

## Bioassay of Parathyroid Hormone.\* (20314)

BETTY L. RUBIN<sup>†</sup> AND RALPH I. DORFMAN.<sup>†</sup>

*From the Departments of Biochemistry and Medicine, Western Reserve University School of Medicine and Lakeside Hospital, Cleveland, O. and the Worcester Foundation for Experimental Biology, Shrewsbury, Mass.*

Tweedy, Chilcote, and Patras(1) demonstrated an increase in radiophosphorus excretion in thyroparathyroidectomized rats following the injection of 5 or more U.S.P. units of parathyroid hormone and 3 mg of phosphorus as disodium phosphate (13.75 mg of disodium phosphate). These studies are concerned with the use of this increase in  $P^{32}$  excretion as the basis of an assay technic for parathyroid hormone.

*Animals, materials and methods.* Parathyroid extract (Lilly and Co.) was administered to 90-160 g, thyroparathyroidectomized Holtzman albino rats maintained on a dog chow diet. The hormone was administered subcutaneously in 1 cc of saline. Optimum conditions with respect to the amount of disodium phosphate given and route of administration were studied, but in all experiments the material was injected in a volume of 2 cc. After both hormone and phosphate had been administered, the animals were immediately placed in glass metabolism cages and the urine collected quantitatively for 3 hours. The method of collection was the same as that used by Dorfman, Potts, and Feil(2). *Experimental.* In order to establish the optimal conditions for the assay, the following variables were investigated: 1) The interval between thyroparathyroidectomy and use of animals for assay of parathyroid activity, 2) the amount of disodium phosphate to administer, 3) the route of administration of disodium phosphate, and 4) the effect of varying the time interval between the hormone and phosphorus and the collection of urine.

*Influence of interval between operation and assay.* The effects of differing intervals between operation and assay are

shown in Table I. In the first 2 experiments, 13.75 mg of disodium phosphate per 100 g of body weight were administered to animals 14 and 32 days after operation, and 1.55 mg of disodium phosphate per 100 g of body weight were administered to the same animals a week later. Five U.S.P. units caused a significant increase in  $P^{32}$  excretion in 2 of the 4 trials. In the third and fourth experiments, 1.2 mg of disodium phosphate were administered to rats one day after operation and again a week later. One-half to 10 U.S.P. units caused a significant increase in  $P^{32}$  excretion in the animals one day after operation. Fisher's  $t$  values(3) were used as criteria of the significance of the differences between the mean excretions of control and experimental groups. Using a total of 10 animals, the  $t$  value must be greater than 2.28 for the probability ( $P$ ) to be less than 0.05. With a total of 20 animals,  $t$  must be greater than 2.09, and for 26 animals,  $t$  must be greater than 2.05. The data also show that 5 U.S.P. units administered to animals 14-39 days after operation caused an increase in  $P^{32}$  excretion of lesser magnitude than the increase caused by 2.5 units administered one day after operation. A tendency for the excretion of  $P^{32}$  in the control group to increase with increasing time after operation was observed. This is especially striking in an experiment (the sixth in Table I) in which animals selected for their low excretion of phosphorus on the first post-operative day (mean =  $1.21 \pm 0.23\%$ ) were tested again on the second day, at which time the mean  $P^{32}$  excretion had increased to  $5.66 \pm 1.21\%$ . No significant difference in  $P^{32}$  excretion of the untreated operated animals could be demonstrated within the first 16 hours post-operatively (Table I). The individual variations were high, as indicated by the large standard errors of the means. In all later experiments animals were used within 24 hours after operation.

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<sup>†</sup> Present address: Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

TABLE I. Effect of Varying the Time Interval between Operation and Start of Test. ( $P^{32}$  administered intraperitoneally, simultaneously with hormone.)

| Time post-op. | $Na_2HPO_4$ admin., mg/100 g | Parathyroid hormone, U.S.P. units | Urinary excretion of inj. $P^{32}$ |                       | No. animals | Exp., % $\pm$ S.E. | % change | t    |
|---------------|------------------------------|-----------------------------------|------------------------------------|-----------------------|-------------|--------------------|----------|------|
|               |                              |                                   | No. animals                        | Control, % $\pm$ S.E. |             |                    |          |      |
| * 32 days     | 13.75                        | 5                                 | 5                                  | 6.57 $\pm$ .84        | 6           | 9.36 $\pm$ 1.02    | + 45     | 2.11 |
| 39 "          | 1.55                         | 5                                 | 5                                  | 3.25 $\pm$ 1.34       | 5           | 11.15 $\pm$ 1.57   | +243     | 4.40 |
| * 14 "        | 13.75                        | 5                                 | 6                                  | 6.87 $\pm$ 1.31       | 6           | 9.42 $\pm$ .69     | + 37     | 1.71 |
| 21 "          | 1.55                         | 5                                 | 6                                  | 5.06 $\pm$ 1.65       | 6           | 9.63 $\pm$ .76     | + 90     | 2.54 |
| *1-2 "        | 1.15                         | 2.5                               | 11                                 | 2.51 $\pm$ 1.03       | 9           | 10.59 $\pm$ 1.80   | +321     | 3.84 |
|               |                              | 5.0                               |                                    |                       | 11          | 11.62 $\pm$ 1.32   | +363     | 5.35 |
|               |                              | 10.0                              |                                    |                       | 9           | 14.22 $\pm$ 1.82   | +466     | 5.57 |
| 8-9 "         | 1.2                          | 2.5                               | 11                                 | 10.66 $\pm$ .89       | 11          | 12.38 $\pm$ 1.45   | + 16     | 1.01 |
|               |                              | 5.0                               |                                    |                       | 10          | 10.26 $\pm$ 1.57   |          | .22  |
|               |                              | 10.0                              |                                    |                       | 10          | 11.73 $\pm$ 1.92   | + 10     | .51  |
| * 1 "         | 1.2                          | .5                                | 10                                 | 2.60 $\pm$ .87        | 11          | 5.91 $\pm$ 1.10    | +127     | 2.36 |
|               |                              | 1.0                               |                                    |                       | 11          | 6.09 $\pm$ 1.08    | +134     | 2.49 |
|               |                              | 2.5                               |                                    |                       | 9           | 10.27 $\pm$ 2.55   | +295     | 3.19 |
| 8 "           | 1.2                          | .5                                | 7                                  | 3.47 $\pm$ .83        | 12          | 5.73 $\pm$ 1.35    | + 65     | 1.41 |
|               |                              | 1.0                               |                                    |                       | 9           | 4.99 $\pm$ .98     | + 44     | 1.17 |
|               |                              | 2.5                               |                                    |                       | 9           | 6.35 $\pm$ 1.33    | + 83     | 1.80 |
| * 1 "         | Tracer                       | 0                                 | 14                                 | 1.95 $\pm$ .67        |             |                    |          |      |
| 8 "           | dose                         | 0                                 | 14                                 | 2.80 $\pm$ .40        |             |                    |          |      |
| 1 "           | .2                           | 0                                 | 6                                  | 1.21 $\pm$ .23        |             |                    |          |      |
| † 2 "         |                              | 0                                 | 6                                  | 5.65 $\pm$ 1.21       |             |                    |          |      |
| * 1 "         | 1.2                          | 0                                 | 8                                  | 3.49 $\pm$ .99        |             |                    |          |      |
| 2 "           |                              | 0                                 | 8                                  | 3.96 $\pm$ .21        |             |                    |          |      |
| 1 1/4 hr      | 1.2                          | 0                                 | 9                                  | 3.82 $\pm$ 1.6        |             |                    |          |      |
| 2 "           |                              | 0                                 | 8                                  | 2.52 $\pm$ 1.38       |             |                    |          |      |
| 4 "           |                              | 0                                 | 8                                  | 1.77 $\pm$ .83        |             |                    |          |      |
| 8 "           |                              | 0                                 | 8                                  | 1.46 $\pm$ .65        |             |                    |          |      |
| 16 "          |                              | 0                                 | 8                                  | 3.65 $\pm$ 1.74       |             |                    |          |      |

\* Same animals used at both time intervals.

† Selected animals showing lowest excretion on day 1.

*Influence of varying amounts of disodium phosphate.* When 13.75 mg of disodium phosphate per 100 g of rat were administered, there was no significant difference in  $P^{32}$  excretion between operated and unoperated control animals, both excreting more than 6% of the injected  $P^{32}$ . Five units caused the excretion in operated animals to increase approximately 40% over the control excretion.

*Influence of varying dose of hormone.* Table II lists the responses to various doses of parathyroid hormone when 1.2 mg disodium phosphate per 100 g of body weight were administered simultaneously with the hormone to animals operated 24 hours previously. Table III lists the responses when tracer  $P^{32}$  and hormone were administered to animals operated 24 hours previously. A straight line log-dose response relationship fits the data

obtained with 1.2 mg disodium phosphate much more closely than that obtained with tracer  $P^{32}$ . The equation of the regression line for the data obtained with 1.2 mg phosphate over the dosage range from 0.5 to 3.0 U.S.P. units, determined by the method of Fisher (1934), is  $y = 8.3 + 8.6 \log x$ . The sensitivity, as indicated by a significant increase in  $P^{32}$  excretion is about the same for both levels of phosphate administration, 0.5 U.S.P. unit causing a significant increase (64-220% in magnitude) in  $P^{32}$  excretion over that of the controls in 4 of 6 experiments. The variation of  $P^{32}$  excretion within groups appears to be somewhat less when 1.2 mg of phosphorus was administered than when tracer  $P^{32}$  was used.

*Influence of time interval between injection of hormone plus injection of phosphorus and*

TABLE II. Influence of Parathyroid Hormone on Phosphate Excretion of Thyroparathyroidectomized Male Rats. 24 hr after operation, 1.2 mg  $\text{Na}_2\text{HPO}_4/100$  g.  $\text{P}^{32}$  and hormone inj. simultaneously I.P.

| Parathyroid hormone, U.S.P. units | No. experiments | No. rats | Mean $\text{P}^{32}$ excretion, % $\pm$ S.E. |
|-----------------------------------|-----------------|----------|----------------------------------------------|
| 0                                 | 11              | 97       | $2.68 \pm .32$                               |
| .10                               | 1               | 10       | $2.90 \pm .50$                               |
| .25                               | 1               | 10       | $2.80 \pm .47$                               |
| .50                               | 3               | 28       | $5.35 \pm .33$                               |
| 1.00                              | 9               | 101      | $8.35 \pm .53$                               |
| 2.50                              | 2               | 18       | $10.43 \pm 1.49$                             |
| 3.00                              | 8               | 90       | $12.60 \pm .80$                              |
| 5.00                              | 1               | 11       | $11.62 \pm 1.36$                             |
| 10.0                              | 1               | 9        | $14.22 \pm 1.82$                             |

TABLE III. Influence of Parathyroid Hormone on Phosphate Excretion of Thyroparathyroidectomized Male Rats. 24 hr after operation, tracer  $\text{P}^{32}$ .  $\text{P}^{32}$  and hormone inj. simultaneously I.P.

| Parathyroid hormone, U.S.P. units | No. experiments | No. rats | Mean $\text{P}^{32}$ excretion, % $\pm$ S.E. |
|-----------------------------------|-----------------|----------|----------------------------------------------|
| 0                                 | 4               | 34       | $2.40 \pm .39$                               |
| .5                                | 2               | 23       | $6.58 \pm .90$                               |
| 1.0                               | 1               | 16       | $3.62 \pm .80$                               |
| 1.5                               | 1               | 16       | $7.91 \pm 1.71$                              |
| 2.0                               | 1               | 16       | $10.78 \pm 1.36$                             |
| 3.0                               | 1               | 13       | $6.17 \pm .63$                               |
| 6.0                               | 1               | 16       | $9.74 \pm 1.58$                              |

TABLE IV. Influence of Parathyroid Hormone on Phosphate Excretion of Thyroparathyroidectomized Male Rats. 2-4 hr after operation, 1.2 mg  $\text{Na}_2\text{HPO}_4/100$  g.  $3\frac{1}{2}$  hr between  $\text{P}^{32}$  (SQ) and hormone.

| Parathyroid hormone, U.S.P. units | No. experiments | No. rats | Mean $\text{P}^{32}$ excretion, % $\pm$ S.E. |
|-----------------------------------|-----------------|----------|----------------------------------------------|
| 0                                 | 7               | 68       | $.15 \pm .05$                                |
| .25                               | 1               | 10       | $.49 \pm .21$                                |
| .50                               | 1               | 7        | $.62 \pm .22$                                |
| 1.00                              | 3               | 30       | $.59 \pm .14$                                |
| 4.00                              | 1               | 10       | $1.92 \pm .39$                               |
| 16.00                             | 1               | 10       | $2.58 \pm .24$                               |

collection of urine. In the experiments described so far, the phosphate was injected into the animals immediately after the administration of the hormone, and the urine was collected in the 3 hours immediately following. The data from another experimental design are presented in Table IV. Animals were used 2 to 4 hours after operation. After the administration of phosphate, the urine was collected for  $3\frac{1}{2}$  hours and discarded. Then the hormone or saline was administered and

the urine collected for 3 hours. In Table IV are listed the excretions of  $\text{P}^{32}$  at various levels of parathyroid hormone treatment. 0.5 U.S.P. unit was the minimum effective dose. A straight line relationship was found to hold over the range of 0.25 to 16 U.S.P. units. The  $\text{P}^{32}$  excretion values are lower and the slope is less steep than that observed when the same amount of phosphate (1.2 mg) was administered, simultaneously with the hormone, to animals operated 24 hours previously.

**Discussion.** Excretion of  $\text{P}^{32}$  by thyroparathyroidectomized rats furnishes a sensitive indicator for parathyroid activity. In spite of the high variation in the  $\text{P}^{32}$  excretion of untreated animals, 0.5 U.S.P. unit caused a significant increase in  $\text{P}^{32}$  excretion as compared to solvent-treated controls. The most sensitive method in the literature (Tepperman *et al.*(4)) requires 12.5 U.S.P. units for a minimum significant effect on the blood phosphate level in the rat. Another advantage of the method described is its comparative ease.

The slope is steeper for the log dose-response curve obtained with the technic in which animals were used 24 hours after operation and hormone and 1.2 mg of disodium phosphate were administered simultaneously than for the technic in which  $3\frac{1}{2}$  hours elapsed between phosphate and hormone injection.

**Summary.** There is an increase in excretion of administered  $\text{P}^{32}$  by thyroparathyroidectomized male rats under the influence of parathyroid hormone. Amounts as small as 0.5 U.S.P. unit may be detected, an increase in sensitivity of 25 times over the method of Tepperman *et al.*(4), and of 200 times over the standard U.S.P. assay method. In addition, there are no tedious chemical determinations of blood phosphate levels to be done.

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