parasite could avoid immune destruction by activating C1, thereby rendering the lytic process inoperative even in the presence of complement fixing antibody. In addition, trypanosome infected hosts are predisposed to secondary infections, perhaps partly as a result of general destruction of lymphoid tissues, but also as a consequence of diminished lytic processes of infective agents. Deprivation of complement has been shown in experimental animals by Pepys et al.[9–12] (mouse) and White et al.[13] (chicken) to cause disorganization of the architecture of lymphoid tissues. This could also be the case in trypanosomiases in some instances of which marked deterioration of the thymus, lymph nodes, spleen and bone marrow has been reported[6]. Further, the decrease in complement levels in trypanosome infected animals along with the reported increases in serum IgM[3] raises some interesting questions with respect to the influence of this parasite on the homeostatic mechanism governing antibody production. Kohler and Mueller-Eberhard[14] in addition to Glovsky and Fudenberg[15] reported that in some individuals with Waldenstroms macroglobulinemia C1 levels were decreased. In fact, the former authors demonstrated a direct correlation between C1q levels and IgG levels. These findings, along with those of Pepys[9,12] and Nielsen and White[16] who reported homeostatic defects in animals treated with cobra venom factor support premises made with regard the necessity of complement for production of antibody of different immunoglobulin classes.

Finally, it becomes essential to study further the mechanism by which trypanosomes are able to activate complement in order to understand the pathogenesis of this disease. Secondly, it will be necessary to ascertain the impact of this organism on immunoglobulin regulatory mechanisms, as it would appear to be essential knowledge not only to research on protective immunity to the parasite but also for its use as a natural animal model for the future elucidation of the homeostatic mechanism of antibody biosynthesis.

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Some properties of the goat placental lactogen

S. Bečka, J. Bílek, J. Slaba, J. Škarda and I. Mikuláš[1]

Institute of Animal Physiology and Genetics, Czechoslovak Academy of Sciences, Praha-Uhříněves (Czechoslovakia), 27 October 1976

Summary. Goat placental lactogen was partially purified from a medium collected after placental tissue incubation. The data obtained by disc electrophoresis and isoelectric focusing experiments, as well as by means of radioreceptor assay methods, provide evidence of the similarity between the goat and ovine placental lactogen.

Recently, ovine and bovine placental lactogens have been purified[2–4] and the existence of goat placental lactogen has been demonstrated[5–7]. In the present paper, some properties of partially purified goat placental lactogen (gPL) are described.

Materials and methods. Placental tissue of Czech white goats at week 17 of pregnancy was used. Explants of about 20 mg were prepared and batches of 10–15 explants were put into Petri dishes containing 4 ml of Waymouth's medium[8]. The medium contained 2 mg/ml glucose, 0.01% penicillin-G, 0.005% streptomycin and 0.002% mycostatin. The tissue explants were incubated at 37 °C in an atmosphere of 95% O_2 and 5% CO_2. The incubation medium was replaced with fresh medium every 24 h. The proteins of the collected medium were gel filtrated on a Sephadex G-25 column, equilibrated with 0.1 M NH_4HCO_3, and freeze-dried. Disc electrophoresis was made on 7.5% polyacrylamide gel (pH 8.9)[9]. Isoelectric focusing was performed on 7.5% polyacrylamide gel columns prepared by photopolymerization[10]. Lactogenic activity of the proteins was estimated using a mouse mammary gland bioassay in vitro[11]. Placental lactogen was monitored by a radioreceptor assay for prolactin (RRA-PRL) using the rabbit mammary gland receptor[12]. Somatotrophin-like activity was estimated by a radioreceptor assay (RRA-GH) employing rabbit liver receptor[13]. The following hormones served as the tracers and the standards: ovine prolactin (30 IU/mg)[14] and bovine growth hormone (1.4 IU/mg: prepared according to the method of Prusík and Braun[15]). The iodinated hormones were prepared by the lactoperoxidase method[16].

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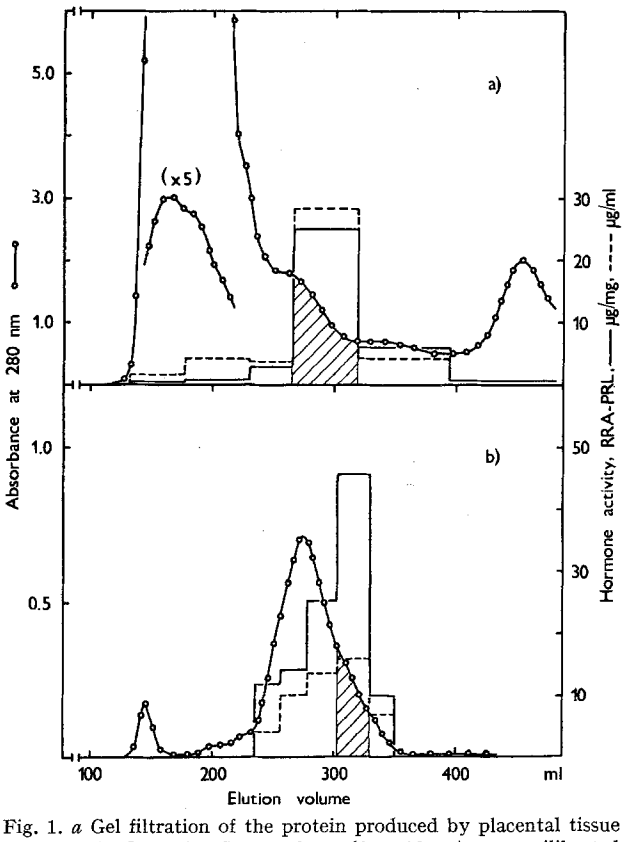

Fig. 1. *a* Gel filtration of the protein produced by placental tissue in vitro. The Sephadex G-100 column (2.5 × 98 cm) was equilibrated and eluted with 0.1 M ammonium bicarbonate, pH 8.0 at 15 ml/h. *b* Gel filtration of the placental lactogen rich fraction (see *a*) under the same conditions. Hormone activity in the radioreceptor assay for prolactin is given in µg of standard ovine prolactin per mg protein (solid line) and on the same scale in µg/ml of eluate (broken line).

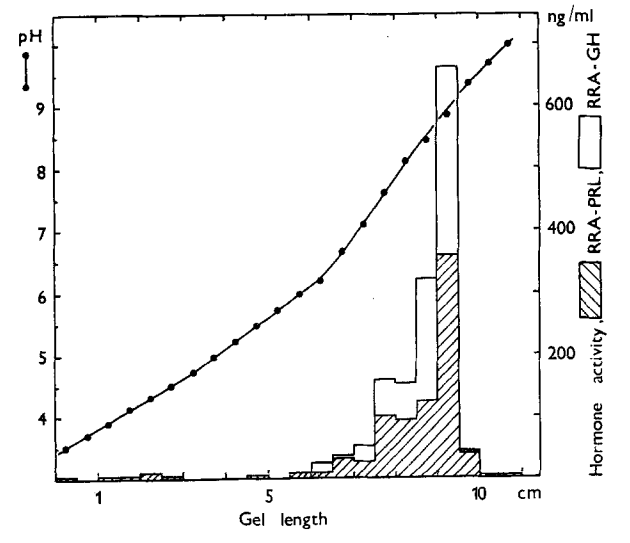

Fig. 2. Isoelectric focusing of the partially purified goat placental lactogen (0.1 mg). The focusing was performed on 7.5% polyacrylamide gel columns (6 × 110 mm) at 4 °C, 200 V, 19 h, using Ampholine (LKB) with a pH range 3–10. The gels were cut in the 0.5 cm segments which were extracted by the solution (2 ml) of 0.025 M-tris, containing 10 mM $CaCl_2$, pH 10, and the extracts were adjusted to pH 7.6. Hormone activity is expressed in ng of standard equivalents (ovine prolactin for RRA-PRL and bovine growth hormone for RRA-GH) per ml extract.

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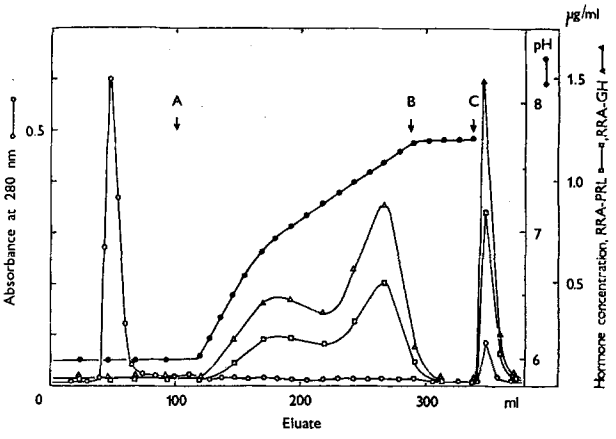

Fig. 3. Ion-exchange chromatography of the partially purified goat placental lactogen (6 mg) on SP-Sephadex C-50 column (1 × 15 cm), equilibrated and eluted by 0.05 M ethylenediamine-acetate, pH 6.0 at + 4 °C. The following buffers were used: *A*, a 200 ml gradient from 0.05 M ethylenediamine-acetate, pH 6.0 (100 ml) to 0.05 M ethylenediamine-acetate, pH 7.7 (100 ml); *B*, 0.05 M ethylenediamine-acetate, pH 7.7; *C*, 0.05 M ethylenediamine-acetate, pH 7.7 containing 0.5 M NaCl. The column was eluted at 8 ml/h.

Results and discussion. In a 4-day incubation, 1340 mg of proteins were obtained from 50 g of placental tissue. The total lactogenic activity of proteins was equivalent to 1530 µg of the standard ovine prolactin (in vitro bioassay). The pooled proteins were subjected to gel filtration on Sephadex G-100 (figure 1, a). Fractions with maximum RRA-PRL (figure 1, a) and lactogenic activities (in vitro bioassay) were pooled, lyophilized and subjected to the second gel filtration on the same Sephadex G-100 column (figure 1, b). The peak of RRA-PRL (figure 1, b) corresponded in its elution volume to a mol. wt of approx. 22,000; this is in accordance with the data of Buttle et al.[5] and Currie[17]. The lactogenic activity (bioassay) of 1 mg of the partially purified gPL (the shaded area on figure 1, b) was equivalent to 45,600 ng of the standard ovine prolactin; activities of 1 mg of the same gPL in RRA-PRL and RRA-GH were equivalent to 45,500 ng of standard ovine prolactin and 40,300 ng of the standard bovine growth hormone, respectively. No separation of the RRA-PRL and RRA-GH activities was observed in both the isoelectric focusing (figure 2) and the ion-exchange chromatography experiments (figure 3). By isoelectric focusing, the maximum of both activities was focused at pH 8.8 and a small part of active material was focused in the more acidic region. After the ion-exchange chromatography, the active material appeared in 2 peaks (similarly as with bovine placental lactogen[3]) and a part of the active material was displaced from the column by 0.5 M NaCl. The ion-exchange chromatography has shown that our preparation of gPL is not homogenous and contains a bulk of inactive proteins. In course of the separation procedure, the ratio of the RRA-PRL vs. RRA-GH activity fluctuated from 1:2 to 1:1. During disc electrophoresis, the maximum RRA-PRL activity was eluted from a segment with $R_F = 0.14$ mobility.

It may be inferred from the isoelectric focusing and ion-exchange chromatography experiments that the RRA-PRL and RRA-GH activities are the properties of a single substance. RRA-PRL vs. RRA-GH activity ratio as well as the isoelectric point pI = 8.8 and mobility $R_F = 0.14$ (disc electrophoresis on 7.5% polyacrylamide gel, pH 8.9) show a similarity of gPL to ovine placental lactogen which was purified and characterized by Chan et al.[2]. Evidently, this similarity is a consequence of the phylogenetic proximity between the goat and the sheep.