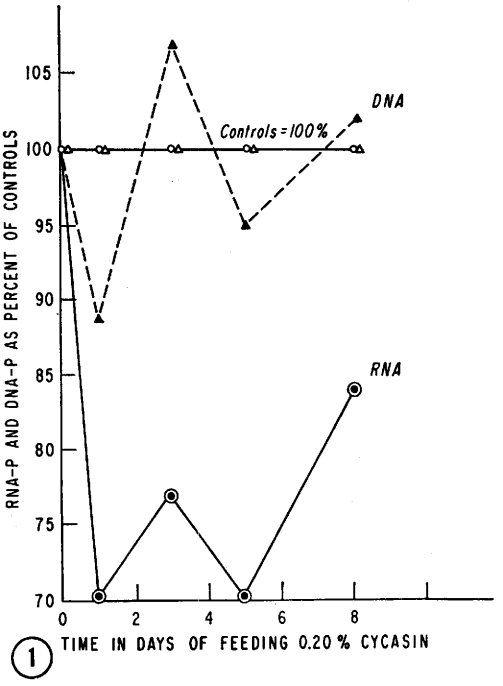
In Fig. 2 are presented results of feeding increasing levels of cycad meal (1-3%) or cycasin (0.01-0.4%) for 48 hours on liver RNA and DNA concentrations. Chemical analysis of the cycad meal indicated that it contained approximately 3% of cycasin. About one-third of the liver RNA per gram of liver was lost by feeding either 3% cycad meal or 0.3-0.4% of cycasin. DNA concentration was not significantly affected by either cycad meal or cycasin. From these results it appears that the cycad meal is considerably more potent in its effects on RNA concentrations, based on its cycasin concentration, than cycasin.

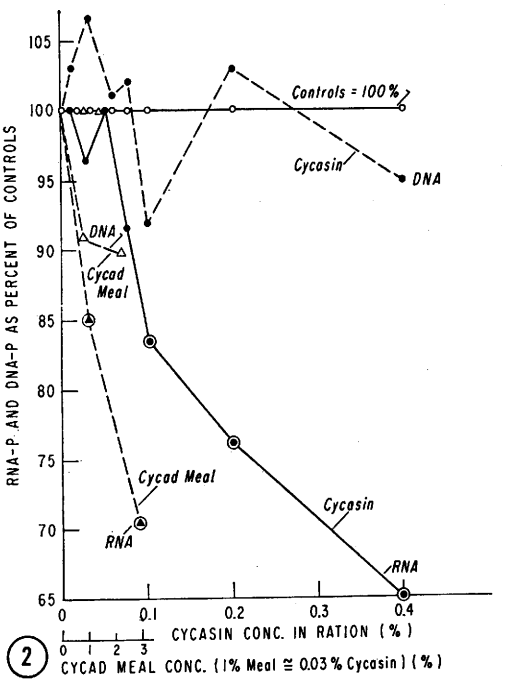
Phospholipids. From Fig. 3 it can be seen that the response of liver phospholipids to cycad meal or cycasin-feeding was almost identical to the results for RNA (Fig. 2).

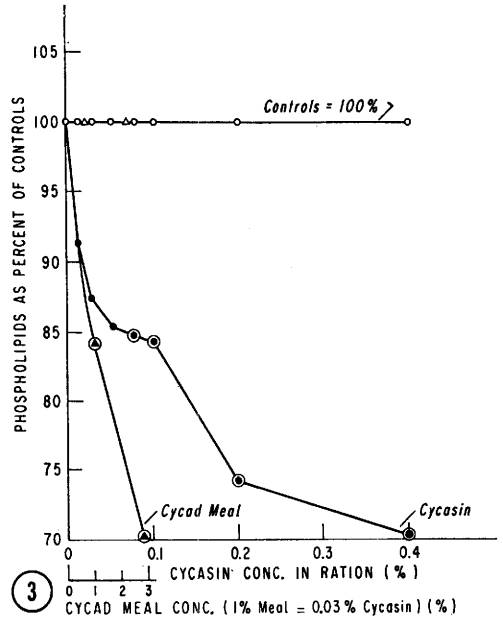
Other lipids. Liver neutral glyceride concentrations were increased by about 40% over the controls by all levels of cycasin tested (0.01-0.4%) (figure not shown). Cycad meal, however, at 1-3% levels had no effect on neutral glyceride concentrations. From Fig. 4 it can be seen that cycasin increases liver cholesterol almost linearly when fed at 0.01-0.4% levels. Cycad meal had no greater effect than would be caused by its estimated cycasin content. No effect on plasmalogen concentration was observed either with cycad meal or cycasin.

Discussion. The dissimilarities in effects from cycad meal compared with cycasin, both in kind and degree, observed in several instances in these studies permit the following conclusions: (1) Cycad meal may contain substances in addition to cycasin that produce losses in cellular RNA and phospholipids. (2) Since cycasin causes elevated neutral glyceride levels while cycad meal does not, it is possible that cycad meal contains substances which reverse the effects of its cycasin content in this respect. (3) The identical effects of cycad meal (based on its cycasin content) and cycasin on liver cholesterol indicate that cycasin is probably the

* The ground stock ration was Purina N.I.H. Chick Startina and contained 23.0% crude protein (min.), 4.5% crude fat (min.), 4.0% crude fiber (max.) and 67% nitrogen-free extract (min.). The exact composition is available upon request.







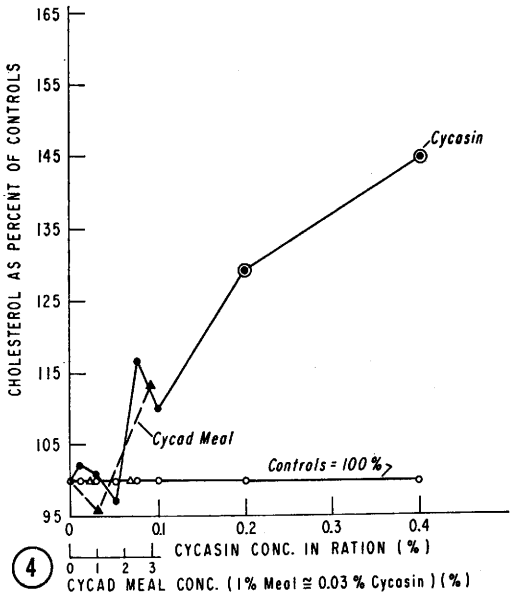


FIG. 1. Effect of 0.2% of cycasin for 0-8 days on liver RNA and DNA concentrations. Results are expressed as per cent of controls. Encircled points indicate that the means are significantly different from controls ($p < 0.005$; Student's t test). Five rats per point.

FIG. 2. Effect of cycasin or cycad meal fed for 48 hours on liver RNA and DNA concentrations. Results are expressed as per cent of controls. Encircling of points indicates that the means are significantly different from controls ($p < 0.005$; Student's t test). Five rats per point.

FIG. 3. Effect of cycasin or cycad meal fed for 48 hours on liver phospholipid concentration. Results are expressed as per cent of control. Encircling of points indicates that the means are significantly different from controls ($p < 0.005$; Student's t test). Five rats per point.

FIG. 4. Effect of cycasin or cycad meal fed for 48 hours on liver cholesterol concentration. Encircling of points indicates that the means are significantly different from controls ($p < 0.005$; Student's t test). Five rats per point.

only component of cycad meal which increases liver cholesterol.

The marked similarity in effects of cycad meal and cycasin on RNA and phospholipid concentrations, which also correlate closely with the histologically observed disintegration of the cytoplasmic basophilia and of the loss of ribosomes from the parallel arrays of the endoplasmic reticulum, is evidence that a primary effect of the cycad toxins in liver cells may be localized at the endoplasmic reticulum. Whether the main effect is on the lipoprotein-rich membrane of the endoplasmic reticulum, whose destruction would cause a loss of positions for the attachment of the ribosomes, or whether the effect is on RNA synthetic pathways or directly on RNA by alkylation of the bases is not clear. This relationship is being studied.

Summary. The feeding of ground *Cycas circinalis* L. endosperm or cycasin, a glycoside isolated from the cycad endosperm, both of which produce liver and kidney tumors in rats and liver tumors in guinea pigs, produces an almost identical loss of RNA and phospholipids from rat liver cells. The ground endosperm is considerably more active, based on its cycasin content, than is cycasin. These changes correlate closely with loss of cytoplasmic basophilia, as observed with the light microscope, and with loss of ribosomes from the parallel arrays of the lipoprotein-rich endoplasmic reticular membrane, as observed with the electron microscope. No effect on DNA concentration occurred. Liver cholesterol and neutral glycerides were increased by cycasin feeding.

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Specific Complement-Fixing Tumor Antigen in SV_{40}-Transformed Human Cells.* (29741)

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The presence of a specific complement-fixing (CF) antigen in SV_{40}-induced tumors and in SV_{40}-transformed cells was demonstrated by Black *et al*(1) through the use of serum antibodies produced in SV_{40} tumor-bearing hamsters. The same antigen was shown to be present not only in hamster tumors but in hamster, rabbit, mouse, pig and bovine cells "transformed" by SV_{40} virus *in vitro*, and their experimental findings suggested that this CF antigen was not identical to structural antigens of the virus. However, its specificity indicated that the information

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