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Method for Determining Oxytocin Concentrations in Unextracted Sera; Characterization in Lactating Cattle (40394)

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Patterns of oxytocin release into blood of dairy cattle in response to conditioning, udder stimulation, and milking, have been described by several workers (1–4). Data from these studies were based on bioassays which were sufficiently sensitive for measuring elevated physiological levels, but were not capable of accurately detecting resting hormone concentrations. The bioassays used were also sensitive to day to day assay variation and to nonspecific agents, some of which stimulate milk ejection.

A radioimmunoassay (RIA) for oxytocin would overcome many problems associated with bioassays, since it is theoretically more sensitive, specific and reproducible. Several reports describing oxytocin radioimmunoassays have appeared in the literature (5–8). These assay techniques, however, involve tedious extraction methods for plasma or sera and have not been applied to studies of physiological responses in lactating animals. Thus, the objective of the present work was to develop a sensitive, specific and reproducible RIA for measuring oxytocin in unextracted sera of lactating cattle during milking and under resting conditions.

Materials and methods. Oxytocin antibody and standard. Oxytocin antiserum was obtained from Calbiochem-Behring Corp. (La Jolla, CA). Lyophilized aliquots of antiserum were diluted 1:200 using normal rabbit serum (1:400) prior to use in assays. The final dilution of antiserum per assay tube was 1:1000.

Synthetic oxytocin (Calbiochem-Behring Corp.) was used as standard in all assays.[1] Standard was tested for biological activity according to the procedures outlined by the United States Pharmacopoeia (9). Standard contained 29.2 ± 0.42 IU/mg oxytocin.[2] Oxytocin was dissolved in double distilled water, pH 8, and stored at −20° to achieve a 1000 μg/ml stock solution for iodination and standard. Dilutions of oxytocin solution were established in assay diluent and used as standard in assays. Assay diluent consisted of 1% bovine serum albumin (BSA) in 0.05 *M* phosphate buffer, pH 7.5, containing 372 μg of ethylene dinitrilotetraacetic acid, 48 μg of cystine, 100 μg merthiolate and 5 μg 1,10-phenanthroline (Sigma Chemical Co.) per ml.

Hormone labeling and purification. Oxytocin was iodinated using ^{125}I as described by Kumaresan *et al.* (10). Iodination proceeded for precisely 20 sec. Reduction was omitted, since oxytocin is sensitive to reduction by sodium metabisulfite (11). After incubation, the reaction mixture was transferred with 100 μl of 10% BSA to a Sephadex G-25 (medium bead) column (1.5 cm × 20 cm) preequilibrated with 1% BSA. The mixture was eluted with 0.25 *M* phosphate buffer, pH 7.5. One ml fractions were collected in 12 mm × 75 mm culture tubes. Radioactive oxytocin eluted with free ^{125}I. Free ^{125}I was removed from column fractions by adding 100 mg of Amberlite CG-50 (ion exchange resin) to tubes containing ^{125}I. Tubes were vortexed for 1 min and centrifuged at 4° for 3 min at 1000*g*. Supernatants were transferred to clean prelabelled 12 mm × 75 mm culture tubes, diluted to yield 10,000 cpm in 100 μl diluent

[1] Amino acid analysis revealed that oxytocin was at least 90% pure with regard to amino acid composition. Standard contained no contaminants (R_f = .60) as shown by thin layer chromatography (20 μg) on silica gel using *n*-butanol:pyridine:acetic acid:water (15:10:3:6v/v). Chromatograms were developed using chlorine and tolidine-reagent. Standard contained mannitol as an excipient.

[2] Biological activity was tested using the chicken depressor assay method against United States Pharmacopoeia (USP) posterior pituitary reference standard as described by the United States Pharmacopoeia. Oxytocin activity is expressed as the mean of 5 animals tested ± SE.

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and tested for immunoreactivity. Fractions displaying the greatest percentage of antibody-bound radioactivity were used for assays.

Assay methods. Standard tubes contained from 0.03 to 1.0 ng oxytocin and were assayed with each lot of unknowns. Serum aliquots of 250 μl were assayed in duplicate. On day one of each assay, assay diluent, standards, unknowns, and rabbit antioxytocin (200 μl), were added to achieve a total volume of 700 μl. Tubes were incubated at 4° for 24 hr. On day 2, ^{125}I-oxytocin (100 μl) was added to tubes and they were incubated overnight at 4°. On day 3, 200 μl of sheep anti-rabbit gamma globulin was added to each tube and incubation continued at 4° for 48 hr. On day 6, 3 ml cold phosphate buffered saline, pH 7.1, was added to each tube except for total count tubes. Tubes were centrifuged at 1000*g* for 30 min. Supernatants were decanted and tubes were inverted to dry for at least 30 min. Radioactivity in precipitates was quantified in a gamma scintillation spectrometer.

Cross-reactivity and recovery studies. Specificity of oxytocin antiserum was assessed by determining the ability of .03 to 150 ng of the following compounds to suppress binding of ^{125}I-oxytocin to antibody: arginine–vasopressin (385 IU/mg, Calbiochem-Behring Corp.); arginine–vasotocin (225 IU/mg, Calbiochem-Behring Corp.); lysine–vasopressin (100 IU/ml, Sandoz Co.); bradykinin (Sigma Co.); gonadotropic releasing hormone (GNRH, Abbott Co.); prostaglandin $F_{2\alpha}$ (Tham Salt, Upjohn Co.); histamine (Sigma Co.); acetylcholine hydrochloride (Sigma Co.); melatonin and serotonin (Aldrich Co.). Recovery of oxytocin from unextracted sera was also determined in these experiments by assaying 200, 250 and 300 μl of pooled serum with and without 0.2 ng of standard oxytocin.

Oxytocin free serum was prepared in order to determine if the RIA was effectively measuring basal concentrations of hormone and to further test assay parallelism and recovery of added oxytocin to unextracted sera. For this purpose, four pools of sera each made up from eight lactating cows, obtained under resting conditions, were used. Each of the four serum pools were then divided into two aliquots. One aliquot was not treated further. The other aliquot was treated with 200 mg of Norit-A charcoal (Fisher Scientific Co.) and mixed at 4° for 4 hr to produce oxytocin free serum. After charcoal treatment, samples were centrifuged at 1000*g* for 30 min. Supernatants were decanted and filtered through a .20 μm disposable plastic filter (Nalge Co.). This method was chosen because it provided the most effective means of removing oxytocin from sera when compared to other treatments tested. These included serum dialysis or exposure of sera to either thioglycolate, nonionic polymeric adsorbents or ion exchange resins. Oxytocin free serum pools and nontreated serum pools were assayed at 200, 250, 300, 350 and 400 μl with and without .2 and .4 ng of standard oxytocin.

Animal experiments: oxytocin in response to milking. Six lactating Holstein-Friesian heifers, entering their first lactation, were fitted with indwelling polyethylene jugular catheters (15 cm in length, .044 mm × .065 mm). On the day following cannulation, blood samples were taken before, during and after milking. Milking lasted for 5 min. Premilking stimulation consisted of an udder wash with warm water lasting 20 sec, followed by hand milking on each teat for an additional 10 sec interval. After premilking stimulation, the milking machine was immediately applied and the 0 time sample was taken. Serum oxytocin was analyzed by RIA and milk yield was recorded at each milking.

Relationship between oxytocin and intramammary pressure. Two additional lactating Holstein-Friesian cows entering their second lactation were fitted with indwelling jugular catheters as previously described. In this experiment however, catheters were precoated to achieve thromboresistance with 7% tridodecylmethylammonium chloride-heparin (TDMAC, Poly Sciences, Inc., Warrington, PA) before use. Catheters were connected to a peristaltic pump and fraction collector. Blood was withdrawn from the jugular vein at a rate of .067 ml/sec. The heparin-TDMAC complex did not prevent blood clotting and subsequent serum formation.

A polyethylene catheter (.044 mm × .065 mm) was inserted into the left forequarter teat and intramammary pressure read using a Grass polygraph (3). Blood sampling was timed and adjusted to recorded pressure changes for graphical representation of data.

Blood samples and intramammary pressure readings were taken before, at, and after

milking of the three noncatheterized quarters. The intramammary catheter was then removed, the udder was rewashed and the left forequarter was milked alone. Blood sampling continued during the second udder wash and milking. Premilking stimulation was as described previously.

Routine procedures. All blood samples were placed immediately in ice after collection and refrigerated for 24 hr at 4° to prevent possible enzymatic destruction of oxytocin. Sera were then recovered at 4° by centrifugation at 1000*g* and frozen at −20° until assay.

Statistical methods. RIA sensitivity, as defined by Midgley *et al.* (12), was determined by a "t" test (6, 7). The statistical significance of premilking and milking stimuli on serum oxytocin concentrations was tested using an analysis of variance (13).

Results. Hormone labeling and purification. Figure 1 illustrates the elution profile of ^{125}I from Sephadex G-25 and percent binding of fractions at 1:1000 dilution of antibody. The first peak of radioactivity (fractions 5 and 6) was associated with the void volume. Fractions associated with this peak did not bind antibody. The second peak of radioactivity, fractions (12–27), contained immunoreactive ^{125}I-oxytocin since fractions eluting in this region bound from 18 to 43% antiserum.

Cross-reactivity and recovery studies. Arginine–vasopressin did not cross-react with oxytocin antiserum up to 150 ng (Fig. 2). Moreover, histamine, bradykinin, acetylcholine hydrochloride, melatonin, serotonin, GNRH and $PGF_{2\alpha}$ did not cross-react. Oxytocin antiserum cross-reacted with lysine–vasopressin inhibiting binding by 3% at 40–80 ng and was maximal, reducing binding by 19% at 150 ng (Fig. 2). Arginine–vasotocin cross-reacted with oxytocin antiserum at a maximum of 13% at 150 ng (Fig. 2). The RIA measured 101.4 ± 0.42% (mean ± SD), 101.0 ± .14% and 101.2 ± .7% of 0.2 ng of standard oxytocin which was added to 200, 250 and 300 μl of pooled serum, respectively. Increasing volumes of pooled serum produced inhibition curves that were parallel to those produced by standard hormone preparation diluted in buffer (Fig. 2).

An additional serum pool, made up from eight lactating cows, had an average oxytocin concentration of 5.6 ± .12 μU/ml (mean ± SE) prior to charcoal treatment and production of oxytocin free serum. After charcoal treatment, volumes of 200, 250, 300, 350 and 400 μl of pool showed no detectable levels of oxytocin. The amount of ^{125}I-oxytocin bound to antibody in oxytocin free serum pools was not significantly different from diluent blanks (Table I). Volumes of pooled serum before charcoal treatment produced inhibition curves that were parallel to those produced by standard hormone preparation diluted in buffer (Fig. 3). Furthermore, volumes (200, 250 and 300 μl) of pooled serum before char-

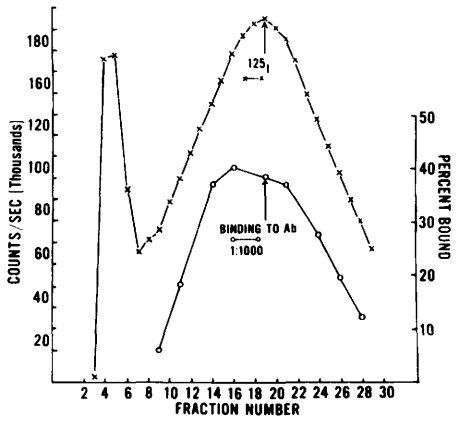

FIG. 1. Elution profile of ^{125}I from Sephadex G-25 and percent binding of label from selected fractions to oxytocin antiserum. Specific activity of oxytocin was 100 μCi/μg.

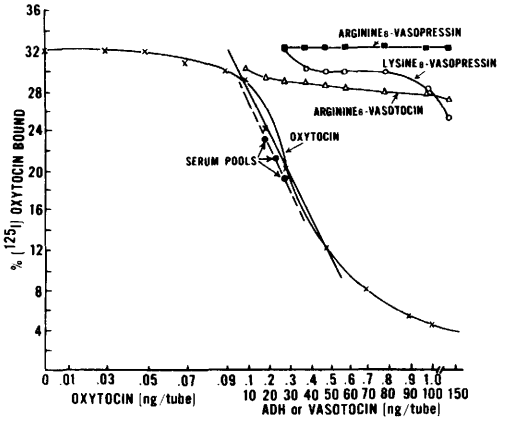

FIG. 2. Cross-reactivity studies. Curves show inhibition of binding of ^{125}I-oxytocin to a 1:1000 final dilution of antioxytocin by oxytocin, arginine-8-vasopressin, lysine-8-vasopressin and arginine-8-vasotocin. Serial dilutions of serum pools (200, 250 and 300 μl) are shown and were parallel to standard oxytocin.

TABLE I. PERCENT ^{125}I-OXYTOCIN BOUND AND PERCENT OF ADDED OXYTOCIN MEASURED FOR VARIOUS VOLUMES OF OXYTOCIN FREE POOLED SERUM.

Volume of pool (μl)	% ^{125}I-Oxytocin bound[a] (Mean ± SD)	% Added oxytocin measured (Mean ± SD)	
		.2 ng	.4 ng
200	28.0 ± .65[b]	100 ± 7.1[b]	95 ± 1.8[b]
250	28.0 ± 1.0	98 ± 5.0	95 ± 2.9
300	26.0 ± 1.5	101 ± 3.5	94 ± 2.9
350	28.1 ± 1.2	99 ± 4.0	96 ± 2.0
400	27.9 ± 1.3	101 ± 3.0	95 ± 2.8

[a] Binding of ^{125}I-oxytocin to diluent blanks (radioactivity bound to antibody) averaged 29% (Fig. 3).

[b] Means in these columns are not significantly different from one another.

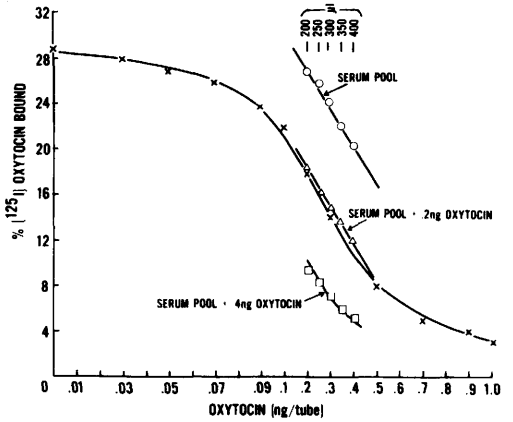

FIG. 3. Standard curve showing inhibition of binding of ^{125}I-oxytocin to a 1:1000 final dilution of antioxytocin. Serial dilutions of serum pools (200, 250, 300, 350 and 400 μl) are shown with and without either .2 or .4 ng of standard oxytocin.

coal treatment, containing either .2 or .4 ng of added oxytocin also produced inhibition curves that were parallel to those produced by standard hormone preparation diluted in buffer (Fig. 3). The RIA measured 98 to 101% of .2 ng standard oxytocin which was added to five volumes of pooled oxytocin free serum. In addition, the RIA measured 94 to 96% of .4 ng standard oxytocin which was added to volumes of the pooled oxytocin free serum (Table I).

Specific assay data. Radioactivity bound to antibody (diluent blanks, corrected for nonspecific binding) averaged 32.4 ± 8.1% (mean ± SE) and ranged from 12 to 42% at antibody dilutions of 1:1000 over 12 assays. Nonspecific bound radioactivity (antibody blanks) averaged 3.02 ± 0.98% and ranged from 1.8 to 4.6% over twelve assays. Nonspecific bound radioactivity, at unlabelled antigen excess (5 ng/tube oxytocin), averaged 2.8 ± 0.03%. Serum nonspecific binding (antibody blank with 250 μl bovine serum pool) averaged 2.8 ± .92% in twelve assays. The sensitivity of the RIA, defined as the minimum amount of oxytocin standard yielding a percent bound significantly different from the diluent blank ($P < 0.05$, "t" test) was 0.07 ng/ml (2.0 μU/ml) over 11 assays. In 12 assays, the within assay coefficient of variation (sd/μ) ranged from .84 to 1.7% and averaged 1.2 ± 0.38% (mean ± SD) for 200, 250 and 300 μl of pooled serum having an oxytocin concentration of 0.9 ng/ml. The between assay coefficient of variation was 6.6% at 200, 250 and 300 μl of pooled serum over twelve assays.

Oxytocin in response to milking. Oxytocin averaged 8.0 ± .11 μU/ml (mean ± SE) prior to milking (Fig. 4). Serum concentrations of oxytocin were significantly ($P < 0.05$) elevated to 18.1 ± 3.8 μU/ml at milking (0 time). Oxytocin concentration reached its peak (25 ± 7.6 μU/ml) 2 min after milking commenced and reached baseline concentrations by 60 min after milking. Variability between animals was seen in the oxytocin response to milking stimuli. Serum oxytocin concentrations seen 2 min after milking for the six animals ranged from 11.2 to 64.5 μU/ml.

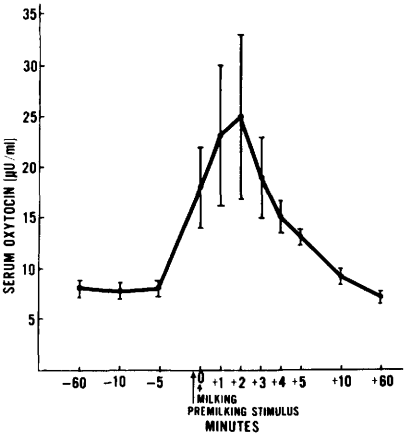

FIG. 4. Oxytocin in response to milking stimuli. Blood samples were taken at −60, −10, and −5 min before milking, at milking (0 time, within 1 min of premilking stimulation and application of the milking machine), during milking at 5 one min intervals and 10 and 60 min after milking. (n = 6, x = mean serum oxytocin ± SE).

Variation was attributed to animal differences with regard to the time of reflex release of oxytocin after udder stimulation and the fact that some animals released more oxytocin than others in response to premilking and milking stimuli. The approximate $T_{1/2}$ for oxytocin, determined by subtracting the average baseline concentrations of oxytocin before milking (8.0 μU/ml) from oxytocin concentrations 2–5 min after milking stimulation (Fig. 4) was 3.6 min.

Relationship Between Oxytocin and Intramammary Pressure. Figure 5 illustrates the relationship between serum concentrations of oxytocin and intramammary pressure before, at and after milking in two cows. Basal serum concentrations of oxytocin ranged from 3.4 ± .14 μU/ml to 5.1 ± .15 μU/ml (mean ± SE) prior to premilking stimulation. Intramammary pressure ranged from 19 ± 0.4 mm Hg to 25 ± .02 mm Hg (mean ± SE) prior to premilking stimulation. Serum concentrations of oxytocin increased at approximately 1 to 1.5 min after udder washing, ranging from 8.0 μU/ml to 12.4 μU/ml for both animals.

Intramammary pressure increased within 1.5 min after the udder wash. Intramammary pressure reached a maximum of 30–40 mm Hg at approximately 1.5 min after cessation of the udder wash. Serum concentrations of oxytocin increased to a maximum of 16.9 μU/ml approximately 5.0 min after premilking stimulation in animal 1562 (Fig. 5). Whereas, serum oxytocin in animal 1715 declined slightly to 7.8 μU/ml approximately 1.5 min after cessation of the wash (Fig. 5). Both cows showed a drop in intramammary pressure from 4.0 to 6.0 min after the udder wash. The fall in pressure paralleled the decline in circulating concentrations of oxytocin. Intramammary pressure reached levels seen prior to milking at 10–15 min after the udder wash. Serum concentrations of oxytocin reached baseline levels by approximately 25 min after cessation of the premilking stimuli. Animal 1562 displayed a pulsatile release of oxytocin at approximately 15 min after premilking stimulation (Fig. 5). Intramammary pressure however, remained stable until the catheter was removed from the udder. No change in serum oxytocin occurred after the second premilking stimulation or during milking (Fig. 5). Animal 1715 did not display a pulsatile release of oxytocin after the initial peak associated with premilking stimulation. Intramammary pressure remained stable until the catheter was removed from the udder. No change in oxytocin was seen after the

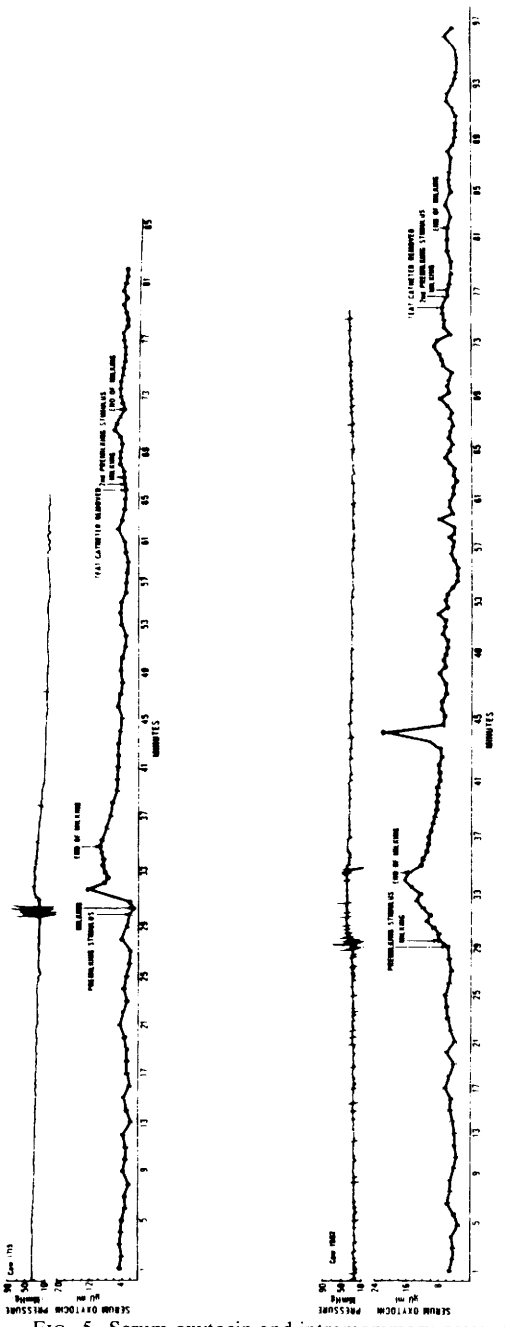

FIG. 5. Serum oxytocin and intramammary pressure for 2 cows before, at and after milking. Blood samples were taken at either 30 sec or 1 min intervals throughout experimentation.

second udder wash and milking.

Discussion. This paper describes an RIA method for measuring oxytocin concentrations from unextracted sera of cattle. The oxytocin RIA described herein is highly specific and sensitive and is capable of measuring basal and elevated physiological concentrations of serum oxytocin in lactating cattle. Antiserum was extremely sensitive and detected as little as 2.0 μU/ml (70 pg/ml) of standard oxytocin in assay buffer. Several reports which described oxytocin antisera for use in RIA's have been published, as reviewed by Chard (14). Very few of the antisera used in these RIA's yield a sensitivity greater than 2 μU/ml plasma.

The RIA methods for oxytocin published by Chard (5, 6), Blank and DeBias (7), and Dawood *et al.* (8) utilized extracts of serum or plasma. Their extraction procedures are tedious and the number of samples assayed per day is limited. Furthermore, extraction efficiency ranged from only 50–60%. Measurement of added oxytocin from serum pools and oxytocin free serum pools in the studies reported herein was virtually 100%. This suggested that serum components other than oxytocin were not significantly binding to the oxytocin antiserum. Assay of unextracted serum contributed less nonspecific assay interference compared with other published oxytocin RIA's and sample values did not need to be corrected for extraction. Binding of ^{125}I-oxytocin to antibody, in the presence of oxytocin free serum obtained from cattle under resting conditions, was not significantly different from binding in blank tubes containing only tracer and antibody. These data suggested that charcoal treatment effectively removed endogenous oxytocin from sera and that the RIA was measuring basal concentrations of hormone in unextracted sera of cattle.

Iodination of oxytocin by methods described in this paper yielded oxytocin labelled at a specific activity of 100 μCi/μg. The specific activity was comparable to the range (20–990 μCi/μg) reported by other workers (7, 14).

Many materials may be present in biological fluids which can give a similar effect to oxytocin in bioassays utilizing smooth muscle or mammary tissue and therefore yield artifactual results. For example, oxytocin bioassays, utilizing mammary tissue, are sensitive to vasopressin, bradykinin, serotonin, histamine, epinephrine, norepinephrine and acetylcholine as well as oxytocin (15). Melatonin (16) and prostaglandin $F_{2\alpha}$ (17) also affect contraction of mammary myoepithelium. Arginine–vasopressin showed no detectable cross-reactivity with oxytocin antiserum up to 150 ng in this RIA, which is well above the physiological concentration (~.5 pg/ml plasma) found in circulating blood of the cow (18) or goat (<20 μU/ml plasma) (19). Histamine, bradykinin, acetylcholine hydrochloride, melatonin, serotonin and $PGF_{2\alpha}$, were without effect up to 150 ng. Although lysine-vasopressin inhibited binding by 19% at 150 ng (Fig. 2), this cross-reaction should present no difficulties when studying oxytocin in animals other than swine (20). Arginine-vasotocin also cross-reacted with oxytocin antiserum by 13% at 150 ng. Its concentration in rat sera has been reported to be only 1.87–2.57 pg/ml (21). Therefore, if vasotocin is present in cattle sera at this concentration range, it should not interfere with the oxytocin RIA. Volumes of unextracted serum pools with and without added oxytocin produced inhibition curves which were parallel to those produced by biologically active standard oxytocin over the entire linear portion of the standard curve (Figs. 2, 3). This suggested that immunoreactive, as well as biologically active oxytocin was being measured in sera.

Gazŕek *et al.* (22) quantified oxytocin concentrations in unextracted plasma of humans with the same standard and antiserum used in our studies with lactating cattle. Plasma oxytocin concentrations were in agreement with RIA estimates for humans reported by workers using other antisera and extraction methods. However, validation of the antiserum was not reported in that study.

The lactating rat mammary strip bioassay technique, as described by Van Dongen and Hays (23), has been employed for measuring serum or plasma oxytocin in cattle. The sensitivity of this assay was reported to be as great as 1.0×10^{-4} μU/ml. More reproducible data were derived at .01–1.0 μU/ml. Fabian *et al.* (24), however, examined the sensitivity of the isolated *in vitro* rat mammary gland assay and found that the optimal sensitivity was 25 μU/ml of oxytocin. The method occasionally responded to 1 μU/ml solutions of oxytocin. The limited sensitivity and lack of

specificity of the *in vitro* mammary strip bioassay has therefore made it difficult to accurately quantify basal serum or plasma oxytocin concentrations in cattle. In the present study, basal concentrations of oxytocin in sera from lactating cattle ranged from 3.5 to 8.0 μU/ml. Similar basal values (1.6–4.7 μU/ml plasma) were reported for nonpregnant goats (7, 25), castrated male goats (<5 μU/ml) (19) and puerperal ewes (1.8 to 3.6 μU/ml) (26) using RIA.

Several reports have described the measurement of oxytocin concentrations in blood of cows and goats during milking (2–4). Determinations were made using bioassay methods. The concentration and pattern of oxytocin release in cattle during milking was examined in this paper. The overall pattern of oxytocin release in these experiments was similar to those reported by Momongan and Schmidt (4), Folley and Knaggs (2), and Lawson and Graf (3) for lactating cows. However, peak concentrations measured in bioassays during the early milking interval ranged from 9 to 2000 μU/ml plasma. Bioassay estimates of oxytocin in excess of 100–200 μU/ml, as a result of milking stimuli, may be due to the lack of reproducibility and nonspecificity of these methods. Bioassay measurements of plasma oxytocin concentrations of cows at milking may be higher than the RIA estimates described in this paper if oxytocic substances, known to interfere with bioassay procedures, are specifically released into blood in response to milking stimuli with oxytocin.

Bioassay methods were unable to detect oxytocin levels for more than 5 min after teat cup application; the RIA method detected concentrations of oxytocin release up to 60 min postmilking. The approximate time required for endogenous serum oxytocin to decrease to one-half its maximal value was 3.6 min. The $T_{1/2}$ for oxytocin is in agreement with the 3–5 min RIA estimate published for humans by Chard (6), but greater than that for cows (.8–1.4 min) published by Momongan and Schmidt (4) using bioassays. Data previously reported for the $T_{1/2}$ of oxytocin at milking, using bioassays, may be shorter than RIA estimates if substances other than oxytocin were affecting the bioassay methods. Many physiological parameters are involved in hormonal metabolism and they must be considered before a precise value for the $T_{1/2}$ of oxytocin after milk ejection can be defined.

The intramammary pressure response to milking was related to changes in jugular vein concentrations of oxytocin (Fig. 5). Intramammary pressure readings before, at and after milking were in agreement with intramammary pressure determinations published by Lawson and Graf (3). Lawson and Graf (3) also reported oxytocin levels higher than baseline values within 1–4 min post milking stimulation. However, peak concentrations of oxytocin at milking measured by bioassay were in excess of 1 mU/ml. The subtle differences in time to peak oxytocin levels in this study and others are probably attributable to differences in sampling techniques. Oxytocin levels were higher than baseline levels after intramammary pressure had reached its premilking value (Fig. 5). Oxytocin concentrations declined within 5–6 min (Fig. 5) after the premilking stimulus as reported by Lawson and Graf (3). Cow 1562 showed a pulse of oxytocin release well after udder stimulation and milking (Fig. 5) with no corresponding increase in intramammary pressure. Lawson and Graf (3) also observed a cow displaying pulsatile spikes of oxytocin occurring after premilking and milking stimulation with no associated intramammary pressure increase. The pulsatile release of oxytocin seen for cow 1562 may be associated with exteroceptive stimuli. A gradual increase of oxytocin concentrations seems necessary to affect an increase in intramammary pressure since the pulsatile release of oxytocin was ineffective in altering mammary pressure. Further experimentation is necessary to define this response. On the basis of this preliminary study, it was difficult to relate a specific concentration of serum oxytocin activity to a specific rise in intramammary pressure during milk ejection. A second udder wash was ineffective in releasing oxytocin (Fig. 5). This suggested that a refractory period occurs before oxytocin can be released at a subsequent milking. There was a great deal of variability associated with peak levels of oxytocin released at milking in all animals studied. Folley and Knaggs (2) reported that no release of oxytocin was detected in cows by bioassays, in 32% of experimental machine milkings, although milk yields were normal. Milk yields for all animals used in this study were monitored. Experimental procedures

employed had no effect on milk yield.

It is hoped that the oxytocin RIA described in this paper will provide answers to many questions related to the role of oxytocin in lactational and reproductive processes.

Summary. A highly sensitive, specific and reproducible double antibody RIA for measuring oxytocin in unextracted serum of cattle was developed. Serum concentrations of oxytocin were significantly ($P < 0.05$) elevated from 8.0 ± .11 μU/ml to 25 ± 7.6 μU/ml (mean ± SE) 2 min after commencement of milking. The range of peak oxytocin concentrations seen 2 min after milking in six animals was 11.2–65 μU/ml. The $T_{1/2}$ for oxytocin, after milk ejection, was 3.6 min. Simultaneous determinations of oxytocin activity in blood sera and intramammary pressure were monitored in two cows before, during and after stimulation of the milk ejection reflex. Oxytocin activity in blood closely paralled changes in intramammary pressure.

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