Solid-Phase Radioimmunoassay for Sheep Growth Hormone[1] (34617)

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A number of radioimmunoassay techniques have been developed for measurement of the primate growth hormones (1–4). The amount of labeled hormone bound to antibody can be determined by the chromatoelectrophoresis method of Yalow and Berson (5), the double antibody technique of Schalch and Parker (2), the coated charcoal method of Herbert *et al.* (6) or the solid-phase radioimmunoassay described by Catt and Tregear (4). In addition to simplicity, the solid-phase technique has one potential advantage in that plasmas or levels of plasma that cause excessive damage to the labeled hormone can be removed from the reaction before adding the labeled hormone if there is little displacement of previously bound hormone from the antibody adsorbed to a solid phase as suggested by Catt *et al.* (7). This communication describes modifications of the solid-phase radioimmunoassay for measuring sheep growth hormone (SGH) in plasma. Because bovine growth hormone (BGH) cross-reacts with antibodies to SGH (8), the method is also applicable to assay of the bovine hormone if BGH is used as a standard.

Methods. Highly purified SGH was prepared by chromatographing a SGH preparation obtained by the method of Wallace and Ferguson (9) on a column of Sephadex G-100 (2.5 × 100 cm) in a 0.075 *M* carbonate buffer, pH 9.4. The growth-promoting activity of the purified SGH was established in hypophysectomized rats. Antisera to the purified SGH were obtained by immunizing rabbits as described by Moudgal and Li (8). In agar plates, only a single precipitation line occurred between extracts of sheep anterior pituitary tissue and the anti-SGH serum absorbed with small amounts of diluted, normal sheep serum. This single line fused completely with a single line between the purified SGH and the antiserum. A gamma-globulin fraction was obtained by bringing a volume of the antiserum to half saturation with ammonium sulfate, dissolving the precipitate in water, dialyzing, and lyophilizing. The SGH was iodinated with $^{125}I^-$ (New England Nuclear Corporation, Boston, Mass.) by the procedure of Greenwood *et al.* (10). The ^{125}I-GH was separated from $^{125}I^-$ on a column of Sephadex G-100 (1 × 40 cm). The ^{125}I-GH was further purified immediately before use in the immunoassay by mixing a small volume of labeled hormone (0.1–0.2 ml) with 0.1 ml of normal sheep plasma and chromatographing on a column of Sephadex G-150 (1 × 40 cm) equilibrated with barbital buffer (pH 8.6, ionic strength 0.1) containing 2.5 mg of bovine serum albumin/ml. The material used in the immunoassay emerged from the column in a symmetrical peak, which followed a small portion of the radioactivity that was not retarded on the column.

The interior of polystyrene or polypropylene 10 × 75-mm tubes was coated with antibody by addition of 0.5 ml of anti-SGH γ-globulin diluted in 0.075 *M* carbonate buffer, pH 9.4. The tubes containing the diluted antibody were then stored at 4° overnight. The gamma-globulin solution was removed by aspiration, and the tubes were washed 4 times with 0.85%(w/v) NaCl. Solutions of diluted antibody have been reused) up to 8 times with no discernible change in the results.

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TABLE I. Effect of Time and Temperature on Coating Plastic Tubes with Antibodies.[a]

	Room temp				4°	
	Time					
Exp.	15 min	60 min	2.5 hr	24 hr	2.5 hr	24 hr
	(% bound[b])					
I	24.0 ± 0.87[c]	27.3 ± 0.88	—	26.0 ± 1.1	—	34.4 ± 0.06
II	—	—	21.8 ± 0.71	—	25.4 ± 0.96	—

[a] Polypropylene tubes used in both experiments. Diluent was barbital buffer, pH 8.6, with 2.5 mg of bovine serum albumin/ml.

[b] Tubes incubated at 4° for 24 hr with 0.1 mμg of labeled SGH. Amount of labeled hormone bound is corrected for adsorption of labeled hormone in tubes not coated with antibody.

[c] Standard error of the mean.

Quadruplicate 50- or 100-μl samples of unknown or standards were pipetted into the washed antibody-coated tubes. Diluent, consisting of 10% centrifuged and filtered human plasma in pH 8.6, 0.1 ionic strength, barbital buffer, was added to make a final volume of 0.5 ml. The tubes were shaken and refrigerated for 3 days. Labeled-GH (0.1 to 0.2 mμg in 50 μl) was added to each tube, followed by mixing and storage in the refrigerator for 2 days. After the second incubation, the contents were removed by aspiration, and each tube was washed 6 times with ice-cold 0.85% NaCl and counted for 10 min in an automatic gamma counter.

Results and Discussion. Table I shows the effect of varying time and temperature for coating the plastic tubes with rabbit antibodies to SGH. Increasing the time of exposure to the antibody solution from 15 min to 24 hr at room temperature did not greatly increase the amount of binding of labeled hormone; however, an increased binding was observed if the tubes were coated at 4°. Increased binding in the tubes coated at 4° would be expected if the attachment of antibody to the plastic is by adsorption as suggested by Catt and Tregear (4). More recent experiments have shown that polystyrene tubes are somewhat superior to polypropylene in that a 15 to 20% greater binding of labeled hormone occurs in antibody-coated polystyrene tubes. No differences in binding were found between polystyrene tubes from two commercial sources.

Table II shows binding of labeled hormone in different diluents in tubes with and without antibody. Similar binding of labeled SGH

TABLE II. Influence of Diluent on Binding and Adsorption of Labeled Hormone.[a]

Additions to buffer	Exp.	Bound to antibody[b]	Adsorbed in blanks	Bound ^{125}I-SGH displaced by unlabeled SGH[c]
			(%)	
0	I	41.6 ± 3.3	12.9 ± 0.53	3.5 ± 1.9
Bovine serum albumin, 2.5 mg	I	44.3 ± 1.4	1.4 ± 0.15	10.6 ± 1.0
Human plasma, 3%	I	37.8 ± 1.2	1.1 ± 0.16	11.5 ± 3.3
	II	39.3 ± 1.2	1.8 ± 0.50	—
20%	I	34.3 ± 1.0	1.1 ± 0.19	10.7 ± 1.7
	II	35.2 ± 0.75	1.6 ± 0.20	—

[a] Polypropylene tubes coated with antibody at room temperature. Incubated with 0.1 mμg of ^{125}I-SGH for 2 days at 4°.

[b] Corrected for adsorption of ^{125}I-SGH in tubes not coated with antibody.

[c] After removal of unbound ^{125}I-SGH by washing the tubes, 200 mμg of unlabeled SGH was added to each tube and the tubes were incubated another 2 days at 4°.

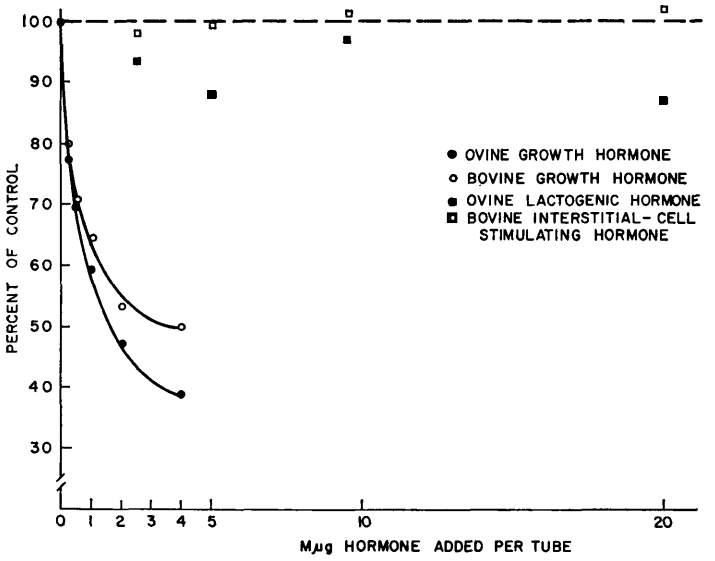

FIG. 1. Standard curves obtained with antibody-coated polystyrene tubes. Tubes were coated with 0.5 ml of 1:5000 dilution of rabbit anti-SGH serum at 4° for 16 hr.

occurred with the barbital buffer and the same buffer containing bovine serum albumin. When no protein was added to the buffer, however, there was considerable adsorption of labeled hormone in tubes without antibody. When protein was added either as bovine serum albumin or human plasma, the adsorption of the labeled hormone to the tubes without antibody was less than 2% There was somewhat less binding of labeled hormone to antibody in the buffer containing human plasma. To reduce nonspecific effects of plasma, the procedure has been standardized to use 10% human plasma in barbital buffer as diluent. The addition of several thousandfold excess of unlabeled-SGH to tubes which contained labeled SGH bound to SGH antibodies adsorbed to the interior surface of plastic tubes displaced about 10% of the labeled SGH during a 2-day incubation at 4° (Table II). These results are in agreement with those of Catt *et al.* (7) and indicate that the unlabeled hormone can be removed from the reaction before the labeled hormone is added without loss of sensitivity.

Dose–response curves demonstrating decreased binding of ^{125}I-SGH in the presence of unlabeled specific antigen (SGH) and a cross-reacting antigen (BGH) are shown in Fig. 1. The use of ^{125}I-SGH and plastic tubes coated with anti-SGH can be used to measure either SGH or BGH; but since the shape of the curve with BGH is different from the curve with SGH, it is necessary to use BGH as a standard when measuring the bovine hormone. The lack of response from ovine lactogenic and bovine luteinizing hormones is also shown. Additional studies have shown that bovine thyroid-stimulating hormone does not react in this assay. The antiserum to SGH used in this study did not bind labeled-sheep lactogenic hormone, and antibodies to sheep lactogenic did not bind the labeled SGH.

Figure 2 shows a response curve with standard SGH and three dilutions of two samples of sheep serum. The responses obtained with the two serum samples were similar to those obtained with the standard SGH. The addition of up to 0.2 mg of heparin/assay tube did not affect the binding of labeled SGH. Similar response curves were obtained in studies in which the tubes were washed before adding fresh diluent and labeled hormone; however, for most plasma samples, this does not appear a necessary step. After adjusting for potency, similar response curves were obtained with SGH preparations iso-

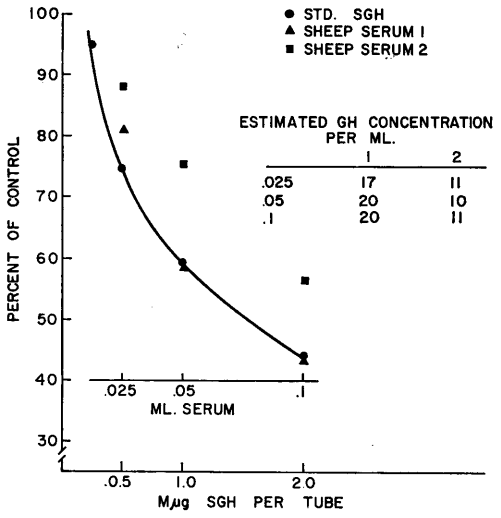

FIG. 2. Standard curves obtained with SGH and sheep sera in antibody-coated polystyrene tubes. Tubes were coated with 0.5 ml of 1:5000 dilution of rabbit anti-SGH serum at 4° for 16 hr.

lated by widely different methods. One preparation with very low biological activity also had very low immunological activity in this assay. Estimates of GH concentrations in plasma samples obtained from a young calf were 13 mμg/ml before hypophysectomy and an undetectable value after hypophysectomy.

The application of this technique to samples from cattle and sheep is shown in Table III. From these limited data, it does not appear that fasting resulted in increased levels of plasma GH in sheep. The decline in blood sugar and the marked increase in plasma free fatty acids indicated that these animals were fasting and mobilizing fatty acids from adipose tissue. The results of this study are quite similar to data obtained from pigs by Machlin *et al.* (11), but quite different from data obtained from humans by Roth *et al.* (12). The reason for differences among species is not obvious.

The 1- to 8-week-old calves had higher levels of plasma GH than the 3- to 5-year-old cows. At 20 weeks of age, the plasma GH in the calves was more similar to that found in the cows. As the calves developed functional rumens, which is associated with the consumption of dry feed as the calf becomes older, levels of blood sugars declined. During this period, rumen volatile fatty acids rather than glucose start to supply a greater proportion of the metabolizable energy. The levels of plasma GH were lowest in the older calves and cows deriving the major portion of their metabolizable energy from rumen vola-

TABLE III. Plasma Growth Hormone in Sheep and Cattle.

	No. of animals	Plasma growth hormone (mμg/ml)	Blood sugar (mg/100 ml)	Plasma free fatty acids (meq/liter)
I. Sheep[a]				
After feeding (hr)				
4	5	5.6 ± 0.58[b]	55.0 ± 1.6	0.077 ± 0.015
12	5	6.8 ± 0.88	53.2 ± 0.8	0.136 ± 0.015
24	5	6.2 ± 0.57	44.4 ± 0.6	0.400 ± 0.039
48	5	4.8 ± 0.38	38.2 ± 1.2	1.470 ± 0.176
72	5	5.7 ± 0.54	45.2 ± 1.6	1.458 ± 0.151
II. Cattle[c]				
Age 1 week	4	22.5 ± 8.2	98.2 ± 7.8	—
4 weeks	4	26.1 ± 5.9	86.6 ± 6.0	—
8 weeks	4	36.2 ± 5.8	66.4 ± 1.9	—
20 weeks	4	16.8 ± 5.2	52.9 ± 3.3	—
3–5 years	4	9.3 ± 1.9	51.3 ± 2.5	—

[a] Crossbred wethers weighing 35 kg were fed 650 g of a diet consisting of corn and alfalfa prior to obtaining samples from the jugular vein.

[b] Standard error of the mean.

[c] Samples were obtained by jugular vein puncture from four Hereford cows and their calves.

tile fatty acids rather than from glucose. The data from the animals used in this study suggest that glucose metabolism has less influence on GH release in ruminants as compared with primates.

Summary. A procedure for solid-phase radioimmunoassay of ovine and bovine GH is described. Fasting sheep with functional rumens did not result in increased plasma GH levels. Young calves had somewhat higher levels of plasma GH than did mature cows.

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