THE CALCIUM CONTENT OF THE KIDNEY AS RELATED TO PARATHYROID FUNCTION

By WILLIAM DONOHUE, M.D., CLIFFORD SPINGARN, M.D., AND ALWIN M. PAPPENHEIMER, M.D.

With the assistance of Nancy Downes

(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York)

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Several considerations prompted this study. Enlargement of the parathyroid glands is of regular occurrence in human cases of chronic renal disease (1-3), and may be experimentally induced in rats by reduction of functional kidney tissue (4, 5). This enlargement is attended by an increase in circulating parathormone (6). Injection of parathormone increases the calcium content of the renal tissue (7-9), and its continued administration in large doses brings about severe histological damage (10). Renal calcinosis and calculus formation are often found in cases of "primary" hyperparathyroidism (11).

With these facts in mind, it seemed logical to predict that the enlarged and presumably hyperactive parathyroid glands would induce an increased calcium content of the kidney tissue remaining after partial nephrectomy; and that in the absence of the glands this would not occur. The following experiments bring support for such assumptions.

Methods

Hooded rats of the Long-Evans strain, bred in the laboratory, were used as experimental animals. Partial reduction of kidney substance was performed according to the technique described in a previous paper, the upper and lower poles of one kidney being ablated, the cut surfaces seared and the opposite kidney removed usually 5 days later.

Parathyroidectomy was performed under ether anesthesia through a median incision. The completeness of the operation was established by serial section of
the excised tissue. In the partially nephrectomized rats, the parathyroids were removed in the interval between the first and second kidney operations.

Calcium determination: The accurately weighed tissue was wet ashed according to the method of Gieseking, Snyder and Gety (12), using nitric and perchloric acids; the calcium determined by the Kramer-Tisdall method (14).

The severity of the renal injury was estimated (a) from the non-protein nitrogen of the blood, determined by micro Kjeldahl from the serum obtained when the animal was killed; and (b) from the histological examination of the kidneys. The volume of the parathyroids was estimated by the procedure described in a previous paper (4). These rats were maintained on the Zucker diet (13).  

**Calcium Content of the Normal Kidney**

The average value of 29 determinations was 7.2 mg. per 100 gm. of wet tissue (maximum 10.1, minimum 4.3). This agrees rather closely with the observations of Olsen (8) (12 determinations—average 6.14, maximum 7.9, minimum 4.9) and with those of Tufts and Greenberg (15) (17 determinations—average 9.4, maximum 12.4, minimum 6.9).

**Effect of Parathormone upon the Kidney Calcium**

The effect of parathormone injections in elevating the calcium content of the kidney is illustrated in the following experiment; 12 rats 8 days old from 2 litters received increasing doses of parathormone from 0.02 to 0.1 cc. (2 to 10 Hanson units).

8 days elapsed between last injection of 10 units. Animals killed 24 hours after last injection.

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1. **Zucker Stock Diet.**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground barley</td>
<td>15</td>
</tr>
<tr>
<td>Ground whole oats</td>
<td>15</td>
</tr>
<tr>
<td>Ground whole wheat</td>
<td>15</td>
</tr>
<tr>
<td>Ground yellow corn</td>
<td>15</td>
</tr>
<tr>
<td>Soy bean meal</td>
<td>15</td>
</tr>
<tr>
<td>Alfalfa meal</td>
<td>2</td>
</tr>
<tr>
<td>Whole dried milk</td>
<td>10</td>
</tr>
<tr>
<td>Meat scraps</td>
<td>10</td>
</tr>
<tr>
<td>NaCl</td>
<td>2</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.5</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>0.5</td>
</tr>
</tbody>
</table>

100
In older rats, ranging between 3 and 6 months, injection of larger doses produced an extreme rise in the kidney calcium, such as has been reported by Olsen in similar experiments. Thus,

Although we do not wish to enter into a detailed description of the histological changes, it is of interest to note that in spite of the great increase in chemically determinable calcium, little or no stainable Ca was found in von Kossa preparations.

### Renal Calcium in Partially Nephrectomized Rats

Excluding 3 animals in which there was reason to suspect technical error in the analyses, we may present observations on 31 rats surviving for various periods, and showing varying degrees of renal insufficiency,
as estimated from the blood N.P.N. and the histologic changes. The individual data are shown on the accompanying scatter chart (Chart 1). As was to have been anticipated from the differences in the severity of the renal damage produced, the data show a wide range of variability. However, the average calcium content of the kidney in the entire series is 16.3, and 66 per cent of the determinations
exceed the maximum normal value, and only 3 fall below the average of the controls. \[ \frac{D}{PE_{M, M}} = 7.8, \] indicating that the difference in the average of the two sets of observations is unquestionably significant.

**Relation between the Severity of the Nephritis, Kidney Calcium and Parathyroid Volume**

The renal lesions in the residual tissue varied greatly in severity. They were graded from the sections as 0 or ±, +, ++ and ++++, without knowledge of the corresponding data on parathyroid volume, renal calcium or non-protein nitrogen of the blood. When the data are assembled (Table I), they indicate a definite correlation. Al-

<table>
<thead>
<tr>
<th>Severity of renal lesions</th>
<th>No.</th>
<th>Mean volume of parathyroids</th>
<th>No.</th>
<th>Renal calcium per 100 gm. of wet tissue</th>
<th>No.</th>
<th>N.P.N. of blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls, unoperated</td>
<td>11</td>
<td>0.1473</td>
<td>8</td>
<td>6.9</td>
<td>10</td>
<td>41.1</td>
</tr>
<tr>
<td>0 or ±</td>
<td>7</td>
<td>0.1690</td>
<td>4</td>
<td>8.8</td>
<td>7</td>
<td>56.0</td>
</tr>
<tr>
<td>+</td>
<td>4</td>
<td>0.1170</td>
<td>3</td>
<td>26.1</td>
<td>2</td>
<td>64.0</td>
</tr>
<tr>
<td>++</td>
<td>9</td>
<td>0.2151</td>
<td>8</td>
<td>15.4</td>
<td>6</td>
<td>72.5</td>
</tr>
<tr>
<td>+++</td>
<td>5</td>
<td>0.3258</td>
<td>8</td>
<td>33.6</td>
<td>5</td>
<td>87.0</td>
</tr>
</tbody>
</table>

though there are individual exceptions, and the number of determinations in each group is small, the general trend is unmistakable. The more severe the nephritis, the greater the volume of the parathyroids, and the higher the calcium content of the remaining kidney tissue.²

**Effect of Parathyroidectomy upon the Kidney Calcium**

Having found that partial nephrectomy leads to a definite increase in the calcium of the remaining kidney tissue, it was of interest to

² It must be pointed out that the figures for parathyroid volume of normal rats are lower than those reported in previous papers (4, 5). The reason for this discrepancy has not been discovered. In the present experiments, the rats were kept on the standardized Zucker diet, whereas in the former experiments they received an ordinary stock diet. This is the only known variable which might explain the discrepancy. The conclusions derived from the data are not affected.
determine whether this was to be ascribed to excessive activity of the enlarged parathyroids. Were this the case, in the absence of the parathyroid glands there should be no appreciable increase in the kidney calcium after partial nephrectomy.

The combined operation was attended by a mortality of over 50 per cent, although with increasing experience, fewer animals were lost. The rats averaged about 15 to 20 days in age at the end of the last operation and were carried on for a period of 65 to 110 days. Analyses of the kidney Ca were obtained on 10 rats with parathyroidectomy alone, and on 9 rats in which the removal of the parathyroids was combined with partial nephrectomy. The data are presented on Chart 1. Parathyroidectomy alone did not notably affect the kidney calcium. In the nephrectomized rats from which the parathyroids were removed, the calcium of the kidney likewise showed only slight elevation. We cannot say, therefore, that the parathyroidectomy has completely prevented the rise in renal calcium, but it has unquestionably restrained it. It is possible that the slight increase as compared with the normal may be due to calcium deposition in the renal tissue directly injured by the operation. One must reckon with the possibility that only those rats with a minor degree of renal damage and insufficiency were able to survive the combined operation, and hence the calcium values of the kidney tissue in the two groups may not have been strictly comparable. But this possibility may be discounted. Taking the N.P.N. as a measure of the severity of the renal injury, we find that the average N.P.N. of 11 rats with parathyroids removed in addition to the partial nephrectomy, was 74.1; 20 rats with partial nephrectomy alone, had an average N.P.N. of 72.1. There has thus been no artificial selection, and one seems warranted in attributing significance to the difference in the renal calcium in the two groups.

DISCUSSION

The above observations seem to forge another link in the chain which functionally connects the parathyroids with the kidneys. The fact that chronic renal insufficiency, however produced, leads to excessive activity of the parathyroids, may be accepted as established. Our experiments suggest that this secondary hyperparathyroidism
reacts adversely upon the kidney by increasing the calcium content of the renal tissue to an abnormal degree. Albright, Baird, Cope and Bloomberg (11) have emphasized the frequency with which diffuse calcinosis of the kidneys is associated with primary hyperparathyroidism in adenomata of the gland. Our experiment demonstrates that the parathyroid hyperplasia which follows experimental renal insufficiency in rats likewise induces an increase in kidney calcium as shown by chemical analysis. That this is due at least in part to excessive parathyroid function, and not to the reduction of kidney substance \textit{per se}, is indicated by the fact that it occurs to but a limited degree when the parathyroids are removed.

Our analyses of the kidney calcium following injections of parathormone confirm the work of Morgan and Samisch (7), Olsen (8) and Molinari-Tossati (9). They show that even small doses are capable of inducing a significant elevation of the kidney calcium. Unfortunately, we have no observations upon the effect of small doses continued over a period comparable to that during which the nephritic rats were under observation. Chown (10) has shown that the prolonged administration of larger doses may produce profound histological changes in the kidney, associated with, and perhaps caused by calcium deposits and tubular obstruction.

CONCLUSIONS

Partial nephrectomy in rats leads to an increase in the calcium content of the residual renal tissue.

This increase is correlated with enlargement of the parathyroid glands, the degree depending upon the severity of the kidney lesions.

Early removal of the parathyroid glands almost completely prevents this increase in kidney calcium.

BIBLIOGRAPHY