PHYSIOLOGICAL RESPONSES IN FOALS WEANED BY ABRUPT OR GRADUAL METHODS

C.A. McCall, PhD,1 G.D. Potter, PhD,2 J.L. Kreider, PhD,3 and W.L. Jenkins, DVM, PhD2

SUMMARY

Physiological responses to weaning procedures were studied in 21 foals assigned to one of five treatments: (1) abrupt, total separation of mare and foal, no preweaning creep feed (TSNC); abrupt, total separation with preweaning creep feed (TSC); partial separation of mare and foal allowing fenceline contact, no preweaning creep feed (PSNC); (4) partial separation with creep feed (PSC); and (5) control (CON) no separation of mare and foal, foals creep fed. Changes in adrenal response to exogenous ACTH, basal and peak plasma cortisol concentrations, plasma triiodothyronine (T3) and thyroxine (T4) concentrations, weight gains and feed consumption were measured. Foals on the total separation treatments had higher adrenal responses (P<.05) and pre-ACTH basal (P<.05) and post-ACTH peak plasma cortisol concentrations (P<.05) than foals on other treatments indicating they were stressed at weaning. The PSNC, PSC and CON treatments did not differ (P>.05) in any cortisol response. No treatment differences were found in thyroid hormone concentrations in this study. On partial separation treatments, creep-fed and non-creep-fed foals consumed similar amounts of feed during the first week postweaning. On total separation treatments, non-creep-fed foals consumed more feed (P<.05) than creep-fed foals. All foals without creep feed gained more weight immediately after weaning (0-2 weeks) than creep-fed foals (P<.05), reflecting higher feed intakes and possible compensatory gains. Total postweaning weight gains (0-8 weeks) of foals were not significantly affected by treatment.

INTRODUCTION

Fraser et al. discussed various concepts of stress and concluded that an animal is in a state of stress if it must make abnormal or extreme changes in its physiology or behavior to cope with adverse effects of its environment or management.7 Weaning has been implicated as a source of emotional anxiety and physiological changes in many species.22 Physiological responses of animals to various stress producing situations have been examined by many researchers,23,24,25 and emphasis is usually focused on the hypothalamic-hypophyseal-adrenal axis.

In normal animals, a rapid increase in adrenocorticotropic hormone (ACTH) and a consequent increase in cortisol secretion follow any major stress.8,18,19 At the onset of stress the cells of the adrenal cortex quickly become depleted of glucocorticoids. Approximately 48 hours after the initiation of a continual stress, the adrenal cortex undergoes hypertrophy and hyperplasia which allows an increased, sustained secretion of glucocorticoids.8,18,19 This increased glucocorticoid secretion continues until the stress is removed, the animal adapts to the stressful situation, or adrenal exhaustion occurs and the animal dies.19

Gribble13 reported that equine adrenal function is similar to that of other species in which cortisol is the major glucocorticoid. Equine plasma cortisol concentrations are elevated by
Mares and foals from the CON treatment were also kept in the same pen. All foals were kept in the weaning pens for 14 days before being turned back to pasture. Mares and foals from the CON treatment were also kept in the weaning pens for 14 days before being turned back to pasture. This insured that CON foals were exposed to the same surroundings and experiences, except actual weaning, as weaned foals. Foals were always weaned in pairs or triplets to ensure that any changes resulted from weaning rather than isolation. Weaning always started between 0700 and 1000 hours.

From weaning until 6 months of age, all foals were fed the same concentrate diet that the creep fed foals had received during the preweaning period. At 8 hour intervals, foals were placed in individual stalls and allowed to eat ad libitum for 1 hour. Refusals were collected and weighed after each feeding. All foals were weighed twice monthly from 2 months until 6 months of age.

Blood sampling

Blood samples were taken by jugular catheter from all foals 5 days before weaning to accustom foals to the procedure and obtain baseline blood concentrations of hormones, and again at 2 days and 9 days after weaning. Catheterization was performed quickly and quietly under a local anesthetic, mepivacaine hydrochloride (carbocaine V), to minimize excitement. On sampling days, foals were catheterized at approximately 1000 hours and blood sampling began at approximately 1300 hours. This allowed adequate time for catheterization and for the foals to become quiet if excited during catheterization. Also, beginning the blood collection at 1300 hours helped protect against detecting physiological differences that were simply due to normal circadian rhythms. Since daily maximum cortisol values occur during morning hours in the horse, the foals’ plasma cortisol concentrations should have passed peak values by 1300 hours.

At each blood sampling time, a sample was taken to determine baseline concentrations of cortisol, T_{3}, and T_{4}. The foals’ adrenals were then challenged with a pharmacological dose (.56 UI/kg .75) of ACTH (porcine, grade II) and the resulting increase in plasma cortisol was measured. ACTH IV results in peak cortisol concentrations 2 hours post injection in the horse. Administration of 10-50 IU of ACTH IV results in peak cortisol concentrations 2 hours post injection in the horse.

The purpose of this phase of the study was to compare methods of weaning on physiological indicators of stress in those same foals.

MATERIAL AND METHODS

Animal Management

Twenty-One Quarter Horse foals were used in this study. All foals were handled prior to weaning to facilitate blood sampling and to minimize the effect of handling as an extraneous source of stress. Handling began at birth and continued twice weekly until the foals were 2 months of age. Each handling session lasted approximately 15 minutes and consisted of catching and rubbing the foal while it was stalled with its dam. All foals were vaccinated and dewormed 1 month prior to weaning.

Foals were blocked by sex and birth date and randomly assigned to one of five different weaning management regimens in a randomized blocked design with two main effects, separation method and preweaning feeding management. Originally 30 foals were assigned to the study (6 per treatment), however, injuries and illnesses reduced the number of foals on each treatment. Weaning management regimens (treatments) and numbers of foals on each treatment were:

- **TSNC** - Total, abrupt separation of mare and foal; no preweaning creep feed (n=5)
- **TSC** - Total, abrupt separation of mare and foal; with preweaning creep feed (n=4)
- **PSNC** - Partial separation of mare and foal allowing visual, auditory, olfactory and tactile contact; no preweaning creep feed (n=5)
- **PSC** - Partial separation of mare and foal allowing visual, auditory, olfactory and tactile contact; with preweaning creep feed (n=4)
- **CON** - Controls, no separation of mare and foal; with preweaning creep feed (n=3)

All foals were weaned at approximately 4 months of age. Mares and foals were maintained on pasture prior to weaning. In TSNC and PSNC treatments, mares were fed their concentrate ration from buckets situated 1.5 m high on posts to prevent foals from eating their dam’s feed. Before weaning, foals on TSC, PSC and CON treatments had access to the mare’s feed, and additional feed was provided free choice in a creep feeder (16% crude protein commercial pelleted foal diet).

After separation, foals were placed in wire mesh pens approximately 15 m x 15 m with 1.2 m high fences. Dams in TSNC and TSC treatments were moved (approximately .8 km away) where their foals could not see, hear or smell them. Dams in PSNC and PSC treatments were kept in pens adjoining the foals’ weaning pens. Fenceline contact between these mares and foals was permitted for 7 days following weaning, then dams were moved out of sight and hearing of their foals. In CON treatments, both foal and dam remained together in the same pen. All foals were kept in the weaning pens for 14 days following weaning, then were turned out to pasture. Mares and foals from the CON treatment were also kept in the weaning pens for 14 days before being turned back to pasture. This insured that CON foals were exposed to the same surroundings and experiences, except actual weaning, as weaned foals. Foals were always weaned in pairs or triplets to ensure that any changes resulted from weaning rather than isolation. Weaning always started between 0700 and 1000 hours.

From weaning until 6 months of age, all foals were fed the same concentrate diet that the creep fed foals had received during the preweaning period. At 8 hour intervals, foals were placed in individual stalls and allowed to eat ad libitum for 1 hour. Refusals were collected and weighed after each feeding. All foals were weighed twice monthly from 2 months until 6 months of age.

Blood sampling

Blood samples were taken by jugular catheter from all foals 5 days before weaning to accustom foals to the procedure and obtain baseline blood concentrations of hormones, and again at 2 days and 9 days after weaning. Catheterization was performed quickly and quietly under a local anesthetic, mepivacaine hydrochloride (carbocaine V), to minimize excitement. On sampling days, foals were catheterized at approximately 1000 hours and blood sampling began at approximately 1300 hours. This allowed adequate time for catheterization and for the foals to become quiet if excited during catheterization. Also, beginning the blood collection at 1300 hours helped protect against detecting physiological differences that were simply due to normal circadian rhythms. Since daily maximum cortisol values occur during morning hours in the horse, the foals’ plasma cortisol concentrations should have passed peak values by 1300 hours.

At each blood sampling time, a sample was taken to determine baseline concentrations of cortisol, T_{3}, and T_{4}. The foals’ adrenals were then challenged with a pharmacological dose (.56 UI/kg .75) of ACTH (porcine, grade II) and the resulting increase in plasma cortisol was measured. ACTH IV results in peak cortisol concentrations 2 hours post injection in the horse.
TABLE 1
Plasma Cortisol During Each Test and For Each Change from Preweaning to Postweaning Test

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>TEST</th>
<th>TSNC</th>
<th>TSC</th>
<th>PSNC</th>
<th>PSC</th>
<th>CON</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preweaning</td>
<td>217.6 ± 20.1^c</td>
<td>253.1 ± 25.3^bc</td>
<td>312.1 ± 20.1^b</td>
<td>230.7 ± 22.5^c</td>
<td>232.0 ± 26.0^c</td>
<td></td>
</tr>
<tr>
<td>2 day postweaning</td>
<td>291.6 ± 24.8</td>
<td>295.7 ± 25.3</td>
<td>268.3 ± 24.8</td>
<td>227.2 ± 27.7</td>
<td>225.2 ± 32.0</td>
<td></td>
</tr>
<tr>
<td>9 day postweaning</td>
<td>210.8 ± 17.3</td>
<td>196.0 ± 28.4</td>
<td>262.3 ± 17.3</td>
<td>200.1 ± 19.3</td>
<td>225.8 ± 19.3</td>
<td></td>
</tr>
<tr>
<td>2 day postweaning-preweaning change</td>
<td>-78.0 ± 21.2^b</td>
<td>42.6 ± 23.2^ae</td>
<td>-45.2 ± 21.2^e</td>
<td>-3.6 ± 23.2^d</td>
<td>-25.8 ± 28.4^ae</td>
<td></td>
</tr>
<tr>
<td>9 day postweaning-preweaning change</td>
<td>-5.4 ± 18.3</td>
<td>-39.2 ± 24.5</td>
<td>-50.7 ± 18.3</td>
<td>-30.7 ± 20.0</td>
<td>-5 ± 24.5</td>
<td></td>
</tr>
</tbody>
</table>

^aExpressed as area under the curve (adrenal response curve area). Values are least squares means ± standard error of mean.
^b,cExpressed in the same row means in the same row differ (P<.05)

stered via jugular catheter. Post-ACTH blood samples were then collected at 30 minute intervals for 4 hours. Plasma was stored at -20°C until assayed for cortisol.

Hormone assays
Prior to cortisol analyses, a 20 μl volume of plasma was pipetted into duplicate tubes and diluted with 380 μl of .1 M phosphate buffered saline plus .1% gelatin, pH 7.2. Tubes were stoppered and heated in a hot-water bath at 70°C for 1 hour to denature cortisol binding globulins. Cortisol was then analyzed by a radioimmunoassay technique modified from that of Abraham et al. A standard curve was constructed in triplicate using 62.5 to 4000 pg cortisol/tube. Cortisol values of the standard curve and interpolation of the unknown samples were determined using a log-logit transformation in order to obtain a linear dose response curve. Binding ranged from 24 to 46% of [1,2-3H] cortisol. Non-specific binding was reported by Pantex for 21-desoxycortisol (70%); corticosterone (30%); 11-desoxycortisol (30%); desoxycorticosterone (5%); 17-hydroxy progesterone (5%); and cortisone (5%).

Assay sensitivity was 125 pg cortisol/tube. Inter-assay and intra-assay coefficients of variation were 13% and 10%, respectively.

Serum concentrations of triiodothyronine (T3) and thyroxine (T4), for blood samples obtained prior to ACTH challenge, were assayed using a Tri-Tab RIA diagnostic kit and Tetra-Tab RIA diagnostic kit, respectively. For the T3 assay, nonspecific binding was reported by Nuclear-Medical Laboratories for thyroxine (.35%), and for L-3,3',5'-triiodothyronine, diiodothyronine, monoiodothyrosine, diiodothyroisine (<.5%). For the T4 assay, nonspecific binding was reported by Nuclear Medical Laboratories for D-Thyroxine (98%) and T4 (<2%). T3 and T4 inter-assay coefficients of variation were 4.3% and 2.9% respectively.

Data analyses
Preweaning weight gains, postweaning weight gains from weaned foals and corresponding gains in unweaned (CON) foals were examined by analysis of variance in a randomized block design with treatment and sex of foal as sources of variation. Where F ratios for treatment were significant, Duncan's new multiple range test was used to separate least squares means of weight gains in weaned and unweaned foals. Effects of separation method and preweaning feeding management on postweaning gain and feed intakes of only the weaned foals (CON treatment excluded) were examined by analysis of variance for the 2 x 2 factorial design. In all analyses, interactions between variables were tested and if P>.20 they were dropped from the model.

During each adrenal challenge, nine cortisol concentrations, plotted against time, were used to form a sinusoidal curve. The area under each curve, termed adrenal response curve area (ARCA), was estimated using the equation reported by Friend et al. A pre-ACTH basal and a post-ACTH peak cortisol value was identified for each foal on each test.

Plasma cortisol concentrations and plasma concentrations of thyroid hormones were examined by analyses of variance in a randomized block design with treatment and sex as sources of variation. When the F statistic for treatment was significant, Duncan's new multiple range test was utilized to separate least squares treatment means. Further examination of cortisol responses from only the weaned foals (CON excluded) was conducted using 2 x 2 factorial analyses of variance with separation method and preweaning feeding management as sources of variation. Interactions between variables were tested and dropped for the model if P>.20. Relationships between plasma cortisol concentrations, thyroid hormone concentrations, weight gains and feed intakes were examined using simple correlation analyses.

EQUINE VETERINARY SCIENCE
TABLE 2

Pre-ACTH Basal (ng/ml) Cortisol Concentrations During Each Test and Each Change from Postweaning to Preweaning Test*

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>TEST</th>
<th>TSNC</th>
<th>TSC</th>
<th>PSNC</th>
<th>PSC</th>
<th>CON</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preweaning</td>
<td>30.1 ± 7.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.6 ± 8.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.5 ± 7.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.2 ± 8.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.2 ± 9.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2 day postweaning</td>
<td>34.2 ± 7.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55.5 ± 7.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.2 ± 7.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.5 ± 7.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.5 ± 9.0&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>9 day postweaning</td>
<td>37.8 ± 6.1</td>
<td>10.7 ± 7.8</td>
<td>38.7 ± 6.1</td>
<td>31.1 ± 6.8</td>
<td>45.1 ± 7.8</td>
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<tr>
<td></td>
<td>2 day postweaning-preweaning change</td>
<td>3.7 ± 6.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.9 ± 7.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-28.2 ± 6.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-6.7 ± 7.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-8.7 ± 8.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>9 day postweaning-preweaning change</td>
<td>6.9 ± 8.5</td>
<td>-19.7 ± 11.4</td>
<td>-15.7 ± 8.5</td>
<td>4.0 ± 9.3</td>
<td>6.7 ± 11.4</td>
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</tbody>
</table>

<sup>a</sup>Values are least squares means ± standard error of mean.
<sup>b</sup>,<sup>c</sup>Means in the same row with different superscripts differ (P<.05).

TABLE 3

Post-ACTH Peak (ng/ml) Cortisol Concentrations During Each Test and Each Change from Postweaning to Preweaning test*

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>TEST</th>
<th>TSNC</th>
<th>TSC</th>
<th>PSNC</th>
<th>PSC</th>
<th>CON</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preweaning</td>
<td>72.8 ± 3.2</td>
<td>86.8 ± 6.4</td>
<td>100.1 ± 6.5</td>
<td>82.1 ± 7.3</td>
<td>80.5 ± 8.4</td>
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<td>2 day postweaning</td>
<td>93.0 ± 6.9</td>
<td>106.1 ± 7.7</td>
<td>96.7 ± 6.9</td>
<td>78.0 ± 7.7</td>
<td>76.0 ± 8.9</td>
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<td>9 day postweaning</td>
<td>78.1 ± 6.6</td>
<td>66.8 ± 8.6</td>
<td>94.6 ± 6.6</td>
<td>76.7 ± 7.4</td>
<td>77.9 ± 8.6</td>
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<tr>
<td></td>
<td>2 day postweaning-preweaning change</td>
<td>21.1 ± 5.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.3 ± 6.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-3.7 ± 5.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-3.9 ± 6.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-10.0 ± 7.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>9 day postweaning-preweaning change</td>
<td>4.6 ± 5.9</td>
<td>-10.8 ± 7.9</td>
<td>-5.6 ± 5.9</td>
<td>-5.2 ± 6.4</td>
<td>-1.5 ± 7.9</td>
</tr>
</tbody>
</table>

<sup>b</sup>Values are least squares means ± standard error of mean.
<sup>c</sup>Means in the same row with different superscripts differ (P<.05).

RESULTS AND DISCUSSION

Cortisol

Initial analysis of variance of the ARCA showed a significant treatment by test interaction. The ARCA for the preweaning test, 2-day postweaning test, 9-day postweaning test, changes in ARCA for the 2-day postweaning minus preweaning test and changes in ARCA for the 9-day postweaning minus preweaning test are shown in Table 1. During the preweaning test, the PSNC foals had higher (P<.05) ARCA than foals on the TSNC, PSC, and CON treatments. The TSNC, TSC, PSC and CON foals did not have significantly different ARCA during the preweaning test. There were no significant treatment differences during either the 2-day or 9-day postweaning tests, however, the ARCA of the foals varied greatly from animal to animal and between tests. The significant treatment differences found during the preweaning test, which occurred before separation of mare and foal, were not expected and probably are indicative of the individual physiological variation in adrenal responsiveness of the foals. It was felt that this individual variation was creating inherent differences among the treatments. Therefore, each individual foal's preweaning ARCA was utilized as an adjustment factor for its postweaning ARCA. The change from the 2-day postweaning ARCA minus the preweaning ARCA represents the foal's initial response to weaning. The change from the 9-day postweaning ARCA minus the preweaning ARCA reflects any long-term treatment effects and represents the time immediately after removal of the dams from PSNC and PSC foals.

Foals on TSNC and TSC treatments had the highest positive changes (P<.05) in ARCA from the 2-day postweaning minus preweaning values indicating that these foals had increased adrenal responses during weaning (Table 1). Access to preweaning creep feed seemed to moderate stress involved in total separation weaning, since TSC foals did not differ (P>.05) from PSC or CON foals. Foals on both PSNC and PSC weaning methods had ARCA changes which were not significantly different from unweaned, CON foals, indicating that foals on these treatments were not stressed excessively during
weaning.

Changes in the 9-day postweaning minus preweaning ARCA were not significantly affected by treatment indicating the weaning stress precipitated in this study was not prolonged, and final removal of dams in the partial separation weaning treatments caused no detectable adrenal responses in PSNC and PSC foals.

Sex of foal had no significant effect on ARCA during the preweaning, 2-day postweaning, 9-day postweaning or during the adjusted 2-day postweaning minus preweaning or the 9-day postweaning minus preweaning tests.

Pre-ACTH basal cortisol concentrations for the preweaning test, 2-day postweaning test, 9-day postweaning test, changes in basal cortisol concentrations from the 2-day postweaning minus preweaning and changes in basal cortisol concentrations 9-day postweaning minus preweaning tests are shown in Table 2. Pre-ACTH basal cortisol concentrations ranged from 5.6 to 70.1 ng/ml, with a mean of 38.6 ng/ml and were similar to mean concentrations reported by Hoffsis et al.13 (51.2 ng/ml), James et al.14 (75 ng/ml) and Bottoms et al.3 (29.9 ng/ml). In 52% of the adrenal challenge tests, the pre-ACTH blood sample contained the lowest plasma cortisol concentration obtained during the test. In the remaining adrenal challenge tests, the sample obtained 4 hours post-ACTH most often contained the lowest plasma cortisol concentration. A possible explanation for post-ACTH samples having lower cortisol concentrations than the pre-ACTH basal sample is that the foals may still have been stimulated from catheterization when the basal blood sample was obtained, causing a high basal cortisol reading. A more probable cause is that pharmacological doses of exogenous ACTH theoretically cause maximum cortisol output, which is often accompanied by a fall in plasma cortisol to below basal concentrations before reverting to pre-ACTH administration concentrations.14

There were treatment by test interactions for pre-ACTH basal cortisol concentrations (P<.01). During the preweaning test, foals on the PSNC treatment had significantly higher pre-

ACTH basal cortisol concentrations than those on TSNC, PSC or CON treatments, and the PSC foals had significantly lower basal cortisol concentrations than TSC, PSNC and CON foals. These treatment effects again reflect large individual variations in cortisol concentrations in the foals. In the 2-day postweaning test, the TSC foals had the highest (P<.05) pre-ACTH basal cortisol concentrations. However, the TSNC, PSNC, PSC and CON foals were not significantly different. There were no treatment differences in pre-ACTH basal cortisol concentrations in the 9-day postweaning test, again indicating weaning methods used in this study caused no prolonged increases in adrenal response.

The pre-ACTH basal cortisol values were adjusted for individual variation by the same method used for the ARCA (subtracting each foal’s preweaning value from the 2-day postweaning value and from the 9-day postweaning value). In the 2-day postweaning versus preweaning adjustment, the TSNC foals had lower (P<.05) changes in basal cortisol concentrations than TSC or TSC foals, but they were not different (P>.05) from PSC or CON foals. This low negative change in the PSNC pre-ACTH basal cortisol change probably represents sampling problems such as excitement during catheterization or preweaning blood sampling, or it may reflect the pulsatile nature of glucocorticoid release15 rather than indicating a true treatment effect. No treatment effects were found in the 9-day postweaning versus preweaning basal cortisol concentrations.

Sex of foal had no effect on pre-ACTH basal cortisol concentrations for the preweaning, 2-day postweaning, 9-day postweaning, or pre-ACTH peak cortisol concentrations. However, the TSNC, PSNC, PSC and CON foals were not significantly different. There were no treatment differences in pre-ACTH basal cortisol concentrations in the 9-day postweaning test, again indicating weaning methods used in this study caused no prolonged increases in adrenal response.

The pre-ACTH basal cortisol values were adjusted for individual variation by the same method used for the ARCA (subtracting each foal’s preweaning value from the 2-day postweaning value and from the 9-day postweaning value). In the 2-day postweaning minus preweaning adjustment, the PSNC foals had lower (P<.05) changes in basal cortisol concentrations than those on TSNC, PSC or CON treatments, and the PSC foals had significantly lower basal cortisol concentrations than TSC, PSNC and CON foals. These treatment effects again reflect large individual variations in cortisol concentrations in the foals. In the 2-day postweaning test, the TSC foals had the highest (P<.05) pre-ACTH basal cortisol concentrations. However, the TSNC, PSNC, PSC and CON foals were not significantly different. There were no treatment differences in pre-ACTH basal cortisol concentrations in the 9-day postweaning test, again indicating weaning methods used in this study caused no prolonged increases in adrenal response.

The pre-ACTH basal cortisol values were adjusted for individual variation by the same method used for the ARCA (subtracting each foal’s preweaning value from the 2-day postweaning value and from the 9-day postweaning value). In the 2-day postweaning versus preweaning adjustment, the TSNC foals had lower (P<.05) changes in basal cortisol concentrations than TSC or TSC foals, but they were not different (P>.05) from PSC or CON foals. This low negative change in the PSNC pre-ACTH basal cortisol change probably represents sampling problems such as excitement during catheterization or preweaning blood sampling, or it may reflect the pulsatile nature of glucocorticoid release15 rather than indicating a true treatment effect. No treatment effects were found in the 9-day postweaning versus preweaning basal cortisol concentrations.

Sex of foal had no effect on pre-ACTH basal cortisol concentrations for the preweaning, 2-day postweaning, 9-day postweaning, or either adjusted test.

Preweaning, 2-day postweaning, 9-day postweaning, changes in 2-day postweaning minus preweaning and changes in 9-day postweaning minus preweaning post-ACTH peak cortisol concentrations are shown in Table 3. The post-ACTH peak cortisol concentration occurred 1.5 hours after ACTH administration in 52% of the tests. In the remainder of the adrenal challenge tests, the peak glucocorticoid concentrations were recorded at 1 hour (24%), 2 hours (21%), and 2.5 hours (2%) after ACTH administration. There was a treatment
postweaning test, suggesting they were not as stressed by higher responses throughout the test. In contrast, partially separated foals had lower ARCA, basal and peak cortisol concentrations during the 2-day postweaning test than during the preweaning, 2-day postweaning adjusted cortisol concentrations (P<.01) and both pre-ACTH basal and post-ACTH peak cortisol concentrations (P<.001; Table 4). Foals in the total separation group generally showed greater positive change in ARCA, pre-ACTH basal and post-ACTH peak cortisol concentrations than partially separated foals.

This indicates that foals in the total separation group generally began the 2-day preweaning test with higher adrenal responses and cortisol concentrations and continued to have higher responses throughout the test. In contrast, partially separated foals had lower ARCA, basal and peak cortisol values during the 2-day postweaning test than during the postweaning test, suggesting they were not as stressed by weaning as the total separation foals.

As shown in Table 5, preweaning feeding management did not affect change in ARCA nor peak cortisol concentrations during the 2-day postweaning test. However, foals without preweaning creep feed had lower (P<.04) changes in basal glucocorticoid concentrations than those with creep feed. Causes for this observation are not obvious.

### Thyroid hormones

The foal’s plasma T3 concentrations ranged from 12 to 137 ng/dl with a mean of 61.2 ng/dl. These concentrations were similar to those reported by Reap et al. 17 for adult horses and by Glade and Luba10 for weaning foals. The T4 concentrations ranged from 1.2 to 6.2 μg/dl with a mean of 2.9 μg/dl. These concentrations are higher than those reported by Reap et al. 17 (1.0 to 2.4 μg/dl) and Glade and Luba10 (means ranged from 2.1-2.8 μg/dl). There were no treatment differences (P>.05) in T3 or T4 concentrations during the preweaning or postweaning tests. Sex of foal did not significantly affect T3 or T4 concentrations during any test.

### Feed Intake and weight gain

Individual feed intakes of the weaned foals (CON excluded) ranged from 4.8 to 48.5 kg/week over the 8 week postweaning period. During the first week postweaning, TSC foals had lower average feed consumption (P<.05) than foals on other treatments. A significant separation method by preweaning feeding management interaction was observed for feed consumption during the first week postweaning. On total separation weaning treatments, non-creep-fed foals consumed more feed per head (17.0 kg/week) than creep-fed foals (9.4 kg/week; P<.05). On partial separation weaning treatments, foals with and without preweaning creep feed had essentially the same feed consumption (21 kg and 20.4 kg/week, respectively).

After the first week postweaning, feed consumption was similar for all foals on all treatments. Also, sex of foal did not affect (P>.05) feed intakes during any period.

Creep-fed foals gained more weight (P<.03) than foals without creep feed over the 56 day preweaning period (57.4 vs. 48.6 kg, respectively), but sex of foal did not significantly affect preweaning weight gains.

Postweaning weight gains for each treatment are shown in Table 6. During the first 2-week postweaning period, weight gains of foals on the weaning treatments did not differ from unweaned, CON foals, but the TSNC and PSNC foals had higher (P<.05) weight gains than TSC or PSC foals. After the first 2-week weighing period, there were no treatment differences (P>.05) in foal weight gains, and total postweaning gain was not affected by treatment (P>.05). Sex of foal did not influence postweaning weight gains.

Weight gains of the weaned foals (CON excluded) during the initial 2-week postweaning weighing period were not significantly affected by separation method (total separation = 8.1 kg, partial separation = 7.9 kg). However, non-creep-fed

### TABLE 6

Average postweaning weight gain (kg)a during each 2 week weighing period

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weeks</th>
<th>Total postweaning gain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-2b</td>
<td>3-4c</td>
</tr>
<tr>
<td>TSNC</td>
<td>11.5d</td>
<td>9.7</td>
</tr>
<tr>
<td>TSC</td>
<td>4.7e</td>
<td>7.6</td>
</tr>
<tr>
<td>PSNC</td>
<td>11.0d</td>
<td>13.7</td>
</tr>
<tr>
<td>PSC</td>
<td>4.8e</td>
<td>9.4</td>
</tr>
<tr>
<td>CON</td>
<td>7.9da</td>
<td>8.4</td>
</tr>
</tbody>
</table>

aValues are least squares means.
bData from 2 foals deleted due to sickness during these periods.
cColumn means with different superscripts differ (<.01).
foals gained more weight (11.2 kg) than creep-fed foals (4.7 kg; P < .01) during the first 2-week postweaning.

These feed consumption and weight gain data probably reflect a high level of curiosity for feed in non-creep-fed foals. During the preweaning period, non-creep-fed foals observed their dams eating and picked up small amounts of spilled feed. At weaning time, non-creep-fed foals had an initial but limited introduction to feed and probably developed an active interest in feed. The relatively faster rate of weight gain in non-creep-fed foals was very likely compensatory in nature and may also account for their higher feed intakes than creep-fed foals. This combination of foals being hungry with a high curiosity for feed may account for higher feed consumptions and weight gains in foals without preweaning creep feed. Total feed intake was positively related to postweaning weight gain (r = 0.50; P < 0.05) indicating that foals which consumed more feed gained more weight. Feed intake during the first week postweaning was negatively correlated with 2-day postweaning minus preweaning pre-ACTH basal (r = -0.44; P < .01) and post-ACTH peak (r = -0.52; P < 0.001) cortisol values, suggesting that foals with high adrenal stress responses had lower feed intake after weaning. Postweaning weight gains tended to be positively related to 2-day postweaning minus preweaning ARCA (r = 0.37; P < 0.10) and post-ACTG peak cortisol levels (r = 0.32; P < 0.05). This tendency was probably caused by the positive relationship between high postweaning weight gains, due to high feed intakes and high glucocorticoid responses of TSNC foals.

These data on glucocorticoid responses, feed intake and weight gain indicate that different weaning management regimens cause different physiological responses in foals. Foals weaned by an abrupt, total separation weaning method exhibited higher cortisol responses than foals managed more intensively.

Different weaning management systems did not precipitate any differences in thyroid hormone concentrations in the foals on this study. Foals on partial separation weaning treatments had higher feed intakes than those abruptly weaned during the first week postweaning. Foals without preweaning creep feed had higher initial postweaning feed intakes than those with preweaning creep feed, and this is very likely related to compensatory weight gain in the non-creep-fed foals.

These physiological data corroborate previously reported behavioral data in which abruptly weaning foals exhibited more signs of emotionality (increased vocalizations, increased activity) than gradually weaned foals. 16 Although a potential exists for differences in feed intake and weight gain due to weaning management, feed intake and weight measurements used in this study apparently were not sensitive enough to detect weaning stress. Reductions in injuries due to relaxed emotional states and reductions in diseases which may result from lowered resistance during stress are the real benefits of a gradual weaning system.

REFERENCES