THE TRANSFER OF IMMUNITY TO THE NEW-BORN CALF FROM COLOSTRUM

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(From the Biological Laboratories, E. R. Squibb and Sons, New Brunswick)

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Some years ago, Theobald Smith and his associates (1) showed that passive immunity acquired by the new-born calf from colostrum is of prime importance in protection against infectious disease. At about the same time, Orcutt and Howe (2) observed that, after the ingestion of colostrum, agglutinins appear in the calf serum associated with a globulin which is precipitable at low concentrations of sodium sulfate. Jameson, Alvarez-Tostado, and Sortor (3) later found by electrophoretic analysis that the serum of the new-born calf does not contain \( \gamma \)-globulin, and that the appearance of slow moving globulin follows the feeding of colostrum. Similar observations have been made by San Clemente and Huddleson (4) for the calf, and by Polson (5) for the new-born foal.

Since it has already been demonstrated that the immune lactoglobulin of the colostrum differs from the \( \gamma \)-globulin of cow serum in electrophoretic mobility and other properties (6-11), it was of interest to ascertain whether the new protein which appears in calf serum after it receives colostrum possesses the same electrophoretic properties as the colostrum globulin, or whether this immune lactoglobulin is modified during its passage from the intestine of the calf to the blood stream. Hyperimmunization was performed with a mixture of antigens: diphtheria toxin (or toxoid), vaccinia virus, and a killed culture of \textit{Hemophilus pertussis}. It was, therefore, possible to follow changes in the acquired immunity of the calf parallel with electrophoretic studies of the serum, and to determine the duration of the passively acquired immunity.

A few observations have also been made on the electrophoretic patterns of the serum of the new-born lamb before and after the ingestion of colostrum, and these have been compared with the pattern obtained with maternal sheep serum.

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1 Studies on the hyperimmunization of bovines with these and other antigens will be reported elsewhere by one of us (A. H.). It should also be noted that previous studies (6-11) of bovine immune proteins were made with animals immunized to these same three antigens.
Electrophoretic Studies on Calf Serum

By fractionation of hyperimmune bovine plasma, two different proteins associated with immune activity have been isolated: a γ-globulin with an electrophoretic mobility of $1.3 \times 10^{-5}$ sq. cm. per volt per second, and a T component with a mobility of $2.5 \times 10^{-5}$ as measured in 0.1 M veronal (diethyl barbiturate) buffer at pH 8.6 (6). Hess and Deutsch (12) have obtained similar proteins from normal bovine serum. The immune lactoglobulin of colostrum has an electrophoretic mobility similar to that of the T component (6). Since both the colostrum and T-globulins have mobilities near that of fibrinogen, our observations were made with serum rather than with plasma. The electrophoretic analyses were performed at 1° in a Tiselius apparatus equipped with the Longsworth schlieren scanning device. The calf was separated from the mother immediately after birth and was fed 2750 cc. of the first colostrum during the next 40 hours; thereafter, the calf was fed on a mixed artificial diet.

Fig. 1 shows the electrophoretic patterns of the sera of the calf at birth and at later intervals. For comparison, the serum of the mother taken 3 days before term is also shown. It is apparent from Fig. 1 that the serum of the new-born calf does not contain γ-globulin and that T-globulin is also absent. Moreover, none of the antibodies present in the serum of the mother could be demonstrated in the serum in the new-born (Table III). This is in agreement with previous observations that there is no placental transfer of antibodies in this species (13, 14). After ingestion of colostrum, a new component appears in calf serum which has an electrophoretic mobility of $2.1 \times 10^{-5}$ sq. cm. per volt per second in veronal buffer of pH 8.4. Thus, this component retains the characteristic mobility of the immune lactoglobulin after passage from the digestive tract to the bloodstream of the calf and is not a γ-globulin. Examination of the data of Jameson, Alvarez-Tostado, and Sortor (3) and of San Clemente and Huddleson (4) shows that in these cases also the new globulin in calf serum possesses the mobility of the colostrum globulin.

Tables I and II present the analytical data for the calf sera at different ages. These data indicate that several striking changes occur during the growth of the young calf. The most obvious is the large amount of immune protein which appears after the feeding of colostrum and its gradual diminution. Also noteworthy is the absence of γ-globulin at birth and its gradual appearance only many weeks later. The α2 component, which decreases in concentration, is probably the fctuin described by Pedersen (15) as the main constituent of fetal α-globulin. In Table II are the values for the absolute concentration of each serum component. These data show clearly that after ingestion of colostrum there is no immediate change in the absolute amounts of the albumin or the α-globulins, but there seems to be an increase in the β2 component. The α3-globulin appears late, and,
although it is first clearly separable at 122 days, it is apparently included with the $\alpha_2$ at 87 days.

Both the $\alpha_2$-globulin and the albumin show a striking decrease in electrophoretic mobility after the ingestion of colostrum. This change may be due to the influence of the large amount of colostrum globulin on the viscosity and the electric properties of the diluted serum, rather than to a sudden change in the character of the $\alpha_2$-globulin or the albumin.

![Electrophoretic patterns of the descending boundaries of the serum of a new-born calf and of the same animal 2, 50, 87, and 122 days later. For comparison, the maternal serum obtained 3 days before term is also shown. The runs were performed at 1° in a veronal buffer of pH 8.4 to 8.6 and at an ionic strength of 0.1. The serum of the new-born is practically devoid of slow moving globulins. The immune component appears after feeding colostrum. The absolute heights of the different serum samples cannot be compared, as electrophoresis was performed at different protein concentrations.](http://www.jbc.org/)

In addition to the data already presented, observations were made on the sera of two other new-born calves. The electrophoretic patterns obtained on the sera before and after feeding were similar to those described above.

**Immune Properties of Calf Serum**

In Table III are given the data for the immune activity of the whole colostrum whey and of the calf serum at different ages. Assays of diph-
theria antitoxin were performed by the rabbit skin test as described by Jensen (16). The values for pertussis antibodies were measured in mouse-

**Table I**

**Electrophoretic Analyses of Calf Serum**

The electrophoretic analyses were performed at 1° in veronal buffer of pH 8.4 to 8.6 and at an ionic strength of 0.1. The mobilities are $\times 10^{-6}$ sq. cm. per volt per second and are negative in sign. The values were obtained from photographs of the descending patterns taken at 150 to 250 minutes. The serum protein was calculated from protein N $\times 0.25$.

<table>
<thead>
<tr>
<th>Age of calf</th>
<th>Protein in serum</th>
<th>$\gamma$</th>
<th>Colostrum globulin</th>
<th>$\beta_2$</th>
<th>$\beta_1$</th>
<th>$\alpha_2$</th>
<th>$\alpha_1$</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>u per cent</td>
<td>u per cent</td>
<td>u per cent</td>
<td>u per cent</td>
<td>u per cent</td>
<td>u per cent</td>
<td>u per cent</td>
<td>u per cent</td>
</tr>
<tr>
<td>days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4.5</td>
<td>2.5 2</td>
<td>3.7 8</td>
<td>5.3 44</td>
<td>6.4 3</td>
<td>7.5 43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8.0</td>
<td>3.1 8</td>
<td>3.6 2</td>
<td>4.5 23</td>
<td>5.6 2</td>
<td>6.4 21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>6.1 1.2</td>
<td>2.8 15</td>
<td>3.6 4</td>
<td>4.5 15</td>
<td>5.4 5</td>
<td>6.3 46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>87</td>
<td>6.2 1.4</td>
<td>2.8 10</td>
<td>3.4 4</td>
<td>4.3 17</td>
<td>5.2 4</td>
<td>6.2 47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>122</td>
<td>5.8 1.3</td>
<td>2.7 11</td>
<td>3.2 2</td>
<td>4.0 10</td>
<td>5.2 4</td>
<td>6.0 46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal</td>
<td>1.4 20</td>
<td>3.0 12</td>
<td>3.6 5</td>
<td>4.7 18</td>
<td>5.6 3</td>
<td>6.3 32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* T-Globulin.

**Table II**

**Composition of Calf Serum at Different Ages**

The values are calculated from the percentage for each component of the total refractive increment and the total protein content of the serum. The concentrations are in gm. per 100 cc. of serum.

<table>
<thead>
<tr>
<th>Age</th>
<th>$\gamma$</th>
<th>Colostrum globulin</th>
<th>$\beta_2$</th>
<th>$\beta_1$</th>
<th>$\alpha_2$</th>
<th>$\alpha_1$</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.0</td>
<td>0.09</td>
<td>0.36</td>
<td>1.98</td>
<td>0.14</td>
<td>1.94</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.52</td>
<td>0.64</td>
<td>0.16</td>
<td>1.84</td>
<td>0.16</td>
<td>1.68</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0.06</td>
<td>0.85</td>
<td>0.24</td>
<td>0.92</td>
<td>0.31</td>
<td>2.81</td>
<td></td>
</tr>
<tr>
<td>87</td>
<td>0.37</td>
<td>0.74</td>
<td>0.25</td>
<td>1.05</td>
<td>0.25</td>
<td>2.91</td>
<td></td>
</tr>
<tr>
<td>122</td>
<td>0.41</td>
<td>0.58</td>
<td>0.12</td>
<td>0.58</td>
<td>0.23</td>
<td>2.67</td>
<td></td>
</tr>
</tbody>
</table>

protective units by a modification of Silverthorne's method (17), and the vaccinia assays were made by a rabbit skin test.2

These data show that the calf may acquire a high level of immunity from

2 Holm, A., unpublished.
the colostrum, as already demonstrated with other antibodies. Since the calf was fed colostrum for only a short time, it is possible to calculate the approximate duration of the passively acquired immunity. For the pertussis and vaccinia antibodies, the time for the activities to decrease to one-half their initial values is about 50 days. The more precise determinations of the diphtheria antitoxin show a half life of about 16 days, and the data in Table II from the Tiselius analyses indicate 20 days. Since the animal was growing rapidly, dilution of the antibodies must have occurred, and the true half times must be even greater than the calculated values.

While there is some variation for the different antibodies, there is no doubt that the passive immunity is effective for a considerable length of time. It may be remarked that humans who are negative to the Schick test are regarded as immune to diphtheria when this test reflects a serum level of about 0.03 antitoxic unit per cc. For the calf described here, the level of circulating diphtheria antitoxin was higher than this 4 months after the feeding of colostrum. This clearly indicates the important rôle that this passive immunity may play in the life of the new-born. Obviously, the persistence of immunity depends greatly on the initial titer of the colostrum and the amount fed. If the calf in this experiment had been permitted to suckle without restriction, circulating antibody concentrations might have been greater and persisted much longer. However, Hansen and Phillips (18) have recently observed that colostrum globulin is absorbed into the serum of the new-born calf only during the first 24 hours of life.

It may be estimated from the data on Lamb 1 of Mason, Dalling, and Gordon (13) that the approximate half life of immunity for lamb dysentery antitoxin acquired from colostrum is 12 to 15 days; this is similar to our data on the calf for diphtheria antitoxin. The duration of the passive immunity acquired by the feeding of colostrum in ruminants shows consider-

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**Table III**

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Diphtheria antitoxin (units per cc.)</th>
<th><em>Hemophilus pertussis</em> antibody (units per cc.)</th>
<th>Vaccinia virus antibody*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;0.001</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>70</td>
<td>6400</td>
</tr>
<tr>
<td>50</td>
<td>5.0</td>
<td>40</td>
<td>3200</td>
</tr>
<tr>
<td>87</td>
<td>1.0</td>
<td>20</td>
<td>1600</td>
</tr>
<tr>
<td>122</td>
<td>0.6</td>
<td>16</td>
<td>800</td>
</tr>
<tr>
<td>Colostrum</td>
<td>80</td>
<td>190</td>
<td>6400</td>
</tr>
</tbody>
</table>

*The vaccinia virus was measured in neutralizing skin test doses per cc.*
able variation from that of other types of passive immunity in different species. The data of Heidelberger et al. (19) on homologous type I pneumococcus antibody injected into a rabbit show that approximately one-half of the activity disappeared in about 36 hours, while the half life of an actively produced antibody molecule was about 2 weeks.

The difference in the duration of the passive immunity may be due more to a species difference than to the route of immunization. Two examples from the older literature may be cited. The data of Smith (20) on the persistence of antitetanolysin injected into a rabbit intraperitoneally or intravenously show a half life of about 1 day, in a goat of about 4 days, and for diphtheria antitoxin in man of about 3 or 4 days. On the other hand, the data of Bulloch (21) on the duration of passively acquired diphtheria antitoxin in the donkey show a half life of about 20 days.

Electrophoresis of Lamb and Sheep Sera

In Fig. 2 are shown the electrophoretic patterns obtained with the sera of a new-born lamb, of a 3 day-old lamb, and of the mother. It is quite
apparent that the serum of the new-born lamb resembles that of the calf, and is devoid of slow moving globulins which appear only after suckling has occurred. Earle (22) has already shown by Howe’s salt fractionation method that the effect of colostrum ingestion on the serum proteins of young foals, kids, lambs, and pigs is similar to that found by Howe in calves.

In Table IV are presented the data for the composition and mobility of the sera shown in Fig. 2. These data were derived from photographs taken at 167 and 250 minutes. The mobility of sheep γ-globulin is quite high, and in this respect resembles the T-globulin usually present in the hyper-immune sera of the cow and the horse. Since the colostrum globulin migrates only slightly faster than the γ-globulin of the maternal serum, it would be difficult in this species to distinguish between the passively acquired globulin and the adult γ-globulin. In addition to the colostrum globulin which appears in the serum of the 3 day-old lamb, the β-globulins show a marked increase, and the relative amounts of the α-globulins change considerably.

In contrast to the absence of γ-globulin from the sera of the new-born calf and lamb, Longsworth, Curtis, and Pembroke (23) have recently found that in the human γ-globulin is slightly higher in the serum of the new-born than in the mother. Since it is well known that in the human there is placental transmission of antibodies, there is good correlation between the presence of γ-globulin and immunity in the new-born.

Electrophoretic examination of fetal or new-born sera of different species taken prior and subsequent to ingestion of colostrum may provide a simple

**Table IV**

*Electrophoretic Analyses of Lamb and Sheep Sera*

These analyses were made at 1° in veronal buffer of pH 8.5 to 8.6 and at an ionic strength of 0.1. The mobilities are $\times 10^{-5}$ sq. cm. per volt per second and are negative in sign.

<table>
<thead>
<tr>
<th>Component</th>
<th>New-born lamb</th>
<th>3 day lamb</th>
<th>Maternal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>u</td>
<td>Concentration</td>
<td>u</td>
</tr>
<tr>
<td>γ</td>
<td>2.4*</td>
<td>26</td>
<td>2.1</td>
</tr>
<tr>
<td>β₂</td>
<td>3.6</td>
<td>10</td>
<td>3.0</td>
</tr>
<tr>
<td>β₁</td>
<td>4.0</td>
<td>2</td>
<td>4.2</td>
</tr>
<tr>
<td>α₂</td>
<td>5.1</td>
<td>31</td>
<td>5.1</td>
</tr>
<tr>
<td>α₁</td>
<td>6.1</td>
<td>10</td>
<td>5.8</td>
</tr>
<tr>
<td>Albumin</td>
<td>7.6</td>
<td>56</td>
<td>6.7</td>
</tr>
</tbody>
</table>

* Colostrum globulin.
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test for ascertaining whether antibodies are transferred through the placenta or the colostrum, or by both of these routes.

The authors gratefully acknowledge the technical assistance of Douglas M. Brown.

SUMMARY

1. The serum of the new-born calf does not possess any slow moving globulin (γ or T components). After ingestion of colostrum, an electrophoretic component appears in calf serum, which is identical in mobility with the lactoglobulin which has previously been demonstrated to carry immunity in bovine colostrum. Similar observations have also been made on new-born lamb serum.

2. During growth of the young calf, changes also take place in other serum components as observed by electrophoretic analysis. The γ-globulin appears some time after birth and gradually increases in concentration. The mobility and concentration of the serum albumin and α-globulin also show striking alterations.

3. Antibodies to three different antigens, two of them foreign to the species, were absorbed by the calf from colostrum. The concentration in the serum of these antibodies gradually decreased, and the time for the activity of these antibodies to drop to half their initial values was estimated as follows: for the diphtheria antitoxin about 16 days, for Hemophilus pertussis and vaccinia antibodies about 50 days, and for the immune component estimated by electrophoretic analysis about 20 days.

BIBLIOGRAPHY


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