PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 462–469 (1975)

Milking, Thyrotropin-Releasing Hormone and Prostaglandin Induced Release of Prolactin and Growth Hormone in Cows[1,2] (38828)

H. ALLEN TUCKER, D. T. VINES, J. N. STELLFLUG, AND E. M. CONVEY

Animal Reproduction Laboratory, Department of Dairy Science, Michigan State University, East Lansing, Michigan 48824

Convey *et al.* reported a single injection of 100 μg thyrotropin-releasing hormone (TRH) into cows markedly elevated serum prolactin (PRL) and growth hormone (GH) concentrations. In a preliminary experiment using four lactating cows we established that constant infusion of 100 μg TRH/hr for 2.5 hr maintained serum PRL concentrations approximately 300% greater than basal concentrations. Furthermore, single injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) also increased serum PRL and GH (2). In addition, serum PRL concentrations, but not GH, increased in response to stimuli associated with milking (3). The primary objective of the following experiments was to determine whether combinations of these stimuli produce additive increases in serum concentrations of PRL and GH.

TRH has potential application for increasing milk yield in cows (4) and body growth in calves (5). Practical usefulness may depend on developing systems whereby increased concentrations of endogenous hormones are maintained for a relatively long time. Therefore, we were also interested in determining the pattern of serum PRL and GH concentrations in response to constant infusion of TRH and $PGF_{2\alpha}$ or to intermittent injections of TRH.

Materials and Methods. Holstein cows or heifers were fitted with polyvinyl cannulae in both jugular veins about 20 hr before the start of each experiment. One cannula was used for infusion and the other for blood collection. Sera from each experiment were stored at −20°C until assayed for PRL (3, 6) and GH (7).

Experiment I. Four cows lactating 4 mo were used. On the first day two cows received a single iv injection of 200 μg TRH/10 ml 0.85% NaCl at 1000 hr followed by constant iv infusion of 333 μg TRH/30 ml 0.85% NaCl/hr until 1600 hr. Thus, each cow received a total dose of 2.2 mg TRH. Two other cows received 10 ml 0.85% NaCl injection followed by infusion of 30 ml of 0.85% NaCl/hr between 1000 and 1600 hr. On the second day treatments were reversed between the two pairs of cows. All cows were milked between 1500 and 1520 hr, with blood samples collected at 30-min intervals between 0830 and 1600 hr, and at 2-min intervals for 12 min beginning at the start of milking; the milking stimulus was applied for 6 min. Blood samples were also collected at various intervals (Fig. 1) between 1600 and 1700 hr.

Experiment II. Blood samples were collected from four diestrous heifers at 30-min intervals between 0830 and 2200 hr. At 1000 hr 200 μg TRH/10 ml 0.85% NaCl were injected iv followed by constant iv infusion of TRH at the rate of 333 μg/30 ml 0.85% NaCl/hr until 2300 hr. The iv injection of 5 mg $PGF_{2\alpha}$ in 5 ml of 0.85% NaCl at 2200 hr was followed by blood sampling, as shown in Fig. 3, until 2300 hr.

Experiment III. Four heifers, 2–4 days postestrus were used. On the first day, two received constant infusion of 30 mg $PGF_{2\alpha}$/30 ml 0.85% NaCl/hr commencing at 1100 hr, with each heifer receiving a single injection of 200 μg TRH in 5 ml 0.85% NaCl at 1600 hr. The other pair received constant infusion of 333 μg TRH/30 ml 0.85% NaCl/hr starting at 1100 hr, and were injected with 5 mg $PGF_{2\alpha}$ in 5 ml 0.85% NaCl at 1600 hr. The $PGF_{2\alpha}$ and TRH infusions were stopped at 1700 hr. On the second day, all heifers received 0.85% NaCl injections (5 ml) and infusions (30 ml/hr) at the same times the $PGF_{2\alpha}$ or TRH had been given on the first day. Day 1 treatments were reversed on the

[1] Journal Article No. 7038 from the Michigan Agricultural Experiment Station.

[2] Supported in part by USPHS Grant AM-15899.

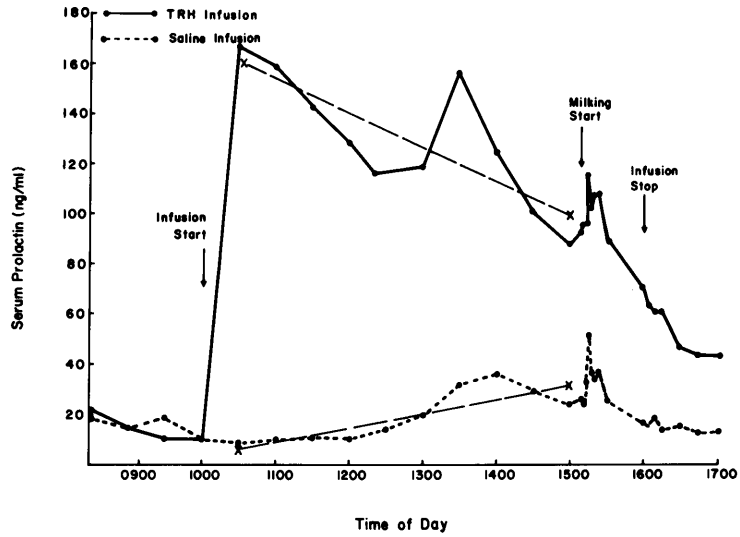

FIG. 1. Serum prolactin response to milking in lactating cows infused iv with 333 μg TRH per hr or infused with 0.85% NaCl. SE between 0830 and 1000 hr was 1.3 ng/ml; SE of TRH- and saline-infused cows between 1030 and 1500 hr were 7.9 and 2.3 ng/ml, respectively.

two pairs of heifers on the third day. Blood samples were collected as shown in Fig. 5.

Experiment IV. Two lactating cows received 10 injections (1 ml iv) of 200 μg each of TRH at 15-min intervals, while two other lactating cows received 10 doses, also 1 ml, of 0.85% NaCl. The treatments were reversed on the second day. The cows were those used in the first experiment. An interval of 42 hr was allowed between the end of TRH infusion in Experiment I and the beginning of TRH injections in Experiment IV. Blood samples were collected at 0830, 0900, 0930, 0945, 0950, 0955, and 1000 hr. TRH or saline injections began immediately after collection of the 1000-hr sample. Sampling was continued at 5-min intervals from 1000 to 1245 hr.

Results. Experiment I. Serum PRL averaged 14.9 and 16.1 ng/ml during the 90-min interval before infusion of TRH or saline, respectively (Fig. 1). The previous day's infusion did not significantly affect ($P > 0.05$) preinfusion PRL concentrations on the second day; serum PRL averaging 14.6 and 16.4 ng/ml on days 1 and 2, respectively. Serum PRL increased to a maximum of 167 ng/ml within 30 min of starting TRH infusion. From this peak serum PRL declined linearly ($P < 0.01$; $Y = 302.5–13.5X$) between 1030 and 1500 hr. However, after 5 hr of TRH infusion PRL was still elevated more than 300% in cows infused with TRH as compared with saline controls. A single cow caused the increased serum PRL at 1330 hr. PRL in saline-infused cows increased linearly ($P < 0.01$; $Y = -53.0 + 5.7X$) between 1030 and 1500 hr.

Milking induced release ($P < 0.01$) of serum PRL in cows concurrently infused with TRH or saline (Fig. 1). Specifically, premilking serum samples for TRH- and saline-infused cows averaged 93 and 23 ng/ml, respectively; milking-induced peak concentrations of serum PRL occurred within 6 min after applying the milking machine to the cow's teats and averaged 116 and 52 ng/ml, respectively.

Average serum GH fluctuated between 2.4 and 6.1 ng/ml in preinfusion samples (Fig. 2). TRH infusion increased serum GH approximately 4-fold ($P < 0.01$) within 30 min. Between 1030 and 1500 hr serum GH decreased linearly ($P < 0.01$; $Y = 18.2 - 0.58X$) despite constant infusion of TRH. However, in saline-infused cows serum GH increased slightly during this interval ($P < 0.01$; $Y = 1.2 + 0.14X$). Despite convergence of these lines, serum GH concentration after 5 hr of TRH infusion was still

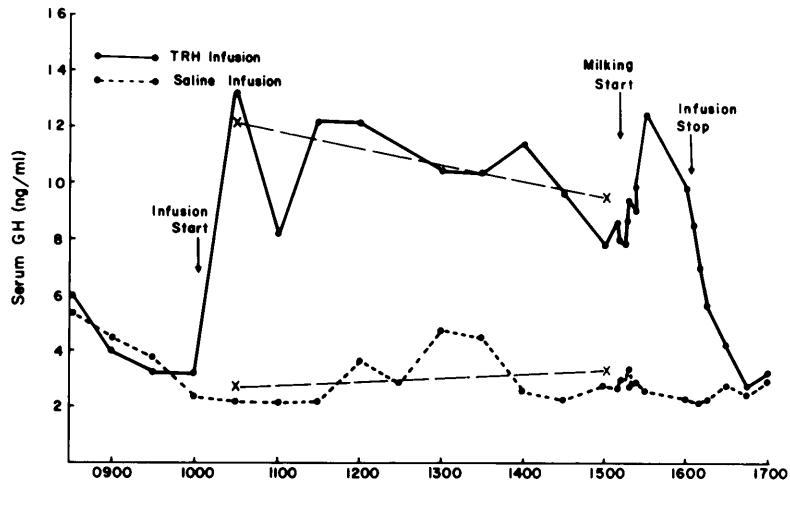

FIG. 2. Serum growth hormone response to milking in lactating cows infused iv with 333 μg TRH per hr or infused with 0.85% NaCl. SE between 0830 and 1000 hr was 0.5 ng/ml; SE of TRH- and saline-infused cows between 1030 and 1500 hr were 1.0 and 0.3 ng/ml, respectively.

300% greater than GH in saline-infused cows.

Serum GH did not change significantly ($P > 0.05$) for 12 min after the milking stimulus was applied to either TRH- or saline-infused cows (Fig. 2). Elevated concentrations of GH at 1530 hr occurred in all TRH-treated cows, but significance of this response is obscure because it did not occur when these same cows were infused with saline. After TRH infusion was stopped at 1600 hr, serum GH concentrations declined to one-half of the 1600-hr value within 12 min.

Experiment II. PRL averaged 22 ng/ml of serum before TRH infusion was initiated, and it increased to 231 ng/ml within 30 min of starting the infusion (Fig. 3). Between 1030 and 2200 hr serum PRL concentration decreased linearly ($P < 0.01$; $Y = 225 - 5.4X$) although it remained at least 500% greater than concentrations observed before TRH infusion commenced. Injection of 5 mg $PGF_{2\alpha}$ at 2200 hr caused an additional increase in PRL to an average maximum of 707 ng/ml at 2240 hr. After this time PRL declined to one-half this value within approximately 19 min despite continued infusion of TRH.

Before infusion of TRH, serum GH concentration averaged 6.5 ng/ml (Fig. 4). During the first 30 min of TRH infusion serum GH concentration rose to 16.8 ng/ml, but it subsequently declined linearly ($P < 0.01$; $Y = 15.4 - 0.38X$) between 1030 and 2200 hr. At 2200 hr and before $PGF_{2\alpha}$ was injected, serum GH averaged 10.1 ng/ml, but GH concentration increased ($P < 0.01$) after $PGF_{2\alpha}$ injection to a maximum of 19.4 ng/ml at 2208 hr (Fig. 4). Serum GH concentrations returned to pre-$PGF_{2\alpha}$ concentrations within 22 min after reaching peak values.

Experiment III. Serum PRL averaged 5.4 ng/ml before infusion of either TRH or $PGF_{2\alpha}$ (Fig. 5). During saline infusion on the second day of the experiment (not shown in Fig. 5), serum PRL fluctuated between 1.8 and 9.6 ng/ml, with an over-all average of 5.4 ng/ml. Maximum concentrations of PRL during initial 60 min of TRH or $PGF_{2\alpha}$ infusions averaged 92 and 384 ng/ml, respectively. As in experiment II, serum PRL declined as infusions continued, yet after 5-hr infusion concentrations were still 5- to 10-fold greater than preinfusion or saline-infusion values. Injection of 5 mg $PGF_{2\alpha}$ during TRH infusion increased PRL from 26 ng/ml at 1600 hr to 708 ng/ml at 1615 hr. Similarly, injection of 200 μg TRH during infusion of

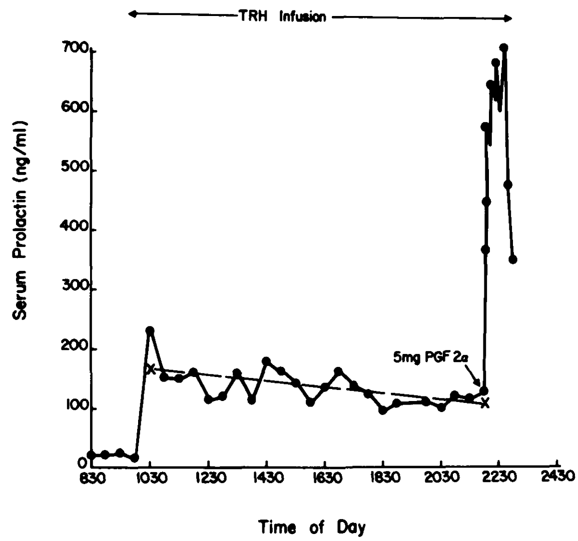

FIG. 3. Serum prolactin response to 5 mg iv $PGF_{2\alpha}$ in heifers infused iv with 333 μg TRH per hr. SE between 0830 and 1000 hr was 1.4 ng/ml; SE between 1030 and 2200 hr was 7.8 ng/ml.

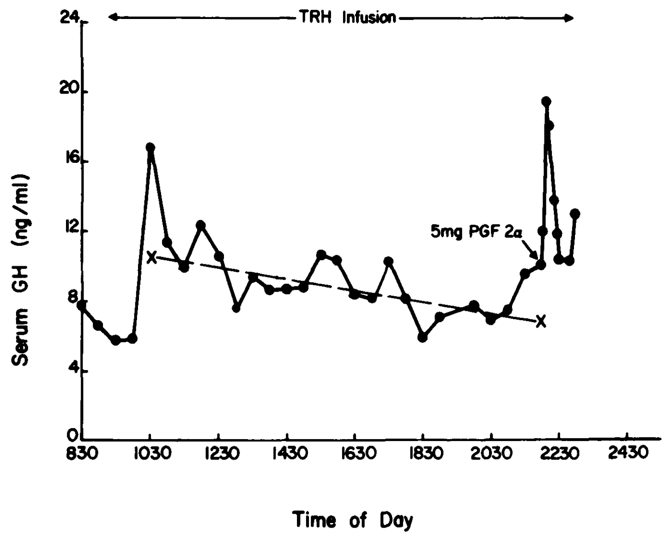

FIG. 4. Serum growth hormone response to 5 mg iv $PGF_{2\alpha}$ in heifers infused iv with 333 μg TRH per hr. SE between 0830 and 1000 hr was 0.6 ng/ml; SE between 1030 and 2200 hr was 0.4 ng/ml.

$PGF_{2\alpha}$ increased serum PRL from 51 ng/ml at 1600 hr to 317 ng/ml within 5 min.

Preceding the start of TRH or $PGF_{2\alpha}$ infusions or during infusion of saline on the second day of the experiment, serum GH averaged 8.8–10.5 ng/ml (Fig. 6). Infusions of TRH or $PGF_{2\alpha}$ alone increased GH to maximums of 30 and 45 ng/ml at 1120 and 1140 hr, respectively. However, serum GH returned to preinfusion baseline values within the first 60 min of TRH infusion and within the first 3 hr of $PGF_{2\alpha}$ infusion. Injection of $PGF_{2\alpha}$ during TRH infusion increased serum GH from 11 ng/ml at 1600 hr to 102 ng/ml at 1615 hr. Likewise, injection of 200 μg TRH at 1600 hr during $PGF_{2\alpha}$ infusion increased GH from 11 to 78 ng/ml at 1610 hr.

Experiment IV. Ten injections of 200 μg TRH at 15-min intervals increased serum PRL to an average of 81.4 ng/ml which

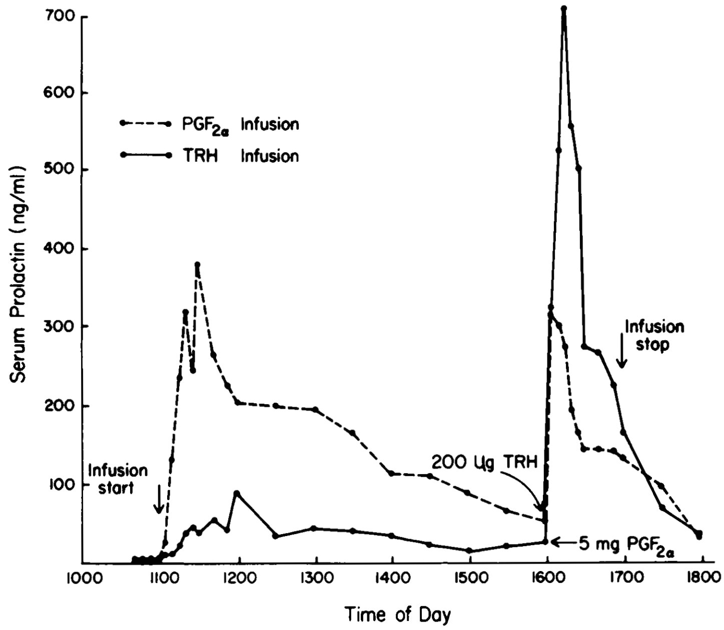

FIG. 5. Serum prolactin response to infusion of 333 μg/hr TRH followed by injection of 5 mg $PGF_{2\alpha}$; or infusion of 30 mg/hr $PGF_{2\alpha}$ followed by injection of 200 μg TRH. SE between peak prolactin response to TRH or $PGF_{2\alpha}$ infusions and 1600 hr averaged 6.6 and 17.4 ng/ml, respectively. Heifers infused and injected with saline averaged 5.4 ± 0.4 ng/ml (not shown).

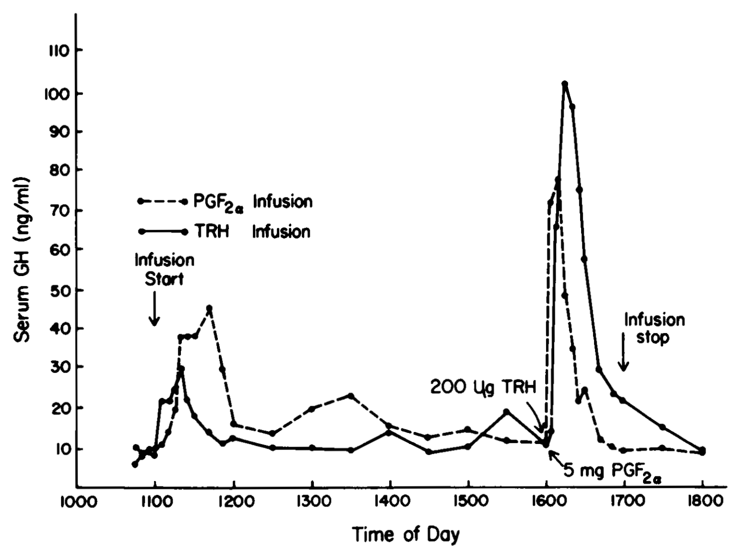

FIG. 6. Serum growth hormone response to infusion of 333 μg/hr TRH followed by injection of 5 mg $PGF_{2\alpha}$; or infusion of 30 mg/hr $PGF_{2\alpha}$ followed by injection of 200 μg TRH. SE between peak GH response to TRH or $PGF_{2\alpha}$ infusions and 1600 hr were 1.2 and 1.9 ng/ml, respectively. Heifers infused and injected with saline averaged 10.5 ± 1.2 ng/ml (not shown).

represented a 9.3-fold increase ($P < 0.01$) over saline-injected controls (Fig. 7). Between 1030 and 1230 hr serum GH averaged 3.4 and 10.1 ng/ml when saline or TRH were injected, respectively ($P < 0.01$; Fig. 8). During the TRH-injection period serum PRL increased only slightly, but it was a statistically linear increase ($P < 0.01$; $Y =$

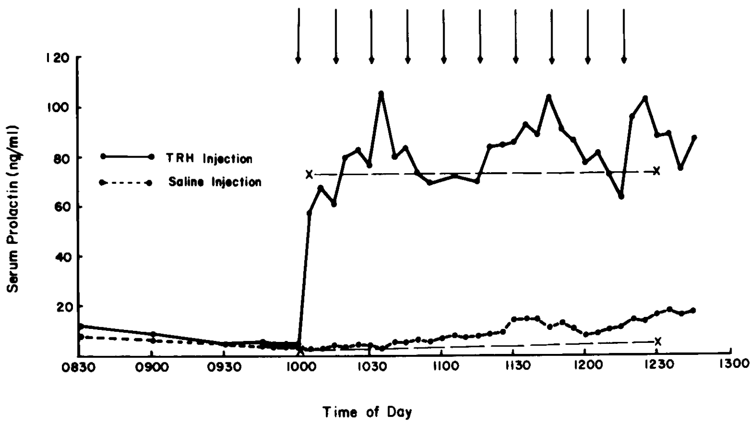

FIG. 7. Serum prolactin response in lactating cows to intermittent (arrows) iv injections of 200 μg TRH or 0.85% NaCl. SE between 0830 and 1000 hr was 0.4 ng/ml; SE of TRH- and saline-infused cows between 1030 and 1230 hr were 4.3 and 0.7 ng/ml, respectively.

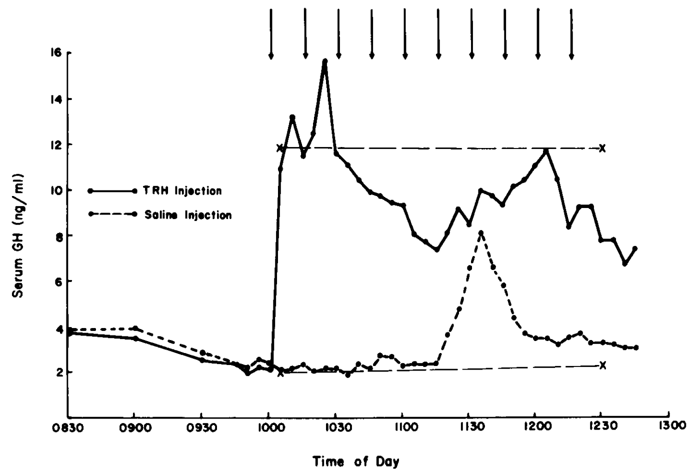

FIG. 8. Serum growth hormone response in lactating cows to intermittent (arrows) iv injections of 200 μg TRH or 0.85% NaCl. SE between 0830 and 1000 hr was 0.2 ng/ml; SE of TRH- and saline-infused cows between 1030 and 1230 hr were 0.6 and 0.3 ng/ml, respectively.

72.1 + 0.11X). Although serum GH appeared to decrease during the injection period (Fig. 8), the slope of the line was not different from zero ($P > 0.05$; $Y = 11.7 - 0.02X$). At 25 min after the last TRH injection, serum PRL and GH concentrations were 80% and 147% greater than respective control values.

The marked increase in serum GH in saline-treated cows, which occurred between 1115 and 1200 hr (Fig. 8), was attributable to two of the four cows with each occurrence on a different day. Thus, significance of this response was obscure.

Discussion. Chronic administration of PRL to rats reduced endogenous pituitary (8, 9) and serum (10) concentrations of PRL. These data were consistent with existence of a mechanism whereby serum PRL concentration may regulate its own secretion and/or release rate. However, in cows we (11) failed to prevent milking-induced release of PRL with constant infusion of NIH-B_3 PRL. In the present study increasing serum concentrations of endogenous PRL 5- to 10-fold above basal concentrations for up to 13 hr did not prevent increases in PRL concentration when new stimuli such as milking (Fig. 1), $PGF_{2\alpha}$ (Fig. 3), or TRH (Fig. 5) were used. Either the auto-feedback mechanism for regulation of PRL secretion does not exist in cattle, or elevated concentrations of PRL in the sera must be maintained longer than 13 hr.

Others have reported that exogenous ovine or human GH (12, 13) or GH secreted from pituitary tumors (14) reduced pituitary content of GH in rats. However, analogous to our findings with PRL, maintaining an elevated concentration of serum GH in cattle with TRH infusion (Fig. 4) did not prevent additional release of GH when $PGF_{2\alpha}$ was injected. Although we were unable to maintain serum GH concentrations substantially above preinfusion basal values with either TRH or $PGF_{2\alpha}$ infusions, these cattle released additional quantities of GH when the TRH or $PGF_{2\alpha}$ infusions were supplemented with an injection of $PGF_{2\alpha}$ or TRH, respectively (Fig. 6).

The increases in serum PRL during saline infusion (Fig. 1) probably were associated with circadian periodicity as previously reported (15). The small, though statistically significant, increases in serum GH in saline-infused cows in the first and second experiments were not in agreement with our previous observation (15) of no circadian pattern of release for GH.

These experiments clearly confirm our work that TRH (1) and $PGF_{2\alpha}$ in cows (2) markedly increased serum PRL and, to a lesser extent, GH. Constant infusion of either TRH or $PGF_{2\alpha}$, however, did not prevent eventual declines in PRL and GH (Figs. 1–6). Whether this represented gradual development of a refractoriness of the cattle to TRH or $PGF_{2\alpha}$, or depletion of pituitary stores of PRL and GH cannot be definitely answered. Circumstantial evidence against the latter concept is that additional quantities of PRL and GH can be discharged in response to an additional different stimulus (Fig. 1, 3–6).

$PGF_{2\alpha}$ administration consistently resulted in greater concentrations of serum PRL and GH when compared with TRH (Figs. 5, 6). The additional responses to combinations of TRH and $PGF_{2\alpha}$ (Figs. 5, 6) suggested that the drugs stimulated PRL and GH secretion via different mechanisms or sites of action. Furthermore, additional increases in serum PRL concentration in response to milking plus TRH infusion (Fig. 1) may suggest that stimuli associated with milking also act through mechanisms different from TRH- or $PGF_{2\alpha}$-induced releases of PRL. Thus, whether TRH or $PGF_{2\alpha}$ play a role in normal control of PRL or GH secretion in cattle must await further studies.

Concentrations of PRL and GH after administration of 2 mg of TRH in 10 discrete injections within 2.5 hr were not greater than those observed with 2.2 mg infused over 6 hr. Furthermore, continuous infusion of TRH did not cause additional net increases in serum PRL or GH concentrations after the first 20–60 min. These data suggested that a ceiling exists for release of PRL and GH in response to TRH. This concept was further supported by our (16) findings that PRL and GH increased with graded doses of TRH, but eventually the hormones attained plateaus after which additional doses of TRH did not further increase PRL or GH. In the present study this ceiling could be overcome with application of a second heterologous stimulus.

Summary. Within 30 min of starting continuous iv infusion of 333 μg thyrotropin-releasing hormone (TRH)/hr into 12 cows, serum prolactin (PRL) increased more than 10-fold and growth hormone (GH) increased 2.6- to 4-fold above basal concentrations. Constant infusions of 30 mg/hr of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) increased serum PRL and GH to maxima within 30–40 min which were 64- and 5-fold greater than basal concentrations. Despite continuous infusion of TRH or $PGF_{2\alpha}$, PRL declined throughout the 6- to 13-hr infusion interval although it remained well above preinfusion or saline-infusion control values. Serum GH declined more rapidly than PRL in the face of TRH or $PGF_{2\alpha}$ infusions, reaching basal concentrations in one experiment within 1–3 hr. Application of milking stimuli during the 5th hr of TRH infusion caused an additional increase of 23 ng/ml of PRL above the TRH-stimulated concentrations. Similarly, intravenous injection of 5 mg $PGF_{2\alpha}$ during the 5th or 12th hr of TRH infusion increased serum PRL an additional 582–682 ng/ml and further increased serum GH 9–91 ng/ml. When 200 μg TRH was injected during the 5th hr of a $PGF_{2\alpha}$ infusion, serum PRL in-

creased another 267 ng/ml and GH increased an additional 61 ng/ml. Administration of 10 doses of TRH of 200 μg each in 2 hr did not increase PRL or GH in the serum above that observed when 2.2 mg TRH was infused over a 6-hr period. Collectively, the data suggest that a ceiling exists in cows for secretion of PRL and GH, but this ceiling may be overcome with application of a second heterologous stimulus.

1. Convey, E. M., Tucker, H. A., Smith, V. G., and Zolman, J., Endocrinology **92,** 471 (1973).
2. Louis, T. M., Stellflug, J. N., Tucker, H. A., and Hafs, H. D., Proc. Soc. Exp. Biol. Med. **147,** (1974).
3. Tucker, H. A., J. Anim. Sci. **32** (Suppl. 1), 137 (1971).
4. Convey, E. M., Thomas, J. W., Tucker, H. A., and Gill, J. L., J. Dairy Sci. **56,** 484 (1973).
5. McGuffy, R. K., Thomas, J. W., and Convey, E. M. J. Dairy Sci. **57,** 607 (1974).
6. Koprowski, J. A., and Tucker, H. A., J. Dairy Sci. **54,** 1675 (1971).
7. Purchas, R. W., Macmillan, K. L., and Hafs, H. D., J. Anim. Sci. **31,** 358 (1970).
8. Sinha, Y. N., and Tucker, H. A. Proc. Soc. Exp. Biol. Med. **128,** 84 (1968).
9. Clemens, J. A., and Meites, J., Endocrinology **82,** 878 (1968).
10. Niswender, G. D., Chen, C. L., Midgley, A. R., Meites, J., and Ellis, S., Proc. Soc. Exp. Biol. Med. **130,** 793 (1969).
11. Tucker, H. A., Convey, E. M., and Koprowski, J. A., Proc. Soc. Exp. Biol. Med. **142,** 72 (1973).
12. Krulich, L., and McCann, S. M., Proc. Soc. Exp. Biol. Med. **121,** 1114 (1966).
13. Voogt, J. L., Clemens, J. A., Negro-Villar, A., Welsch, C., and Meites, J., Endocrinology **88,** 1363 (1971).
14. MacLeod, R. M., and Abad, A., Endocrinology **83,** 799 (1968).
15. Koprowski, J. A., Tucker, H. A., and Convey, E. M., Proc. Soc. Exp. Biol. Med. **140,** 1012 (1972).
16. Vines, D. T., Tucker, H. A., and Convey, E. M., J. Dairy Sci. **56,** 652 (1973).

Received Nov. 8, 1974. P.S.E.B.M., 1975, Vol. 149.