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Nadine Martinat, Elisabeth Hall, J. P. Ravault, M. P. Dubois. Purification of rat prolactin : Development of an homologous radioimmunological assay and comparison with the NIAMDD system. *Annales de biologie animale, biochimie, biophysique*, 1979, 19 (6), pp.1771-1788. hal-00897608

HAL Id: hal-00897608

<https://hal.science/hal-00897608>

Submitted on 11 May 2020

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Purification of rat prolactin : Development of an homologous radioimmunological assay and comparison with the NIAMDD system

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Summary. This paper describes the isolation and purification of prolactin (PRL) from rat pituitaries. Six-month old male rats were castrated and given subcutaneous implants of progesterone (20 mg) and estradiol (2 mg). This treatment resulted in the accumulation of amounts of PRL in the pituitary. After 4 to 6 weeks of treatment, the rats were killed and the pituitaries dissected. Using a thorough fractionation procedure, the other pituitary hormones were eliminated. To obtain a PRL preparation with high biological activity, gel filtration on Sephadex G 100 was used. The prepared PRL was studied using physicochemical, biological and immunological methods. From gel filtration data the molecular weight of the PRL thus prepared was approximately 26 000. Using a radioreceptor assay the activity of the preparation was two times higher than the NIAMDD preparation of PRL (NIAMDD-RP₁). Immunofluorescence studies revealed that rabbit antiserum raised against this PRL had affinity for the PRL cells in ovine, bovine and rat pituitaries but none for the other types of pituitary cells. A double antibody radioimmunoassay was developed using the above hormone and its antiserum. Standard curves were plotted over the range of 16 to 400 pg. Rat FSH, LH, TSH and GH did not react in this assay nor did plasma proteins. The intra-assay coefficient of variation was 10 p. 100 and the between assay 16 p. 100. The rat prolactin radioimmunoassay kit distributed by the National Institute of Arthritis and Metabolic Diseases (NIAMD) was also examined. Prolactin levels in rat serum measured by the NIAMD-radioimmunoassay were twice those measured by our system. The apparent differences in serum levels of PRL simply reflected the lower immunologic activity of the NIAMD prolactin.

Introduction.

Pituitary, hypothalamic or external factors as light, which affect the regulation of prolactin secretion and synthesis, are not well known in the rat.

Radioimmunological assay (RIA) measurement of the quantities of circulating hormones or of pituitary content is a necessary step in understanding some of the mechanisms. Many research teams have purified rat prolactin (rPRL) in an attempt to

develop an homologous RIA (Kwa and Verhofstad, 1967 ; Kwa *et al.*, 1967 ; Groves and Sells, 1968 ; Niswender *et al.*, 1969 ; Neill and Reichert, 1971 ; Kuo and Gala, 1972).

However, the only assay available is that distributed by the National Institute of Arthritis and Metabolic Diseases. Some of its characteristics, such as high antibody concentration and weak assay sensitivity (250 pg), are not very satisfactory, requiring relatively large volumes of plasma.

These drawbacks led us to develop an homologous RIA for rPRL using the prolactin extracted in our laboratory. Employing a method based on that of Ellis *et al.* (1969), large quantities of the hormone were taken from the pituitaries of castrated rats treated with estrogens, and were purified.

Our RIA system and that of the NIAMDD have been compared.

Material and methods.

1. — Hormone preparation.

Animals. — Adult Wistar rats (INRA strain O3) about 6 months old, castrated and treated with subcutaneous estrogen implants containing 20 mg of progesterone and 2 mg of estradiol (Implix « bm », Sovetal) were used as pituitary donors. They were killed by decapitation 4 to 6 weeks after the treatment began.

Extraction and purification. — The pituitaries were taken immediately and were weighed (about 40 mg ; mean weight of an untreated, intact rat pituitary: 12 mg) and ground with a potter at 4 °C in a 0.37 M Tris-glycocolle buffer, pH = 9.5 (0.5 ml buffer/

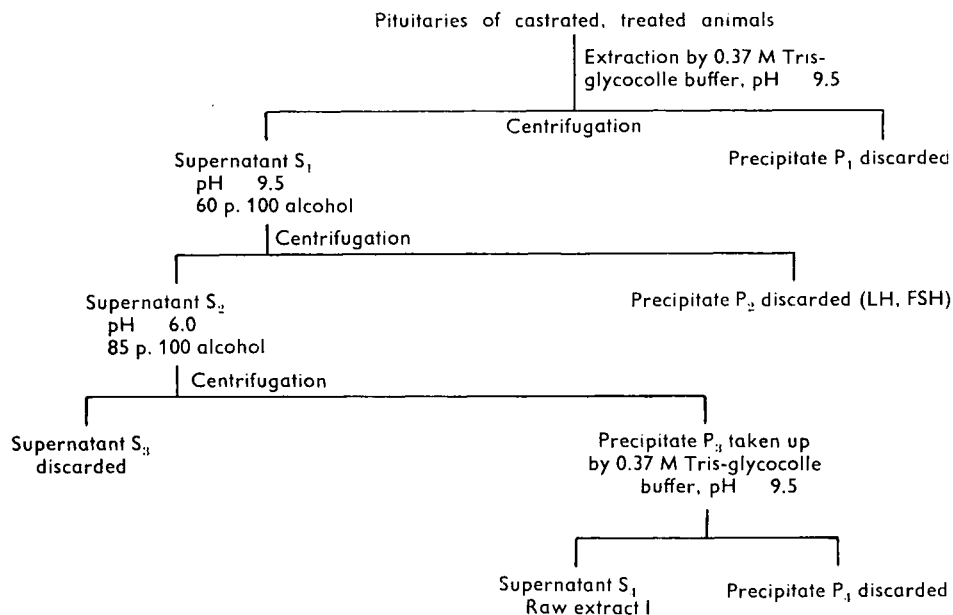


FIG. 1. — Fractionated precipitation.

pituitary). The homogenate was subjected to supersonic treatment (low-power MSE 150 W supersonic disintegrator, amplitude 4, 2 times 10 seconds) to disrupt the cells and release the maximum PRL.

The technique of Ellis *et al.* (1969) using fractionated ethanol precipitation was employed at the beginning of purification. The stages are shown in figure 1.

The PRL was precipitated at pH = 6.0 using an 85 p. 100 ethanol concentration. The supernatant was discarded after centrifugation and the precipitate dried under nitrogen was taken up by 600 to 1 000 μ l of 0.37 M Tris-glycocolle buffer at pH = 9.5. The insoluble part at alkaline pH was eliminated by centrifugation, and the soluble fraction constituted our raw extract I.

At a later stage, the PRL was separated from the other components of extract I by gel filtration on Sephadex G 100 (fig. 2a). The fractions composing each peak were regrouped and freeze-dried. By precipitation to the interface, the different lyophilisates were tested against specific antisera and peak C was identified as the PRL. The electrophoretic migration of each of the peaks on the acrylamide gel was determined using the technique of Hjerten *et al.* (1965) (fig. 3).

The biological activity of our preparation (peak C = rPRL INRA) was assayed *in vitro* to compare it with the NIAMDD RP₁ PRL using the rabbit mammary gland radioreceptor assay (Djiane *et al.*, 1977). NIH ovine prolactin PS₇ (23 IU/mg) was used as a reference and as a radioactive tracer. We searched for a GH contaminant of PRL using the rabbit liver radioreceptor technique of Tsuchima and Presin (1973).

Immunological activity was tested by immunofluorescence on rat pituitary sections; the criterion was the aptitude (or the inability) of the PRL to inhibit the reaction caused by the specific sera in the corresponding cell types.

2. — Antibody preparation.

Antibodies were obtained from 4 Fauve de Bourgogne rabbits by repeated injections of an emulsion of INRA rPRL (v/v) 200 μ g in physiological serum with Freund's complete adjuvant enriched with the dead bacterial bodies of mycobacterium (5 mg of *M. Phlei*/ml adjuvant) (Institut Mérieux, Lyon, France). The first injection was done intrasplenically on an anesthetized animal, and the following ones intradermally (2 series of injections: 1 per week for 4 weeks followed by a month of rest before recommencing the series) at many points on the animal's back. Blood samples were taken after each injection and the antisera were stored in aliquot fractions at -20 °C. The sera obtained were tested by gelose immunoelectrophoresis with the micromethod of Scheidegger (1955) (fig. 4), by immunodiffusion according to the technique of Ouchterlony (1948), and by immunofluorescence for their aptitude (or inability) to reveal identified cell types.

3. — RIA.

Hormone labelling. — Hormones were labelled with ¹²⁵iodine (¹²⁵I) according to the technique of Greenwood, Hunter and Glover (1963).

5 μ g of INRA PRL dissolved in 5 μ l of a 0.05 M phosphate buffer, pH = 7.4, reacted with 500 μ Ci of ¹²⁵I Na (5 μ l; IMS 300 Amersham, France, previously buffered by a 0.5 M phosphate buffer) in the presence of 7.5 μ g of chloramine T (5 μ l). After

20 seconds the reaction was stopped by the addition of 60 μg (10 μl) of sodium metabisulfite. 500 μg of potassium iodide were added to the reactive medium before filtration on Sephadex G 50 (Pharmacia column K9-30 ; elution by a 0.025 M veronal buffer, pH = 8.6, containing 2.5 p. 1 000 HSA and 1 p. 1 000 azide). The labelled hormone thus separated from the free iodide was purified again by filtration on a Sephadex G 100 column (Pharmacia K9-30).

The iodation yield was about 75 p. 100. The B/T immunoreactivity of the different fractions (B : bound radioactivity ; T : total radioactivity introduced) of the G 100 filtration was tested in the presence of antibodies at 1/100. We labelled the NIAMDD I₂ PRL in the same conditions.

Assay conditions. — RIA of rat PRL was carried out in 0.025 M veronal buffer, pH = 8.6 ; HSA 2.5 p. 1 000. The 500 μl of reactive volume in each tube was distributed as follows :

Reactive	Volume (in μl)	Quantity
Anti-rPRL	50	working dilution
PRL or plasma for assay ...	50	between 16 pg and 4 ng
Radioactive antigen	400	5 000 cpm, 7 pg

The antibody was diluted in a buffer containing 1 p. 100 of normal rabbit serum. After the tubes were incubated at 4 °C for 3 or 4 days, the antigen-antibody complex was precipitated for one night at 4 °C by adding 50 μl of sheep serum raised against rabbit gamma globulin (SMAL).

After the addition of 2 ml of buffer, the tubes were centrifuged 30 min at 4 000 g, the supernatant was discarded and pellet radioactivity was measured on a gamma counter. The NIAMDD assay conditions for the use of the kit were maintained.

Collection of assay samples. — To study the possible effect of anticoagulants on circulating PRL content, the blood of the same animal was collected on different supports : heparin (50 μl of liquemine), Na heparinate (vacutainer), Na citrate (Biotrol) and Na₂ EDTA (vacutainer).

We killed young normal male rats at 2 months, castrated males 1 month after castration, and implanted, castrated males 1 month after castration and following a treatment using a solid implant of 2 mg of estradiol + 20 mg of progesterone. The blood was collected immediately after decapitation ; the serums were collected and stored at -20 °C, and the pituitaries treated according to the same experimental procedure as for PRL extraction.

Young rats 21 to 25 days old were hypophysectomized through the ear and killed 15 days later. The serums were collected ; only those animals whose body weight did not increase by more than 10 to 15 g were used (increase of body weight during the same period in intact animals of the same age : 50 to 60 g).

The serums of female rats in proestrus and diestrus were collected from animals having a normal estrous cycle (checked by vaginal smear) of 4 days during 3 successive cycles. All sampling was done between 9 and 10 hrs.

Expression of the results. — The standard curves were expressed in two ways :

— in B/B_0 in terms of the logarithm of the dose of unlabelled hormone (B_0 : radioactivity bound to the antibody in the absence of an unlabelled antigen ; B : radioactivity bound to the antibody for each dose of reference antigen). This mode of expression was used in determining sample PRL concentrations according to the Hewlett Packard calculator program ;

— by logit transformation : the effect of the reactive medium was determined by curve linearization after a study of the regression curves. rPRL contents were presented as the mean plus or minus the standard deviation of the mean ($M \pm SEM$).

Results.

A. — Hormone characteristics.

PRL purification in the conditions described yielded 1.5 to 2 mg per 1 lot of pituitaries (12 to 15), or about 150 μ g of PRL/pituitary or 4 mg of PRL/g of fresh tissue. This preparation seemed homogeneous on the molecular sieve and with acrylamide gel electrophoresis. On Sephadex G 100 (fig. 2b), the peak C had a relative elution volume

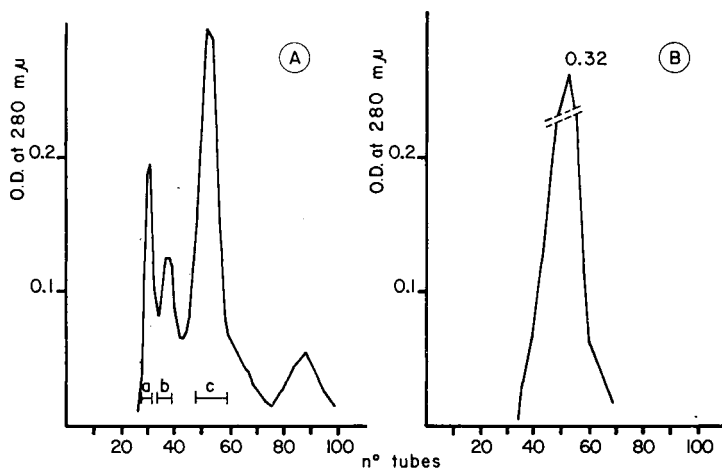


FIG. 2. — A) Elution pattern of raw extract I on Sephadex G 100 column (1×110 cm). $0.1 M NH_4 HCO_3$ buffer. Output : 4.5 ml/h. — 1 ml fraction. — The column was previously tested with the following standard substances : α -chymotrypsinogen (MW = 25 000), VE = 51 ml ; human albumin (MW = 68 000), VE = 36 ml ; dextran blue (MW 2.10^6), V_0 = 30 ml ; alanine DNP (MW = 225), V_t = 84 ml.

B) Elution pattern of a lot of INRA PRL lyophilized in the same conditions as A). VE = 52 ml.

of $V_e/V_o = 1.73$ and a $K_{av} = 0.41$. From graphs of K_{av} variation in terms of the logarithm of the molecular weight of a given type of gel (Andrews, 1964), the extracted protein had a molecular weight of about 26 000.

In electrophoresis, the extracted PRL only gave an anodic single band (fig. 3).

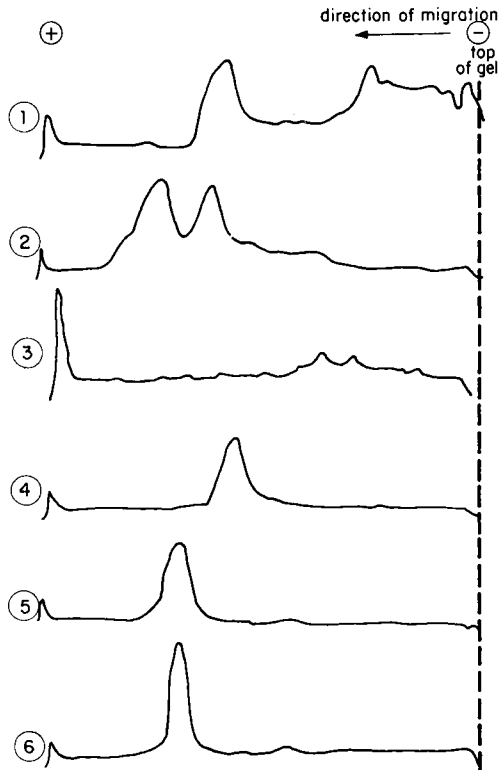


FIG. 3. — Analytical polyacrylamide gel electrophoresis at $pH = 9.5$. The gels ($l = 45$ mm ; $\varnothing = 5$ mm) had only one acrylamide (7.5 p. 100) and bisacrylamide (0.2 p. 100) concentration. A 0.37 M Tris-glycocolle buffer, $pH = 9.5$, was used. Migration required 1 h 30 at the rate of 2 mA/tube. After staining for 30 min in 1 p. 100 amidoschwartz in 7 p. 100 acetic acid, the excess stain was discarded under electric voltage. The gels were recorded at 620 nm on an ISCO densitometer.

1) 50 μ l of rat serum at 1/10 dilution ; 2) raw extract I before passage on G 100 ; 3) 100 μ l of F31 of G 100 ; 4) 100 μ l of F38 of G 100 ; 5) 100 μ l of F52 of G 100 ; 6) 20 μ g of INRA PRL (peak C).

In the biological rabbit mammary gland receptor test *in vitro*, the following activities were found : NIAMDD RP₁ : 1.8 IU/mg compared to NIH PS₇ ; INRA PRL 27/1 : 4.3 IU/mg ; INRA PRL 3/12 : 3.3 IU/mg. (27/1 and 3/12 correspond to 2 lots of PRL). In this test the activity of the extracted prolactin was two times higher than that of the NIAMDD RP₁ PRL (fig. 5).

No GH contaminant was found in our preparation.

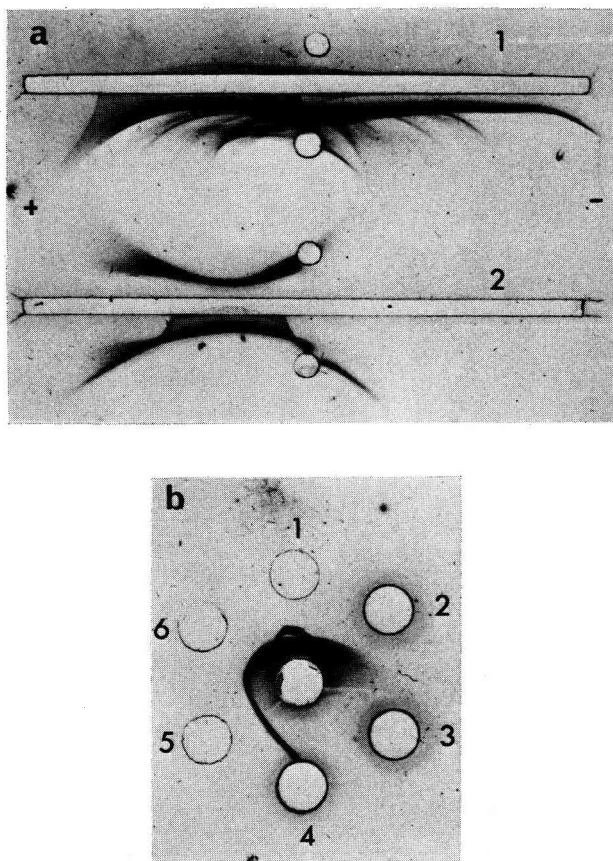


FIG. 4. — a) *Gelose immunoelectrophoresis* : 0.05 M veronal buffer, pH = 8.6. 45-min migration under 45 V (6 V/cm). Precipitating arc developed 24 h at 4 °C. Amidoshwartz staining. 1) Migration of a serum and immunological reaction against a total antiserum. 2) Rat PRL migration (1 mg and 2 mg/ml ; 3 μ l) and immunological reaction against a rat antiprolactin serum.

b) *Immunodiffusion* : 1.5 p. 100 gelose plates. 24 to 48-hour diffusion at 4 °C. Amidoshwartz staining. 10 μ g of pure antibody were placed in the middle well and 10 μ l of antigen in each of the peripheral wells. 1) 20 μ g of rat PRL ; 2) Serum of castrated, estrogen-treated male rat ; 3) Serum of castrated, estrogen-treated female rat ; 4) Male rat serum ; 5) 3 μ g of rat PRL (3/3/76) ; 6) 3 μ g of rat PRL (16/6/75).

From an immunological viewpoint, the extracted PRL specifically inhibited the PRL-anti-PRL reaction when immunofluorescence was used on rat or calf pituitary sections (tables 1 and 2).

B. — *Antibody characteristics.*

Using immunoelectrophoresis and immunodiffusion the antibodies obtained gave a single precipitating arc for the PRL and the serum proteins (fig. 4).

TABLE 1

Ovine, bovine and murine inhibition of prolactin cell immunofluorescence (observed immunofluorescence)

	Pituitary of	
	Calf	Rat
A) 1. Rat anti-prolactin serum (1/50) (19 601)	+++ *	
2. Rat anti-prolactin serum (1/50) + NIH bovine prolactin PB ₂ (58 µg/0.2 ml Ac. 1/50)	—	
3. Rat anti-prolactin serum (1/50) + Clin-Byla ovine prolactin (20 U/mg) (277 µg/0.2 ml Ac. 1/50)	—	
4. Rat anti-prolactin serum (1/50) + rat prolactin (19-11-76) (15 µg/0.1 ml Ac. 1/50)	—	
B) 1. Ovine anti-prolactin serum (1/50) (Ref. G 6) (Gabrion G 1976)		+++
2. Ovine anti-prolactin serum (1/50) + rat prolactin (19-11-76) (15 µg/0.1 ml Ac. 1/50)		—
3. Ovine anti-prolactin serum (1/50) + rat prolactin (19-11-76) (1 µg/0.1 ml Ac. 1/50)		—

The Ag. inhibitor is preincubated for 1 h with the antibody before carrying out the immunofluorescence reaction.

* Reaction intensity is indicated by the number of plus signs.

TABLE 2

Immunofluorescence of different cell types of the rat pituitary : Rat prolactin inhibition tests (7.5 mg of rat prolactin/ml of antiserum at 1/1 dilution)

Antiserum raised against	Cells observed by immunofluorescence		Inhibition
H-STH (Begeot <i>et al.</i> , 1977)	somatotropic	+++	—
Ovine LH (Dubois and Mauléon, 1969) (Dubois, 1971a, 1972b)	gonadotropic	+++	—
Porcine β LH (Dubois, 1971a, 1972b)	gonadotropic	+++	—
Bovine β TSH (Dubois, 1971a, 1972b)	thyreotropic	+++	—
ACTH β (1-24) (Dubois, 1971b, 1972a, c) ..	corticotropic	+++	—
Porcine ACTH (17-39) (Dubois, 1972c) ...	corticotropic	+++	—
α MSH (Dubois, 1972a, c)	intermediary lobe	+++	—
β MSH (Dubois, 1972a, c)	intermediary lobe	+++	—

NB : The amount of the Ag inhibitor used is equal to 15 times the minimum quantity needed for 100 p. 100 inhibition of prolactin cell immunofluorescence in the presence of rat antiprolactin serum.

With immunofluorescence :

— on contiguous sections of calf pituitary the antibody raised against rat PRL showed the same cells as the antibody raised against ovine PRL ;

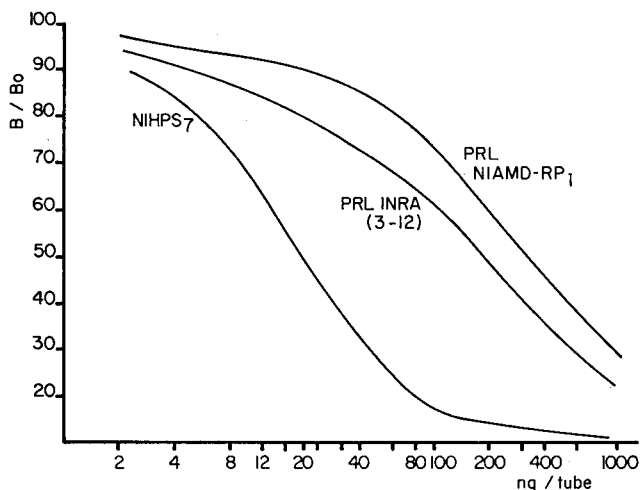


FIG. 5. — Radioreceptor assay. Curve shows competition of ^{125}I -labelled ovine PS₇ prolactin and ovine PS₇ and rat NIAMD-RP₁ and INRA (3/12) prolactins for rabbit mammary gland receptors.

— on sections of rat pituitary the cells revealed by the antibody raised against the INRA rPRL were different from those shown by an antibody to GH (fig. 6) and antibodies raised against other pituitary hormones LH, pLH, TSH, ACTH, MSH).

C. — RIA.

1. — *Labelling and iodized PRL binding to the antibody.* — In the conditions described we obtained a ^{125}I -labelled PRL having a specific activity of about $75 \mu\text{Ci}/\mu\text{g}$. The immunoreactivity of the different fractions of the G 100 filtration varied little through the elution peak (65 to 80 p. 100 of the introduced radioactivity). However, it was slightly lower in the ascending part (65 p. 100). The most radioactive fraction was used in the assays.

For antibody 19 601 (P8), about 50 p. 100 of the introduced radioactivity (B/T) was precipitated at a final dilution of 1.10^{-6} .

2. — *Criterion of assay quality.* — The standard curve expressed in B/B₀ in terms of the logarithm of the amount of non radioactive PRL is shown in figure 7.

a) Specificity.

— *For plasma proteins.* — The standard curves (expressed by logit transformation), plotted in the presence or the absence of the serum of hypophysectomized animals, were superposable.

Serum dilutions of normal or of estrogen-treated, castrated male rats, as well as

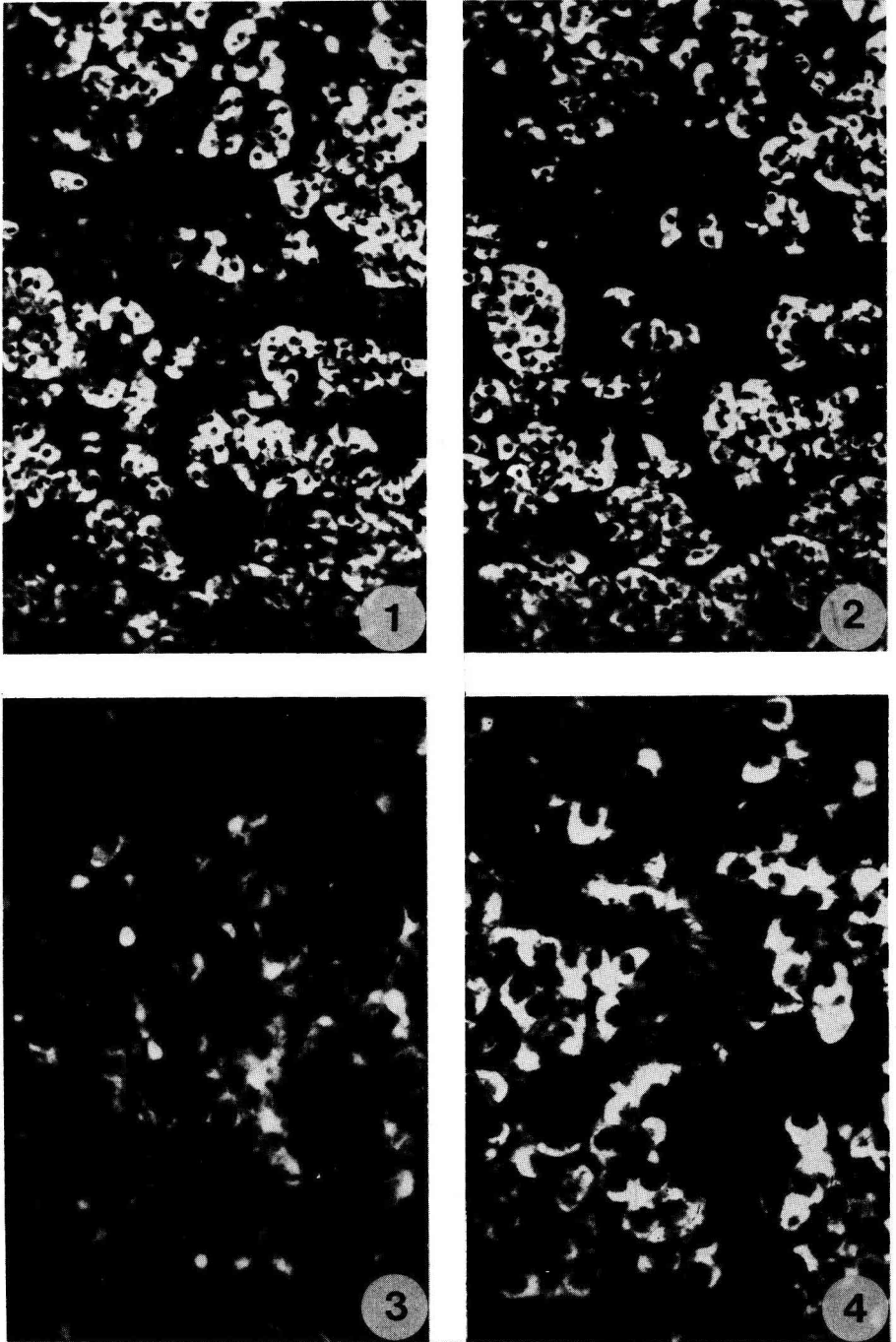
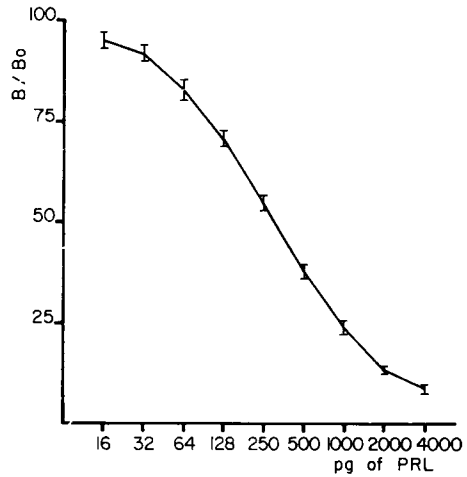


FIG. 6. — *Immunocytologic reaction.*

Above : serial sections of calf pituitary treated with : 1) ovine PRL antibody, 2) rat PRL antibody.
Below : serial sections of rat pituitary treated with : 3) rat PRL antibody, 4) human STH antibody.

FIG. 7. — Standard curve expressed in B/B_0 ($M \pm SEM$; $n = 10$ per point) in terms of logarithm of increasing amounts of PRL.



dilutions of pituitary extracts, gave straight lines parallel to the straight reference line (fig. 8). Table 3 summarizes the preceding results.

— For pituitary hormones. Assay specificity was studied in relation to some pituitary hormones of the rat (LH, FSH, TSH, GH). The percentage of interference was expressed according to Abraham (1969) by the formula :

$$\text{p. 100 of interference} = \frac{\text{mass of Y which decreases the ratio } B/B_0 \text{ from 100 to 50 p. 100}}{\text{mass of X which decreases the ratio } B/B_0 \text{ from 100 to 50 p. 100}} \times 100$$

X = assay hormone ; Y = reference hormone.

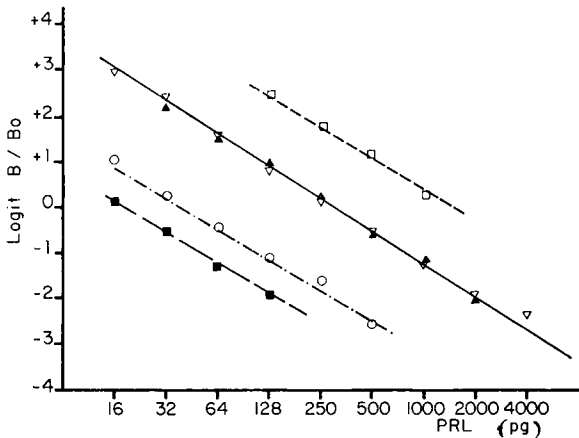


FIG. 8. — Dose-response curve expressed in B/B_0 logit in terms of logarithm of the dose or dilution of the serum or the pituitary extract.

△ range in HSA medium ; ▲ range in H-serum medium ; ○ dilution of castrated, implanted male rat serum ; ■ pituitary dilution ; □ dilution of male rat serum.

The following percentages were found : FSH $R_6 Sx_2$: 0.025 p. 100 ; LH $R_6 G_4$: 0.025 p. 100 ; TSH RP_1 : 0.1 p. 100 ; GH RP_1 : 0.05 p. 100.

TABLE 3
Assay specificity for proteins

	Slope of the straight line (a)	Coefficient of correlation (r)
Reference range HSA medium	a = -2.26	r = -0.998
Serum medium (serum of hypophysectomized animals).....	a = -2.35	r = -0.999
Dilution serum of castrated, estrogen-treated male rat	a = -2.27	r = -0.994
Male rat serum	a = -2.34	r = -0.994
Pituitary extract	a = -2.33	r = -0.999

b) *Sensitivity.*

Sensitivity corresponded to the smallest amount of non radioactive hormone for which B/Bo was significantly different from Bo.

The maximum standard deviation obtained (for $n = 10$) all along the curve was 2.5. The amount of hormone corresponding to B/Bo = 95 p. 100 (100 p. 100-2 SD) was about 16 pg.

c) *Reproductibility.*

— *Intra-assay reproductibility* expressed by the coefficient of variation of n repeats of the same assay was less than 10 p. 100 for B/Bo values between 75 and 25 p.100 (table 4).

TABLE 4
Intra-assay reproductibility expressed by the coefficient of variation of n repeats of the same assay

PRL x in pg/tube	SD	n	Coef. var = 100 SD/x (p. 100)	B/B ₀ (p. 100)
1 702	255	10	17	17.1
974	99	10	10	25.2
480	36.5	10	7.5	39.5
233	12	10	5	56.5
110	9.8	10	8.7	74.2

— *Interassay reproductibility* was determined by assaying the PRL of the same serum in 6 consecutive assays. The amount found was 239 ng/ml with a 16 p. 100 coefficient of variation.

3. — *Comparison with the NIAMDD system.* — The INRA PRL and the NIAMDD I₂ PRL were labelled in the conditions described, and were purified on Sephadex G 100. The behavior of the antibodies (INRA : 19 601 and NIAMDD = S₄) in relation to labelled antigens in the presence or the absence of non radioactive antigen is shown in figure 9.

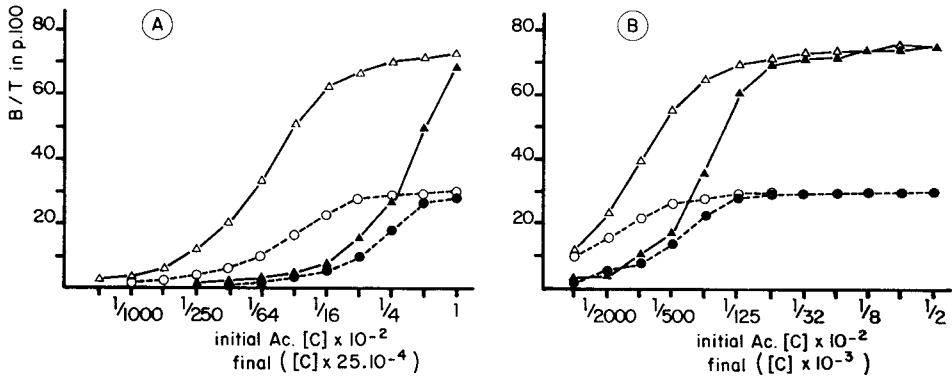


FIG. 9. — *Binding of NIAMDD (AC NIAMD) and INRA (AC INRA) antibodies against prolactin in respect to labelled NIAMDD (Ag I₂) and INRA (Ag, INRA) hormones in the presence or the absence of non radioactive hormone. Curves express the B/T binding percentage (B : bound activity ; T : total introduced activity) in terms of antibody dilutions.*

- A) Δ Ac NIAMD Ag*INRA ; \blacktriangle Ac NIAMD Ag*INRA 40 ng Ag INRA ; \circ Ac NIAMD Ag*I₂ ; \bullet Ac NIAMD Ag*I₂ 40 ng RP₁.
 B) Δ Ac INRA Ag*INRA ; \blacktriangle Ac INRA Ag*INRA 5 ng Ag INRA ; \circ Ac INRA Ag*I₂ ; \bullet Ac INRA Ag*I₂ 5 ng RP₁.

The initial antibody dilutions used to plot the standard graphs were 1/100 000 and 1/2 500 for the 19 601 and the S₄, respectively. System sensitivity was 16 and 250 pg, respectively.

The amounts of PRL (in $\mu\text{g/ml}$) estimated in the rats serums of each system are shown in table 5.

TABLE 5

Amounts of PRL (in $\mu\text{g/ml}$) estimated in the rat serums of each system

Rat No	INRA system M \pm SEM	NIAMD system M \pm SEM	NIAMD/INRA
1	18.2 \pm 0.4	34.6 \pm 0.9	1.9
2	27.9 \pm 1.2	47.7 \pm 3.9	1.7
3	13.7 \pm 0.8	29.2 \pm 1.0	2.1
4	8.6 \pm 0.5	16.3 \pm 0.5	1.9
5	10.7 \pm 0.5	18.9 \pm 0.2	1.8
6	21.2 \pm 0.6	34.2 \pm 1.6	1.6

The quantities of PRL detected in our system were 1.6 to 2.1 times lower than those detected with the NIAMDD system.

4. — *Experimental results.*

The effect of anticoagulants on the amount of circulating PRL is shown in table 6. The mode of sampling had no significant effect on the quantities of prolactin.

TABLE 6
Effect of anticoagulants on the amount of circulating PRL

Rat No	Heparinate	Citrate	Serum	Na ₂ EDTA	Heparin
1	3.8 ± 0.25	4.8 ± 0.3	4.2 ± 0.3	3.7 ± 0.25	4.3 ± 0.3
2	5.8 ± 0.3	4.4 ± 0.45	6.0 ± 0.3	5.6 ± 0.25	4.7 ± 0.2
3	19.4 ± 1.3	—	18.1 ± 0.5	16.3 ± 1	19.3 ± 0.3
4	—	3.2 ± 0.2	5.3 ± 0.4	4.3 ± 0.3	5.4 ± 0.2

The amounts of circulating prolactin in different experimental animals are shown in figure 10. The quantities of prolactin found in intact males or in those 1 month after castration (4.4 ± 0.5 and 2.7 ± 0.5 ng/ml) were not significantly different at 5 p. 100.

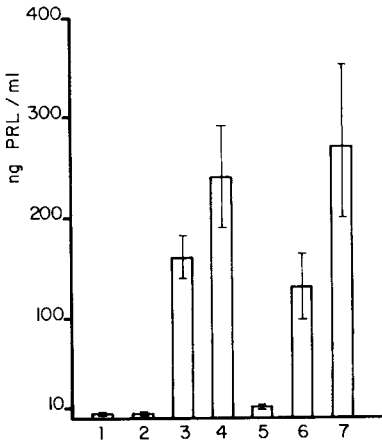


FIG. 10. — *Diagram showing the serum PRL rates (in ng/ml) of : 1) young male rats, 2) young castrated male rats, 3) young castrated, estrogen-treated male rats, 4) old castrated, estrogen-treated male rats, 5) diestrous female rats, 6) proestrous female rats, 7) nursing female rats.*

The quantities of pituitary PRL are shown in table 7.

TABLE 7
Pituitary PRL content

	PRL in µg/pituitary	PRL in µg/mg of pituitary
Males.....	31.6 ± 2.2	3.4 ± 0.2
Castrated males	39.7 ± 3.2	3.9 ± 0.3
Castrated, treated males ..	535 ± 54.6	21.8 ± 0.4

Discussion and conclusion.

This study shows that highly purified rat prolactin can be prepared in large quantities from PRL-enriched pituitaries obtained by appropriate treatment of the rats. Meites (1974) showed that estrogens strongly stimulate PRL secretion at two levels : (i) by direct action where the number and the activity of PRL cells is increased, and (ii) by acting on the hypothalamus and thus decreasing the PIF in the pituitary portal circulation. Progesterone and testosterone, which have little or no effect on pituitary PRL level when used alone, appear to have a potentializing influence when associated with estrogens (Meites, 1974). The extracted PRL having a molecular weight of about 26 000, and thus in the monomer state, presented a very anodic singlet band migrating in front of the albumin on acrylamide gel at pH = 9.5. Similar characteristics have been reported by Groves and Sells (1968), Ellis *et al.* (1969), and Cheever and Lewis (1969).

The extracted rat prolactin has not been biologically studied *in vivo*. Radioreceptor assay carried out on rabbit mammary gland membranes showed that the activity of our preparation was two times higher than that of the NIAMDD RP₁. The values given in IU/mg of NIAMDD RP₁ (1.8) and of INRA PRL 27/1 (4.3), using the NIH PS₇ as a reference, were much lower than those reported by other authors employing different tests of biological activity. The NIAMDD gave 11 IU/mg for the RP₁ in the assay on murine deciduoma, Kwa *et al.* (1967) finding an activity of 13.6 IU/mg for their preparation in the assay on cultured murine mammary gland using NIH PS₄ as a reference. The apparent weak activity of our preparation might be explained by a difference in rabbit mammary gland receptor site affinity for ovine and murine prolactin.

The immunological purity of our antigen, which showed no pituitary contamination, was confirmed by the specificity of the antibodies raised against that protein.

This study reports a technique for the extraction of highly purified PRL with pituitary yields 10 times higher than those obtained by Ellis *et al.* (1969). An antibody obtained by repeated immunization showed a high titer and was used for radioimmunoassay at a final dilution of 10⁻⁶ (B/T = 50 p. 100). A sensitive, specific and reproducible assay has been developed. The detection limit of 16 pg per tube is 8 times higher than that of the NIAMDD (125 pg). This sensitivity, permitting detection of the PRL in 50 μ l of serum (as against 400 for the NIAMDD), makes it possible to assay serial samples in the rat. Plasma protein and pituitary hormones (LH, FSH, TSH and GH) interfered only when extraphysiological doses of hormones (1 μ g/50 μ l) were used.

The prolactin « extracted » from the pituitaries of normal rats or « secreted » in the peripheral circulation produces straight lines parallel to the standard curve. The immunological behavior of these prolactins was thus identical to the « tumoral PRL » induced by estrogens in the radioimmunological system developed. Farmer *et al.* (1976) in a comparative study of the PRL « extracted » from the pituitary or « secreted » in culture found no biological or immunological difference. Stevens and Lanson (1977) reported a single form of PRL in the serum and several types in the pituitary, but all were assayed by RIA.

Asawaroengchai, Russell and Nicoll (1978) found several types of PRL in the

pituitary and the incubation media, but reported a difference in the activity ratio between biological (BA) and immunological (RIA) activity, depending on the types separated by polyacrylamide gel electrophoresis. However, at least 75 p. 100 of the PRL activity (band corresponding to RP_1) gave a constant ratio of BA/RIA. The type of PRL migrating well in front of the « PRL band » and having a BA/RIA ratio = 80, only appeared in 4-day pituitary cultures. It may be hypothesized that this type corresponded to a fragment of the PRL molecule supporting biological activity but no longer including the antigenic sites corresponding to the major antibody determinants of the serum used in RIA. More recently, the same research team (Leung, Russell and Nicoll, 1978) showed that serum content measured by RIA only represented 25 p. 100 of that found by BA. However, in those cases, the plasma contents assayed by RIA and BA were highly correlated.

Absolute percentages of PRL are difficult to define ; they agree for a given type of assay with a defined reference and in precise conditions. To validate our assays, we evaluated the serum PRL of different rats in our system and in the NIAMDD system. The PRL contents detected in the same animal were two times weaker in ours than in that of the NIAMDD. These results would favorize a PRL about two times purer than that of the NIAMDD.

This study confirms the results of Niswender *et al.* (1969), Neill and Reichert (1971) and Kuo Gala (1972) who showed a high prolactin content in proestrous females. The quantities reported by different teams are not comparable since the references are different.

No significant difference in circulating and pituitary PRL was found between immature castrated animals 1 month after castration and intact animals of the same age. In rats castrated when adult, Shin *et al.* (1974), and Shaar *et al.* (1975) showed a decrease of circulating PRL 7 to 13 days after castration ; on the other hand, Winters and Loriaux (1978) reported an increase of circulating PRL in male rats 24 hrs after castration. These results are not comparable, the animals at the time of castration being at different stages of sexual maturation (impuberal and adult) and the times after castration varying in the different studies.

Reçu en mai 1978.

Accepté en juin 1979.

Acknowledgments. — We wish to thank Dr. Djiane (INRA) who did the radioreceptor assays of PRL and GH, the National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD) which provided the prolactin RIA kit for rats as well as the TSH- RP_1 and the GH- RP_1 , Dr. Jutisz (Gif-sur-Yvette, France) for the FSH $R_6 Sx_1$ and the LH $R_6 G_4$, the Laboratoires Pfizer (Amboise, France) for material help and Mr. Terriot for making the photographic plates.

Résumé. Le présent article expose l'isolement et la purification de la prolactine hypophysaire de rat. Des rats mâles de 6 mois sont castrés et reçoivent des implants sous-cutanés de progestérone (20 mg) et d'œstradiol (2 mg) provoquant l'accumulation de la prolactine hypophysaire. Quatre à six semaines plus tard, les rats sont sacrifiés et les hypophyses disséquées ; les hormones hypophysaires autres que la prolactine sont éliminées grâce à une technique poussée de purification. Une préparation de prolactine à haute activité biologique est obtenue par filtration sur Sephadex G 100.

La prolactine ainsi purifiée est étudiée à l'aide de méthodes physico-chimiques, biologiques et immunologiques. Son poids moléculaire, apprécié par calibration sur tamis moléculaire, est d'environ 26 000. Son activité, mesurée par un système de radiorécepteurs, est deux fois plus élevée que la préparation NIAMDD-RP₁. En immunofluorescence, les anti-sérums obtenus sur lapin contre cette prolactine reconnaissent les cellules à prolactine au niveau des hypophyses de mouton, de bœuf et de rat, mais non les autres types cellulaires.

Un dosage radioimmunologique utilisant le double système anticorps a été réalisé à partir de cette prolactine et de ses antisérums, couvrant au mieux une gamme comprise entre 16 et 400 pg. Les coefficients de variation intra et interdosages ont été respectivement de 10 p. 100 et de 16 p. 100.

Le « kit » de dosage radioimmunologique de la prolactine de rat distribué par le NIAMDD a été étudié comparativement. Les taux de prolactine de rat, mesurés dans le sérum par le système de dosage du NIAMDD, ont été le double de ceux appréciés par notre système. Les différences apparentes dans les taux de prolactine sérique ne font que refléter une activité immunologique inférieure de la prolactine du NIAMDD.

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