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Development of Sheep Embryos *in vitro* in a Medium Supplemented with Different Batches of Serum Albumin

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Abstract

Variability in different lots of commercial serum albumin affects mammalian embryo development in culture. The composition of commercial preparations of ovine, bovine and defatted bovine serum albumin and a fraction of ovine serum containing proteins with a mean molecular weight of 65 kDa (fraction 3) was examined by polyacrylamide gel electrophoresis. All preparations were heavily contaminated with serum proteins other than albumin. Day-6 sheep morulae were cultured for 48 h in a basal bicarbonate-buffered salt solution supplemented with the commercial preparations of ovine, bovine or defatted bovine serum albumin. These three albumin preparations differed in their abilities to support the development of morulae into expanded blastocysts, but these differences disappeared when the basal medium was also supplemented with a component of ovine serum containing substances with molecular weights of less than 10 kDa. In the latter case, the three commercial albumin preparations and fraction 3 of ovine serum all supported full development in about 40–60% of morulae.

Introduction

Mammalian embryos will generally develop to the blastocyst stage *in vitro* only in culture media supplemented with serum or commercial preparations of serum albumin. Commercial preparations of serum albumin are contaminated with proteins other than albumin (Peters 1975; Kane and Headon 1980), as well as fatty acids and many other small molecules (Kane and Headon 1980). The latter include an embryotropic growth factor, which stimulates cell division and growth in rabbit morulae and blastocysts (Kane 1985). Different batches of bovine serum albumin differ in composition, especially in regard to amounts of bound fatty acid (Chen 1966), growth-promoting factor and ability to support embryo development in several species (Kane 1983). In the absence of added fatty acids or other energy substrates, albumin-bound fatty acids are essential for rabbit embryo development *in vitro* (Kane and Headon 1980).

Sheep morulae develop into expanded blastocysts in a simple bicarbonate-buffered salt solution supplemented with ovine serum or a fraction isolated from ovine serum that contains a group of proteins with a mean molecular weight of about 65 kDa (fraction 3, Batt and Miller 1988). The aims of the present study were to (*a*) contrast the protein composition of fraction 3 from serum with that of commercial preparations of ovine, bovine and defatted bovine serum albumin, and (*b*) examine the development of sheep morulae in a medium supplemented with these three commercial preparations of serum albumin.

Materials and Methods

The procedures used to collect and assess day-6 embryos from superovulated Merino ewes have been described (Batt and Miller 1988). The maintenance and experimental use of animals in this study was in accordance with the guidelines issued by NH & MRC/CSIRO/AAC in 1985.

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Culture of Embryos in vitro

A total of 238 day-6 embryos were cultured *in vitro* in two experiments. In each experiment, embryos were allotted at random to different treatment groups. The basal culture medium was a bicarbonate-buffered salt solution, pH 7·6, supplemented with penicillin and streptomycin (Moore 1982). The component of serum containing substances with a molecular weight of less than 10 kDa (kilodaltons), and protein fraction 3 from serum containing a group of proteins with a mean molecular weight of about 65 kDa, were prepared by ultrafiltration and Sephadex gel filtration, respectively, from the same batch of ovine serum (Batt and Miller 1988). The culture media used in the experiments contained components of serum and/or commercial preparations of serum albumin. Embryos were cultured for 48 h at 37°C in 1·0 ml of medium under a layer of paraffin oil and an atmosphere of 5% CO_2 in air (Batt and Miller 1988).

Experiment 1

Protein fraction 3 from serum and commercially prepared ovine serum albumin were contrasted in terms of composition and ability to support the development of day-6 embryos *in vitro*.

Aliquots of fraction 3 and the commercial preparation of ovine serum albumin, each containing 30 μg protein, were fractionated in homogeneous 7% polyacrylamide slab gels (70 × 70 × 3 mm) for 3 h at 100 V and 4°C in a 0·09 M Tris/0·003 M Na_2EDTA/0·09 M boric acid buffer, pH 8·0 (Peacock and Dingman 1967). Electrophoresis was performed in a Gradipore Multi Cell Electrophoresis Unit (Gradient Laboratories Pty Ltd, Pyrmont, N.S.W.) and proteins in the gel were stained with amido black. Equivalent fractionations of individual polypeptides were carried out in the same manner, except that aliquots of fraction 3 and the commercial preparation of ovine serum albumin, each containing 30 μg protein, were first incubated at 80°C for 10 min in the presence of 2% sodium dodecyl sulfate (SDS) and 3·3% β-mercapto-ethanol, and both the gels and buffer used for electrophoresis were 0·1% with respect to SDS. Proteins in these gels were stained with Coomassie blue.

Embryos were cultured in one of two treatment media (Table 1). Protein concentrations were determined by the method of Hartree (1972).

Table 1. Development of embryos in media supplemented with components of serum and ovine serum albumin

Both media also contained the <10 kDa component of serum at a concentration equivalent to 5% serum

Group	Composition of medium	Concn of protein in the medium (mg ml^{-1})	Number of embryos showing: Full development	Partial development	No development	Total
1	2·5% protein fraction 3[A]	0·45	17	2	9	28
2	Ovine serum albumin[B]	0·45	17	3	6	26

[A] A protein fraction from ovine serum containing proteins with a mean molecular weight of about 65 kDa, at a concentration similar to that of these proteins in 2·5% serum.

[B] A commercial preparation of ovine serum albumin (Fraction V Powder, Cat. No. A3264, Lot: 122F9335, Sigma Chemical Company, St Louis, U.S.A.).

Experiment 2

The abilities of commercial preparations of ovine, bovine and defatted bovine serum albumin to support the development of sheep morulae into expanded blastocysts were examined in the presence and absence of the <10 kDa component of ovine serum. The sources of these preparations are given in Table 2. A further group of embryos was cultured in medium containing fraction 3 and the <10 kDa component of ovine serum, as a control. Embryos were cultured in one of nine treatment media.

Embryos were assessed for stage of development after culture *in vitro* and scored as having undergone full, partial or no development, according to previously described criteria (Batt and Miller 1988). Briefly, those classed as fully developed showed full or near full expansion of the blastocyst with

Table 2. Development of embryos in media supplemented with different preparations of serum albumin

The media for Groups 1, 2, 3, 4, 6 and 8 also contained the <10 kDa component of serum at a concentration equivalent to 5% serum

Group	Composition of medium	Concn of protein in the medium (mg ml^{-1})	Number of embryos showing: Full development	Partial development	No development	Total
1	2·5% protein fraction 3[A]	0·45	13	2	6	21
2	Ovine serum albumin[B]	0·45	12	1	7	20
3	Ovine serum albumin	0·23	8	1	12	21
4	Ovine serum albumin	0·12	3	1	16	20
5	Ovine serum albumin	0·45	5	6	10	21
6	Bovine serum albumin[C]	0·45	8	3	9	20
7	Bovine serum albumin	0·45	11	1	8	20
8	Defatted bovine serum albumin[D]	0·45	11	1	9	21
9	Defatted bovine serum albumin	0·45	2	7	11	20

[A] See Table 1.

[B, C, D] Commercial preparations of serum albumin purchased from Sigma Chemical Company, St Louis, U.S.A. (B) Fraction V Powder, Cat. No. A3264, Lot: 122F9335; (C) Fraction V Powder, Cat. No. A4503, Lot: 20F0231; (D) prepared from Fraction V Powder, Cat. No. A6003, Lot: 114F9391, essentially (<0·005%) free of fatty acid.

marked thinning of the zona pellucida, translucent trophoblast cells and a clearly visible inner cell mass. Within each experiment the proportions of embryos showing full development in different media were compared, using the Chi-square test (Steel and Torrie 1980). Individual polypeptides in the three commercial albumin preparations were also contrasted by electrophoresis in the presence of SDS, as in experiment 1.

Results

Experiment 1

In the absence of SDS, the protein in both fraction 3 of serum and the commercial preparation of ovine serum albumin migrated essentially as a major band (presumably albumin) and a slower moving minor band. In the case of the commercial ovine serum albumin preparation only, two further faintly stained bands that moved more slowly than the minor band were detected (Fig. 1*A*). In the presence of SDS, the proteins or polypeptide subunits in both fraction 3 and the commercial ovine albumin preparation migrated essentially as a major band and a much slower moving minor band. Additional slow moving, faintly stained bands were detected (Fig. 1*B*), but the number and density of staining of these faint bands from fraction 3 and the commercial ovine albumin preparation differed.

When the medium contained the <10 kDa component of serum there was no difference between the proportions of day-6 embryos that developed to expanded blastocysts *in vitro* in the presence of fraction 3 protein or ovine serum albumin (Table 1; 17/28 *v.* 17/26, $\chi^2 = 0 \cdot 1$). The concentration of protein in these media was chosen on the basis of the

results of a previous study, where media containing concentrations of fraction 3 of 0·90, 0·45 and 0·23 mg protein ml^{-1} plus 5% of the <10 kDa component of serum permitted full development in about 85%, 60% and 0% of ovine morulae, respectively (Batt and Miller 1988).

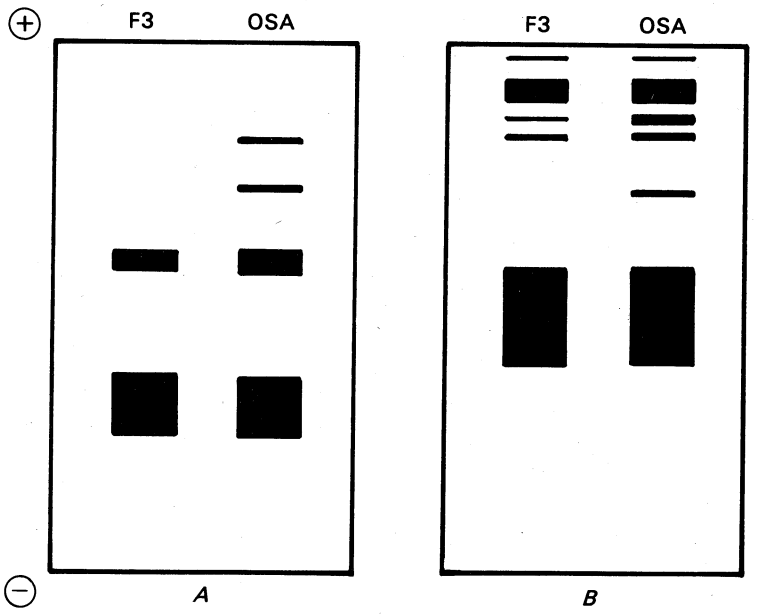

Fig. 1. Homogeneous polyacrylamide gels after electrophoresis of protein fraction 3 from ovine serum (F3) and a commercial preparation of ovine serum albumin (OSA). Electrophoresis was carried out in the absence (*A*) and presence (*B*) of 0·1% sodium dodecyl sulfate. (See Table 1 for details of the commercial preparations.)

Experiment 2

When the concentration of protein in medium containing the <10 kDa component of serum was maintained at 0·45 mg ml^{-1}, there were no significant differences between the proportions of embryos showing full development in media supplemented with fraction 3 of serum and commercial preparations of ovine, bovine and defatted bovine serum albumin (Groups 1, 2, 6 and 8; 13/21, 12/20, 8/20 and 11/21 respectively). The proportion of embryos developing into expanded blastocysts in medium supplemented with ovine serum albumin decreased with decreasing concentration of protein (Group 2, 12/20 *v.* Group 4, 3/20, $\chi^2 = 8{\cdot}6$, 1 d.f., $P < 0{\cdot}01$). The omission of the <10 kDa component of serum reduced the proportion of embryos showing full development in media supplemented with ovine (Group 5, 5/21 *v.* Group 2, 12/20, $\chi^2 = 5{\cdot}5$, 1 d.f., $0{\cdot}01 < P < 0{\cdot}05$) and defatted bovine serum albumin (Group 9, 2/20 *v.* Group 8, 11/21, $\chi^2 = 8{\cdot}5$, 1 d.f., $P < 0{\cdot}01$) but not in medium supplemented with bovine serum albumin (Group 7, 11/20 *v.* Group 6, 8/20, $\chi^2 = 0{\cdot}9$).

In the presence of SDS, the proteins or polypeptide subunits in all three commercial albumin preparations migrated essentially as a major band and a much slower moving minor band, and the relative amount of staining of these two principal components was constant (Fig. 2). Additional slow moving, faintly stained bands were detected in all preparations, and in the case of defatted bovine serum albumin, two further faintly stained bands moved ahead of the major band. The bovine serum albumin preparation contained no minor component that was not also present in the ovine and defatted bovine serum albumin preparations.

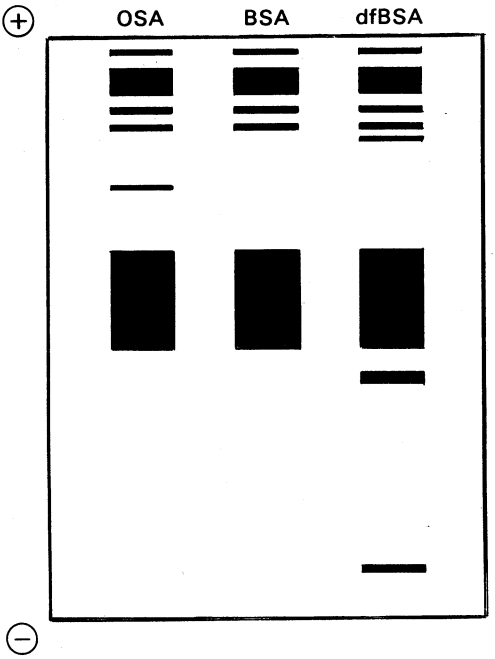

Fig. 2. Homogeneous polyacrylamide gel after electrophoresis of commercial preparations of ovine (OSA), bovine (BSA) and defatted bovine (df BSA) serum albumin in the presence of 0·1% sodium dodecyl sulfate. (See Table 2 for details of commercial preparations.)

Discussion

The principal protein in fraction 3 from ovine serum was albumin. The compositions of fraction 3 and the three purchased preparations of serum albumin were broadly similar, at least in terms of the amounts of the various proteins present. Attempts were made to separate ovine albumin from the other serum proteins present in fraction 3 by chromatography. Albumin may be separated from other proteins of similar molecular weight in human plasma by using columns of Cibacron Blue-Sepharose (Travis *et al.* 1976). However, the mechanisms of binding of human and ruminant albumins to this gel medium differ (Leatherbarrow and Dean 1980), and we were unable to obtain satisfactory preparative separations in the case of ovine albumin. Hence, it remains unclear whether albumin and/or other proteins present in fraction 3 and the commercial serum albumin preparations supported the development of morulae into expanded blastocysts.

Other points of interest arise from the results of experiment 2. Although relative potencies were not determined, it seems likely that fraction 3 and the commercial preparations of ovine, bovine and defatted bovine serum albumin all had similar potencies in this *in vitro* culture system, provided the media were supplemented with 5% of the <10 kDa component of serum. The inclusion of this component increased the proportion of ovine morulae that developed into expanded blastocysts in media supplemented with the >10 kDa component and fraction 3 of serum (Batt and Miller 1988), and the present batches of ovine and defatted bovine serum albumin, but not in a medium supplemented with the present batch of 'normal' bovine serum albumin. Without further studies, the significance of this anomaly is unclear. Perhaps the proteins in this batch of 'normal' bovine serum albumin contained a larger amount of bound low-molecular-weight substances, so that there was no requirement for the <10 kDa component of serum as a source of these or equivalent small molecules. The anomaly cannot be explained by the presence of a unique protein(s) in this preparation of bovine serum albumin.

In the absence of added fatty acids or other energy substrates, very few rabbit embryos develop to expanding blastocysts in culture media that contain commercial preparations of

defatted bovine serum albumin as the sole source of macromolecules (Kane and Headon 1980). In experiment 2 the presence of bound fatty acids *per se* made no difference to the proportion of sheep morulae that developed fully in media containing bovine serum albumin, provided that the media were supplemented with 5% of the <10 kDa component of serum. This result could be interpreted to indicate that the <10 kDa component of serum is a satisfactory source of energy substrates for ovine morulae. This component may also provide a source of specific embryo growth factors. Nevertheless, 10% of morulae (2/20 in Group 9) did develop to expanded blastocysts in the apparent absence of any energy source. Since the charcoal treatment used to remove fatty acids from this preparation of defatted bovine serum albumin also removes a variety of other bound small molecules (Chen 1966), further experiments are required to fully interpret these results. At this stage, it seems reasonable to conclude that different lots of commercially prepared serum albumin commonly differ in their abilities to support the development of ovine morulae into expanded blastocysts when added to a simple bicarbonate-buffered medium, but these differences disappear when the basal medium is also supplemented with the <10 kDa component of serum. This suggests that it is variation in the nature and amount of bound low-molecular-weight material that explains the varying ability of different serum albumin preparations to support embryo development.

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