

The Effect of Stress upon the Determination of Serum Prolactin by Radioimmunoassay¹ (35342)

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(Introduced by D. H. Solomon)

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Stress of many varieties results in alterations in brain catecholamine concentration (1, 2). Furthermore, the adrenergic pathways have been implicated in the release of some anterior pituitary hormones (3-6). It is thus not surprising that pituitary hormone secretion may be affected by stress. It has been known for many years that plasma cortisol and adrenocorticotropin (ACTH) concentrations rise in response to trauma, surgery, fever, and hypoglycemia (7-10). An increased secretion of growth hormone (GH) has also been observed as a result of stress (11, 12). Thyrotropin (TSH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion does not appear to be altered by stress such as surgery; studies in our laboratory in humans undergoing elective surgery failed to reveal any consistent changes (13).

It has been implied that serum prolactin may also be affected by stress in the rat (14), but this suggestion has received little attention. No study of the effect of stress upon serum prolactin levels has appeared. We have described a specific and sensitive radioimmunoassay for bovine prolactin. In studies designed to assess the fluctuations in blood prolactin during various reproductive situations, we were surprised to find concentrations were high and mean concentrations similar under various conditions, at all ages and in both sexes. Further study has revealed

that stress of handling and bleeding produces a great increase in blood prolactin in cattle; changes of such magnitude that any changes due to reproductive alterations are obscured.

Materials and Methods. Fifteen dairy heifers of 4 to 5 years of age were studied in detail; and random single blood samples were obtained from 50 other cattle of both sexes at different ages. Serum prolactin was determined by a highly specific double antibody radioimmunoassay capable of detecting 0.67 μg of NIH-P-B2 (bovine) prolactin/ml of serum. In this assay, bovine and ovine growth hormone, bovine luteinizing hormone, and bovine and ovine thyrotropin, at concentrations in excess of 5000 times the detectable amount of prolactin, produced no response. Dose-response curves of serum from cattle of both sexes and crude bovine pituitary extracts in serial dilution were parallel to the curves obtained with pituitary prolactin standard. There was good agreement between the bioassay (systemic crop sac) and immunoassay potencies of *purified prolactin* preparations. In some studies, LH concentrations were also determined by a previously described double antibody radioimmunoassay technique developed in the laboratory (15). The prolactin assay had a within assay coefficient of variation of 3.0%, and a between assay coefficient of variation of 9.6% for samples determined in the steep portion of the dose-response curve. Details of the prolactin radioimmunoassay are published separately (16).

Two methods of bleeding animals were employed and the resultant prolactin and LH profiles were contrasted. In the first case, the

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animals were restrained via a halter, the head was turned sideways, and blood samples were drawn by puncture of the jugular vein. Daily samples were obtained for 21 to 35 days from nine cycling heifers. In addition, random samples were similarly obtained from

prepubertal cattle of both sexes, from lactating adult females and from three sexually mature males. In the second method, a long indwelling catheter was placed into the jugular vein and left in place 24 hr before the beginning of the experiment. Blood was

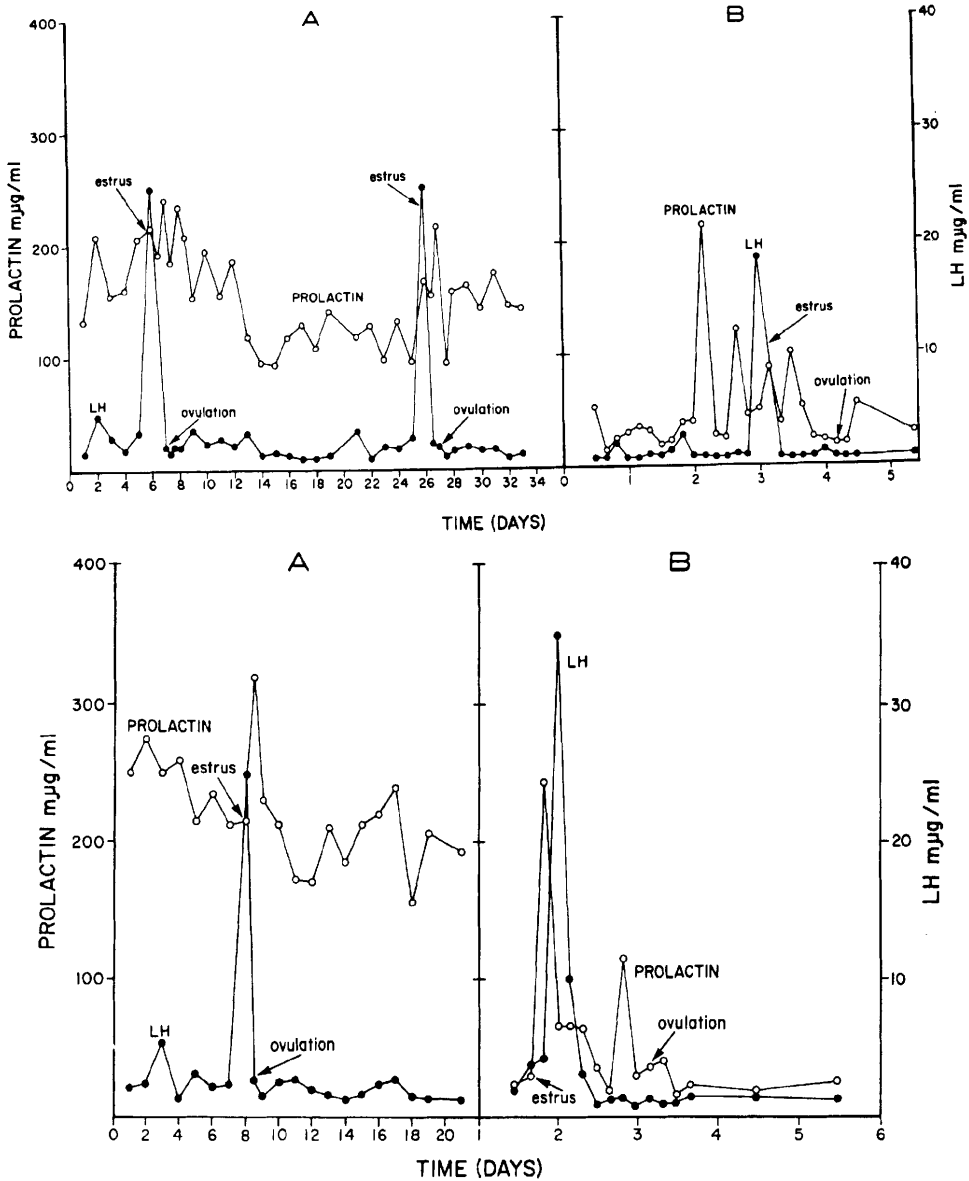


FIG. 1. Effect of bleeding technique upon serum prolactin and luteinizing hormone levels in two adult cycling cows: (A) Blood drawn by jugular puncture taking no precautions to avoid stressing the animals. (B) Blood drawn from a long indwelling jugular catheter implanted 24 hr before the beginning of the experiment. Note the considerably lower and less erratic prolactin levels in the catheterized animals. Luteinizing hormone levels were unaffected by either method.

drawn without apparent animal disturbance or awareness at frequent intervals over several days' time. After each sample, the catheter was flushed with sterile saline and then kept filled with saline until the next sample. A sample equal to the value of the catheter was taken and discarded just prior to sample collection. After each observation, studies were performed under conditions of controlled duration and severity of stress. In one study, after a resting period of 24 hr, blood was drawn on an hourly basis for 6 hr from an animal with an indwelling catheter to establish the base-line level. Subsequently, the animal was restrained and one investigator stood in front and shouted during a 10-min period. Blood was obtained at frequent time intervals during and following the acute stress stimuli. In another study, a heifer was forcibly restrained and deprived of water over a 30-hr period.

Results. Prolactin concentrations in blood drawn from animals of both sexes and all ages under noncontrolled conditions ranged between 100 and 400 $\mu\text{g}/\text{ml}$. No consistent patterns emerged from any groups of animals studied; lactating females did not have greater circulating prolactin concentrations than prepubertal or adult males. Figure 1A is an illustration of characteristic prolactin and LH profiles in two of the nine heifers whose blood was sampled by jugular puncture under noncontrolled bleeding conditions. Mean prolactin concentrations \pm SEM at diestrus in the two animals were 113.8 ± 4.6 and 202.8 ± 5.2 $\mu\text{g}/\text{ml}$ of serum, respectively. In the other seven animals mean diestrus prolactin concentrations ranged from 113 to 485 $\mu\text{g}/\text{ml}$ (mean 298.8 ± 41.7). In contrast, the diestrus prolactin levels of four animals bled from an indwelling jugular catheter under controlled conditions ranged from 31 to 64 $\mu\text{g}/\text{ml}$ (mean 37.8 ± 9.3). Data from exemplary studies are shown in Fig. 1. When the entire population of nine heifers bled by jugular puncture was compared to the catheterized group of four animals, a highly significant ($p > 0.01$) difference in mean diestrus prolactin concentration was noted between the two groups. The preestrus-estrus rise in the animals stressed

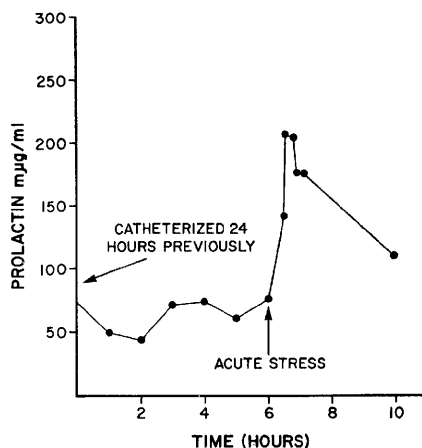


FIG. 2. Effect of acute stress (noise and restraint applied for a 10-min period) upon serum prolactin levels in an adult cow catheterized 24 hr before the beginning of the experiment. Note the low basal levels and the sharp increase in serum prolactin immediately following the application of the stress stimulus.

by jugular puncture was not nearly as sharp or well defined as that seen in the heifers which were subjected to controlled bleeding (Fig. 1). Thus, the average midcycle elevation of prolactin in the stressed animals was 185% of the mean diestrus level, while the mean peak value for the catheterized group was 928% of that of diestrus. In both groups (controlled and uncontrolled bleeding conditions), a peak of prolactin occurred during proestrus or estrus.

The technique of bleeding had no apparent effect on serum luteinizing hormone concentration, either during diestrus or at midcycle, in any of the animals studied. A rapid enhancement of serum prolactin from a mean diestrus level of 64.3 ± 5.4 $\mu\text{g}/\text{ml}$ to a peak value of 206 $\mu\text{g}/\text{ml}$ occurred in 30 min in a heifer stressed for a 10-min period by noise and restraint (Fig. 2). Subsequently, there was a gradual drop in prolactin concentration which approached base-line levels in 4 hr following application of the stress stimulus. The effect of a different type of stress is depicted in Fig. 3; water deprivation and restraint by a rope for a 30-hr period resulted in a gradual but greater increase in serum prolactin from a low level of 86 $\mu\text{g}/\text{ml}$ to a peak value of 400 $\mu\text{g}/\text{ml}$.

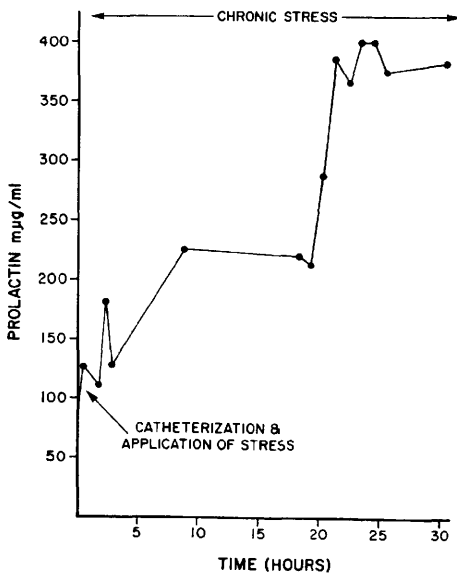


FIG. 3. Effect of chronic stress (water deprivation and restraint applied for 30 hr immediately following catheterization) upon serum prolactin levels in an adult cow. Note the continuous increase in serum prolactin during the course of the stress period.

Discussion. It is evident from these studies that, at least in the cow, serum prolactin concentrations are profoundly influenced by stress of several etiologies. Meaningful physiological data are difficult to obtain unless carefully controlled conditions are employed. One may not conclusively state that stress produces these effects by increase on the secretory mechanism since metabolic clearance rate and volume of distribution under presence and absence of stress have not been determined. It would appear likely that the stimulus is central nervous system mediated; very major and rapid alterations in MCR and/or volume of distribution would have to occur to account for the 300 to 1000% increase in serum prolactin observed.

The practical significance of these findings should not be discounted. Since stress associated with bleeding appears to be a major stimulus to prolactin secretion, any physiological study of prolactin secretion must be carried out under carefully controlled conditions. Small but significant increases or decreases in prolactin secretion may be obscured due to the high base-line levels associated with the stress stimulus. This becomes

apparent when one considers that in our population of heifers the midcycle elevation of prolactin in the stressed group was 1.85 times the mean diestrus level (base-line level), while the animals which were catheterized and carefully handled gave a peak which was 9.28 times the mean diestrus concentration. Thus, physiological stimuli which may be quite important in the control of prolactin secretion, but which may be "weaker" than the stress associated with the method of bleeding may be observed. Furthermore, even relative prolactin concentrations could probably not be accurately compared, since differing degrees of stress during bleeding could quite conceivably modify the basal level of secretion. Such reasoning may explain the wide range of "normal" values obtained by jugular puncture.

Further studies are required to determine conclusively whether stress also affects serum prolactin concentrations in other species such as the laboratory rat, although there are indications that it does (14).

Summary. Serum prolactin concentrations were determined by radioimmunoassay during estrus cycles in 15 dairy heifers bled by repeated jugular punctures or from a long, indwelling jugular catheter. The method of bleeding was found to influence the serum prolactin concentration and was attributed to nonspecific stress. Further confirmation for the stress effect was provided by a controlled study in other heifers, submitted to specific forms of acute and chronic stress stimuli. In cattle, studies of blood prolactin concentrations under various physiological conditions may be performed only when environmental conditions are carefully controlled.

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