LACTOGENESIS IN ORGAN CULTURES OF HEIFER MAMMARY TISSUE

J. DJIANE, C. DELOUIS AND THE LATE R. DENAMUR

Laboratoire de Physiologie de la Lactation,
Institut National de la Recherche Agronomique,
78350-Jouy-en-Josas, France

(Received 23 December 1974)

Hormonal induction of milk synthesis by the ruminant mammary gland in organ culture has received little attention. M. A. Cannata, C. Delouis, C. Jeulin & R. Denamur (unpublished observation) demonstrated the lactogenic effect of insulin + cortisol + prolactin \textit{in vitro} on ewe mammary tissue with lobulo-alveolar development at the time of explantation, but there are no data on the induction of milk secretion in bovine mammary tissue cultured on a synthetic medium. Therefore, we have studied the culture conditions necessary to maintain bovine mammary tissue and the combination of hormones needed to induce milk secretion.

Biopsy samples were obtained from 2-year-old French Friesian heifers at various times during the oestrous cycle, after i.m. injection of the tranquillizer, xylazine (Rompun, Bayer; 2% solution, 1·5 ml/100 kg body weight). Explants (1 mm$^3$) were cultured in organ culture dishes (Falcon Plastics). The culture medium was medium 199 (Microbiological Associates) and Eagle’s minimum essential medium (MEM, Eurobio) in equal amounts. The tissue was not so well maintained on Medium 199 or MEM alone. Final amino acid concentration was adjusted to five times that of Medium 199 by adding a concentrated amino acid solution (Eagle’s basal medium amino acids, ×100, Microbiological Associates); sodium bicarbonate (2·2 g/l) and sodium acetate (5·44 g/l) were also added to the medium. Cultures were incubated at 37°C in 57% O$_2$:5% CO$_2$:38% N$_2$ (by vol.).

Histological examination of biopsy samples revealed poorly developed mammary tissue; in most cases ducts with small end buds only were present. Cultured on medium containing amorphous insulin (Endopancrine, 24·5 i.u./mg; 5 μg/ml) this tissue was well maintained as judged by light microscopy for up to 10 days. In an atmosphere of 95% O$_2$:5% CO$_2$ (v/v) maintenance was very poor, even if the medium contained cortisol (Microfine, Roussel; 5 μg/ml) in addition to insulin and/or 20% serum from hypophysectomized ewes. These results agreed well with observations of M. A. Cannata et al. (unpublished results) on the survival of pregnant ewe mammary tissue \textit{in vitro}. Bovine mammary tissue was also well maintained on a synthetic medium supplemented with insulin if the atmosphere was 95% air:5% CO$_2$, but the cells of ducts cut across during the preparation of the tissue for culture tended to migrate around the surface of the explants.

Results presented in Table 1 show that insulin + cortisol did not induce secretion after 5 days \textit{in vitro}. Addition of ovine prolactin (NIH-P-S9, 30 i.u./mg, 5 μg/ml) to this medium induced secretion in most of the explants. Epithelial cells had abundant cytoplasm and their nuclei were basal. The enlarged lumina were filled with an eosinophilic secretion containing numerous fat droplets. The addition of ovine growth hormone (NIH-GH-S10, 0·86 i.u./mg, 5 μg/ml) did not modify the response observed with insulin + cortisol + prolactin, while insulin + prolactin did not induce milk secretion \textit{in vitro}.

As in most mammals (see Denamur, 1969, 1971; Forsyth, 1971) insulin + cortisol + prolactin is thus the minimal hormonal requirement for lactogenesis in bovine mammary tissue \textit{in vitro}. Histological results correlated well with those obtained on the appearance of lactose synthetase activity during lactogenesis in heifer mammary tissue in organ culture (J. Djiane & C. Delouis, unpublished observations).
Table 1. Secretory activity of heifer mammary explants

<table>
<thead>
<tr>
<th>Hormones (5 µg/ml)</th>
<th>No. of explants (No. of cultures)</th>
<th>Number of explants presenting histological secretory grade shown</th>
</tr>
</thead>
<tbody>
<tr>
<td>I+C</td>
<td>39 (6)</td>
<td>1 1.5 2 2.5 3 3.5 4</td>
</tr>
<tr>
<td>I+C+P</td>
<td>49 (6)</td>
<td>- 3 5 8 12 17 4</td>
</tr>
<tr>
<td>I+C + P + G</td>
<td>28 (5)</td>
<td>- - 3 7 7 6 5</td>
</tr>
</tbody>
</table>

I, Insulin; C, cortisol (Microfine, Roussel); P, ovine prolactin (NIH-P-S9); G, ovine growth hormone (NIH-GH-S10).

Explants were examined histologically after 5 days of culture. Secretory activity was graded, using the 1–4 plus scale of Barnawell (1965), and is the mean of estimations by two different experimenters. Secretory activities in uncultured biopsy samples were grade 1·0 for 5 biopsies and grade 2·0 for 1 biopsy.

The authors wish to thank Dr I. A. Forsyth for much helpful advice. Cortisol was a gift from the Roussel Laboratories, prolactin and growth hormone were gifts from the National Institute of Health, Bethesda, Maryland, U.S.A. This work was carried out with the excellent technical assistance of Miss M. L. Combaud and Mr P. Chesné, supported in part by the Délégation Générale à la Recherche Scientifique et Technique, convention no. 72-7-0029. Miss J. Ménage, Mrs A. Daifuku and Mrs D. Molitano are acknowledged for help in preparation of the manuscript.

REFERENCES