CXXIV. PARATHYROID HORMONE

I. ASSAY

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Collip's original method of assay of parathyroid hormone [Collip & Clark, 1925] presents certain serious disadvantages for routine laboratory use. Not only are dogs expensive and troublesome to maintain, but in practice it is impossible to obtain a sufficiently large group of animals of one breed. As a result, individual differences will be greater in a group of dogs than in an equally numerous group of inbred laboratory animals, such as the mouse and rat colonies available in every biological laboratory. Methods based on prevention of the toxic effects of injections of Na oxalate or $MgSO_4$ into mice or rats suffer from the disadvantage of requiring extremely numerous groups of animals, in order to give significant results [cf. Burn, 1937]; moreover, individual variations in susceptibility to both the toxic factor and the hormone tend still further to increase the variance. Dyer's rat urine method [1932; 1933; 1935] appeared to afford the most hopeful basis for the elaboration of a practicable and relatively trustworthy method of assay, an attempt at which is described in the present paper.

Experimental

Material and methods. The animal material consisted of male albino rats, of the State Hygiene Institute's inbred Wistar strain, weighing 150–200 g. each. Groups of 5 rats were placed in glass metabolism cages, of a type similar to that used by Dyer [cf. Burn, 1937], but with certain minor improvements. The cage (Fig. 1) consists of an inverted glass bell-jar, with a 0·5 cm. mesh Ni-plated brass wire netting floor and ceiling. The neck of the bell-jar is fitted with a thin glass rod, serving for suspension of a glass bulb, the drawn-out part of which dips into a 100 ml. Erlenmeyer flask, standing in a petri dish.

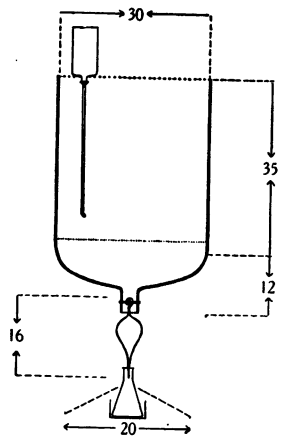

Fig. 1. Metabolism cage (to scale).

The dish is covered with a piece of fine-mesh wire gauze, in the form of a truncated cone. The cage, dish and outside of the flask are rinsed with small amounts of hot acetic acid once daily, the washings being added to the urine. The rats are fed once daily in a separate cage, the urine voided during this time being filtered immediately after feeding, and added to the main portion. Distilled water is allowed *ad lib.*

The diet consists of a mixture of wholemeal rye flour 70, evaporated skim milk 10, commercial casein 6, linseed cake 15, dried liver 3, $CaCO_3$ 0·5 and NaCl 0·5 parts, made into a stiff paste with 50 g. of butter and one raw egg per kg. of

mixture, together with the necessary amount of hot water. The mass is kneaded into ovoids, each weighing about 15 g., five of which are placed in the feeding cage. Feeding usually lasts about 1 hr., and scattering is practically absent. The combined urine + washings are diluted to 100 ml. in measuring flasks, and Ca is determined by the method of Truszkowski *et al.* [1938]. The values in all cases refer to urinary Ca output per 100 g. of rat per 24 hr.

Results

The appraisal of the potency of parathyroid preparations by a rat urine method requires that the standard deviation in urinary Ca output be determined for uninjected animals, in order to know what rise in urinary Ca might be regarded as being significant. Typical daily Ca excretion curves are given in Fig. 2,

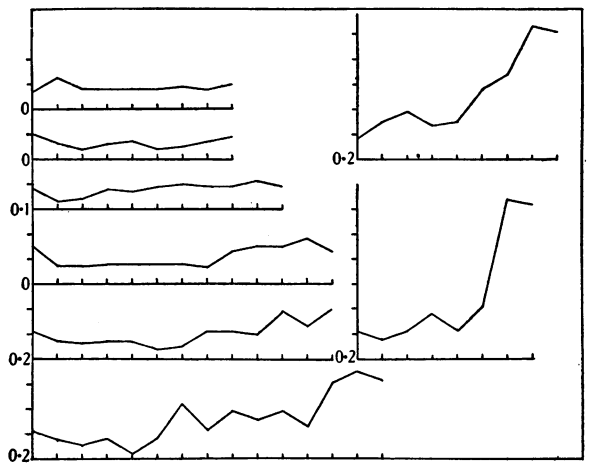

Fig. 2. Daily Ca excretion curves. Ordinates: Ca in 0·1 mg. Abscissae: days.

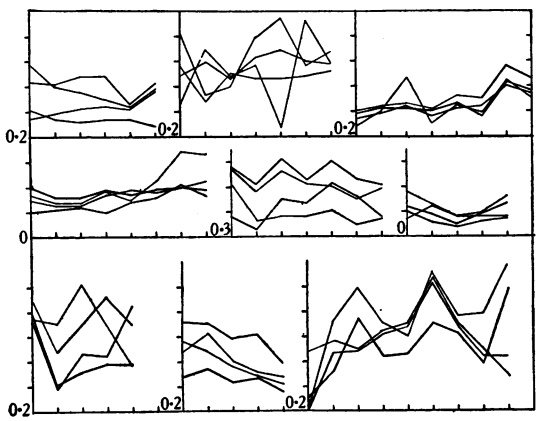

Fig. 3. Daily Ca excretion curves, for four groups studied simultaneously. Ordinates: Ca in 0·1 mg. Abscissae: days.

from which it appears that considerable variations in 24 hr. urinary Ca output may exist (see also Fig. 3), in spite of every precaution to avoid loss or contamination of urine, and of maintenance of standard feeding and living conditions.

Fig. 3 represents a number of groups of curves obtained for four groups of rats studied simultaneously. A general tendency towards parallel fluctuations is evident, suggesting that some factor responsible for variation in urinary Ca output affects all four groups simultaneously; the fluctuations are due not so much to individual group variability as to the action of some external, uncontrolled factor. As to the nature of this factor no basis for speculation is available. The phenomenon described may, however, be of practical importance for assay purposes, in as much as whilst the fluctuations for a given group of rats may be considerable, yet the differences between this group and a second control group may vary within narrower limits.

Fig. 4*a* represents the distribution of results of 792 determinations of urinary Ca (mg./100 g./24 hr.), under standard conditions, and Fig. 4*b* the same, for differences (283 values) between test and arbitrarily selected control groups. In

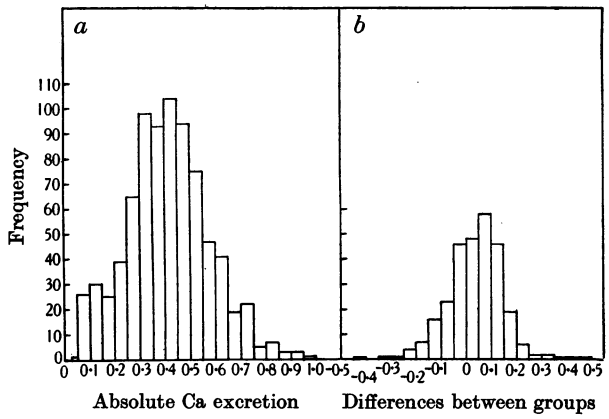

Fig. 4. Distribution of daily Ca excretion for groups of rats.

both cases the results correspond with a normal distribution. The standard deviation was calculated for both groups of results, from the equation

$$\sigma = \lambda \sqrt{\frac{\Sigma a^2 d}{n} - \left(\frac{\Sigma ad}{n}\right)^2 - \frac{\lambda}{12}},$$

where λ is the class interval (0·05 mg.), a is the deviation, d the frequency and n the number of group-days. On this basis, Ca excretion, in mg./100 g. live wt./24 hr., was found to be 0·404, with $\sigma = 0{\cdot}166$, whilst the difference between one group and another amounted to 0·064, with $\sigma = 0{\cdot}066$. It follows that the standard deviation is 2·5 times as great for absolute Ca excretion as for the differences between groups. In other words, a rise or fall in Ca excretion equal to 0·166 mg./100 g./24 hr. could be expected once in 3 days, and in the difference between two groups on a given day once in 100 days. The conclusion is that a rise in the difference between two groups, of which one had received parathyroid hormone, is a more trustworthy indication of the activity of the hormone than is the absolute Ca rise in one group only.

Effect of injection of parathyroid hormone

Solutions of parathyroid hormone were prepared by a method based substantially on that of Tweedy [1930], from different batches of ox glands. Owing to technical difficulties, both in procuring large batches of glands, and in work-

ing them up in one operation, our preparations varied considerably in potency. Fig. 5*a* represents the effect of an injection, and Fig. 5*b* shows how the quantitative effect of this injection is evaluated. The curves of Fig. 5*a* represent daily variations in Ca excretion in two groups of rats. An injection of parathyroid hormone was given to one of the groups (curve 2) at the beginning of the 24-hr.

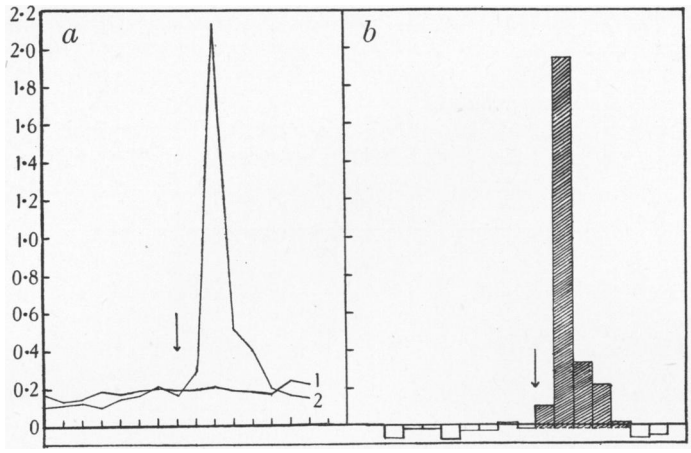

Fig. 5. Effect of injection of parathyroid hormone. Ordinates: Ca in 0·2 mg. Abscissae: days.

period marked by an arrow. The curve then rises and falls steeply, the effect being completed within 72 hr. The histogram (Fig. 5*b*) consists of a series of columns, each being the difference between the values of curves 1 and 2 on a given day; these differences may be positive or negative. The response to the injection is evaluated as being the sum of the areas of the columns (shaded) above the level of the pre-injection column. This procedure involves the arbitrary assumption that the level on the day preceding injection remains constant over the test period; actually, since the standard deviation is 0·066, it may vary considerably. The response is, however, considerably greater than these possible variations, which should not involve an error >10 %; such a margin of error is generally regarded as permissible in biological assay methods. The original histograms are drawn on millimetre square paper, to a scale of 1 cm./0·1 mg. Ca and per 24 hr. period, so that the sum S is expressed in sq. cm. We would propose that with this method of plotting 1 sq. cm. (=0·1 mg. Ca) be tentatively taken as being equivalent to 1 rat unit of parathyroid activity, pending the production and adoption of a reference standard. The potency of solid preparations of parathyroid hormone would then be best represented as $S/\log D$, where D is the dose in mg.

The groups of rats were discarded after each experiment, in view of the possibility of development of immunity in previously injected animals.

Uniformity of response of different groups of rats to an identical dose of hormone

An injection of 11·26 mg./100 g. of prep. A_c was given simultaneously to three groups of rats. The histograms representing the effect are shown in Fig. 6*a*. Fig. 6*b* illustrates the result of a similar experiment, taking 19 mg./100 g. of prep. Y. The values of S for prep. A_c at the given dosage level were $S_1=1·3$,

$S_2 = 2{\cdot}4$, $S_3 = 2{\cdot}3$; for prep. Y the values were $S_1 = 22{\cdot}6$, $S_2 = 25{\cdot}2$, $S_3 = 22{\cdot}9$ sq. cm. It follows that the responses differ considerably qualitatively, those to prep. A_c being distributed over 2, 3 and 1 days respectively for groups 1, 2 and 3, or

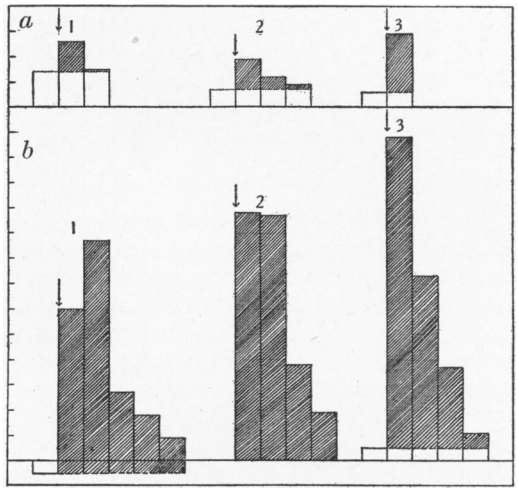

Fig. 6. Effect of equal doses of hormone prep. A_c (Fig. 6*a*) and Y (Fig. 6*b*) given to three groups of rats. Ordinates: Ca in 0·1 mg. Abscissae: days.

rising gradually to a low maximum for group 1 (prep. Y), and abruptly to higher ones in groups 2 and 3. The maximum level is maintained for 48 hr. in group 2, and for 24 hr. only in groups 1 and 3. Return to pre-injection levels is most gradual in group 3, less so in group 2 and least so in group 1. Yet the values of S differ relatively little for identical doses.

Gradation of response to various doses of a given preparation

It might be expected that the magnitude of S should bear some simple relation to the logarithm of the dose, were the assay method described to give a

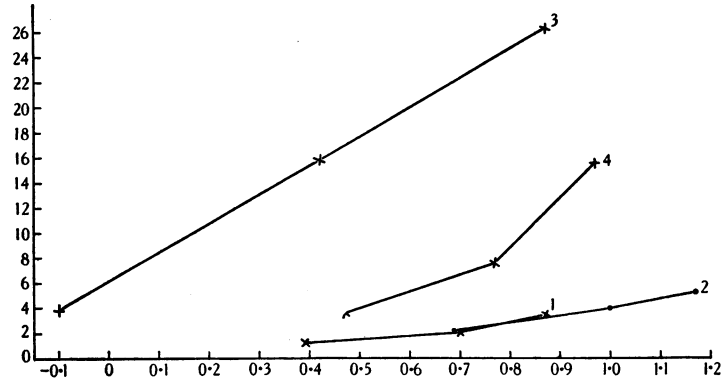

Fig. 7. Relation of dose of hormone to response. Ordinates: Response S. Abscissae: Logarithm of dose in mg.

true representation of the potency of the hormone preparations. The results obtained are shown in Fig. 7, in which curves 1 and 2 represent the effect of

injections of 2·5, 5·0 and 7·55, and of 5, 10 and 15 mg. of prep. N_2/100 g. of rat, made at different times into three groups of rats. Curve 2 is a straight line, and two of the points of curve 1 fall on the same line, the lowest dosage giving an aberrant effect. This is due probably to the magnification of the experimental error at this low dosage level. Curve 3 is a straight line joining the points obtained with doses of 0·78, 2·68 and 7·55 mg. of the more active prep. D. Curve 4 is for doses of 2·97, 5·95 and 8·87 mg. of prep. Z, and deviates to some extent from the rectilinear.

On the whole, the results confirm the validity of the assay method proposed.

Effects of multiple or single injections

It is known that for a number of hormones a better response is obtained when the dose is divided into a number of smaller ones, administered at intervals, and Dyer [1933] has reported the same for parathyroid hormone. A test made with one of our preparations showed that S was 20·1 when a given dose was administered in two injections on 1 day, and 26·0 when the same dose was spaced over 3 days. Whilst this result confirms Dyer's findings, yet the advantage does not in our opinion counterbalance the inconvenience of multiple injections, and of the prolongation of the test period. In subsequent work the hormone was administered in two injections at an interval of 4 hr.

Units

The present units are the Collip dog unit (one-hundredth of the amount of hormone causing a 5 mg. rise in dog serum Ca, after 15 hr.), or the Hanson unit, being one-hundredth of the amount of hormone raising the serum Ca of a parathyroidectomized dog by 1 mg. within 6 hr. These units are not very satisfactory, but no better ones have been proposed. Dyer bases his assays on comparison with the declared potency of a commercial preparation; such a procedure presents obvious disadvantages. The most satisfactory methods are those based on comparison with a standard preparation, but no such preparation is available in the present case. A sample of hormone was prepared in this laboratory about a year ago, and its stability is now being studied, with a view to the ultimate adoption of this or a similar preparation as a reference standard. In the meantime, the assay method proposed can be applied, if possible taking some reliable commercial preparation for comparison. In a test made in our laboratory it was found that a dose of 10 Collip units/100 g. of rat gave a response of $S = 19·3$, whence it would follow that 1 Collip unit is equivalent to about 2 of our rat units. The most potent preparation so far obtained in our laboratory had an activity of about 6 rat units/mg.

Summary

1. Fairly considerable fluctuations in urinary Ca output are established for groups of rats kept under standardized conditions.

2. A tendency towards parallel variation in Ca output of four different groups during given periods of time is evident; the standard deviation of differences between the various groups is 0·064 mg./100 g. live wt./day (283 values), as compared with 0·166 mg. for absolute urinary Ca output (792 values, of which the mean is 0·404 mg.).

3. The response to various doses of a given preparation of parathyroid hormone is proportional to the logarithm of the dose. For a given dose approximately the same response is given by different groups.

4. A graphical method of evaluating the potency of parathyroid preparations, based on that of Dyer, is described. Pending the establishment of a reference standard, it is proposed that activity be expressed in terms of excess of urinary Ca excretion following injection.

5. The proposed rat unit is defined as being one-tenth of the amount of hormone giving a total rise in urinary Ca output of 1 mg.

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