The Effects of Cold Stress on Neonatal Calves
II. Absorption of Colostral Immunoglobulins

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ABSTRACT

Newborn calves were subjected to cold stress and made hypothermic by immersion in water at 15 to 17°C. Cold stress delayed the onset and significantly decreased (P < 0.05) the rate of absorption of immunoglobulins (IgM, IgG, and IgG1) up to 15 hours after first feeding of pooled colostrum. However, the net absorption of colostral immunoglobulins was not affected. The possible deleterious effect of cold stress on absorption of colostral immunoglobulins by newborn calves under range conditions is discussed.

RÉSUMÉ

Les auteurs ont provoqué le stress du froid, chez des veaux nouveau-nés, et les ont rendus hypothermiques, en les immergeant dans un bassin d'eau dont la température variait de 15 à 17°C. Ce stress retarda le début de l'absorption des immunoglobulines IgM, IgG, et IgG1; il en diminua aussi de façon appréciable (P < 0.05) le taux d'absorption, jusqu'à 15 heures après le premier repas d'un mélange de colostrum. L'absorption nette des immunoglobulines colostrales ne s'en trouva cependant pas affectée. Les auteurs commentent les effets dommageables possibles du stress du froid sur l'absorption des immunoglobulines colostrales par les veaux nouveau-nés, dans les conditions naturelles du vêlage au champ.

INTRODUCTION

Newborn calves are usually born either hypogammaglobulinemic (12, 13) or agammaglobulinemic (20, 22) due to the absence of transplacental transfer of antibodies from the maternal to the fetal circulation during normal gestation (2). The calf, like many other neonates, is passively immunized by the ingestion and absorption of colostral immunoglobulins during the early postnatal period. Nonselective absorption of immunoglobulins and other macromolecules in colostrum by the intestine of the suckled neonate normally begins within three hours after ingestion and continues for only 24 to 36 hours after birth (8, 11). Consequently, suckled calves may die from a variety of infectious diseases due to failure of absorption and the resulting persistent hypogammaglobulinemia or agammaglobulinemia (3, 5).

Range calves, born during late winter and early spring, are often cold stressed and may become hypothermic due to exposure to harsh environmental conditions (15, 16, 23, 24). Work with dogs has shown that hypothermia is responsible for decreased venous outflow from the small intestine, decreased intestinal motility and a net reduction in transport of substances from intestinal lumen into the blood (10). Therefore, it seemed possible that hypothermia might impair absorption of colostral immunoglobulins by calves. This work was undertaken to determine the effect of cold stress on the ability of neonatal calves to absorb colostral immunoglobulins.
MATERIALS AND METHODS

GENERAL PROCEDURES

Procedures for cold stress treatment of 26 Holstein-Friesian calves have been described (18). Briefly, calves in groups 1 and 2 were cold stressed by immersion in water at 15 to 17°C until the core body temperature (CBT) was lowered 10°C. Calves in group 1 were euthanatized two hours after cold stress. Calves in group 2 were allowed to recover (after cold stress) for 72 to 96 hours in a cold (4°C) wet environment and then euthanatized. Calves in group 3 remained normothermic during immersion in water at thermoneutral temperature (35 to 37°C) for two hours. They were then maintained in a 25°C dry environment for 72 to 96 hours and then euthanatized. Calves in groups 1 and 2 were fed one liter of pooled colostrum by stomach tube when the CBT was lowered 2°C or 5°C, respectively. Calves in group 3 were fed after one hour of immersion.

COLLECTION OF SPECIMENS

A polyethylene catheter (PE 90) was implanted into the jugular vein of each calf before immersion. Blood was collected before immersion, hourly for nine hours after the start of immersion and approximately every 12 hours thereafter. Serum was separated from clotted blood and stored (−20°C) until analyzed for immunoglobulin. Rennin precipitated and defatted whey was also prepared from the pooled colostrum fed to calves and stored (−20°C) until analyzed for immunoglobulins.

IMMUNOLOGICAL PROCEDURES

Bovine IgM, IgG, and IgG₂ were isolated and purified by minor modifications of previously described procedures (6). Immunoglobulin G₁ was prepared from whole blood serum whereas IgM and IgG₂ were prepared from colostral whey.

Gel filtration chromatography was performed for semipurification of IgM by gravity flow elution at 4°C on a single column (2.5 by 90 cm) using 0.1M tris (hydroxymethyl aminomethane) buffer (pH 8.18) to which sodium azide (400 mg/mL) had been added as a preservative (6).

Ion exchange chromatography was performed on diethylaminoethyl cellulose columns (1.5 by 30 cm) at 25°C. Immunoglobulin G₁ was semipurified by single step elution with phosphate buffer solution (PBS) (0.01M, pH 7.4) (6). Immunoglobulin G₁, from gel filtration chromatography was further purified by stepwise elution with PBS. Buffer concentrations of phosphate and pH value used were 0.01M, 0.02M and 0.05M at pH 6.3 (6).

Samples of fractions of IgM, IgG, and IgG₂ from column chromatography were tested by spectrophotometry, immunoelectrophoresis (21) and double diffusion in agar gel (19) to determine purity.

Guinea pig antiwhole bovine serum and monospecific antiligulbins against bovine IgM, IgG, and IgG₂ were prepared as previously described (17). Antiligulbins were stored (−20°C) until used.

QUANTITATION OF IMMUNOGLOBULINS

Concentrations of IgM, IgG, and IgG₂ in pooled colostral whey and calf serum were determined by single radial immunodiffusion (14). A stock solution of 2% (w/v) agar gel was prepared in 0.05M barbital buffer (pH 8.6) to which sodium azide (400 mg/mL) had been added as a preservative. Antiserum was diluted with 0.05M PBS (pH 7.4), heated to 55°C, and thoroughly mixed with an equal volume of heated (60°C) agar solution. The final dilutions of antiglobulins for bovine IgM, IgG, and IgG₂ were 1:25, 1:15 and 1:20, respectively. Preliminary tests were conducted to determine the optimum final dilution of the sample to be used in the analysis.

Calibration curves were obtained by testing fractions of bovine IgM, IgG, and IgG₂ of known concentrations. All pooled colostral whey and calf serum samples were tested in duplicate and incubated at 25°C in a moist chamber for 48 hours. Ring dia-

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1Sephadex G-200, Pharmacia Fine Chemicals, Piscataway, New Jersey.
2Whatman DE 52, Reeve Angel, Clifton, New Jersey.
3Noble Agar, Difco Laboratories, Detroit, Michigan.
4Miles Laboratories, Inc., Elkhart, Indiana.
meters were read in two dimensions on a calibration viewer and the mean diameter calculated. An equation for the calibration curve for each immunoglobulin class (concentration vs. the square of the mean ring diameters) was determined by linear regression. The concentrations of IgM, IgG1, and IgG2 in pooled colostral whey and in calf serum were calculated using the appropriate regression equations.

**RESULTS**

The concentration of immunoglobulins (mg/mL) in whey from pooled colostrum fed to the calves was: IgM=9.10, IgG1=60.73 and IgG2=7.87. Mean concentrations of IgM, IgG1, and IgG2 in serum of calves in groups 2 and 3 are summarized (Table 1, Figs. 1-3). Data from calves in group 1 are not included since the mean concentrations of immunoglobulins measured did not differ significantly (P > 0.05) from those of calves in group 2 by three hours after first feeding and euthanasia at an earlier age prevented a full length comparison with the noncold stressed calves.

There was a delay of from one and one-half to three and one-half hours in first appearance of immunoglobulins in serum of calves in group 2 when compared to calves in group 3. Furthermore, the mean concentrations and thus the rate of absorption of IgM and IgG1 in the serum of calves in group 2 were significantly lower (P ≤ 0.05) than the respective concentrations in the calves in group 3 during the period three to 18 hours after feeding of colostrum. Similarly, the mean concentration of IgG2 in the serum of calves in group 2 was significantly lower (P ≤ 0.05) than that found in calves in group 3 during the period of three to 15 hours after feeding of colostrum. In all cases, the rate of absorption of immunoglobulins by noncold stressed calves was exponential and near-maximal concentrations were attained by

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Transdyne General Corporation, Ann Arbor, Michigan.
six to nine hours after first feeding.

The net absorption of colostral immunoglobulins by calves was not affected by cold stress. Data show that the mean concentrations of IgM and IgG1 in the serum of calves in groups 2 and 3 did not significantly differ (P \( \geq 0.05 \)) by 21 hours after feeding of colostrum. Also, there was no significant difference (P \( \geq 0.05 \)) between calves in groups 2 and 3 in concentration of serum IgG2 by 18 hours after feeding.

### DISCUSSION

Data from the present investigation show that cold stress of newborn calves caused a delay in onset and a significant decrease in the rate of absorption of colostral immunoglobulins up to 15 hours after the first feeding. The reasons for the delay in onset and the decrease in rate of absorption of colostral immunoglobulins were not determined, although they may have been the same as those previously described in cold stressed dogs (10).

Resistance of newborn calves against diseases such as colibacillosis is dependent in part on ingestion and absorption of sufficient quantities of protective colostral antibodies (1, 4, 7, 9). Factors which cause a delay in onset and a decrease in rate of absorption of colostral antibodies could have a deleterious effect on calf survival, particularly when natural exposure to infectious organisms occurs within the first few hours of life. Data from the present investigation suggest that severe cold stress of newborn calves under range conditions could cause a delay in onset and a decrease in rate of absorption of colostral antibodies and enhance the susceptibility of these animals to infectious organisms.

The apparent absence of an effect on net absorption of colostral immunoglobulins by calves may have been due to the short duration of the cold stress. A previous report (18) showed that the core body temperature of these calves had returned to normal by eight hours after the first feeding of colostrum, whereas the present report indicates that absorption of immunoglobulins continued for 18 to 21 hours. Consequently, cold stressed calves were hypothermic for less than half the time period during which immunoglobulin absorption occurred. Prolonged hypothermia would probably have decreased net absorption.

Muscular weakness and reluctance to stand and nurse are behavioral changes which were observed in the cold stressed calves (18). Similar alterations in clinical behavior by cold stressed calves under range conditions could cause a delay in onset of nursing activity and thus cause a decrease

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**TABLE I. Mean Concentrations (mg/mL) of IgM, IgG1, and IgG2 in the Serum of Cold Stressed (group 2) and Noncold Stressed (group 3) Calves at Selected Times After Ingestion of Colostrum**

<table>
<thead>
<tr>
<th>Immunoglobulin Concentrations (Mean ± SD)</th>
<th>Time (hours) after Ingestion of Colostrum</th>
<th>IgM</th>
<th>Groups</th>
<th>IgG1</th>
<th>Groups</th>
<th>IgG2</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
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<tr>
<td></td>
<td>3</td>
<td>0.00 ± 0.16a</td>
<td>0.00 ± 1.99a</td>
<td>0.00 ± 0.20a</td>
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<td></td>
<td>6</td>
<td>0.06 ± 0.63a</td>
<td>0.55 ± 4.09a</td>
<td>0.06 ± 0.36a</td>
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<tr>
<td></td>
<td>9</td>
<td>± 0.12 ± 0.24</td>
<td>± 1.07 ± 2.08</td>
<td>± 0.12 ± 0.13</td>
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<tr>
<td></td>
<td>15</td>
<td>± 0.25 ± 0.27</td>
<td>± 1.43 ± 4.25a</td>
<td>± 0.15 ± 0.41a</td>
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<td></td>
<td>18</td>
<td>± 0.27 ± 0.29</td>
<td>± 1.50 ± 2.03</td>
<td>± 0.14 ± 0.13</td>
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<tr>
<td></td>
<td>21</td>
<td>± 0.23 ± 0.29</td>
<td>± 1.37 ± 2.10</td>
<td>± 0.12 ± 0.12</td>
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<tr>
<td></td>
<td>2</td>
<td>± 0.21 ± 0.26</td>
<td>± 1.44 ± 1.55</td>
<td>± 0.12 ± 0.14</td>
<td></td>
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</tr>
</tbody>
</table>

\( a < 0.005 \)
\( b < 0.01 \)
\( c < 0.025 \)
\( d < 0.05 \)
\( e \) Not significant
in the total amount of colostrum ingested and absorbed.

ACKNOWLEDGMENTS

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REFERENCES