THE EFFECT OF PARATHYROID HORMONE ON PHOSPHATE EXCRETION IN THE RAT

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SUMMARY

The magnitude of the decrease in serum inorganic phosphate and the increase in urine phosphate of parathyroidectomized rats caused by injected parathyroid hormone has been studied. About 3 U.S.P. units of this hormone have been found to cause maximum changes in both serum and urine phosphate. Both responses depend on the respective initial phosphate level. Urine phosphate levels too high for more than minimal changes in phosphate excretion have been reduced by diets containing aluminium sulphate.

Previous work on the effect of the parathyroid hormone on the serum phosphate level of rats [Tepperman, L'Heureux & Wilhelmi, 1947] has indicated a possible method for the estimation of the hormone. Since Tepperman et al. were able to obtain only a 21% fall in the serum inorganic phosphate of normal rats by the injection of 100 U.S.P. units, it was decided to examine the response of thyro-parathyroidectomized animals in the expectation that they would be more sensitive. This proved to be the case. When the rats were first investigated in the spring, the injection of only 2 U.S.P. units of hormone resulted in an average fall of about 35% in the serum phosphate level. Unfortunately, the magnitude of this effect was found to be subject to considerable variation; when similar animals were tested in the autumn, a fall of only 15% was obtained. Later it was found that such variations can largely be correlated with differences in the initial level of the serum phosphate of the rats. Although it may thus be possible to select animals which will show a considerable decrease in serum phosphate after injection of hormone, the magnitude of this effect must necessarily be limited by compensatory release of phosphate from the tissues and possibly by conversion of organic to inorganic forms of phosphate in the blood. Since the last two factors do not apply to the phosphate excreted in the urine, it seemed worth while to investigate whether increase in urine phosphate of parathyroidectomized rats might form a useful measure of injected parathyroid hormone. This method seemed especially hopeful because of the previous results of Tweedy, Chilcote & Patras [1947], who showed that thyro-parathyroidectomized rats 2–3 hr after operation are sensitive to only 2.5–5 units of hormone, and those of Stoerk & Silber [1949] who found that parathyroidectomized rats give a maximal response when injected with only 20 units of hormone.

METHODS

Diet. The stock diet was M.R.C. cube diet no. 41 [Bruce & Parkes, 1949]. In certain experiments aluminium-containing diets were used to reduce the amount of absorbable phosphate. These were fed as a paste made of 100 g diet no. 41 and 125 ml. of aluminium sulphate solution, containing 0.25–0.75 atom aluminium per atom of dietary
phosphorus (see below, p. 297). Both types of diet were fed ad lib. and the animals had free access to drinking water. The animals were not fed or given drinking water in the metabolism cages.

Parathyroid hormone. 'Parathormone' (Eli Lilly) was used throughout; the hormone units mentioned below are U.S.P. units.

Metabolism cages. Cylindrical aluminium or stainless steel cages and funnels were used; \( \frac{1}{2} \) in. wire meshing formed the floors of the cages. The funnels had a short neck of 1 in. diameter, and fitted into glass separators, similar to those of Gross & Connell [1923], which allowed urine to be collected uncontaminated by faeces. The cages were not washed down.

Estimation of phosphate. Blood in amounts of 0.5 ml. was obtained by heart puncture from animals under light ether anaesthesia. Inorganic phosphate was estimated by the method of Allen [1940] on samples of 0.2 ml. serum after removal of protein with trichloracetic acid. Urine inorganic phosphate was estimated similarly, except that trichloracetic acid was not used. Samples of 20 \( \mu l \). of urine were delivered from a semi-automatic constricted pipette as described by Levy [1936].

**Observations**

Serum phosphate

The rats used for this part of the work were of the black and white (hooded) strain kept at Mill Hill. They were maintained on diet 41. Males weighing between 150 and 250 g were thyro-parathyroidectomized as described by Ingle & Griffith [1942]. In later experiments, the parathyroids alone were destroyed by cauterization. These organs occur in the rat as a pair of small white glands usually lying embedded in the upper third of the thyroid in a dorso-lateral position. No search could be made for accessory parathyroids, but according to a survey of the neck region in rats by Hoskins & Chandler [1925], they occur in this part of the body in less than 10% of animals. In preliminary work, a histological check of the thyroid showed no trace of parathyroid tissue either immediately after operation or three weeks later. The urinary phosphate response to 20 units of hormone in parathyroidectomized rats was the same as in thyro-parathyroidectomized animals. Thus, using twelve experimental and twelve control rats, the increased phosphate excretion due to hormone was identical, irrespective of the type of operation employed. Thyro-parathyroidectomy was discarded in favour of parathyroidectomy, because the latter was a quicker operation and caused less loss of body weight.

As reported by Engfeldt [1950], parathyroidectomy caused a gradual increase during the next 10 days of approximately 2 mg/100 ml. in the serum inorganic phosphate level. Specimen figures for rats before and after thyro-parathyroidectomy are given in Tables 1 and 2. As already mentioned, these elevated serum inorganic phosphate levels were found to fall sharply after injection of small doses of parathyroid hormone. The time course of this effect was investigated by taking blood samples from a group of four rats at intervals during 3 hr after each had received 20 units of hormone.

As shown in Table 1, the maximum decrease at this dosage took place about 60–90 min after injection. Using 1½ hr after injection as a standard time for blood sampling, an experiment was carried out to ascertain whether the animals remained
sensitive to repeated doses of hormone. Table 2 shows that no loss of sensitivity was apparent up to 24 days after operation. With this information available, it became possible to examine the relationship between dosage and response. For this purpose

Table 1. Acute effect of a single dose of 20 units of parathyroid hormone on serum inorganic phosphorus of rats thyro-parathyroidectomized 14–15 days previously

<table>
<thead>
<tr>
<th>Min after injection</th>
<th>30</th>
<th>50</th>
<th>60</th>
<th>90</th>
<th>135</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease in serum inorganic phosphorus (mg/100 mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-1</td>
<td>0-9</td>
<td>—</td>
<td>1-3</td>
<td>—</td>
<td>0-7</td>
<td></td>
</tr>
<tr>
<td>9-2</td>
<td>2-8</td>
<td>—</td>
<td>3-8</td>
<td>—</td>
<td>1-2</td>
<td></td>
</tr>
<tr>
<td>7-4</td>
<td>—</td>
<td>0-9</td>
<td>—</td>
<td>1-0</td>
<td>1-3</td>
<td></td>
</tr>
<tr>
<td>8-0</td>
<td>—</td>
<td>1-3</td>
<td>—</td>
<td>1-8</td>
<td>1-2</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8-4</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Effect of 20 units of parathyroid hormone on serum inorganic phosphorus of thyro-parathyroidectomized rats at various times after operation

<table>
<thead>
<tr>
<th>Days after operation</th>
<th>No. of rats</th>
<th>Before operation</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>20</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>F</td>
<td>I</td>
<td>F</td>
<td>I</td>
<td>F</td>
<td>I</td>
<td>F</td>
</tr>
<tr>
<td>2</td>
<td>6-3</td>
<td>9-2</td>
<td>2-4</td>
<td>—</td>
<td>—</td>
<td>9-4</td>
<td>2-9</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>5-5</td>
<td>—</td>
<td>—</td>
<td>7-7</td>
<td>1-8</td>
<td>—</td>
<td>—</td>
<td>7-6</td>
</tr>
<tr>
<td>2</td>
<td>6-1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>7-3</td>
<td>1-7</td>
<td>—</td>
<td>8-6</td>
</tr>
<tr>
<td>Mean</td>
<td>6-0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

I = initial value; F = fall observed 1-5 hr after injection of hormone.

three groups of four rats each were used which had been thyro-parathyroidectomized 18–27 days previously. To allow sufficient recovery from the necessary loss of blood, each group was tested on 6 days spaced irregularly over a period of 19 days, varying doses being given so that no group always obtained either the highest or lowest dose. The results given in Fig. 1 indicate a maximum response to a dosage of approximately 2 units.

Fig. 1. Relationship of fall in serum inorganic phosphorus level of thyro-parathyroidectomized rats to dosage of hormone. Each point is an average of values obtained from four animals.
Urine phosphate

For this part of the work parathyroidectomized male or female rats weighing 150–200 g were used; no differences due to sex were detected. Both black and white (hooded) and albino rats were employed; no difference in sensitivity was found. As both the present investigations of the serum phosphate levels and the results of Stoerk & Silber [1949] indicated the desirability of a short period of urine collection, irregularities due to incomplete collection of urine had to be minimized. An obvious means to accomplish this was to increase the volume of urine. Following a suggestion of Stoerk & Silber, the effects of giving water by stomach tube simultaneously with hormone were examined. In an experiment designed to ascertain a suitable urine collection period, 10 ml. of water were given to each of twelve rats. Immediately after this treatment each of six animals received a subcutaneous injection of 20 units of hormone. Next, the rats were placed in four metabolism cages, there being three rats of similar total weight in each cage. Measurement of the hourly volume of urine showed that, apart from about 30% of the administered water which was retained, almost all the rest was excreted by the end of 3 hr. After 4 hr the collecting vessels were changed and each animal received a further 10 ml. of water. Collection was continued for a further 2 hr. The results of this experiment, shown in Fig. 2, indicated the suitability of the shorter collection period, because the ratio of phosphate excreted in response to the hormone compared with the control excretion was higher than in the subsequent 3 hr. An experiment was next carried out on twelve rats to make sure that the sensitivity in respect of the excretion of urinary phosphate does not quickly disappear after operation. Although the 3 hr period of urine collection had been shown to be adequate, in this instance, in the hope of minimizing differences due to incomplete collection, urine was collected for 5 hr. Twenty units of hormone and

Fig. 2. Comparison of increased urinary phosphorus excretion due to parathyroid hormone during two consecutive periods of urine collection. Six parathyroidectomized rats were used in each group. Each received 10 ml. water by stomach tube at 0 and 4 hr.
two additions of water by stomach tube at 0 and 4 hr were used. As shown in Table 3, no loss of sensitivity could be demonstrated up to 21 days after operation.

The relationship of 3 hr phosphate excretion to dosage of hormone was investigated in forty-eight female albino rats divided into groups of three. After giving 10 ml. water per rat by stomach tube, 3 hr urine was collected from all groups at similar times of day (morning) on 4 consecutive days. The first and fourth days served as controls; on the second and third days the animals were arranged in four larger groups of twelve, and doses of 0·1, 0·33, 1 and 3 units of hormone per rat were injected into twelve animals respectively. Those which received the high doses on the second day were given the low ones on the third and vice versa. The results, together with certain other ones obtained with another set of animals, are shown in Fig. 3.

Table 3. Effect of 20 units of parathyroid hormone on the urinary phosphate of parathyroidectomized rats at various times after operation

(Each group consisted of six male albino rats)

<table>
<thead>
<tr>
<th>Days after operation</th>
<th>Mg urinary phosphorus per rat/5 hr</th>
<th>Ratio: Experimental/control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental group</td>
<td>Control group</td>
</tr>
<tr>
<td>2</td>
<td>3·75</td>
<td>1·80</td>
</tr>
<tr>
<td>6</td>
<td>2·00</td>
<td>0·95</td>
</tr>
<tr>
<td>14</td>
<td>2·65</td>
<td>1·40</td>
</tr>
<tr>
<td>21</td>
<td>2·45</td>
<td>1·30</td>
</tr>
</tbody>
</table>

Fig. 3. Relationship of increase of urinary phosphorus excretion of parathyroidectomized rats to dosage of hormone. Each point is an average of values obtained from twenty-four animals. Different symbols represent independent experiments.

At this stage it seemed advisable to investigate whether the maximum response to hormone depended on the amount of phosphate excreted in the absence of hormone. To achieve this the control and test periods were carried out immediately following one another, rather than on consecutive days. Each collection period lasted for 3 hr and followed the usual administration of 10 ml. of water by stomach tube. For experiments thus designed to be valid, a steady rate of phosphate excretion during the two consecutive collection periods must be assumed. This point was investigated using sixteen sets of rats, each set consisting of twenty-four animals placed in eight
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metabolism cages. The work was carried out during 3 months and included rats on diet 41 and on diet 41 + aluminium sulphate (see below). Neither date nor diet had any detectable influence on the results. Fortunately, over the range of control values studied, the mean control values in the morning and afternoon were similar and there was no statistical difference between the results obtained in the two periods in all sixteen experiments (see Table 4). Another advantage of such experiments is that the overall time required is reduced by one-half. Using this method, further experiments were carried out in which 3 units of hormone per rat were injected immediately after the second administration of water. The results given in Fig. 4 show a clear relationship between control level and response.

Table 4. Comparison of phosphate excreted in urine of parathyroidectomized rats during two consecutive 3 hr periods

(The figures below were derived from sixteen experimental values (expressed as mg phosphorus/rat/3 hr). Each experimental value was the mean of eight urine samples obtained from twenty-four rats used in groups of three.)

<table>
<thead>
<tr>
<th>Average morning excretion: 0.664 ± 0.130 (s.e.)*</th>
<th>Range: 0.89-0.35.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average afternoon excretion: 0.635 ± 0.128 (s.e.)</td>
<td>Range: 1.12-0.18.</td>
</tr>
<tr>
<td>t-tests on individual experiments always gave values of P &gt; 0.1.</td>
<td></td>
</tr>
</tbody>
</table>
* Average s.e. of means of individual experiments.

Fig. 4. Relationship between initial urine phosphorus level and response to parathyroid hormone.

- stock diet no. 41; ○, stock diet no. 41 + aluminium sulphate.

At this time (April) it became apparent that fewer and fewer rats were showing control phosphate levels < 0.7 mg phosphorus per 3 hr. Since this was seriously reducing the response, convenient means had to be sought to reduce the excretion of urinary phosphate. Following the work of Street [1942], by the use of between 0.25 and 0.75 m proportions of aluminium sulphate per atom dietary phosphorus, it was possible to reduce the control urinary phosphate to the required range of values. This range of phosphate excretion could sometimes be maintained in one set of rats for several weeks on a diet containing a particular proportion of aluminium sulphate, but with other sets of rats the amount of aluminium sulphate in the diet had to be adjusted to control changes in the trend of phosphate excretion.


**DISCUSSION**

Using normal rats, Tepperman *et al.* [1947] examined initial serum phosphate levels and the corresponding decreases in serum phosphate resulting from the subcutaneous injection of 50 units of parathyroid hormone, and reported no significant relationship. That this negative finding does not apply to rats deprived of their parathyroid glands is demonstrated by the results given in Fig. 5, which shows the decreases in serum phosphate obtained by the injection of 2 and 1.5 units. The experiment is the one quoted above on the relationship between dosage and response. Only values for 1.5 and 2 units are plotted because they lie near the peak of the curve. From the slope of this graph, it appears that on the average only those animals having a serum inorganic phosphorus above 8 mg/100 ml. can be expected to show a fall. In view of this correlation it is not surprising to find the relationship between the level of urinary excretion and the increase due to injection of hormone (Fig. 4). Although in this figure only values between 0.27 and 1.1 mg phosphorus per rat are shown, it has also been demonstrated that when the initial phosphate level approaches zero, the injection of hormone does not lead to the excretion of any detectable amount of phosphate in the urine. Therefore it seems certain that this curve must actually commence at the origin and pass through a maximum somewhere below 0.3 mg phosphorus control value.

The dependence of response to hormone on the initial phosphate level in serum and urine of the parathyroidectomized rat undoubtedly decreases the usefulness of this animal in relation to parathyroid hormone assay. In the present work, attempts at assays of unknown hormone preparations were made using both serum and urine responses. The former method had two advantages. First, the variation was some-
what lower. The index of precision ($\lambda$) for a serum phosphate experiment was 0.36 and for a urine phosphate experiment 0.84. In these experiments the serum phosphate response was the fall in mg P/100 ml. serum per rat, using twelve rats at each dose level. The urine response was mg P excreted per three rats per 3 hr. Twenty-four rats were used per dose. Secondly, the losses connected with urine collection were obviated. On the other hand, the percentage serum phosphate fall is much smaller than is the percentage rise in urine phosphate, and it is not known how serum phosphate levels likely to give large decreases can be achieved with certainty. Whether this can be done by feeding high phosphorus diets has not been tested.

The chief disadvantages of the urine method are the high variation, and the fact that at certain seasons rats must be specially dieted to bring their excretion of phosphorus in the urine to a level (0.3–0.8 mg/rat/3 hr) where useful responses to hormone are obtained.

As a basis for possible assay methods the greatest advantage of both the serum and urine effects is their considerable sensitivity. As mentioned above, this was first established by Tweedy et al. [1947] for the excretion of phosphate in the urine of thyro-parathyroidectomized rats immediately after operation. The present work shows that such animals remain sensitive for many weeks and that maximum effects are obtained with a dose of approximately 3 units of hormone containing some 20 $\mu$g N. The marked dependence of the response on the initial phosphate level suggested that it would not be a simple matter to obtain adequate dose response curves for either effect. Thus the chief interest of Figs. 1 and 3 is probably that the maximum response is obtained in each case with almost the same dose. No reason is so far apparent for the marked falling off of the serum phosphate response at dosages above 3 units.

The question naturally arises whether parathyroidectomized rats which give unduly small decreases in serum inorganic phosphate after the injection of hormone, because of an initial serum phosphate level little higher than that of normal rats, are likely to have urinary phosphate levels low enough to lead to large urinary responses. That this is not true, at least for rats some weeks after operation, was indicated in preliminary tests which showed an inverse relationship between serum and urinary phosphate levels. The complexity of the relationship was suggested by the wide scatter of the values obtained.

These effects of the hormone on serum and urinary phosphate levels must be considered in relation to its ability to increase the concentration of serum and urinary calcium. Unfortunately, this has been little studied in the parathyroidectomized rat, but the work of Tweedy & Chandler [1929] on the increase of serum calcium suggests that two or three times the response obtained in the normal rat is to be expected. If this finding is taken in conjunction with the results of Biering [1950] who was only able to obtain an increase of 3 mg/100 ml. in the serum calcium of normal rats after the injection of just over 1000 units of parathyroid hormone, it would seem probable that some 300 units must be required to obtain a maximum response in the thyro-parathyroidectomized rat. On this assumption about one hundred times as much hormone would be required to give a maximum serum calcium response as is necessary to bring about the maximum increase in the excretion of urinary phosphate. This

* $\lambda = \sqrt{(error\ variance) \div slope\ of\ log\ dose-response\ curve.}$
wide difference of effective hormone concentration and the much slower serum calcium response compared with the development of the serum phosphate effect brings considerable support to the theory of the independent action of the hormone on bone and kidney, as recently stressed by Törnblom [1949].

The authors wish to thank Sir Charles Harington, F.R.S., for his interest in this work and Miss M. V. Mussett for carrying out the statistical analyses.

REFERENCES

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