Cycling of Cycasin from Newborn Rats to Their Mother and Back to the Newborn* (33822)

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Magee (1) and Hirono *et al.* (2) demonstrated that day-old rats injected subcutaneously with a single dose of 2.5 mg of cycasin developed tumors in various organs. This observation was unique and seemed contrary to what is known about the mechanisms of action of cycasin. All of the previous work suggested cycasin requires deglucosylation and its aglycone, methylazoxymethanol (MAM), is the principal carcinogenic moiety (3,4). Deglucosylation is accomplished by beta-glucosidases present in gastrointestinal tracts. This was first suggested by Nishida *et al.* (5) when they reported cycasin is toxic when fed but nontoxic when injected into rats. These investigators (5) proposed that the explanation involved the presence of beta-glucosidases in the microflora of the gastrointestinal tract. The absence of toxicity in germfree rats fed cycasin confirmed the essential role of bacterial beta-glucosidases (6). Further evidence for the essentiality of beta-glucosidases came when toxicity was produced in monocontaminated rats fed cycasin (7).

The present studies were designed to determine whether cycasin injected into newborn rats is excreted and then consumed by their dams. This would permit the bacterial enzymes of the dam's gastrointestinal tract to split the cycasin. The MAM thus liberated would be absorbed and secreted via the mammary glands back to the newborn rats thus exposing them to the active carcinogen. This route, even though circuitous, is a likely one because rats groom their pups and induce them to urinate by licking. The transmission of toxicity via the mammary gland has already been shown to occur in several species including rats when the young nursed mothers fed cycasin (8). The urinary excretion rates of injected cycasin by newborn rats were also studied and compared to those obtained from older rats.

Methods. To determine whether cycasin is consumed by the dam and MAM returned to the pups via the mammary glands, part of each of 14 litters of newborn rats were injected subcutaneously with saline and the remainder with either 2.5 or 5.0 mg of cycasin in 0.05 ml of sterile saline. All pups were then returned to their respective dams thus allowing saline and cycasin-treated pups to nurse together. If the toxin were consumed by the dam and secreted into the milk, then all pups including the saline-treated ones should become affected. Of the 14 litters, 1 litter each was killed 2 and 3 days after injection, 4 litters were killed after 10 days and another 3 after 11 days. The remaining 5 litters were weaned at 22 days of age. The weanlings were housed in individual suspended wire cages and fed a laboratory rat diet. Three of the last 5 litters were weighed periodically. The rats that were weaned were killed only when they became moribund. All rats were autopsied and kidneys and liver were fixed in formalin and examined histologically using eosin and hematoxylin stains.

In a separate study, 70 rats were given a single subcutaneous injection of cycasin either on 1, 2, 7, or 14 days of age. The 1- and 2-day-old rats were injected each with 5 mg whereas 7- and 14-day-old rats were injected with 10 and 20 mg of cycasin, respectively. The cycasin was dissolved in saline at a concentration of 250 mg/ml. After injection the rats were placed individually in small beakers until killed at intervals ranging from 1 to 8 hours after the injection of cycasin. At least 3 rats were killed by decapitation at each in-

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terval. Urine was obtained quantitatively from the bladder which had been exposed by an incision through the abdominal wall.[1] The urine from each rat was analyzed for cycasin by liquid-gas chromatography after trimethylsilylation (9).

Results and Discussion. The increases in body weights of newborn rats injected with cycasin were less than those injected with saline. The average body weights at 22 days of age were 45.9 g for the saline-treated rats and 41.9 and 40.5 g for those injected with 2.5 and 5.0 mg of cycasin. Except for 2 litters killed at 2 and 3 days of age, all rats including those injected with saline had liver lesions. In the 2 litters killed at 2 and 3 days of age only the pups injected with 2.5 mg of cycasin had lesions. These included widespread degenerative changes such as albuminous and fatty degeneration with loss of nuclei of the liver cells. The seven litters of rats that were killed 10 and 11 days after the injections not only had some hepatic degeneration and vacuolation but also some necrosis. These findings strongly suggest that cycasin was consumed by the dams and that MAM or a comparable compound was returned to the pups via the dam's milk. This is the only apparent means whereby the pups injected with saline could have been exposed to the metabolites of cycasin. Extramedullary hemopoeisis also occurred in both the saline-and cycasin-injected rats but this was considered normal.

Of the 5 litters of rats that were weaned, 21 were injected with 5 mg of cycasin, 8 with 2.5 mg and 14 with saline. Nearly all of these rats died or were killed because they were moribund between 6 and 12 months of age. During this period of time 15 or about 50% of the rats injected with cycasin had kidney tumors. The tumors were identified as carcinoma, fibrosarcoma, and hemangiosarcoma, all of which were found in earlier studies where rats were fed cycasin or cycad products containing cycasin (10, 11). One of 14 saline-injected rats had renal carcinoma. A second had a large kidney hematoma and nearby, a focus of neutrophils in the interstitial tissues. The latter suggested an early nephritis. Another 5 saline-treated rats had liver lesions similar to those found in rats fed cycasin (10). However, the lesions observed in these 5 rats were not as severe as those previously reported (6, 10). The lesions observed were also much less severe when compared to those found in the rats injected with cycasin in the present study. Six of the 29 injected with cycasin had extramedullary hemopoiesis in the liver. Except for those litters killed 2 or 3 days after injection, all other litters indicate that cycasin or its metabolites was cycled from the pups to their mothers and then back to the pups via the mother's milk. One of the reasons the saline injected pups of the 2 litters killed 2 and 3 days after injection did not have lesions could be insufficient time for the cycasin to complete the pup–dam–pup cycle.

When rat pups of different ages were injected with cycasin, the fraction of the dose recovered in the urine increased with the age of the pups at the time of injection. There was a marked increase in excretion rate about 24 hr after birth and again sometime between days 7 and 14 of life (Fig. 1). These rates were based on the amounts excreted in the urine during the first 7 hr after injection. It was not possible to extend the study beyond that time since the bladders of these rats became so full thereafter that urine leaked out. When this happened, it was impossible to recover cycasin quantitatively from the bodies of the animals.

In 7 hr, the 1-day-old pups excreted about 33% of the injected dose of cycasin as such; the 2-and 7-day-old pups excreted 50–60%. When 14-day-old rats were injected with cycasin, they excreted almost 100% of the injected dose in the urine within about 7 hr. This rate for the 14-day-old rats is comparable to that reported by Kobayashi and Matsumoto (12) for adult rats. Within 12 hr, their rats excreted into the urine 86–103% of 138–150 mg of cycasin which had been injected intraperitoneally.

[1] Young rats for unknown reasons do not urinate unless properly induced by their dams. Artificial induction of urination in these rats is possible but cumbersome and imperfect.

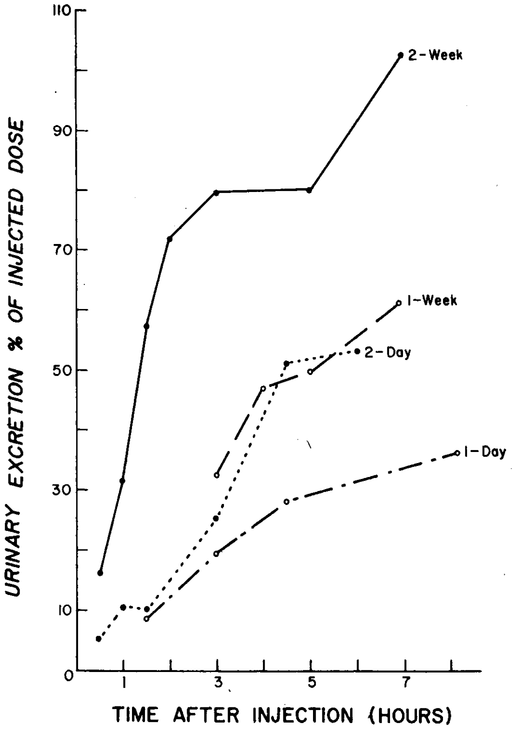

Fig. 1. Urinary excretion rates of cycasin injected as a single dose subcutaneously into rats when they were 1, 2, 7, or 15 days old.

By extrapolation of the curve in Fig. 1 the day-old rats would require more than 1 day to excrete the injected dose of cycasin in contrast to the 14-day olds which required only 7 hr. This difference is probably a reflection of different kidney functions of the 2 age groups, for other workers have previously demonstrated older rat pups to have better kidney functions than younger pups (13, 14). Whether some of the retained cycasin is secreted into the gastrointestinal tract is doubtful but possible. If this should happen, cycasin in the gastrointestinal tract can thus be cleaved and its toxic aglycone liberated. This aspect has not been studied, but in 14-day-old rats the complete recovery in urine of injected cycasin would indicate that cycasin was not secreted into the gastrointestinal tracts of these rats.

Summary. Day-old-rats injected subcutaneously once with saline, 2.5 or 5.0 mg of cycasin and returned to their dam developed kidney and liver tumors later in life. It is suggested that the lesions seen in these rats resulted from the ingestion by the dam of the cycasin excreted in the pups' urine. The urine was probably consumed by the dam when she licked and groomed her pups. The cycasin was then degraded to MAM, the presumed carcinogen, by the bacteria in the dam's intestinal tract. MAM and the cycasin that escaped deglucosylation were then returned to the pups via the dam's milk. By this means, the saline as well as the cycasin injected pups were exposed to the carcinogen. In separate studies, day-old rats excreted within 7 hrs up to 33% of 5.0 mg of cycasin injected subcutaneously. This rate increased to 50–60% in 2-day-old pups and sometime after day 7 of life, 100% of a 20-mg dose could be recovered in the urine within 7 hr.

1. Magee, P. N., Fourth Conference on the Toxicity of Cycads, (M. G. Whiting, ed.), Natl. Inst. Health, Bethesda, Maryland (1965).

2. Hirono, I., Laqueur, G. L., and Spatz, M., J. Natl. Cancer Inst. **40**, 1003 (1968).

3. Laqueur, G. L. and Matsumoto, H., J. Natl. Cancer Inst. **37**, 217 (1966).

4. Matsumoto, H. and Higa, H. H., Biochem. J. **98**, 20C (1966).

5. Nishida, K., Kobayashi, A., Nagahama, T., Kojima, K., and Yamane, M., Seikagaku **28**, 218 (1956).

6. Laqueur, G. L., Federation Proc. **23**, 1386 (1964).

7. Spatz, M., Smith, D. W. E., McDaniel, E. G., and Laqueur, G. L., Proc. Soc. Exptl. Biol. Med. **124**, 691 (1967).

8. Mickelsen, O. and Yang, M. G., Federation Proc. **25**, 104 (1966).

9. Wells, W. W., Yang, M G., Bolzer, W., and Mickelsen, O., Anal. Biochem. **25**, 325 (1968).

10. Yang, M. G., Sanger, V. L., Mickelsen, O., and Laqueur, G. L., Proc. Soc. Exptl. Biol. Med. **127**, 1171 (1968).

11. Laqueur, G. L., Mickelsen, O., Whiting, M. G., and Kurland, L. T., J. Natl. Cancer Inst. **31**, 919 (1963).

12. Kobayashi, A. and Matsumoto, H., Arch. Biochem. Biophys. **110**, 373 (1965).

13. Hall, S. M. and Zeman, F. J., J. Nutr. **95**, 49 (1968).

14. McCance, R. A., "Age and renal function, in Renal Disease" (D. A. K. Black, ed.) Blackwell, Oxford (1962).

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