enzyme systems has not yet been determined.

Summary. Administration of nikethamide or phenobarbital to rats increases the rate of degradation of the organophosphorus insecticide, EPN, by a microsomal oxidase system in the liver. Induction of the activity of this system by nikethamide provides a possible explanation for the previously demonstrated ability of this drug to decrease the susceptibility of animals to the acute toxicity of EPN. In the present study there was good correlation between the extent of increase in activity of the detoxification enzyme system by nikethamide and phenobarbital and the ability of these compounds to reduce the acute toxicity of EPN to rats with phenobarbital being superior to nikethamide in both respects. It appears that the microsomal enzyme system that catalyzes the degradation of EPN to p-nitrophenol provides a practical means of testing various chemical agents for ability to induce synthesis of microsomal enzyme activity.

1. DiStefano, V., Hurwitz, L., Neuman, W. F., Hodge, H. C., Proc. Soc. Exp. Biol. and Med., 1951, v78, 712.

2. Rosenberg, P., Coon, J. M., J. Pharm. Exp. Therap., 1960, v128, 289.

3. Neal, R. A., DuBois, K. P., ibid., 1965, v148, 185.

4. Brazda, F. G., Baucum, R., ibid., 1961, v132, 295.

5. Conney, A. H., Burns, J. J., Adv. Pharm., 1962, v1, 31.

6. Litchfield, J. T., Wilcoxon, F., J. Pharm. Exp. Therap., 1949, v95, 99.

Received September 9, 1965. P.S.E.B.M., 1966, v121.

Human Growth Hormone Immunoassay: Two-Antibody Method Using I-125 Tracer.* (30698)

CARL R. MORGAN

Department of Anatomy, Indiana University Medical Center, Indianapolis

Methods for immunoassay of human growth hormone using an I-131 tracer have been reported(1,2). Experience with insulin immunoassay by the 2-antibody technique has demonstrated that this procedure can be conveniently and reliably used(3-7). The present report describes a two-antibody procedure for immunoassay of human growth hormone utilizing a commercially prepared I-125 human growth hormone tracer.

Materials and methods. Each of 6 rabbits received a subcutaneous injection of 1 mg of human growth hormone† (HGH) on days 0, 7, 14, and 21. The HGH was prepared in a solution of 0.15 M NaCl, phosphate buffer (pH 7.4); the final concentration was 1 mg/ml. Equal volumes of HGH solution and lanolin-oil were mixed to a thick emulsion(8). After the 4 injections, each animal was bled *via* cardiac puncture on days 32 and 35, *i.e.*, at 11 and 14 days after the fourth injection. Two of the six rabbits responded with anti-human growth hormone (AHGH-R) serum which, at a final dilution of 1:4,000, was capable of binding approximately 50% of 0.5 mμg of I-125-HGH.

The I-125-HGH was prepared commercially.‡ The specific activity of the product was 390 millicuries per milligram.

Anti-rabbit serum (ARS-S) was obtained from sheep which had received subcutaneous injections of rabbit serum plus lanolin-oil adjuvant. One of three sheep responded with

* This investigation was supported in part by Am. Cancer Soc. Grant IN 46-F in part by U.S.P.H.S. Grant HE06308, and in part by facilities of the Research Laboratories of the Steno Memorial Hospital, Gentofte, Denmark.

† This human growth hormone was kindly made available to me by Dr. Henning Andersen, Director, Children's Hospital, Fuglebakken, Denmark. This part of the project was supported by the Crippled Children's Fund, Boston, Mass.

‡ Abbott Laboratories, Oak Ridge, Tenn. This was Andersen's HGH preparation, which was also used to immunize the rabbits.

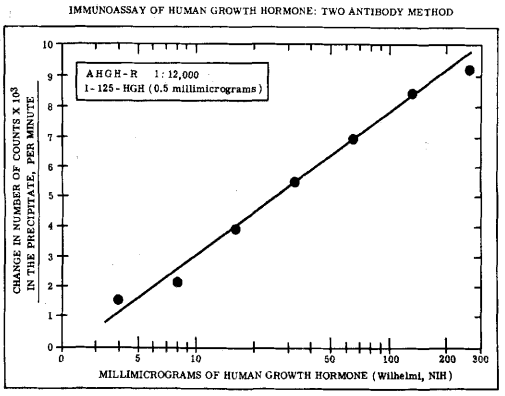

FIG. 1. A standard curve for immunoassay of human growth hormone. Each point is mean value of 3 determinations. This assay standard was used to obtain the results shown in Fig. 2.

suitable antiserum after 4 injections of 5 ml each.

For immunoassay standards a HGH preparation (NIH-HS-612B)§ was used which was different from that used for antigen and tracer.

A 1% bovine serum albumin—0.15 M NaCl-phosphate buffer, pH 7.4, (1% BSA) was used as the diluent for the standards. A 0.044 M EDTA solution in 1% BSA was used as the diluent for the I-125-HGH(6).

The reactions were carried out as follows. *First antibody reaction:* (a) 1.0 ml 1% BSA, or standard, or assay sample was mixed with (b) 0.5 ml I-125-HGH, 0.5 mμg, and (c) 0.5 ml of a dilute AHGH-R. This reaction mixture was placed at 4°C for 24 hours. *Second antibody reaction:* a sufficient amount of ARS-S was added to insure maximum precipitation in 16 hours (in this case 0.3 ml was used). When dilutions of AHGH-R greater than a final concentration of 1:4,000 in 2 ml were used, 0.1 ml of dilute normal rabbit serum sufficient to bring the concentration of rabbit serum to 1:4,000 was used. This reaction mixture was placed at 4°C for 24 hours. Then the tubes were centrifuged at 2,000 rpm for 15 minutes. The resultant supernatants were decanted and discarded. The precipitates were counted in a Packard 410 autogamma counter with a window setting at 15 to 50 KEV. The standard curve was plotted, and the amount of HGH in each assay sample was determined from the standard curve.

Results. Fig. 1 shows a typical assay curve obtained by this procedure. The total number of counts was greater than 10,000 cpm at each concentration shown on the curve. The change in the number of counts in each precipitate was obtained by subtracting the number of counts in each precipitate from the mean of the counts in the precipitate when no unlabeled HGH was present. There was a linear log dose response over the range from 4 to 250 mμg of added unlabeled HGH. With other dilutions of antiserum the assay range was 1 to 100 mμg, an example is shown in Table I.

As can be seen in Fig. 2, R.C. (a hypopituitary dwarf) did not respond with elevated HGH levels during the hypoglycemia (9) following i.v. insulin nor during the fall in blood glucose at 45 to 60 minutes after oral glucose. The two acromegalics and the one non-treated hyperpituitary giant had elevated HGH levels as compared to the one normal and one hypopituitary dwarf. Table II shows the correspondence of duplicate determinations for human growth hormone in 12 serum samples.

Discussion. The 60 day half-life of I-125 recommends it as an isotope for use as a radiotracer for tyrosine containing proteins. Its low energy gamma emission presents no problem with modern gamma counting equipment. The combination of long half-life and high specific activity precludes the necessity of counting both supernatant and precipitate;

TABLE I. Assay Standard for Human Growth Hormone Using 1:16,000 Anti-Human Growth Hormone (Rabbit Source).

HGH standard (mμ)	cpm I-125 ppt	Δ cpm ppt
0	30,200	—
1	28,800	1,400
2	27,800	2,400
4	25,800	4,400
8	23,400	6,800
16	20,800	9,400
32	17,200	13,000
64	14,800	15,400
128	12,800	17,400

§ Kindly supplied by Dr. A. E. Wilhelmi, Emory Univ., Atlanta, Ga.

consequently, counting time and calculations are minimized. Further improvement can be made by making all calculations with a computer(10). The assay results reported here indicate that this method can be used to distinguish hypo- and hyper- levels of human growth hormone. Also, these results are in agreement with values reported by others (1,11).

Further studies are in progress to extend the effectiveness of this assay method. The preliminary results reported here indicate that two-antibody immunoassay of human growth hormone using I-125 tracer may be an effective tool in clinical diagnosis and biomedical research.

Summary. Immunoassay of human growth hormone with a 2-antibody method utilizing commercially prepared I-125 tracer provides a simple procedure for determining the concentration of human growth hormone in plasma. The method is sensitive to as little as 1 mμg.

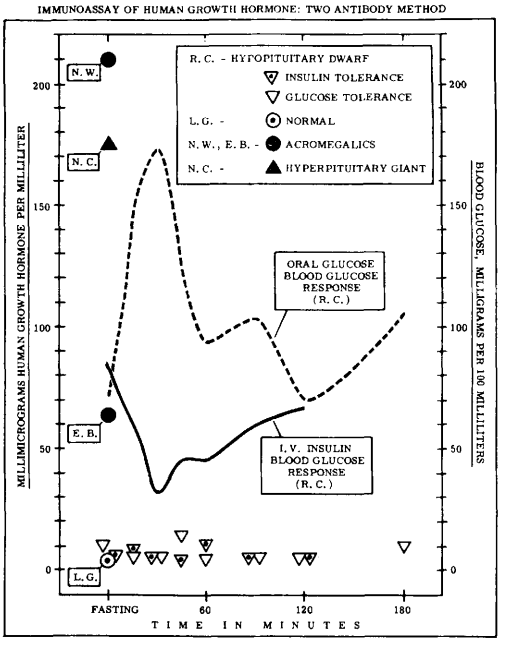

FIG. 2. Each point represents a serum concentration of human growth hormone, scale on the left. The 2 lines represent blood glucose concentrations for R.C., scale on the right. Each growth hormone value represents mean of duplicate determinations.

TABLE II. Correspondence of Values for Human Growth Hormone in Duplicate Determinations of Serum Samples.

Sample	Δ cpm I-125 ppt	HGH (mμg)
N.C.	8712	155 (177)
	9377	200
N.W.	9577	225 (205)
	9199	185
E.B.	7145	70 (61)
	6569	52
L.G.	897	<4 (<4)
	1393	4
R.C. 8:48	4876	23 (15)
	2279	7
R.C. 9:15	2163	6 (5)
	1300	4
R.C. 9:25	1311	4 (4)
	1495	4
R.C. 9:40	4392	18 (12)
	2208	6
R.C. 9:55	1788	5 (5)
	1650	5
R.C. 10:27	1782	5 (5)
	1715	5
R.C. 10:55	2387	7 (6)
	2002	6
R.C. 11:58	3700	11 (9)
	2631	8

1. Utiger, R. D., Parker, M. L., Daughaday, W. H., J. Clin. Invest., 1962, v41, 254.

2. Glick, S. M., Roth, J., Yalow, R., Berson, S. A., Nature, 1963, v199, 784.

3. Morgan, C. R., Lazarow, A., Proc. Soc. Exp. Biol. and Med., 1962, v110, 29.

4. ———, Diabetes, 1963, v12, 115.

5. Morgan, C. R., Sorenson, R. L., Lazarow, A., ibid., 1964, v13, 1.

6. ———, ibid., 1964, v13, 579.

7. Samols, E., Bilkus, D., Proc. Soc. Exp. Biol. and Med., 1964, v115, 79.

8. Robinson, B. H. B., Wright, P. H., J. Physiol., 1961, v155, 302.

9. Roth, J., Glick, S., Yalow, R. S., Berson, S. A., Science, 1963, v140, 987.

10. Brunfeldt, K., Jørgenson, K., Rosenbrands, B., Acta Endocrinologica, 1965, v48, in press.

11. Roth, J., Glick, S. M., Yalow, R. S., Berson, S. A., Metabolism, 1963, v7, 577.

Received September 9, 1965. P.S.E.B.M., 1966, v121.