the serosa and that preventive heparinization arrested implantation in this early stage.

1. Goldie, H., *N. Y. Acad. Sci.*, 1956, v63, 727.
2. Goldie, H., Felix, M. D., *Cancer Res.*, 1951, v11, 73.
3. Goldie, H., Jeffries, B., Maxwell, M. D., Hahn, P., *ibid.*, 1952, v12, 92.
4. Goldie, H., Walker, M., Graham, A. R., Mitchell, G., *ibid.*, 1957, v17, 374.
5. Goldie, H., Walker, M., Graham, T., Williams, F., *J. Nat. Cancer Inst.*, 1959, v23, 891.
6. Straube, R., *Cancer Res.*, 1958, v18, 57.
7. Warner, P., Kroeker, H., Lederman, J., *Brit. J. Cancer*, 1957, v11, 93.
8. Jones, F. S., Rous, P., *J. Exp. Med.*, 1914, v20, 404.
9. Sampson, A., *Am. J. Path.*, 1931, v7, 423.
10. Goldie, H., Kelly, L., Ellington, A., Parahoo, R., *Proc. Am. Assn. Cancer Res.*, 1956, v2.
11. Bailiff, R. N., *Cancer Res.*, 1954, v14, 554.
12. Quick, A. J., *The Physiology and Pathology of Hemostasis*, Lea & Febiger, Phila., 1951.

Received June 9, 1961. P.S.E.B.M., 1961, v107.

A Method for Determination of Parathyroid Secretion Rate in the Rat.* (26772)

B. N. PREMACHANDRA AND HERMAN T. BLUMENTHAL
Institute of Experimental Pathology, The Jewish Hospital, St. Louis, Mo.

While secretion rates of some endocrine glands, notably thyroid, have been measured by a variety of technics, virtually no attempt has been made to estimate parathyroid hormone secretion rate (PSR) in individual small laboratory animals. This is partly due to the difficulty in obtaining adequate serial samples of blood in the same animal and, in part, to a failure to develop technics comparable to those utilized in determining the thyroxine secretion rate. The availability of an ultramicro method for determination of serum calcium(1) has led to an attempt to determine PSR in individual rats, based on a principle similar to that used for measuring thyroxine secretion rate(2). This report presents the feasibility of such a technic.

Experimental. One hundred and thirty-three female rats of a local strain, each weighing about 200 g, were housed in cages maintained at uniform temperature (78 ± 2°F) and illuminated only during daylight hours. They were fed Purina Laboratory chow *ad libitum* and water was available at all times. The rats were thyroparathyroidectomized (TPEx) surgically, under ether anesthesia. Completeness of TPEx was checked by administering I^{131}, and when radioactivity over the neck region was less than background this indicated an absence of thyroid I^{131} pick-up. One day prior to TPEx the laboratory chow was replaced by a diet in which calcium content was less than .016%, in order to prevent calcium in the feed from affecting serum calcium levels following TPEx. Serum calcium was determined by a chelating procedure utilizing ethyelene diamine tetra-acetate (EDTA) as the complexing agent. As little as 20 lambda (20 μl) of serum was found to be sufficient for each calcium determination. Blood samples were collected from the tail vein directly into polyethyelene microcentrifuge tubes and centrifuged in a Beckman microfuge. Twenty lambda of serum were titrated against EDTA in a microtitrator in a highly alkaline medium utilizing calcein as a indicator. The endpoint of titration was represented by the disappearance of the blue-green color. With little practice it was possible to distinguish this endpoint clearly. An ultraviolet light source may also be used to aid in distinguishing the endpoint sharply. Serum calcium determinations by this technic checked against classical flame photometry methods were found to be well within limits of expected experimental error. Re-

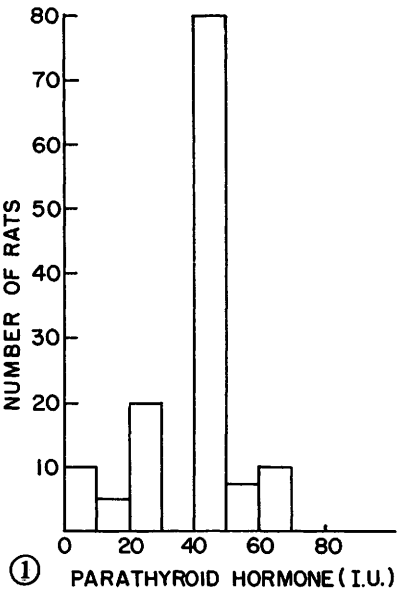
* This investigation was supported in part by grant from the Louis Monheimer-Clara K. London Memorial Research Fund and from U.S.P.H.S. Grant No. A 5107.

peated serum calcium determinations (at least 6-8) with 20 lambda of serum from the same rat yielded a variation of only ± 0.4 mg %.

Experimental procedure consisted in determining serum calcium levels prior to TPEx followed by serial determinations thereafter at 3, 6, 9, 12 and 24 hours after operation. After TPEx, when serum calcium had reached its lowest level, parathyroid hormone (PTH)† was injected subcutaneously into each rat in graded amounts of 25 units. PTH administration was discontinued when the post TPEx serum calcium reached the pre TPEx level. At this point it was assumed that sufficient PTH had been administered to maintain the serum calcium at a normal level. However, in many individuals serum calcium again declined within 24 hours after final PTH injection. Thus, the duration of PTH replacement was also determined in each animal.

Results. Serum calcium in individual rats before TPEx varied between 9 and 12 mg %. After TPEx serum calcium generally declined to its lowest level in about 6 hours, although periods up to 18 hours were noted in some rats. The percentage decline in serum calcium following TPEx varied from 0 to 51% of the initial value. In 10 rats as little as 5 I.U. of PTH were sufficient to elevate post TPEx serum calcium to the pre TPEx level while 25 I.U. were required in 20 other animals. In the majority of rats (80), 45 I.U. were necessary, while in another 10 animals the requirement was as high as 65 I.U. When the frequency distribution of the PTH requirement was plotted (Fig. 1), a 13-fold variation in individual rats was found. The final injection of PTH replacement (*i.e.*, the amount necessary to equate pre and post TPEx levels) lasted for an average of 10 hours, with a range from 8 to 12 hours. This was shown by the precipitous decline of serum calcium as the effect of PTH was lost. In certain rats in which excess PTH had been administered, serum calcium soared to levels far in excess of the pre TPEx value (Fig. 2). In such cases the average of the terminal and the preceding PTH injections was taken as the level of adequate replacement.

Discussion. As far as we are aware, this represents the first attempt to determine the quantity of PTH necessary to maintain normal serum calcium levels following TPEx. Individual estimations are of greater signifi-



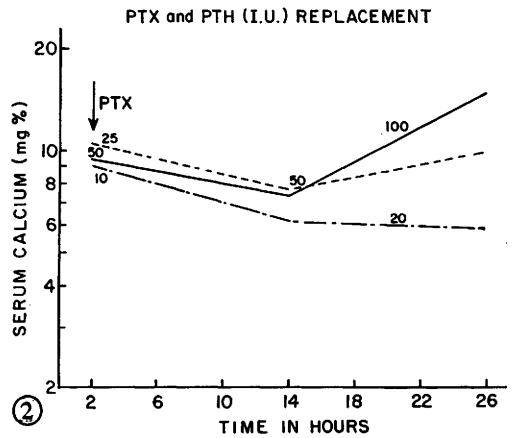


FIG. 1. Frequency distribution of PTH replacement in I.U./100 g body wt in individual rats following TPEx. While there was a 13-fold individual variation, mean PTH replacement was 40 I.U.

FIG. 2. Illustration of the principle of PTH replacement in individual rats. PTH is administered in increasing amounts following TPEx until post and pre TPEx serum calcium levels become equal. Administration of excess or insufficient amounts of PTH will not equate post and pre TPEx serum calcium levels.

† Obtained from Eli Lilly Laboratories, Indianapolis, Ind.

cance than the mean in interpretation of physiological variations as well as the influence of interrelated hormones and associated phenomena under controlled conditions of temperature and nutrition. This is particularly evident since a 13-fold variation was found in the individual PTH requirement following TPEx. Such variability can be compared with a 6-fold variation in the thyroxine secretion rate of rats(3) and with an 11-fold variation in the same determination in cattle(4). In certain rats the decline in serum calcium following TPEx was almost negligible.

Four possible reasons for such variations are: (a) Presence of accessory parathyroid tissue; (b) individual differences in parathyroid hormone secretion rate; (c) absorption of calcium from the gut, and (d) interaction of parathyroid hormone with other hormones to nullify its effect. Since the completeness of TPEx was checked in each individual, and since accessory parathyroid tissue is seldom present in rats(5), the first reason appears unlikely. The third possibility is not likely, since the commercial chow was replaced by a low calcium diet prior to TPEx and continued to the end of experiment. As regards the interaction of other hormones, the fact that these animals are athyroid is of some significance, since it has been observed that thyroxine may influence reabsorption of phosphate in the kidney and thus indirectly affect serum calcium level(6). A secondary influence of other hormones on serum calcium levels, notably those of the adrenal cortex, has not been eliminated in these studies. Since PTH was administered in increments of 25 I.U. the possibility also exists that slightly smaller increments of PTH replacement might give more accurate results. Increments of 25 I.U. were chosen to minimize the number of blood samples required to determine replacement levels. Furthermore, different levels of replacement might be obtained if routes other than the subcutaneous route were employed. Different values might also be obtained with hormone of greater purity. Despite these limitations, the present experiments indicate the validity of the method, although further studies are necessary to establish more absolute values.

Since it was found that a single adequate injection of PTH in TPEx rats maintained the serum calcium level for an average of only 10 hours, this hormone would appear to have only a short half-life. While the level of PTH replacement as determined in this study is in the general range reported in rats by Munson(7) and by Berswordt and Turner (8), if one considers the average duration of effect of parathyroid hormone (10 hours), it would appear necessary to multiply the estimated values in the present study by a factor of 2.4 to arrive at a 24-hour parathyroid hormone output. The range of PTH secreted over a 24-hour period would then be 12-156 I.U. per day with a mean of 96 I.U. Since these rats received a low calcium diet for only one day prior to TPEx, it can be assumed that the parathyroids were in an essentially normal state at time of removal and that the data represent normal individual physiological variations.

Summary. A technic is described in rats for individual determination of the 24-hour parathyroid hormone secretion rate (PSR). This method utilizes the subcutaneous administration of increasing amounts of parathyroid hormone (PTH) following thyroparathyroidectomy (TPEx) until pre and post TPEx levels of serum calcium are approximately equal. There were individual variations in the time required for serum calcium to reach its lowest level following TPEx and also in the quantity of PTH required to elevate serum calcium to the pre TPEx level. These probably represent individual variations in PSR. The average duration of effect of a single adequate PTH replacement was 10 hours, and from this the average PTH secretion over a 24-hour period was calculated to be 96 I.U.

1. Beckman/Spinco Ultramicro Analytical System, Model 150. Tech. Bull., 6071C, 1960.

2. Pipes, G. W., Premachandra, B. N., Turner, C. W., 2nd U. N. Geneva Conference, 1958, p811.

3. Grosvenor, C. E., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 517.

4. Premachandra, B. N., Pipes, G. W., Turner,

C. W., *Univ. Mo. Agri. Exp. Sta. Res. Bull.*, 1960, 727.

5. Burrows, R. B., *Am. J. Anat.*, 1938, v62, 237.

6. Bortz, E. E., Bowers, C. Y., Pont, M., *Ann. Int. Med.*, 1961, v54, 610.

7. Munson, P. L., *Ann. N. Y. Acad. Sci.*, 1955, v60, 776.

8. Berswordt, R. V., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 113.

Received June 12, 1961. P.S.E.B.M., 1961, v107.

Plasma Deoxyribonuclease and the Quantitative Effects of Injected DNA.* (26773)

J. PHILIP SAVITSKY† (Introduced by Bernard A. Sachs)

Lucy and Henry Moses Research Laboratories, Medical Division, Montefiore Hospital, N. Y.

It has been reported from this laboratory that a single injection of purified, undenatured deoxyribonucleic acid (DNA) produced in rabbits a significant decrease in serum cholesterol lasting at least 2 years(1,2,3). DNA preparations from homologous and heterologous mammalian tissues were both effective. The lowered mean serum cholesterol largely reflected the decline in cholesterol levels of those animals with high normal values. Quantitative studies of varying the amount of DNA injected over a 50-fold range showed a significant dose-response relationship(3).

One problem which arises in interpreting the quantitative data is that these animals have a circulating, potent plasma deoxyribonuclease. Some of the injected DNA molecules must be inactivated, denatured, or even degraded prior to their absorption by the cells of the organism. The enzyme circulating in the blood is similar to the pancreatic deoxyribonuclease I in its properties and requirements for activity(4). Mg^{++} ion is an essential activator and citrate is an effective inhibitor which acts by binding the Mg^{++}(5). The present study describes the inhibition *in vivo* of the plasma deoxyribonuclease by sodium citrate and the effect of this inhibition on the physiologic activity of injected DNA.

Methods. DNA used for injection was prepared from fresh beef liver by the mild procedures previously described(1), *i.e.*, extraction with high ionic strength salt and deproteinization with chloroform. The N/P ratio was 1.75. The fibres were dissolved in either isotonic saline or .015M sodium citrate plus isotonic saline, and injected immediately. The DNA used as the substrate for the enzyme determinations was prepared in identical fashion and dissolved in isotonic saline to a concentration of 0.15%. This was heated to 60°C for 30 minutes to destroy any residual deoxyribonuclease, placed in 10 ml aliquots and stored frozen at –15°C. The relative viscosity was 4.2.

The bloods were drawn into heparin in an ice bath, then centrifuged at 3000 r.p.m. for 30′ in a refrigerated centrifuge at 2°C. The supernatant plasma, apparently free of formed elements, was removed and stored frozen at –15°C. Deoxyribonuclease activity was determined by adding 1 ml of the heparinized rabbit plasma and 0.1 ml .02M $MgCl_2$ to 2 ml of the DNA substrate solution in an Ostwald viscosimeter at 37°C. The run-through time of the viscosimeters was approximately 20 seconds. Following a mixing period of one minute, viscosity measurements were made at 3 minute intervals for 30 minutes. Under these conditions, the straight-line portion of the curve lasted 18 minutes.

Total serum cholesterol was determined by a Bloor(6) procedure with 10 ml of the Bloor's reagent containing the extracted serum being taken to dryness.

Albino rabbits of the New Zealand strain weighing 2-3 kg were kept in temperature-controlled, air-conditioned, separate quarters and fed a standard chow *ad libitum*. All the

* This research was aided by grant from Nat. Inst. of Arthritis and Metab. Dis.

† Established Investigator, American Heart Assn.