Chromatographic, immunochemical and electrophoretic studies of human uterotrophic placental factor (hUTPF)

M P BORIC, G IÑIGUEZ, I ROJAS, A SALINAS, E PEREZ and F BEAS

Instituto de Investigaciones Materno Infantil (IDIMI), Facultad de Medicina, Universidad de Chile, Santiago, Chile.

The human uterotrophic placental factor (hUTPF) is a protein obtained from human term placentae and acts on uterine growth, mammary gland, and blastocyst development and implantation. In the present work, we further define some molecular characteristics of hUTPF using chromatographic, electrophoretic and immunochemical methods.

It is concluded that in human term placenta a high molecular weight hUTPF is present, bound to albumin and immunoglobulins, which could represent a storage or transport form of this factor. hUTPF presents several molecular forms, one of them of 270 kDa and others of approximately 90 kDa and 27 kDa.

INTRODUCTION

Beas *et al.* (1) have reported in extracts of human term placenta the existence of a protein factor which is capable of provoking the growth of the uterus of prepuberal female mice. It has been called human uterotrophic placental factor (hUTPF).

It is now known that hUTPF is a protein obtained from human term placenta that stimulates growth and DNA synthesis in the uterus (2). This substance exerts also a similar action to that of prolactin on the pigeon crop sac and it inhibits the development of the mammary gland induced by estrogens and progesterone in rats (6, 7, 8). These effects are not due to possible contaminants such as estrogen, progesterone and chorionic gonadotrophin, substances that stimulate the organs mentioned above. In addition, hUTPF has been shown to produce effects both on the blastocyst and on implantation (17, 18, 19). The presence of a similar protein in bovine term placenta has been recently demonstrated (4, 5). So far, the active placental fraction has been obtained from normal term placentae by acetic acid extraction and filtration in Sephadex G-75.

The present work shows the results of experiments with native hUTPF, using chromatographic and electrophoretic analyses, as well as immunochemical and biologic activity studies, which allow further characterization of hUTPF.

METHODS

Columns C10/40, C26/100, K50/100, XK50/100, K9/60, K16/70 from Pharmacia-LKB (Uppsala, Sweden) were used in chromatographic fractionations. All chromatographic separations were done at room temperature with descending flow maintained with a peristaltic pump. UV detectors, power sources, 6520-8, 6420-4 and 2112 recorders, fraction collectors Ultrorac 7000 and Helirac 2210, and peristaltic pumps were used, all from Pharmacia-LKB.

Protein determinations were done according to Lowry *et al.* (14).

The molecular weight (MW) standards were from Pharmacia-LKB and from Sigma. Sephadex, Sephacryl, Sepharoses, octadecyl silica gel, Protein A-peroxidase and 4-Cl-1-naphthol were supplied by Sigma. Immunoglobulin antiserum was obtained from

Correspondence to: Dr Francisco Beas, Instituto de Investigaciones Materno Infantil (IDIMI), Hospital San Borja-Arriarán, Casilla 226-3, Santiago, Chile. Fax: (56-2) 551-6301.

Instituto de Salud Pública de Chile. The rest of the reagents were of analytical grade.

Preparation of native hUTPF

Native hUTPF was obtained in our laboratory from human term placenta as described elsewhere (1), with the following modifications: sections of similar size from 3 term placentae were washed and homogenized with 0.15 M NaCl (4 ml/g tissue); this saline homogenate was lyophilized and subjected to extraction with 0.1 N acetic acid (40 ml/g dry powder). The suspension was left under continuous horizontal stirring at 4° C for 16 h; it was then filtered and lyophilized and a placental acetic extract (A.E.) was obtained. The A.E. (2 g), dissolved in 0.1 N acetic acid, was chromatographed on Sephadex G-75. The active fractions obtained were lyophilized.

Preparation of anti-UTPF sera

Antiserum to native hUTPF was obtained using the method of Vaitukaitis *et al.* (22), by immunization of New Zealand virgin female rabbits with native hUTPF. The serum obtained was absorbed with a pool of non-pregnant women sera to remove antibodies against non-specific antigens of gestation. Immunological studies against hUTPF were carried out by double immunodiffusion (15) using this antiserum, and the presence of a single precipitation band was observed. The antiUTPF Sephadex G-200 serum was obtained using UTPF-1 Sephadex G-200 as the antigen (see below); this antiserum was absorbed with non-pregnant woman serum and it was purified by the caprylic acid method (20). The IgG fraction obtained from antiserum antiUTPF-1 Sephadex G-200 was purified by immunoadsorption with octadecyl silica gel, according to the method of Chiong *et al.* (9).

Uterotrophic action

The biological effect of the placental protein on uterus weight was determined by the method of Klinefelter *et al.* (12). Prepuberal 19-21 day-old female mice (Balb/c strain) were injected intraperitoneally with three doses of the placental protein at 24 h intervals and sacrificed 72 h after the first injection. The uteri were carefully dissected and immediately weighed on a precision microtorque balance to 0.2 mg accuracy.

Chromatographic methods

The fraction called native hUTPF was subjected to the following chromatographic analyses: ion exchange chromatography in DEAE Sephadex A-25, affinity chromatography (Sepharose 4B-CNBr and Concanavalin A-Sepharose 4B), exclusion chromatography on Sephacryl S-300 sf and Sephacryl S 300 HR, and on Sephadex G-200. Fractions designated as UTPF-1 Sephadex G-200 and UTPF-2 S-300 HR (see Results) were chromatographed on Concanavalin A Sepharose. The fractions thus obtained were dialyzed and lyophilized.

Polyacrylamide gel electrophoresis (PAGE)

The method of Laemmli (13) with some modifications was followed. 10 x 9.5 cm plates with 1.2 mm thick spacers were used. The resolution gel was 12% acrylamide and the concentration gel was 4%. The sample was applied in 6 M urea.

Gels in continuous linear gradients were performed according to Hashimoto *et al.* (11). The acrylamide concentration ranged from 4% to 12%; reducing and non-reducing conditions were used.

Western-blot analysis

Electroimmunotransference was carried out according to the method of Towbin *et al.* (21) with some modifications: transfer time, 20 h; saturating solution, 5% lactose - 1% casein; conjugate, Protein A bound to peroxidase.

RESULTS

Native hUTPF preparation

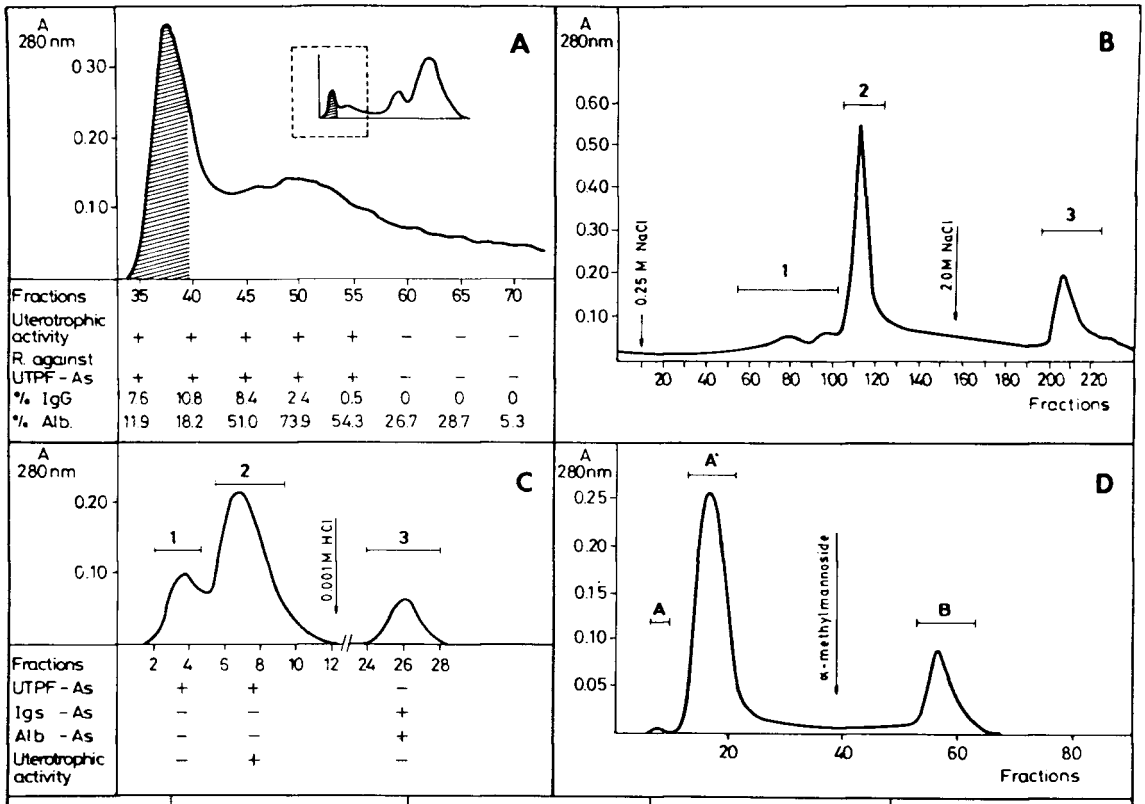
Figure 1 A shows the first part of a Sephadex G-75 elution profile of a placental extract, as

described in Methods. We previously demonstrated that this material produces uterine growth (2). Each fraction was assayed for uterotrophic activity and reaction against native UTPF antiserum. Both the uterotrophic response and the reaction against UTPF-As were positive in fractions 36-57. These fractions also contained variable amounts of immunoglobulin G (IgG) and albumin, which were determined in each fraction (radial diffusion). The pooled fractions 34-39 (shaded area) have been called "native hUTPF" and in this pooled fractions the uterotrophic activity was also determined. The uteri weights were 6.5 ± 0.8 mg in control animals (n = 14) and 9.7 ± 1.1 mg in native hUTPF treated animals (n = 19) ($p < 0.001$).

Ion exchange chromatography

Figure 1B shows the fractionation of native hUTPF on DEAE Sephadex A-25. Two fractions were eluted with ionic strength 0.25 M NaCl (UTPF-1 DEAE and UTPF-2 DEAE) and one with 2.0 M NaCl (UTPF-3 DEAE). The immunochemical study of these fractions showed that all of them precipitated with the native UTPF antiserum.

When mice were injected with the three fractions, either separately or together, all presented uterotrophic action (Table I).



	A	B	C	D
Colum size	5.0 x 100 cm	1.6 x 42.5 cm	0.9 x 9 cm	0.9 x 55 cm
Flow rate	135 ml/h	0.9 ml/min	35 ml/h	1 ml/min
Fraction volume	14.0 ml	3.2 ml	10 ml	3.2 ml

Fig. 1: Elution profiles of hUTPF subjected to different chromatographic separations. A. Sephadex G-75; 2 g of acetic extract in 0.1 N acetic acid. Shaded zone corresponds to so called native hUTPF. B. DEAE Sephadex A-25; 8 mg native hUTPF in 0.01 M sodium glycinate, 0.25 M and 2.0 M NaCl, pH 9.5. C. Sepharose 4-B CNBr coupled to immunoglobulin antiserum; native hUTPF, 2.5 mg protein, in 0.001 M phosphate buffer, pH 7.6. D. Concanavalin A Sepharose; 40 mg of native hUTPF in 0.02 M tris HCl, 0.2 M NaCl, pH 7.4, followed by 0.2 M alpha-D-methylmannoside in the buffer.

TABLE I

Uterotrophic activity of chromatographic fractions of native hUTPF on DEAE-Sephadex A-25

Fraction	Uteri weight (mg)	n	P *vs.* control*
Control	6.5 ± 1.0	16	
1	9.3 ± 1.0	6	< 0.005
2	9.9 ± 1.0	18	< 0.005
3	10.3 ± 0.4	4	< 0.005
1 + 2 + 3 (pooled)	8.1 ± 0.4	6	< 0.005

Means ± SD's.
Control = NaCl 0.15 M.
* Student's *t*-test.

Sepharose 4B-CNBr affinity chromatography

Figure 1C shows the chromatography of native hUTPF on Sepharose 4B-CNBr coupled to immunoglobulin antiserum (Igs-As). Both peaks obtained, UTPF-1 CNBr and UTPF-2 CNBr, showed immunological activity against UTPF-As, and no reaction to Igs-As or albumin-As (double immunodiffusion). The Sepharose-bound protein fraction (UTPF-3 CNBr), later eluted with 0.001 M HCl, reacted against Igs-As and albumin-As but not against UTPF-As. Assays of uterotrophic activity showed that fraction UTPF-1 CNBr had no detectable biological activity. Fraction UTPF-2 CNBr had an uterotrophic response similar to that of the original batch (native UTPF). Uteri weights were: 5.7 ± 0.4 mg in control animals (n = 3), and 7.3 ± 0.8 mg in UTPF-2 CNBr treated animals (n = 14) ($p < 0.001$).

Affinity chromatography on Concanavalin A-Sepharose 4 B

Figure 1D shows the elution profile of native hUTPF chromatographed on Concanavalin A-Sepharose 4 B (Con A). Three peaks were obtained: UTPF-A Con A and UTPF-A' Con A are not retained by Con A and correspond mainly to immunoglobulins and albumin, respectively. The third peak, UTPF-B Con A was desorbed by the addition of alpha-methyl-D-mannoside. UTPF-B Con A did not present immunological reaction against immunoglobulin or albumin antisera (data non shown) and this fraction retained significant uterotrophic activity. Uteri weights were: 5.6 ± 0.6 mg in controls (n = 5) and 9.7 ± 1.7 mg in UTPF-B Con A treated animals (n = 7) ($p < 0.001$). The UTPF-B Con A fraction subjected to electrophoresis in the presence of SDS resolved into 6 bands: one that did not enter the gel and 5 fractions with MW's of approximately 10 to 70 kDa (Fig 3, lane 2).

Native hUTPF fractionation on Sephacryl S-300 sf

Figure 2A shows the elution profile of native hUTPF on Sephacryl S-300 sf. Two peaks were resolved; one of them is excluded from the gel. The identification of the eluate by double immunodiffusion showed that the first peak contained hUTPF and immunoglobulin G (IgG) and the second peak, hUTPF and albumin (data not shown).

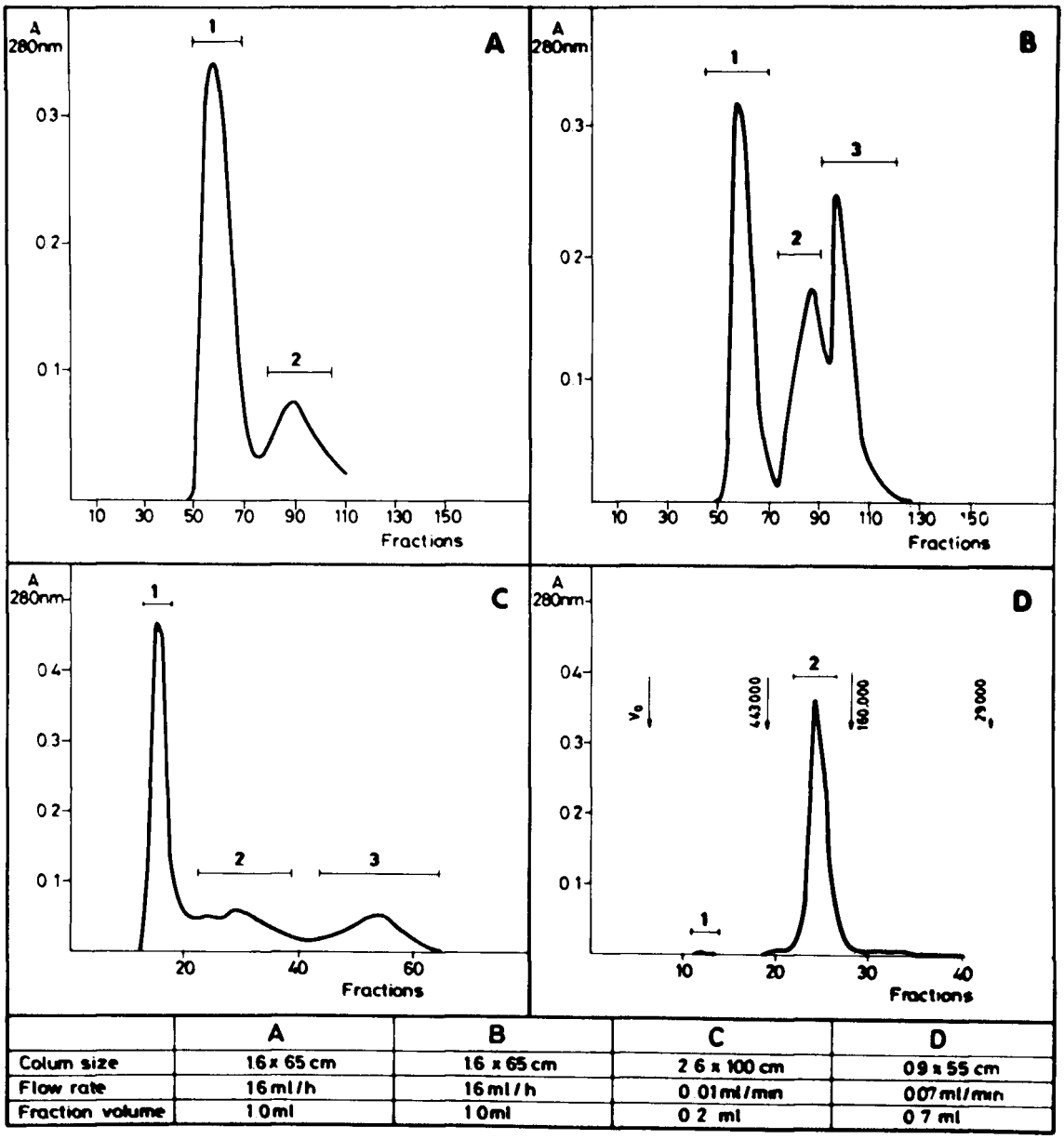
Fractionation of UTPF-B Con A on Sephacryl S-300 sf

When fractionating on Sephacryl S-300 sf the fraction B eluted from the Con A column (subjected to dialysis, lyophilization and dissolution in PBS), three elution peaks were obtained (Fig 2B). The MW's of these fractions estimated from their Kav were > 440, 60 and 25 kDa, respectively. The eluates collected were separated into frac-

tions designated as: UTPF-B Con A, 1 S-300; UTPF-B Con A, 2 S-300, and UTPF-B Con A, 3 S-300, which were dialyzed and lyophilized. The material obtained from lyophilization was redissolved and its immunochemical reaction to specific antisera was determined. In all cases one single precipitation band was obtained against the native antiUTPF serum (data not shown).

Fractionation of native hUTPF on Sephadex G-200

When native hUTPF was chromatographed on Sephadex G-200 and eluted with PBS pH 7.4, an elution profile with 3 peaks was obtained (Fig 2C). The first peak called UTPF-1 Sephadex G-200 was excluded from the gel and its MW was > 200 kDa. The



	A	B	C	D
Colum size	1.6 x 65 cm	1.6 x 65 cm	2.6 x 100 cm	0.9 x 55 cm
Flow rate	16 ml/h	16 ml/h	0.01 ml/mn	0.07 ml/mn
Fraction volume	1.0 ml	1.0 ml	0.2 ml	0.7 ml

Fig. 2: Elution profiles of hUTPF subjected to different chromatographic separations. A and B. Sephacryl S-300 sf in 0.01 M sodium phosphate buffer, pH 7.8, 0.5 M NaCl, 0.02 % NaN_3; 38 mg of native hUTPF were charged in A, and 7.6 mg of UTPF-B Con A in B. C. Sephadex G-200; 100 mg of native hUTPF in 0.01 M phosphate buffer, pH 7.4. D. Sephacryl S-300 HR in 0.1 M acetic acid; 20 mg of native hUTPF.

antiserum prepared with this fraction (antiUTPF Sephadex G-200) did not show reaction to albumin or to immunoglobulin on double diffusion plates.

Fractionation of native hUTPF on Sephacryl S-300 HR

Figure 2D shows native hUTPF chromatographed on Sephacryl S-300 HR and eluted with 0.1 M acetic acid. A very small fraction which elutes on a volume close to Vo and a main fraction called UTPF-2 S-300 HR with a MW of approximately 270 kDa were observed.

Polyacrylamide gels

Figure 3 shows that native hUTPF presents at least 10 bands on polyacrylamide gels (PAGE) with MW's ranging from 10 to 200 kDa (lane 1); UTPF-B Con A has 6 bands with a non-resolved zone (lane 2), and UTPF-1 Sephadex G-200 shows a large band on top and at least three bands that enter the gel (lane 3).

Figure 4 summarizes the results obtained in gels on gradients for native hUTPF, UTPF-1 Sephadex G-200 and UTPF-2 S-300 HR. At non-reducing conditions, it is observed that UTPF-1 Sephadex G-200 enters the fractionating gel and is resolved giving a MW of approximately 270 kDa (Fig 4A1). Under the same conditions, UTPF-2 S-300 HR presents a band of higher intensity whose electrophoretic mobility is equal to that of UTPF-1 Sephadex G-200 (approximately 270 kDa) and several bands of lower MW's (Fig 4 A3).

When these gels are subjected to reducing conditions, it may be observed that the 270 kDa band persists both in native hUTPF and in UTPF-1 Sephadex G-200, as well as in UTPF-2 S-300 HR, but at lower intensity, while two bands of higher intensity appear with approximate MW's of 90 kDa and 27 kDa, respectively (Fig 4B1, B2, B3).

Reaction of UTPF to antiUTPF-1 Sephadex G-200 antiserum

On double immunodiffusion, antiUTPF-1 Sephadex G-200 serum purified by precipitation with caprylic acid was reactive only to native hUTPF, but did not react to the remaining fractions eluted from Sephadex G-75. Furthermore, in double immunodiffusion precipitation reaction, this antiserum reacts to its antigen (UTPF-1 Sephadex G-200) and reacts to UTPF-2 S-300 HR, and a continuous and single precipitation line was observed (data non shown).

On PAGE, the antiUTPF-1 Sephadex G-200 serum presents several bands. When purifying this antiserum with octadecyl silica gel, a single band is observed that coincides with one of the bands of the non-purified antiserum (155 kDa), and would correspond to IgG. In Western-blots, this antiserum recognizes mainly a high MW band of native hUTPF (Fig 5 B1). Also, in Western-blot of

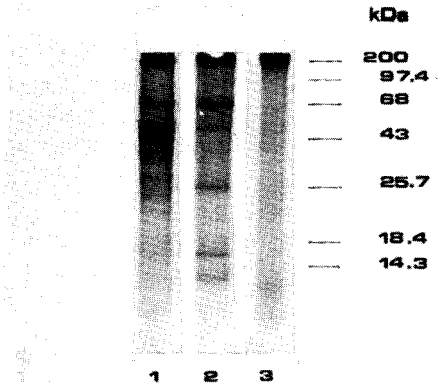


Fig. 3: SDS polyacrylamide gels of different fractions of hUTPF. **1.** Native hUTPF. **2.** UTPF B Con A. **3.** UTPF-1 Sephadex G-200.

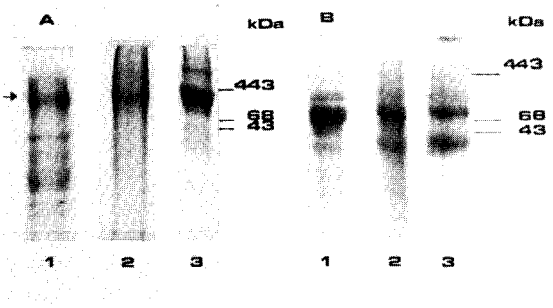


Fig. 4: 4-12% gradient PAGE-SDS of several fractions of hUTPF. **A.** Without ß-mercaptoethanol. **B.** With 5% ß-mercaptoethanol. **1.** Native hUTPF. **2.** UTPF-1 Sephadex G-200. **3.** UTPF-2 S-300 HR. Arrow indicates a band that corresponds to 270 kDa.

the fraction UTPF-1 Sephadex G-200 retained by Concavalin A (UTPF-1 Sephadex G-200, B Con A), and of the fraction of UTPF-2 S-300 HR retained by Concavalin A (UTPF-2 S-300 HR, B Con A), it is observed that the purified antibody antiUTPF-1 Sephadex G-200 also recognizes in both preparations the band that corresponds approximately to 270 kDa (Fig 6B1, B2).

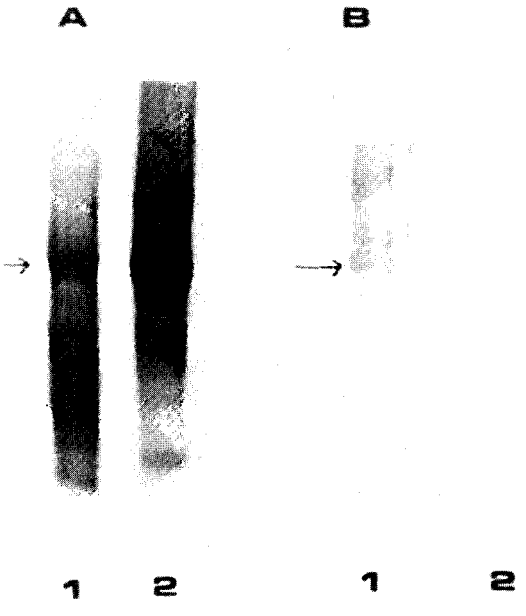


Fig. 5: Characterization of different fractions of hUTPF. A. PAGE-SDS. B. Western-blot with antiserum UTPF-1 Sephadex G-200. 1. Native hUTPF. 2. UTPF-2 S-300 HR. Arrow indicates a band that corresponds to 270 kDa.

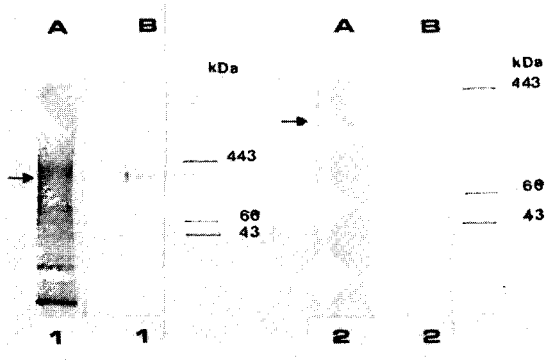


Fig. 6: Characterization of several fractions of hUTPF. A. 4-12% gradient PAGE-SDS without ß-mercaptoethanol. B. Western-blot with antiserum UTPF-1 Sephadex G-200. 1. UTPF-2 S-300 HR, B Con A. 2. UTPF-1 Sephadex G-200, B Con A. Arrow indicates a band that corresponds to 270 kDa.

DISCUSSION

Since its description and in keeping with the methods used for its preparation, hUTPF has been shown to correspond to a polypeptidic substance (1, 2). The present results were intended to further establish its molecular characteristics through chromatographic, electrophoretic and immunochemical methods. All the experiments were run using a pool of only the chromatographic fractions 36 to 39 from placental extract fractionated on Sephadex G-75, a pool designated as native hUTPF. Uterotrophic and immunochemical activity was detected from fractions 35 to 56 of the mentioned preparation. The presence of serum albumin was lesser in the fractions selected.

The studies with ion exchange chromatography (Fig 1B) showed that native hUTPF can be fractionated into three components. All these fractions show a similar uterotrophic activity and reactivity to the antiserum antiUTPF. Neither the uterotrophic activity nor the immunochemical reactivity were affected by the process itself. This was demonstrated by mixing, dialyzing and lyophilizing the three fractions: they presented similar biological activity and reactivity against the antiUTPF serum as the native hUTPF.

Chromatography on Sepharose 4B-CNBr, though with a low yield, achieved a good separation of the UTPF from serum albumin and immunoglobulins.

Chromatography on Concavalin A, as well as producing preparations free from serum albumin and immunoglobulins, gave a better yield and preservation of uterotrophic and immunochemical activity. The fraction retained by Concavalin A showed several bands on 12% PAGE-SDS gels: one that did not enter the gel and at least 5 more of MW's ranging from 10 to 70 kDa.

Native hUTPF is obtained from exclusion chromatography on Sephadex G-75, in the fraction corresponding to Vo. Hence, its MW is > 70 kDa. PAGE-SDS of native hUTPF shows a heterogenous pattern which is not due exclusively to the presence of albumin and immunoglobulins, since the hUTPF fraction obtained by chromatography on Concavalin A –free of albumin and immuno-

globulins– is also heterogenous, though to a lesser extent.

Chromatography of the native hUTPF on Sephadex G-200 eluting with PBS yields three fractions: one of MW > 200 kDa and two smaller ones. When subjecting the fraction of high molecular weight to PAGE-SDS, it is possible to observe a zone which is not resolved unless using gradient, in which case a band of 270 kDa and another of lower MW can be recognized.

Using Sephacryl S-300 HR, another high resolution gel, native hUTPF in 0.1 M acetic acid elutes like a fraction of high MW (approximately 270 kDa) which on gradient PAGE-SDS shows a main band with similar electrophoretic mobility and of higher intensity as compared with the fraction of high MW obtained on Sephadex G-200. It may then be stated that the fraction of high MW obtained at pH 3.0 by fractionation on Sephacryl S-300 HR and that of high MW obtained at pH 7.4 on Sephadex G-200 share common components. This is confirmed by applying gradient PAGE-SDS in reducing conditions. Both preparations showed bands of higher intensity and with MW of 90 kDa and 27 kDa, also present in native hUTPF.

Of the eluted fractions from Sephadex G-75, only that called native hUTPF was recognized by an antiserum prepared against the fraction of high MW obtained by fractionation on Sephadex G-200. This antiserum, which recognizes its own antigen, also reacts to the fraction of higher MW obtained on Sephacryl S-300 HR. Using immunodiffusion, we observed the continuity of the precipitation lines of these three preparations with their antiUTPF serum. Thus, its immunochemical identity becomes evident.

When using Western-blots, the antiUTPF serum 1 G-200 obviously recognizes its antigen but it also recognizes several bands of the native hUTPF, which is a more heterogenous fraction, indicating that the antibody is polyspecific. Nevertheless, when the purified antiserum is subjected to gradient PAGE-SDS, it presents one main band, while on Western-blot it recognizes now in the native hUTPF the same band of 270 kDa which is present in the fraction of high MW obtained on Sephadex G-200. This antibody also recognizes the same band on the fractions retained by Con A both from the high MW fraction obtained on Sephadex G-200 and from the high MW fraction obtained on Sephacryl S-300 HR, indicating that this purified antibody is more specific.

Both the fractions of high MW obtained on Sephadex G-200 and on Sephacryl S-300, as well as native hUTPF, presented a fraction that was retained by Con A. On gradient PAGE-SDS, the fraction of high MW obtained on Sephadex G-200 and retained by Con A showed a band of 270 kDa and a smaller one. On the same electrophoretic system, the fraction of high MW obtained on Sephacryl S-300 HR and retained by Con A presented a band of 270 kDa and several more of smaller size. It is then postulated that the so-called native hUTPF is an aggregate of high MW formed by peptides with uterotrophic action, plus albumin and immunoglobulins. Fractionation by Con A would release the peptides with UTPF biological activity from the albumin and immunoglobulins; in this way, it would be further exposed on electrophoresis to the denaturating action of SDS and urea, giving rise to the bands of lower MW.

The presence of albumin and immunoglobulins in the hUTPF preparation obtained by Sephadex G-75 filtration could be ascribed to contamination. However, after applying different separation methods such as Sephadex chromatography and ion exchange chromatography, we always obtained several fractions with immunological and uterotrophic actions of UTPF, more or less aggregated with albumin and immunoglobulins, except when using Con A and Sepharose 4B-CNBr affinity chromatography. In the last case the yield was very low. These findings suggest that the albumin and immunoglobulins found would not be just contaminants, easily removable by one of the methods used. In the light of these facts there could be two possible interpretations: (a) that the complex UTPF-albumin-immunoglobulins may be an artifact of the method; this is unlikely since in spite of applying a variety of methods and experimental conditions efficient in separating other protein mixtures, we always obtained an aggregate product; (b) that the

association UTPF-albumin-immunoglobulins would correspond to a form of biological storage in the placenta and/or of UTPF transport. The latter hypothesis seems more attractive, since the UTPF immunological reaction has been found not only in the maternal but also in the foetal side (3). The passage of UTPF into the foetus could be explained by the mechanism of pinocytosis described for the transport of high MW proteins (10). This idea would be supported by the experiments of Rachman *et al.* (16) who reported that, in rats, maternal immunoglobulins are gradually transported toward the foetal tissues during the first stages of embryo development.

In conclusion, we have shown that in human term placenta there is an uterotrophic placental factor of high MW bound to albumin and immunoglobulins, which could represent a storage or transport form for this factor. hUTPF presents several molecular forms, one of them of 270 kDa and others of approximately 90 kDa and 27 kDa, which could correspond to different molecular forms of the same substance. This molecular heterogeneity has been described for several proteic hormones and factors. The argument gains support from the evidence that different molecular forms of UTPF, obtained by using different methods, keep immunological and uterotrophic activities similar to those of the native preparation. The elucidation of exactly how these smaller molecular forms are released from the high MW complex, so they can circulate in the plasma and act on target cells, is crucial to the understanding of the UTPF biological action and awaits further studies.

ACKNOWLEDGEMENTS

Thanks are due to C M Barros, M Arcil, F Cacho and S Urquiaga for their contributions to this work. Work supported by grants 91-1009 from FONDECYT, # 9/132/2 and 89004 from OMS, and M-2702 from DTI, Univ. Chile.

REFERENCES

1. BEAS F, FLORES H (1969) Characterization and properties of a new placental protein. Nature 221: 574.
2. BEAS F, SALINAS A, GONZALEZ F, TERAN C, SZENDRO P (1975a) A human placental hormone (UTPH) with uterine growth and DNA promoting effects. Horm Metab Res 7: 515-520.
3. BEAS F, ROJAS I, YAÑEZ L, AGUILERA L (1975b) Niveles plasmáticos de hormona uterotrófica placentaria (UTPH) en embarazadas y recién nacidos. Resúmenes V Jornadas Nacionales de Pediatría, Santiago, Chile.
4. BEAS F, BORIC MA, IÑIGUEZ G, FERRANDO G, MUÑOZ B, MARAGAÑO M (1990) Una nueva proteína placentaria bovina. Monografías Med Vet 12: 59-63.
5. BEAS F, BORIC MA, IÑIGUEZ G, FERRANDO G, MUÑOZ M, MARAGAÑO M (1991) A new bovine placental protein: A possible pregnancy marker. Arch Zootecnia 147: 197-200
6. CALAF G, CAPURRO MT, MENDEZ MC, BEAS F (1987) DNA-synthesis under the effect of a placental protein in mammary gland in culture. Horm Metab Res 19: 43-44.
7. CASTILLO C, BEAS F (1971) Inhibition of estradiol-progesterone-induced mammary development by a human placental protein. Horm Metab Res 3: 361-362.
8. CASTILLO C, BEAS F (1973) Crop sac stimulating activity and mammary inhibition produced by a human placental protein. Horm Metab Res 5: 129-133.
9. CHIONG M, LAVANDERO S, RAMOS R, AGUILLON JC, FERREIRA A (1991) Octadecyl silica: a solid phase for protein purification by immunoadsorption. Anal Biochem 197: 47-51.
10. GITLIN JD, GITLIN D (1973) Cell receptors and the selective transfer of proteins from mother to young across tissue barriers. Pediatr Res 7: 290-298.
11. HASHIMOTO F, HORIGONE T, KANBAYASHI M, YOSHIDA K, SUGANO H (1983) An improved method for separation of low-molecular-weight polypeptides by electrophoresis in sodium dodecyl sulfate-polyacrylamide gel. Anal Biochem 129: 192-199.
12. KLINEFELTER HF, ALBRINGHT F, GRISWOLD GC (1943) Experience with a quantitative test for normal or decreased amounts of follicle stimulating hormone in the urine in endocrinological diagnosis. J Clin Endocrinol 3: 529-544.
13. LAEMMLI UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.
14. LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265-275.
15. OUCHTERLONY O, (1953) Antigen-antibody reactions in gels. IV. Types of reactions in coordinated systems of diffusion. Acta Pathol Microbiol Scand 32: 231-240.
16. RACHMAN F, CASIMIRI V, BERNARD O (1984) Maternal immunoglobulins G, A and M in mouse uterus and embryo during postimplantation period. J Reprod Immunol 6: 39-47.
17. ROBLERO L, BEAS F, ARRAU J (1981a) Embryo development under the effects of a new placental protein (UTPH) and its antiserum. Horm Metab Res 13: 124-125.
18. ROBLERO L, BEAS F, ARRAU J (1981b). Uptake and incorporation of uridine-^{3}H, leucine-^{3}H and thymidine-^{3}H by delayed mouse blastocysts under the effect of a human placental protein (UTPH) and its antiserum. Horm Metab Res 13: 188-189.
19. ROBLERO L, BEAS F, ARRAU J, ROJAS I (1983) Induction of implantation by a human uterotrophic

placental protein (UTPH) in female mice and rats with delayed implantation. Horm Metab Res 15: 53-55.
20. STEINBUCH M, AUDRAN R (1969) The isolation of IgG from mammalian sera with the aid of caprylic acid. Arch Biochem Biophys 134: 279-284.
21. TOWBIN H, STAEHELIN T, GORDON J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76: 4350-4354.
22. VAITUKAITIS J, ROBBINS JB, NIESCHLAG E, ROSS GT (1971) A method for producing specific antisera with small doses of immunogen. J Clin Endocrinol Metab 33: 988-991.