Calculi and Kidney Calcification from Feeding Milk Diets to Rats and Hamsters

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FOR CENTURIES men have sought methods to prevent the formation of calculi in the kidneys and bladder. Much effort has also been devoted to discover methods of dissolving calculi after they are formed.

If more rapid progress is to be made in this field, better use must be made of suitable test animals. The rat and the Syrian hamster seemed worthy of study to determine if they might be useful for such research.

During the past two decades, the calcification of the kidneys of rats has been given some attention in our laboratory, especially by Barnes,[1] Sperling *et al.*,[2] and Saxton *et al.*[3] The kidneys of female rats have proved to be more vulnerable to calcification than those of the males. Diets composed chiefly of whole milk supplemented with trace minerals have proved especially effective in producing calcification of the female kidneys. In the course of these studies numerous observations have been made of calculi in the bladders of white rats dying in old age.

Numerous reports in clinical medicine attempt to relate calculi to the long continued use of Sippy diets. These diets, formerly much used in ulcer treatment, involved the ingestion of substantial amounts of milk and sodium bicarbonate. In a modern text such as that of Ivy *et al.*[4] summaries are given which indicate that the evidence is not entirely convincing that milk and bicarbonate may lead to an increased incidence of calculi.

Since milk itself tends to produce calculi in rats, one may ask if this is related to the high calcium phosphate level provided by milk, to the influence of milk diets upon the pH of the urine or to the low magnesium content of milk. Many studies have implicated magnesium in the formation of calculi,[5] but no consideration was given to it in these experiments.

Although little attention has been given to hamsters in the course of long-term experiments, this species might seem more useful than the rat for such studies because it excretes much more calcium in the urine than does the rat. Hence, one might expect the kidney of the hamster to suffer from deposition of calcium. However, the hamster is much less efficient than the rat in the absorption of calcium from the intestinal tract.[6] It is also more difficult to feed liquid milk diets to the hamster since it tends to ingest less fluid.

The present studies were undertaken to extend earlier observations with rats and to compare the effects of feeding milk to the two species. Attempts were also made to determine the time needed to produce calcification of the kidneys. Since some claims have been made by clinicians that calcification can be reversed in patients, an initial attempt was made to change diets and determine if calcification could be reversed.

Earlier studies had indicated that milk diets tend to protect teeth against decay, help maintain denser bones in old age, and often lead to the formation of balls of hair (trichobezoars) in the stomach of rats. Therefore, attention was paid to these problems in the course of these experiments.

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This study was supported in part by Grant No. H-1658 from the National Heart Institute of the U. S. Public Health Service, in part by a grant from the Dental Branch of the Office of Naval Research and in part by a grant from the Rockefeller Foundation.

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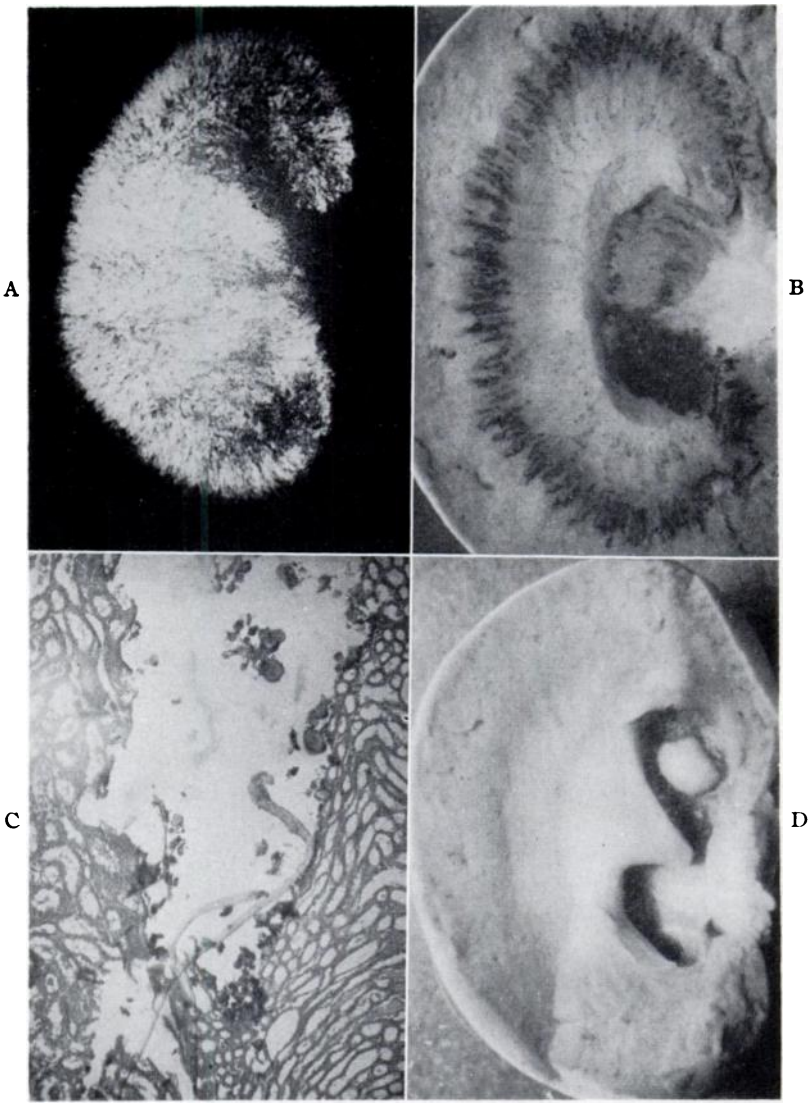

Fig. 1. A. Calcification in medulla of kidney of female rat as shown by x-ray. B. Calcification in medulla of kidney of female rat shown by von Kossa's silver nitrate stain. Small calculi also are present in pelvis. C. Calculi of microscopic size in calyx of male rat's kidney. D. Large calculus in pelvis of kidney of male rat. Small calculi are seen behind the large one.

(*A*) *First Study with Rats and Hamsters*

Thirty weanling rats were divided into three groups of ten each, with the sexes evenly divided. The diet consisted only of fresh, pasteurized, homogenized, vitamin D-milk supplemented with the following trace elements/100 ml: Mn 1.5 mg as $MnCl_2$, Cu 0.8 mg as $CuSO_4$, Fe 5.0 mg as $FeCl_2$ and I 2 mg as KI. The milk had the usual addition of 400 units of vitamin D per quart.

The three groups of rats were fed the following diets: (1) milk; (2) milk containing 0.25 per cent $NaHCO_3$; (3) milk and bicarbonate with an additional daily allowance of 4 g of a solid mixture consisting of 1 g of finely ground cellophane mixed with 3 g of sucrose.

In order to determine the number of months needed to produce calcification in the kidneys of females, one rat in each group of this sex was killed at two, four, and eight months after

weaning. After eight months the calcification was detected readily by staining with either eosin-hematoxylin or silver nitrate (von Kossa).

After 12 months of feeding the diets, two male rats from each group were killed. Four rats from group 1 and 3 from group 2 were then fed a complete stock diet until 16 months from the start of the experiment. All were then killed.

The degree of calcification of the kidneys was determined by three different methods: roentgenograms, gross observation and microscopic study of sections. The bladders were examined for calculi (Fig. 1).

Calcification was found in the medulla of the kidneys of the females with no evidence that this had been alleviated by stopping the milk diet after 12 months. Calcification was found in the pelvis of the kidneys of both sexes. More calcification seemed to occur in animals that had been fed bicarbonate in the milk. Calculi were found in the bladders of one female and four males with no relation to groups.

Dental caries were observed only in group 3 which had been fed sugar and cellophane. No hair-balls were found in the stomachs of the rats of this group but two instances were found in the rats in group 1 which were fed milk only.

An experiment with 30 hamsters similar to that with rats was started at the same time. Due to external circumstances, this experiment could not be completed, but did yield some useful information. At the start of the study the trace elements were not added to the milk. After two months the hemoglobin concentration of the hamsters had dropped to 60 per cent of the normal and the hair of the animals turned gray. Soon after addition of trace elements the hemoglobin level became normal and the fur returned to its golden color. However, the animals died in the course of a few months until only three survived in group 1, none in group 2 and three in group 3 at 11 months after the start.

Only one hamster in group 3 showed very slight calcification in the kidneys. Two of the three animals from group 1 had hair-balls in the stomach while none of the three fed cellophane had these. One female from the group 3 fed sucrose had carious teeth.

(*B*) *Second Study Using Hamsters*

Twenty-four hamsters were divided into two groups, with the sexes evenly distributed. The first group was fed mineralized milk like those in the first study with 0.25 per cent of sodium bicarbonate dissolved in the milk. The second group was given the milk, but 10 per cent sucrose was dissolved in it. Bicarbonate was added to this milk at a level so that the ingestion of the bicarbonate was equal to that of the animals of group 1. The animals of group 2 drank less instead of more of the sweetened milk.

At the end of six months there were only eight hamsters alive in group 1 and four in group 2. These were all killed by etherizing, for study. No calculi were found in the bladder. Very slight calcification could be detected in the pelvis of 40 per cent of the animals. The kidneys of the hamsters were free of casts and the degenerative changes commonly observed in the kidneys of rats. Five of the eight animals in group 1 and one of the four in group 2 had hair-balls in the stomach. Only one animal, in group 2, had carious teeth.

These exploratory studies indicated that the hamster was very resistant to kidney changes while the rat was subject to them.

(*C*) *Third Study Using Rats and Hamsters*

Thirty weanling rats were divided into three groups with the sexes evenly distributed. They were fed as follows: Group 1: fresh mineralized milk; group 2: fresh mineralized milk containing 0.25 per cent $NaHCO_3$; group 3: same as group 2 except that the milk contained 10 per cent sucrose.

Since the males drank more milk than the females and the animals in group 3 drank less milk than the others, the bicarbonate was equalized to that consumed by the females of group 2.

At the end of six months all rats were killed. In Table I the milk consumption per 100 g of live body weight is given for a series of representative weeks. On the basis of body weight the females consume more milk than the males. This has been observed previously in other experiments

TABLE I

Milk Consumption in cc/100 g of Body Weight/Day. Mean Milk Intake with Standard Deviation (See Text, Section C)

Time on Diet	4th week	8th week	12th week	16th week	20th week
Rat					
Group 1 Milk					
F	56 ± 1.7	40 ± 2.0	34 ± 1.4	30 ± 0.6	28 ± 1.1
M	56 ± 4.0	39 ± 2.7	31 ± 2.6	27 ± 0.6	23 ± 2.3
Group 2 Milk and $NaHCO_3$ 0.25%					
F	57 ± 1.9	42 ± 2.8	38 ± 1.7	34 ± 2.1	32 ± 1.3
M	55 ± 5.3	39 ± 3.3	32 ± 2.7	30 ± 3.4	26 ± 1.6
Group 3 Milk, $NaHCO_3$ 0.25% and sucrose 10% dissolved in it					
F	46 ± 4.3	31 ± 3.7	29 ± 2.0	25 ± 3.9	23 ± 3.6
	46 ± 2.3	31 ± 1.5	27 ± 2.5	21 ± 1.1	21 ± 2.8
Hamster					
Group 1 Milk					
F*	49 ± 0.0	47 ± 0.0	39 ± 0.0	41 ± 0.0	...
M	48 ± 1.3	45 ± 4.3	31 ± 11.6	36 ± 10.0	...
Group 2 Milk and $NaHCO_3$ 0.25%					
F	53 ± 3.0	53 ± 4.0	48 ± 3.0	36 ± 13.0	...
	48 ± 1.0	49 ± 5.0	41 ± 4.0	42 ± 1.0	...
Group 3 Milk, $NaHCO_3$ 0.25% and sucrose 10% dissolved in it					
F	31 ± 3.0	35 ± 3.0	29 ± 3.0	33 ± 2.0	...
M	29 ± 2.9	30 ± 2.1	26 ± 1.8	24 ± 3.8	...

* Only one animal was left in this group; no standard deviation was obtained.

F = female; M = male.

The same sex differences were found as in the first study. Calcification was found in the medullas of the kidneys of the females but not in those of the males. In five of the females given bicarbonate, the calcification was also marked in the pelvis. Calcification seemed more marked especially from the x-ray evidence in animals given bicarbonate and seemed somewhat worse in group 3 which received sucrose in the milk.

Calculi were found only in the bladders of males. Ten of the fifteen males had these calculi with even distribution among the three groups. The extra case was in group 2. No females had calculi in the bladder.

No carious teeth were observed and only one hair-ball.

Thirty-six weanling hamsters were divided into three groups and given the same diets as the rats. Only 14 hamsters survived for five months when they were killed. No calculi were found in the bladders of any animal. Only very slight evidence of calcification was found in the pelvis of the kidneys of 7 hamsters. Two of the animals had hair-balls and none had carious teeth.

DISCUSSION

From these studies it seems that the rat is an excellent animal for use in the study of calcium deposits and calculi. The female is more subject to calcium deposits and the male to calculi. The hamster is not a satisfactory animal for such research because its survival is poor on milk diets. Furthermore, its kidneys seem much more resistant to deterioration including calcification than are those of the white rat. In such studies it is evident that both

species suffer from hair-balls when given a diet of liquid milk unless some additional source of roughage is made available.

Although there was no evidence of a reversal of calcification by changing the diet in a single attempt, it seems that rats fed diets rich in liquid milk may afford a useful method for devising methods of reversing calcification of the kidneys and dissolving calculi in the bladder.

SUMMARY

Three studies in which rats and hamsters were fed diets composed of fresh milk supplemented with trace minerals, sodium bicarbonate, and in some cases, sucrose, indicate that the female rat is very subject to calcification of the kidneys while the male suffers from calculi of the bladder. The Syrian hamster is difficult to maintain on fresh milk diets but develops neither calcium deposits in the kidneys nor calculi in the bladder. No evidence was found that these deposits could be dissolved after rats were changed to another diet for three months. The oldest rats were continued on the diets for a year while the hamsters in the longest study were fed the milk for 11 months. The white rat seems to be a very useful species for the study of the effects of Sippy diets and methods of reversing calcification of kidney tissues and calculi of the bladder.

To prevent complications from hair-balls in the stomachs of experimental animals, it seems useful in studies of liquid milk diets to feed a gram of cellulose per day to each animal. This can be mixed with sucrose and fed separately but such animals suffer from decay of the teeth. In neither rats nor hamsters are the teeth affected if sucrose is dissolved in the milk and not fed separately.

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