Somatotropic Activity of U. S. Pharmacopeia Growth Hormone Reference Standard in Alkali or Acid.* (22970)

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A United States Pharmacopeia (USP) Growth Hormone Reference Standard was released within the last year. One unit is defined as the activity of 1 mg of the Standard (1). Since the Standard was prepared by the U. S. Pharmacopeia as a service to those interested in it, and not because it is required for any pharmacopeial preparation, no specific information was made available regarding choice of assay methods or injection vehicles. It is our purpose to report 3-fold differences in potency as a result of dissolving growth hormone preparations in alkaline or acid solution.

Methods. Assays for somatotropic activity were made by the 10-day growth test in hypophysectomized, male, albino, 100 g rats. The animals were received from Hormone Assay Laboratories and were fed a diet containing 25% casein.† Plateaued rats failing to gain or lose over 5 g during the third postoperative week were selected for assay. The rats were distributed at random into groups of 5 to 7. Injections were made subcutaneously, 0.5 cc/rat/day, for 10 days. Final body weights were determined on the day after last injection. A comparison of the USP Standard against itself when injected in alkaline or acid solution was made by suspending 10 mg in distilled water and adding enough alkali or acid to dissolve. Final dilution was made with distilled water. The pH of injected solutions was pH 3.5 or pH 9.5. This experiment was also done with a pork pituitary preparation, STH 8, prepared at The Wilson Laboratories by the method of Raben and Westermeyer(3). The somatotropic potency of STH 8 was standardized against the USP Standard. In tests 1 and 2, 10 mg of each preparation were suspended in 8 ml of 0.9% saline, 3 drops of 0.1 N NaOH added, and diluted to 10 ml with saline. Further dilution to injected concentrations was also made with saline. The injected solutions had a pH range of 8.0-9.5. Identical procedure was followed in test 3, except that 0.5% phenol was used as diluent instead of saline. In tests 4 and 5, 10 mg each of STH 8 and USP Standard were dissolved in 0.5 ml of 0.05 N HCl, immediately diluted to 10 ml, and then to injected concentrations with 0.5% phenol. The pH of final solutions was between 3.5 and 4.0. The final solutions in all cases were refrigerated during the 10-day test period, except for one hour each day when they were brought to room temperature to make the injections. Since after the first day of refrigeration, the solutions made with alkali became turbid in various degrees, all solutions were vigorously shaken before injection. The acidified solutions remained clear during the course of the assay.

Results. The results of comparing each preparation against itself when injected in alkaline or acid solution are presented in Table I. These data show that the USP Standard shows a much smaller effect when injected in acid. On the other hand the activity of STH 8 in alkaline or acid media is the same.

TABLE I. Somatotropic Effect of USP Growth Hormone Reference Standard and STH 8, Pork Preparation Obtained by Method of Raben, Injected in Alkaline or Acid Solution to Hypophysectomized, Male, 100 g Rats (10-Day Test). Dose: 25 μg/day.

Somatotropic preparation	Mean gain in g ± S.E.		Statistical diff.,* alkaline *vs* acid
	Alkaline sol.	Acid sol.	
USP Std.	17.6 ± 1.1	10.8 ± 1.8	Signif. at $P_{.05}$
STH 8	11.6 ± 1.2	12.2 ± .5	Not signif.

* Calculated by Fisher's "t" test.

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† The composition of the diet in percent was: casein 25, milk powder 10, yeast 10, desiccated liver 3, salts IV(2) 2, sucrose 45 and corn oil 5.

TABLE II. Somatotropic Potency of STH 8, Pork Preparation Obtained by Method of Raben, when Assayed against the USP Growth Hormone Reference Standard (1 Unit = 1 mg) in Hypophysectomized, Male, 100 g Rats (10-Day Test).

Test No.	STH 8 Dose, μg/day	STH 8 Gain, g/day	USP standard Dose, μg/day	USP standard Gain, g/day	pH of vehicle	Potency, USP units per mg	95% confidence limits	Index of precision
1	20	.65	15	.73	Alkaline	.32	.20- .51	.17
	80	1.10	60	1.92				
2	40	1.37	20	1.59	"	.32	.21- .49	.26
	120	1.76	60	2.17				
3	40	.80	20	1.30	"	.18	.12- .28	.19
	120	1.35	60	1.94				
4	30	1.08	20	.68	Acid	1.05	.68-1.62	.21
	90	2.02	60	1.62				
5	40	1.40	30	.90	"	.83	.52-1.33	.30
	120	1.68	90	2.05				

The somatotropic potency of STH 8 in terms of the USP Standard is shown in Table II. The pork preparation, STH 8, exhibited an activity slightly less than one-third that of the USP Standard (0.32, 0.32 and 0.18 unit/mg), when an alkaline solution was used, but when an acid solution was utilized the activity of STH 8 was equivalent to that of the USP Standard (1.05 and 0.83 USP unit/mg).

Discussion. The data in Table I indicate that the USP material appears less active in acidic than in basic solution, while STH 8, the pork preparation obtained by the Raben method, produces equivalent responses on both sides of neutrality. This method employs glacial acetic acid extraction, while the USP Growth Hormone Standard was prepared from beef according to the method of Wilhelmi, Fishman and Russell(4), which employs alkaline extraction. The two preparations reported here, therefore, differ in two factors: the method of preparation and the animal source, and their differential reactivity in alkali or acid could be due to either factor. The decreased stability in acid of the USP material reported here, is in agreement with work by Li and Papkoff(5) who reported definite destruction of activity with accompanying changes in electrophoretic behavior when alkali extracted beef growth hormone was dissolved in 0.1 N acetic acid and maintained at 25°C for 24 hours.

Conclusions. 1. The somatotropic activity of a pork pituitary preparation, obtained by the method of Raben was compared with that of the newly released USP Growth Hormone Reference Standard. Some results were dependent on the pH of the injection vehicle. When alkaline solutions were used, the Raben preparation was about one-third as active as the USP material, but when injections were made in an acid vehicle both preparations exhibited equi-potent activity. 2. When somatotropic preparations are assayed against the USP Growth Hormone Reference Standard an alkaline solution must be used, since the USP material shows decreased activity when dissolved in acid.

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