

Continuous Elevation of Blood Growth Hormone Concentrations by Beeswax Implant¹

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ABSTRACT

We examined constancy of release of purified ovine growth hormone from an implant containing soybean oil and beeswax. Implants contained an amount of growth hormone that was sufficient to increase concentrations in blood plasma by 20 and 40 ng/ml and to maintain those concentrations over 1 wk. Growth hormone in plasma increased to approximately 65 ng/ml in lambs receiving low dose implants the 1st day after implantation, returned to 31 ng/ml on day 2, and remained near this concentration for the remainder of the week. Pulse release of growth hormone was not similar in the high dose lambs where growth hormone concentration in plasma averaged 45 ng/ml 1 day after implantation, then gradually increased to 60 ng/ml on day 6. Unimplanted control lambs had mean growth hormone concentrations of 2.9 to 3.9 ng/ml throughout the 6-day observation. This approach should interest investigators studying the chronic influence of purified or synthetic growth hormone on dairy cows, beef steers, or lambs.

INTRODUCTION

The potential use of growth hormone (GH) to increase growth rate or milk production of domestic livestock is coming closer to practical application following observed increases of milk production for dairy cows treated with GH (5) and the advent of GH production by genetic engineering methods. If GH is efficacious for

increasing livestock productivity, a major problem is method of administration. Because GH is a protein, it is not likely to be administered orally. Therefore, an implant appears to be the mode of administration. Although not fully resolved, recent studies suggest that continuous infusion is as biologically effective as pulse injections of GH for increasing nitrogen retention of calves (4) and milk production of dairy cows (D. E. Bauman, personal communication). The problem is then to determine methods of implanting GH to maintain elevated concentrations of the hormone in plasma over extended time. This report describes one approach to maintenance of elevated GH concentrations in peripheral blood of the lamb by beeswax pellet containing purified GH.

MATERIALS AND METHODS

Nine crossbred wether lambs (82 to 96 kg) were assigned to three treatment groups of three lambs each. We assumed that the control group would have approximately 5 ng GH/ml plasma. The objective was to treat the other groups to achieve GH of 25 and 45 ng/ml plasma and to maintain these concentrations over 1 wk. All lambs were in individual metabolism crates for 2 wk prior to implantation and 1 wk after implantation. Lambs received 2.2 kg of a pelleted alfalfa, barley ration at 0730 h daily. Water was available free choice.

Estimation of GH to Implant

For a constant and proportional daily rate of GH delivery from the implant, the amount of GH required in each implant was estimated by:

$$\begin{aligned} & \text{Release rate (RR) (ng/min) =} \\ & \text{Metabolic clearance rate (MCR) (ml/min)} \\ & \times \text{GH concentration in plasma (ng/ml)} \end{aligned}$$

This equation has been used to estimate endogenous secretion rates of prolactin (2), cortisol

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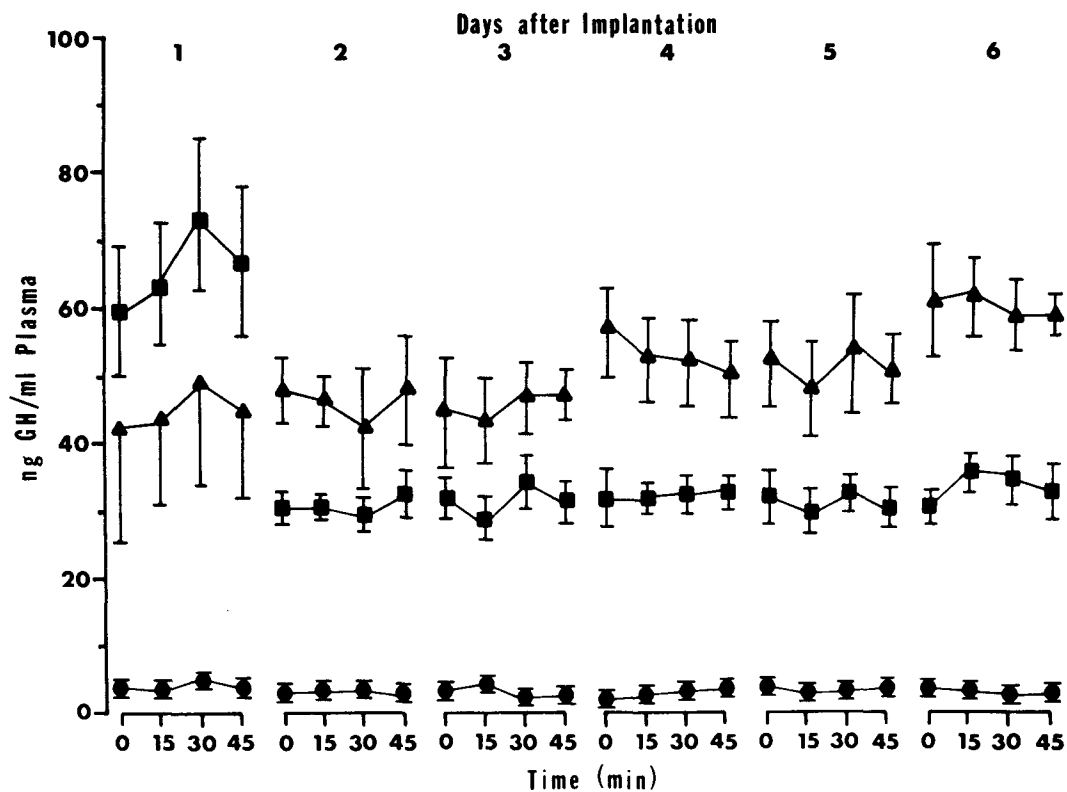


Figure 1. Mean concentrations of growth hormone in plasma at 15-min intervals over a 45-min sampling period for each of the 6 days after implant insertion in the control lambs (●—●), low growth hormone dose (■—■), and high growth hormone dose (▲—▲).

(6), and GH (3). By our inserting the desired increase of GH concentration in plasma (20 ng/ml) and published MCR (7) of ovine GH (150 ml/min) into the equation, an estimated release rate of 3 μ g/min or 30.2 mg/wk was obtained for each lamb. Similarly, the high dose of GH was intended to increase GH concentration in plasma by 40 ng/ml. Therefore, for the same MCR the estimated release rate was 6 μ g/min or 60.4 mg/wk for each lamb.

These estimates assumed that such a release rate would be required to maintain GH in plasma at the desired concentrations. We had no evidence that GH would be released from the implants at the estimated rate.

Preparation of Beeswax Implant

For the low dose group, 90.6 mg of crystalline oGH⁴ (30.2 mg \times three lambs) was added to 3 ml of soybean oil, mixed thoroughly, then added to .135 g of yellow beeswax at 42°C. Similarly, for high dose implants, 181.5 mg of oGH was added to 6 ml of soybean oil, then mixed with .270 g of melted (42°C) beeswax. The heated mixture was decanted into 3-ml plastic disposable syringes and allowed to cool to 4°C overnight. The hardened beeswax then was cut into three 1-ml pellets for the low and three 2-ml pellets for the high doses. Pellets were inserted subcutaneously (over the withers) at 0800 h. Blood samples were drawn from indwelling jugular catheters (inserted the previous day) at 15-min intervals from 1400 through 1445 h on days 1 through 6 after implant insertion. Blood samples were placed in heparinized tubes, stored on ice during collection and at

⁴Merck and Co., Rahway, NJ. Purified (.8 U/mg) by L. E. Reichert, Jr., Department of Biochemistry, Albany Medical College, Union Univ., Albany, NY.

4°C overnight. The following day plasma was collected by centrifugation and frozen at -20°C until assayed for GH (1).

RESULTS

Mean GH concentrations in plasma for each of the treatment groups (Figure 1) were relatively constant over the 6-day observation. For controls, mean GH in plasma was 2.9 to 3.9 ng/ml, whereas the low and high dose GH infusion groups had 30.3 to 65.9 and 45.0 to 60.3 ng/ml. For the low dose, there appeared to be a large pulse release of GH to 65.9 ng/ml on day 1, but mean concentrations decreased to 30.8 ng/ml on day 2 and remained in that range for the remainder of the week. A pulse release of GH was not observed for the high dose. Plasma GH averaged 45.0 ng/ml on day 1 and gradually increasing to 60.3 ng/ml on day 6.

From Figure 1 GH concentrations in plasma remained remarkably constant within treated lambs over the entire 6 days, particularly in the lambs receiving the low GH dose. Although not tested statistically, mean GH concentrations appeared to increase gradually from days 1 to 6 in lambs receiving the high GH dose.

DISCUSSION

These results demonstrate that the beeswax implants provided a relatively continuous release of GH that was proportional to the amount implanted. That GH concentrations still were elevated 6 days after implant insertion suggests that release of GH from these implants was more nearly constant than anticipated.

Plasma GH concentrations still were being maintained at the desired concentrations 6 days after implant insertion. In retrospect, it would have been advisable to continue blood sampling for an additional week to determine when GH concentrations returned to control.

In summary, a GH implant in beeswax and

oil has been prepared that delivered a reasonably constant rate of release and elevated immunoreactive GH concentrations in plasma to a predictable concentration over 6 days. This may be a reasonable approach to continuous delivery of purified or synthetic GH for growth and lactation studies in vivo although our assumption of biological activity of the circulating GH released from such implants should be confirmed prior to growth or lactation studies.

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