ANTIBODY TO ESTROGEN-INDUCED PROTEIN (IP) AND QUANTIFICATION OF THE PROTEIN IN RAT UTERUS BY A RADIOIMMUNOASSAY

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SUMMARY. Antibodies to a rat uterine estrogen-induced protein (IP) have been prepared in rabbit and characterized by gel diffusion and immunoelectrophoresis. A radioimmunoassay for IP has been developed using this antibody and (^{125}I)-labelled IP. Using this assay, significant levels of IP were detected in uteri of both immature and unstimulated castrated adult rats. Within 12 hours after injection of estradiol, the content of IP increased by almost 100 per cent. The amounts of the protein in mature rats varied according to the stage of estrous cycle.

In 1966, Notides and Gorski (1) detected by starch gel electrophoresis of rat uterus cytosol a protein component, the rate of synthesis of which was increased by estradiol. This protein was called 'induced protein' (IP); its synthesis increased within 30-40 min after estrogen administration (2,3) and was inhibited by cycloheximide (1) and actinomycin D (3,4). IP represents a minor and transient component of the total newly synthesized uterine soluble proteins that can be only demonstrated by a double labelling technique. Synthesis of IP has also been demonstrated in uteri of immature animals incubated in the pre-

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sence of physiological amounts of estradiol (5), and in mature differentiated uteri in response to circulating ovarian estrogens However, the double labelling technique only measures changes in the rate of the synthesis and does not answer the question of whether IP is present in unstimulated uteri. Therefore, it was of interest to raise antibodies against IP in order to devise a radic immunoassay for quantification of this protein in uteri under various physiological conditions. We report here (a) the productio of a specific antiserum against IP in the rabbit (b) characterization of the antibody by immunodiffusion, immunoelectrophoresis and electrophoresis of the specific immunoprecipitate, and (c) the measurement of IP in uterine tissue by radioimmunoassay.

IP was prepared following the described general procedures (7,8 involving DEAE chromatography and electrophoresis on cellulose acetate strips. Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis of the IP preparation showed a major component migrating in the range of 43,000 molecular weight and two minor additional components (molecular weight 88,000 and 26,000) by comparison with protein reference run in parallel (Fig. 1).

Specific precipitating antiserum was prepared in New Zealand albino rabbits as follows. Each animal received an emulsion containing 150 µg of IP, 0.5 ml saline and 0.5 ml complete Freund's adjuvant by intradermal injection distributed equally among four sites in the back. Twenty-one days later a similar emulsion containing half the amount of IP was injected i.m. in the posterior limbs. Forty-five days after the first injection, the rabbits were bled by the ear vein. Antisera formed specific immunodiffusion lines against partially purified IP (DEAE cellulose fraction) and IP further purified by electrophoresis on cellulose acetate strips but did not react against rat serum or rat liver. The pre-immunisation sera did not react with any of the IP-containing extracts, rat serum or rat liver. Upon immunoelectrophoresis, one precipitin line was observed which corresponded to the distance of migration

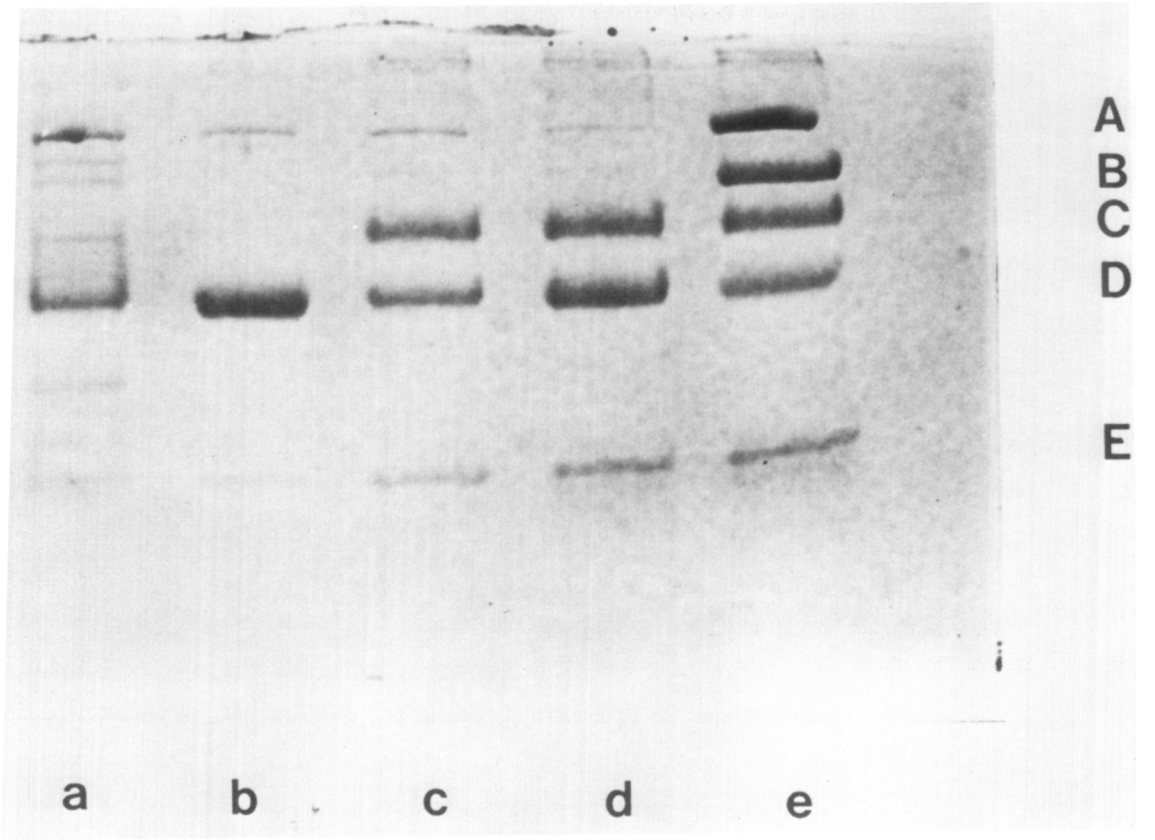

Fig.1

Electrophoresis on SDS-polyacrylamide gel of partially purified IP (DEAE cellulose fraction), purified IP, and immunoglobulin-IP complexes. Samples were boiled for 2 min in 0.05 M Tris, 2% SDS, 0.1 M mercaptoethanol, 10% glycerol, 0.001% bromophenol blue (pH 6.8) and fractionated according to molecular weight on 12% polyacrylamide using a vertical gel slab apparatus (Hoefer Instruments Inc., San Francisco, Calif.). After electrophoresis, the slab was stained with 0.2% Coomassie Blue in 50% methanol and destained in 7% acetic acid. Gel (a) was the DEAE cellulose fraction; gel (b) was the purified IP; gel (c) was an immunoprecipitate obtained by incubating the DEAE cellulose fraction with the antiserum; gel (d) was an immunoprecipitate obtained by incubating purified IP with the antiserum; gel (e) was a mixture of phosphorylase A (A), serum albumin (B), gamma globulin heavy chain (C), ovalbumin (D), and gamma globulin light chain (E).

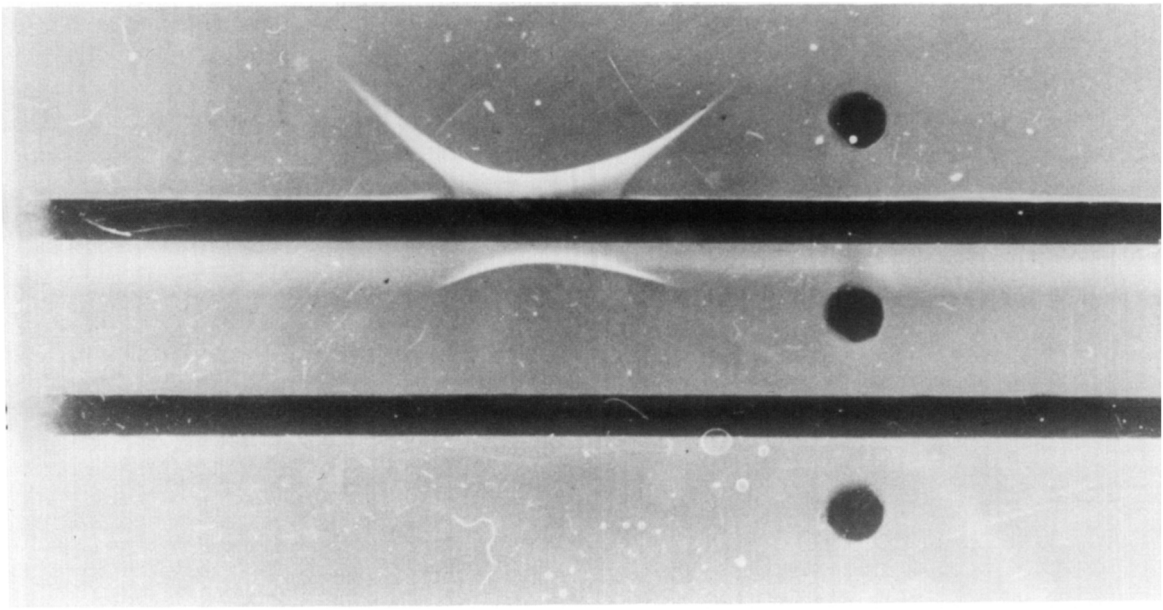

Fig.2

Immunoelectrophoresis of purified IP and crude extract of rat uterus. Electrophoresis was performed on microscope slide coated with 1.5% agarose in Barbital buffer (μ= 0.04, pH 8.6) at 12 V/cm for 90 min. The volume of sample was 10 μl and the amount of antiserum added after electrophoresis was 150 μl. Samples electrophoresed (a) IP, 4 mg/ml; (b) concentrated extract of rat uterus, 45 mg/ml. Sera in immunodiffusion throughs: (1), serum containing specific antibody to IP; (2) pre-immunisation rabbit serum.

of IP, when purified IP or a concentrated extract of rat uterus were used (Fig. 2). Precipitates of immunoglobulin-IP complexes were prepared for electrophoresis by incubating the IP-containing solutions with antisera for 1 hour at 37° followed by 20 hours at 4°. The precipitates were pelleted by centrifugation at 2,000 g for 20 min in the cold, washed twice in saline and prepared for electrophoresis as described in the legend of Fig. 1. As illustrated in Fig. 1, immunoprecipitates obtained by mixing the DEAE cellulose fraction and IP, respectively, with the antiserum showed the major 43,000 dalton IP protein, the 88,000 molecular weight minor component, the 53,000 molecular weight heavy chain and the

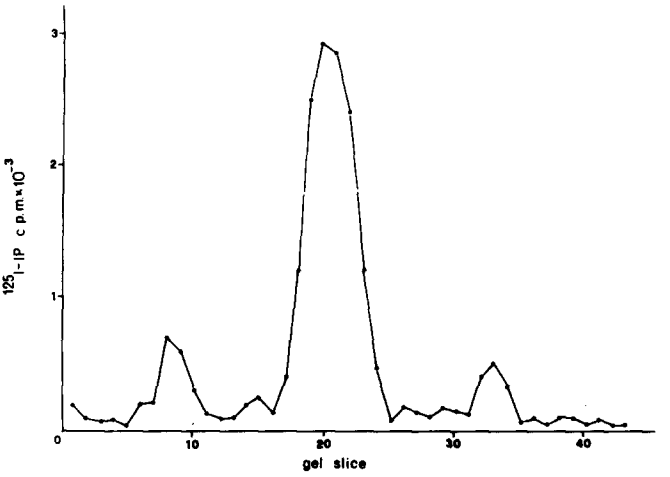

Fig.3

^{125}Electrophoresis on SDS-polyacrylamide gel of immunoprecipitated ^{125}I-labelled IP. Precipitate was solubilized by boiling for 2 min with a solution containing Tris, SDS, and mercaptoethanol and subjected to electrophoresis on 12% polyacrylamide gel slab as described in the legend of Fig. 1. At the termination of the run, the gel was sectioned onto 2 mm-slices for counting.

25,000 molecular weight light chain of dissociated immunoglobulin. The 26,000 dalton component co-migrates with the light chain immunoglobulin. These electrophoretic analyses demonstrated that the antiserum reacts specifically with the major and the two minor components of the IP preparation used as immunogen in the rabbit.

Iodine labelling of IP was performed by a modification of the method of Hunter and Greenwood (9). To approximatively 1 mCi of Na^{125}I (The Radiochemical Center, Amersham) in a small plastic tube were added in turn, 10 µl of 0.4 M Na phosphate buffer (pH 7.6), 10 µg of IP and 25 µg of chloramine T in a final volume of 35 µl. Sodium metabisulphite (50 µg in 0.2 ml) was then added and the reaction mixture was immediately passed over a Sephadex G 50 column (0.7×15 cm) pre-coated with 0.1 per cent bovine serum albumin (BSA). Fractions (0.2 ml) were collected into tubes containing 0.5 ml of 0.1 per cent BSA in phosphate buffered saline, pH 7.3. The most immunoreactive ^{125}I-IP found in fractions 8 and 9 was used as antigen in the following radioimmunoassay. SDS-gel analysis

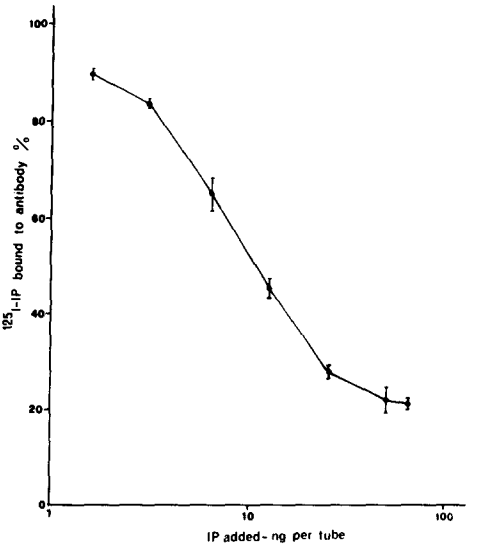

Fig.4

Radioimmunoassay standard inhibition curve for IP. The incubation mixture consisted of 100 µl anti-IP antiserum (1:5,000 in 2.5% normal rabbit serum in PBS) and increasing quantities of IP solutions made up to 50 µl with PBS. After 30 min at 37°, 50 µl of ^{125}I-labelled IP (11,500 c.p.m.) was added and the incubation continued at 37° for 4.5 hours. Hundred microliters of 1:10 dilution of anti-rabbit gamma globulin made in PBS containing 2 mM Na_2EDTA was then added to the tubes. Tubes were incubated for 60 m at 37° followed by 15 min at 2° and centrifuged at 10,000 g for 5 mi Precipitates were counted in a Nuclear Chicago Automated gamma spectrometer. Means ± s.e.m. are shown.

of this ^{125}I-labelled IP after incubation with anti-IP serum, followed by anti-rabbit gamma globulin (see legend of Fig. 4) revealed approximatively 80 per cent of the counts in the 43,000-dalton IP protein (Fig. 3).

Radioimmunoassay of IP was utilized for quantification of the IP content in uteri under different hormonal conditions. As shown in Table 1, significant levels of IP can be demonstrated in uteri of both immature and unstimulated castrated adult rats even 120 day after ovariectomy. Thus, the relative stability of IP in estrogen-deprived rat uteri is at variance with the rapid decline following

Table 1. IP content of immature and ovariectomized rat uterus

Tissue	IP (ng/mg protein)
Immature rat uterus	560 ± 22
Ovariectomized rat uterus (48 hours)	487 ± 20
Ovariectomized rat uterus (120 days)	424 ± 16
Ovariectomized rat uterus plus estradiol (12 hours)	958 ± 22
Immature rat uterus plus estradiol (12 hours)	1025 ± 15

Values are mean ± s.e.m. of triplicate analyses on tissues from 3 rats. Numbers in parentheses indicate time from ovariectomy or administration of estradiol.

Table 2. Variation of IP content in the rat uterus during the estrous cycle

Estrous cycle stage	IP (ng/mg protein)
Metestrus	627 ± 21
Diestrus	689 ± 16
Proestrus	1012 ± 16
Estrus	510 ± 11

Values are mean ± s.e.m of triplicate analyses on tissues from 2 rats.

ovariectomy of the bulk of other uterine proteins (10). Whithin 12 hours after injection of 5 μg estradiol, the uterine content of IP had increased by almost 100 per cent in both immature and castrated adult animals. Furthemore in mature cycling rats, the

level of IP varied according to the stage of the estrous cycle (Table 2), in agreement with previous findings (6) of fluctuactior of the rate of synthesis of IP.

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