Pergamon
0742-8413(95)00013-5

Comp. Biochem. Physiol. Vol. 111C, No. 2, pp. 175–181, 1995

0742-8413/95 $9.50 + 0.00

Purification and characterization of progesterone-binding globulin in the pregnant Cape porcupine, *Hystrix africaeaustralis*

V. Van Wyk, A. W. H. Neitz,* A. I. Louw* and R. J. Van Aarde

Mammal Research Institute, Department of Zoology, University of Pretoria, Pretoria 0002, South Africa; *Department of Biochemistry, University of Pretoria, Pretoria 0002, South Africa

Progesterone-binding globulin (PBG) was purified from pooled pregnant Cape porcupine (*Hystrix africaeaustralis*) plasma by SP-Sephadex chromatography, followed by Sephacryl S-200 gel filtration. The final product was homogeneous according to high performance capillary electrophoresis, SDS–polyacrylamide gel electrophoresis and sedimentation equilibrium centrifugation. A molecular mass of 65,300 Da was calculated from sedimentation equilibrium centrifugation data. Scatchard analysis indicated a dissociation constant of 0.82×10^{-9} M, and PBG concentration of 18.4 nmole/mg protein.

Key words: Progesterone-binding; Globulin; Cape porcupine; *Hystrix africaeaustralis*; Purification; Characterization.

Comp. Biochem. Physiol. 111C, 175–181, 1995.

Introduction

The production of progesterone-binding globulin (PBG) by the cytoplasma of the syncytiotrophoblast of the chorioallantoic placenta (see Perrot and Milgrom, 1978; Perrot-Applanat and David-Ferreira, 1982) is a characteristic of pregnancy in hystricomorph rodents. Various biological functions have been allocated to this protein of which the most readily accepted is that it serves to reduce the rate of removal of progesterone from plasma and hence its metabolism in pregnant females (Westphal *et al.*, 1977).

Progesterone-binding globulin occurs in relatively high levels in plasma of guinea-pigs, *Cavia porcellus* (New World hystricomorph rodent) during pregnancy (Stroupe and Westphal, 1975). Its association constant for progesterone is greater than that of other plasma proteins (Westphal *et al.*, 1977). Purified guinea-pig PBG has been characterized as a polydisperse and anionic glycoprotein with a high carbohydrate content (Burton *et al.*, 1974). Although PBG has a high affinity for 5α- and 20α-substituted steroids and androgens, it has a low affinity for the corticosteroids (Milgrom *et al.*, 1973; Heap and Illingworth, 1974). Moreover, PBG is heat stable (Heap, 1969; Milgrom *et al.*, 1973; Louw *et al.*, 1992) and relatively inert to pH changes (pH 2–11; Harding *et al.*, 1974). Also, each molecule of PBG binds one molecule of progesterone (Milgrom *et al.*, 1973; Heap and Illingworth, 1974). The molecular mass of PBG varies considerably among different hystricomorph species, ranging from 75,000 Da in the coypu, *Myocastor coypus*, and 88,000 Da in the guinea-pig to values between 135,000 and 145,000 Da in the cuis, *Galea musteloides*, degu, *Octodon degus*, and viscacha, *Lagostomus maximus* (Heap *et al.*, 1981).

Plasma progesterone-binding proteins (PPBP) of porcupines (Old World hystricomorph rodent) have a high affinity (10^9 M^{-1}) and a saturable binding capacity for

Correspondence to: A. I. Louw, Department of Biochemistry, University of Pretoria, Pretoria 0002, South Africa.
Received 4 June 1994; revised 20 December 1994; accepted 20 December 1994.

progesterone (Van Aarde and Potgieter, 1986; Louw *et al.*, 1992). However, no information is available on the physico-chemical properties of purified Cape porcupine PBG. The principal methods applied for the purification of guinea-pig PBG were ion-exchange chromatography, gel filtration, ammonium sulphate precipitation, and electrophoresis (Lea, 1973; Milgrom *et al.*, 1973; Burton *et al.*, 1974; Stroupe and Westphal, 1975; Cheng *et al.*, 1976; Heap *et al.*, 1981). Stroupe and Westphal (1975) were the first to use Sulphopropyl (SP)-Sephadex, which is a strong cation exchanger, to separate the acidic PBG molecule from CBG and albumin in a single step. Using a similar protocol we now report on the isolation of PBG from Cape porcupine plasma and on some of its physico-chemical characteristics. Hereby it is hoped to make a contribution to our understanding of the role of PBG in the maintenance of pregnancy in Cape porcupines.

Materials and Methods

Collection of plasma

Plasma aliquots, collected from mid- to near-term pregnant Cape porcupines, were pooled and used for the purification of PBG. Collection of plasma was as described by Louw *et al.* (1992). Aliquots were stored at −20°C until used.

Determination of binding activity

After each step of the purification procedure, PPBP binding activities in aliquots of fractions were determined by the equilibrium dialysis method of Reinard and Jacobsen (1989). Triplicate assays were conducted at 4°C for 24 hr using 15 nM [1,2,6,7-^{3}H] progesterone (Code TRK 413, spec. act. 96 Ci/mMole; Radiochemical Centre, Amersham, U.K.) and protein concentrations of < 0.1 mg/ml.

Determination of protein concentration

Protein concentrations were estimated from the absorbance at 280 nm using a value, $E^{1\,cm}_{1\,mg/ml} = 1.0$ (Milgrom *et al.*, 1973).

Ion-exchange chromatography

Sulphopropyl-Sephadex C-50 (SP-Sephadex; Pharmacia Fine Chemicals, Uppsala, Sweden) was prepared according to the specifications of the manufacturers. The pH of 10 ml plasma was adjusted to 4.5 with 50% acetic acid during continuous stirring, left to stand for 4 hr (4°C) and centrifuged at 40,000 *g* for 30 min at 4°C (Beckman L7-55 ultracentrifuge, Beckman Instruments, Palo Alto). Minimal activity was lost by this treatment (results not shown). The supernatant was then applied to the SP-Sephadex C-50 column and proteins were eluted with 0.02 M sodium acetate buffer (pH 4.5). Fractions containing progesterone binding activity were pooled and neutralized with 0.2 M sodium hydroxide and stored at 4°C before loading onto a Sephacryl S-200 column at 4°C.

Gel filtration

Sephacryl S-200 gel (Pharmacia Fine Chemicals, Uppsala, Sweden) was equilibrated with 0.01 M Tris–0.11 M KCl buffer, pH 8.6 (Tris–KCl buffer). The pooled fractions containing progesterone binding activity, collected after SP-Sephadex chromatography, were applied to the column. Proteins were eluted at 4°C with Tris–KCl buffer, pH 8.6. Fractions containing PPBP were pooled, concentrated with Millipore immersible-CX ultrafilters (M_r cutoff = 10,000) (Millipore, Bedford), after which the concentrate was divided into 0.1 ml aliquots and stored at −20°C.

SDS–Polyacrylamide gel electrophoresis

The electrophoresis was performed according to the method of Laemmli (1970) using a 15% separation gel.

Capillary electrophoresis

High performance capillary electrophoresis (HPCE) separation of proteins was carried out on a Beckman P/ACE System 2100, utilizing System Gold software for control and data collection. A polyimide-coated, fused silica capillary with 75 μm inside diameter and 57 cm length from inlet to detector was used. Borate buffer (high pH, Beckman Instruments) was used for the separations, which were performed at 30 kV constant voltage. Capillary temperature was maintained at 25°C, and detection was by absorbance at 214 nm.

Sedimentation equilibrium ultracentrifugation

Sedimentation equilibrium ultracentrifugation studies were performed with a Spinco Model E analytical ultracentrifuge equipped with an ultraviolet photo-electric scanner (Beckman Instruments, Palo Alto). Sedimentation equilibrium studies were carried out on 0.7 mg protein/ml in phosphate buffer (pH 7.4) with a An-HTi rotor at 8310 and 12,159 rpm.

Amino acid analysis

The amino acid composition was determined using a Waters PicoTag amino acid analyser (Millipore, Bedford). Protein samples (10 μl) were hydrolysed for 24 hr at 105–112°C with 6 M hydrochloric acid containing 1% phenol in the gas phase in vacuum sealed tubes, and derivatized with phenylisothiocyanate

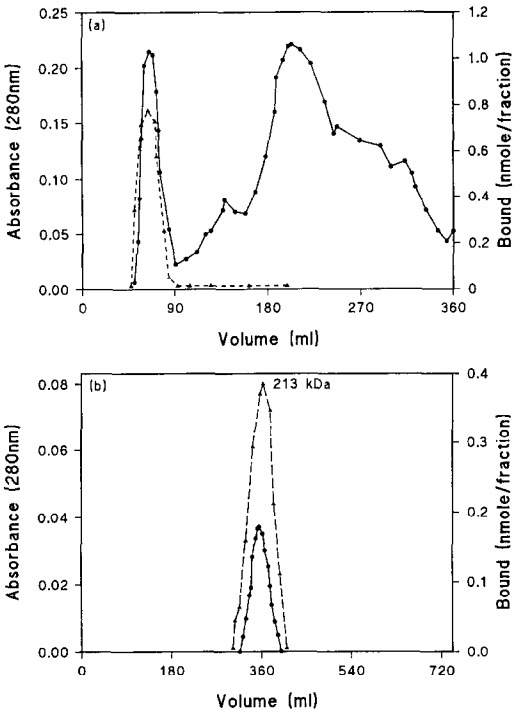

Fig. 1. Separation of plasma proteins by (a) SP-Sephadex C-50 chromatography. Column dimensions, 35 × 2.5 cm; temperature, 4°C; flow-rate, 19 ml/hr. Each fraction contained 3.1 ml. Separation of fractions containing PBG activity by (b) Sephacryl S-200 chromatography. Column dimensions, 38 × 5 cm; temperature, 4°C; flow-rate, 32 ml/hr. Each fraction contained 5.3 ml. The solid line denotes absorbance at 280 nm; ▲-----▲ denotes [1,2,6,7-^{3}H]progesterone binding activity.

according to the method described in the operator's manual (Millipore, Bedford).

Carbohydrate analysis

The carbohydrate content or PBG, was determined by the phenol–sulfuric acid procedure of Dubois *et al.* (1956) using glucose as standard.

Scatchard analysis

Binding properties of purified PBG were determined by equilibrium dialysis (see above) using [1,2,6,7-^{3}H]progesterone concentrations ranging from 1.25 to 22 nM. A protein concentration of 0.25 μg/ml incubation buffer was used and triplicate assays were analysed according to Scatchard (1949).

Results

Chromatographic purification of plasma progesterone-binding globulin

SP-Sephadex chromatography resulted in the absorption of most plasma proteins, except PBG which was eluted in the void volume of the column (Fig. 1a). A single protein peak which coincided with PBG binding activity, was obtained when pooled fractions were re-chromatographed on a Sephacryl S-200 column (Fig. 1b). The highly enriched PBG fraction (Table 1) was used for further investigations of the physico-chemical properties of PBG.

Characteristics of progesterone-binding globulin after purification

Molecular mass and homogeneity. The molecular mass of PBG was calculated as 213,000 Da on a calibrated Sephacryl S-200 column.

SDS–PAGE of the fraction obtained from the Sephacryl S-200 column showed a broad band corresponding to a molecular mass of 200,000 Da at the highest density in the smear (Fig. 2).

An average molecular mass of 68,000 Da for purified PBG was calculated from sedimentation equilibrium centrifugation data when the partial specific volume, v, was assumed to be 0.725 ml/g. However, when using a value of $v = 0.7$ ml/g to compensate for the carbohydrate content (see below) a molecular mass of 65,300 Da was calculated. The straight line relationship of ln a (Naperian logarithm of recorder pen deflection) versus x (radial distance) indicated a homogeneous sample preparation (Fig. 3). HPCE of purified PBG showed a peak with only slight indication of contamination (Fig. 4).

Amino acid analysis. Results of amino acid analyses are presented in Table 2. There are differences with respect to certain amino acids between porcupine and guinea-pig PBG, most

Table 1. Progress of purification of PBG from pooled plasma of mid- to near-term pregnant Cape porcupines. Protein concentration was determined by absorbance at 280 nm ($E^{1\,cm}_{1\,mg/ml} = 1$)

Purification step	Volume (ml)	Protein (mg/ml)	Specific activity pmole/ml	Specific activity pmole/mg	Total activity (pmole)	Yield (%)	Enrichment
Plasma (pH 9.17)	12	44.3	7757	175	93,084	100	1
Plasma (pH 4.5)	11	40.8	7205	177	79,425	85.39	1.01
SP Sephadex (before dialysis)	50	0.15	886	5910	44,300	47.64	33.77
SP Sephadex (after dialysis)	44	0.10	1019	10,198	44,836	48.23	58.27
Sephacryl S-200	75	0.046	494	10,743	37,050	39.83	61.39

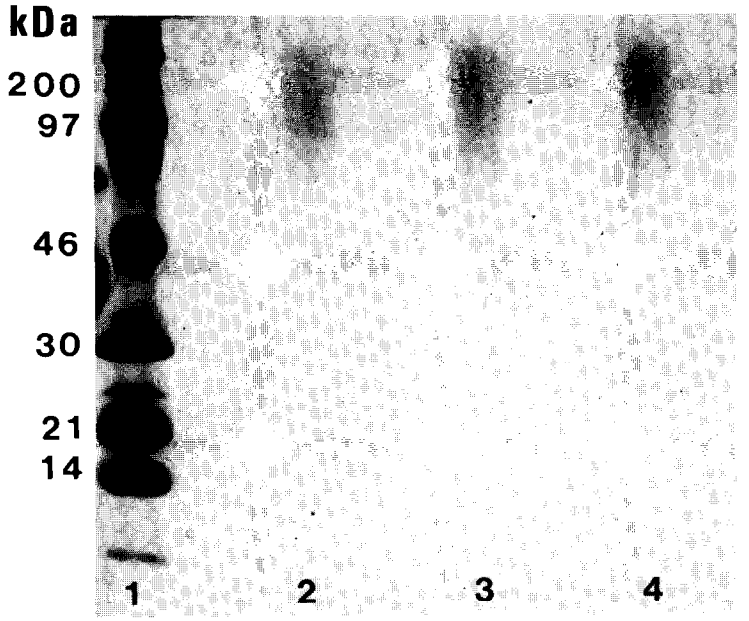

Fig. 2. SDS-gel electrophoresis of PBG after Sephacryl S-200 chromatography. A broad band with relative molecular mass of 200,000 Da is indicated (lanes 2–4, in triplicate). Lane 1 represents standards with indicated molecular masses.

notably serine, alanine and leucine. Only a small quantity of cysteine was detected.

Carbohydrate content. Samples appeared brown after acid hydrolysis which indicated the presence of carbohydrate. A carbohydrate content of 5.13% was calculated.

Scatchard analysis. The K_d was calculated as 0.82×10^{-9} M and the PBG concentration (R_m 18,394 pmole/mg protein (Fig. 5). It should be noted that the latter value was obtained assuming $E^{1\,cm}_{1\,mg/ml} = 1.0$.

Discussion

In the present study the bulk of plasma proteins remained adsorbed to the SP-Sephadex column, while PBG was eluted in the void volume (Fig. 1a). This is in agreement with results reported for plasmas from some other hystricomorph rodents (Stroupe and Westphal, 1975; Heap *et al.*, 1981). The behaviour of PBG on SP-Sephadex provided evidence for its anionic character (Heap *et al.*, 1981) and extremely low iso-electric point (pH < 4.5). Westphal *et al.* (1977) found that a single run of guinea-pig plasma on SP-Sephadex gave a preparation free of albumin and CBG. The latter protein was presumably inactivated by the acidic conditions.

Progesterone-binding globulin was purified ~60-fold (Table 1) but this value may be an underestimate since PBG was not saturated with

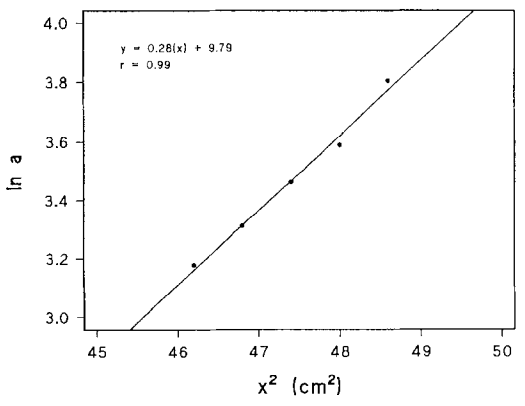

Fig. 3. Plot of sedimentation equilibrium centrifugation data for calculation of molecular mass. a = recorder pen deflection; x = radial distance.

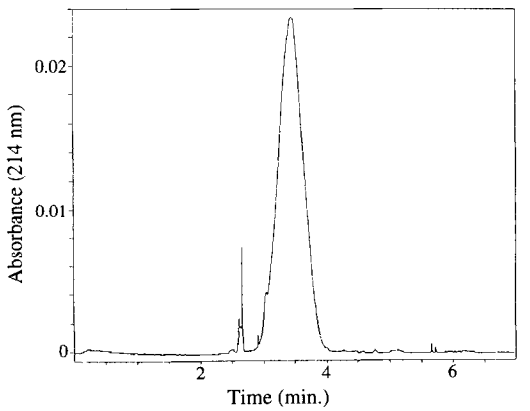

Fig. 4. High performance capillary electrophoresis of PBG after Sephacryl S-200 chromatography using a Beckman P/ACE System 2100 and System Gold software for control and data collection.

Table 2. Amino acid composition of Cape porcupine PBG. Values from Burton *et al.* (1974) for PBG I and PBG II and Milgrom *et al.* (1973) for PBG of guinea-pig are included for comparison. Amino acid composition is expressed in mole ratios with respect to lysine

Amino acid	PBG	PBG†	PBG I*	PBG II*
Aspartic acid	2.29	1.85	2.30	2.00
Glutamic acid	2.13	1.59	2.64	3.42
Serine	3.35	1.26	2.32	2.32
Glycine	1.43	0.91	1.68	1.90
Histidine	0.78	0.45	0.25	0.22
Arginine	0.47	0.61	0.81	0.72
Threonine	1.72	1.28	1.44	1.31
Alanine	1.50	0.72	1.42	1.45
Proline	1.08	0.77	1.18	1.14
Tyrosine	0.74	0.52	0.39	0.33
Valine	1.32	0.98	1.34	1.22
Isoleucine	0.76	0.71	0.83	0.71
Leucine	1.50	2.01	2.26	2.04
Phenylalanine	1.16	1.13	1.48	1.19
Lysine	1.00	1.00	1.00	1.00
Methionine	0.33	0.31	—	—
Cysteine	0.17	—	—	—

*Burton *et al.* (1974).
†Milgrom *et al.* (1973).

progesterone in the binding assays. The concentration of purified PBG was 10,743 pmole/mg protein which should be compared to a concentration of 18,394 pmole/mg protein obtained by Scatchard analysis (Fig. 5). Homogeneity of PBG was suggested by the single band obtained by either SDS–PAGE or high performance capillary electrophoresis and a straight line relationship between ln a and x, after sedimentation equilibrium centrifugation.

A molecular mass of 213,000 Da was calculated for porcupine PBG from the elution volume (V_0) of the Sephacryl S-200 column. The carbohydrate content, determined by the qualitative phenol–sulphuric acid method, was 5.13%. Westphal *et al.* (1977) determined unusually high (71%) carbohydrate values for

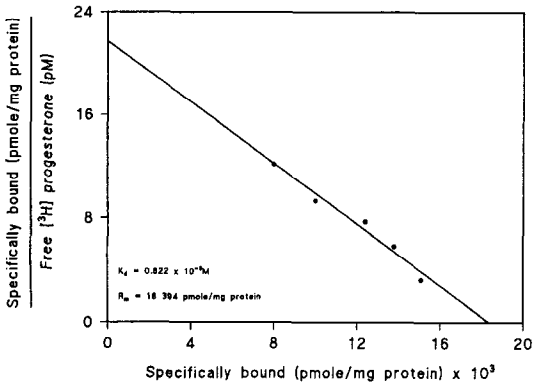

Fig. 5. Scatchard analysis of purified PBG. Equilibrium dialysis of purified PBG with various amounts (1.25–15 nM) of [1,2,6,7-^{3}H]progesterone in Tris–NaCl buffer (pH 7.4). Protein concentrations were determined by absorbance at 280 nm, using $E^{1\,cm}_{1\,mg/ml} = 1.0$.

pure guinea-pig PBG, while 48.7% and 42% were determined by Milgrom *et al.* (1973) and Lea (1973), respectively. Differences could be due to variant glycoproteins present in different guinea-pig strains (Westphal *et al.*, 1977). The carbohydrate content of porcupine PBG was considerably lower. It should be noted that in the present study Sephacryl S-200 was used in the final isolation step, thus eliminating the possibility of contribution of carbohydrates from Sephadex. Furthermore, cleavage of labile carbohydrate–peptide bonds during isolation and the carbohydrate used as standard as well as the method used in the determination may also account for differences in carbohydrate content of glycoproteins. Progesterone-binding globulin I contains almost 24% polypeptide, while PBG II has 33% with the remainder being accounted for by carbohydrates (Burton *et al.*, 1974). The latter authors suggested that the polypeptide–carbohydrate relationship is similar to that of other polydisperse glycoproteins.

Burton *et al.* (1971) estimated the molecular mass of guinea-pig PBG after Sephadex G-200 chromatography at 100,000 Da. MacLaughlin *et al.* (1973) identified two classes of PBG in guinea-pig with molecular masses of 117,000 Da and 78,000 Da based on the same method. Polydispersity of guinea-pig PBG, a unique feature among steroid-binding proteins, is ascribed to different forms of PBG, probably due to various carbohydrate moieties on a constant protein core (~27,000 Da; Westphal *et al.*, 1977). Burton *et al.* (1974) found a near continuous series of molecular sizes of PBG, of guinea-pigs in an unfractionated serum pool, as well as in the sera from individual pregnant animals. Since a full range of differently sized PBG species were present in serum of individual guinea-pigs it was concluded that polydispersity is not a result of pooling of plasma (Burton *et al.*, 1974). It should be borne in mind, however, that during the present study proteins were employed to calibrate the gel permeation column. Due to the greater hydrodynamic radius of carbohydrates as compared to proteins (Squire, 1964), this method is expected to give an erroneously high molecular mass for PBG. It is not clear, however, why the molecular mass of porcupine PBG determined by gel permeation chromatography, would be so much higher than those of other hystricomorph rodents.

After chromatography on Sephacryl S-200 a single, broad band was obtained by SDS–gel electrophoresis suggesting a molecular mass of 200,000 Da for PBG of porcupines. The faintness of bands after Coomassie staining may be ascribed to the presence of carbohydrate, whereas the broadness of the bands is probably due to micro-heterogeneity. For PBG of cuis,

degu and viscacha, and PBG I of guinea-pigs a molecular mass of 135,000–145,000 Da were reported, while for PBG of coypu a molecular mass of 75,000 Da was determined (Heap *et al.*, 1981). SDS–gel electrophoresis resulted in a single broad band for pure PBG of cuis, degu, coypu and viscacha, but two distinct bands for guinea-pigs (PBG I and PBG II) when using the Coomassie staining method (Heap *et al.*, 1981). This interspecific difference in molecular mass may once again be related to carbohydrate heterogeneity (Heap *et al.*, 1981).

A molecular mass of 68,000 Da for porcupine PBG was obtained by sedimentation equilibrium ultracentrifugation. Milgrom *et al.* (1973) and Lea (1973) determined molecular masses of 76,000 and 82,800 Da, respectively, for guinea-pig PBG. Heap *et al.* (1981) found the molecular mass of guinea-pig PBG to be 88,000 Da using the same method. Some uncertainty exists with respect to the molecular mass calculated from the sedimentation equilibrium data, since the partial specific volume was assumed to be 0.725 ml/g, a value typical for proteins (Bowen, 1970). Carbohydrates on the other hand possess lower values (Sober *et al.*, 1970). Using a value of 0.7 ml/g to compensate for the carbohydrate moiety, a molecular mass of 65,300 Da was calculated. Taken together, this value may at present be regarded as reflecting the molecular mass of PBG from porcupines. It is evident that the true molecular mass will only be known after the complete amino acid and carbohydrate compositions of this glycoprotein have been determined.

The values determined for the stable amino acids compare well with those documented by Milgrom *et al.* (1973) and Burton *et al.* (1974), i.e. the amino acid composition is similar for porcupine and guinea-pig (see Table 2). Differences in serine values are probably due to the instability of this amino acid during acid hydrolysis. The data on amino acids for PBG I and PBG II of guinea-pigs are very similar and are consistent with the existence of a common polypeptide chain in PBG molecules of different sizes (Burton *et al.*, 1974).

A value of 0.82×10^{-9} M was determined for the dissociation constant of porcupine PBG (Fig. 5). This value is in the same order of magnitude as that determined on Day 35 (1.82×10^{-9} M) and Day 68 (2.1×10^{-9} M) of pregnancy, for PPBP of porcupine (Van Wyk, 1991).

Through continued research regarding the structure of PBG in Cape porcupines, the function of these globulins in maintaining pregnancy in old world hystricomorph rodents may eventually be elucidated.

Acknowledgements—Mrs S. van Wyngaardt and Mr J. N. Taljaard of the Department of Biochemistry assisted with the gel electrophoresis and the analysis of the amino acid composition of the purified protein. Professor P. C. van Berge of the Department of Physical Chemistry, Rand Afrikaans University, determined the molecular mass of the purified protein. Financial support was provided by the Foundation for Research Development.

References

Bowen T. J. (1970) *An Introduction to Ultracentrifugation.* Wiley–Interscience, London.

Burton R. M., Harding G. B., Aboul-Hosn W. R., Maclaughlin D. T. and Westphal U. (1974) Progesterone-binding globulin from the serum of pregnant guinea pigs, a polydisperse glycoprotein. *Biochemistry* **13,** 3554–3561.

Burton R. M., Harding G. B., Rust N. and Westphal U. (1971) Steroid–protein interactions. XXIII. Nonidentity of cortisol-binding globulin and progesterone-binding globulin in guinea pig serum. *Steroids* **17,** 1–16.

Cheng S. L., Stroupe S. D. and Westphal U. (1976) Steroid–protein interactions. Purification of progesterone-binding globulin by affinity chromatography. *FEBS Lett.* **64,** 380–384.

Dubois M., Gilles K. A., Hamilton J. K., Rebers P. A. and Smith F. (1956) Colometric method for determination of sugars and related substances. *Analyt. Chem.* **28,** 350–356.

Harding G. B., Burton R. M., Stroupe S. D. and Westphal U. (1974) Steroid protein interactions. Isoelectric point and pH stability of progesterone-binding globulin. *Life Sci.* **14,** 2405–2412.

Heap R. B. (1969) The binding of plasma progesterone in pregnancy. *J. Reprod. Fert.* **18,** 546–548.

Heap R. B., Ackland N. and Weir B. J. (1981) Progesterone-binding proteins in plasma of guinea-pigs and other hystricomorph rodents. *J. Reprod. Fert.* **63,** 477–489.

Heap R. B. and Illingworth D. V. (1974) The maintenance of gestation in the guinea-pig and other hystricomorph rodents: Changes in the dynamics of progesterone metabolism and the occurrence of progesterone-binding globulin (PBG). *Symposia of the Zoological Society of London* **34,** 385–415.

Laemmli U. K. (1970) Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature (Lond.).* **227,** 680–685.

Lea O. A. (1973) Isolation and characterisation of a progesterone- and testosterone-binding globulin from pregnant guinea pig serum. *Biochim. biophys. Acta.* **317,** 351–363.

Louw A. I., Van Wyk V. and Van Aarde R. J. (1992) Properties of proteins binding plasma progesterone in pregnant Cape porcupines (*Hystrix africaeaustralis*). *J. Reprod. Fert.* **96,** 117–125.

Maclaughlin D. T., Burton R. M., Aboul-Hosn W. and Westphal U. (1973) Progesterone-binding globulin (PBG) and uterine progesterone receptor in the pregnant guinea-pig. *Federation Proceedings. Federation of American Societies for Experimental Biology* **32,** 1962A.

Milgrom E., Allouch P., Atger M. and Baulieu E.-E. (1973) Progesterone-binding plasma protein of pregnant guinea pig. *J. biol. Chem.* **248,** 1106–1114.

Perrot-Applanat M. and David-Ferreira J. F. (1982) Immunocytochemical localization of progesterone-binding protein (PBP) in guinea-pig placental tissue. *Cell Tiss. Res.* **223,** 627–639.

Perrot M. and Milgrom E. (1978) Immunochemical studies of guinea pig progesterone-binding plasma protein. *Endocrinology* **103,** 1678–1685.

Reinard T. and Jacobsen H.-J. (1989) An inexpensive small volume equilibrium dialysis system for protein-ligand binding assays. *Analyt. Biochem.* **176,** 157–160.

Scatchard G. (1949) The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.* **51,** 660 672.

Sober H. A., Harte R. A. and Sober E. K. (1970) *Handbook of Biochemistry* 2nd ed. Chemical Rubber Co., OH.

Stroupe S. D. and Westphal U. (1975) Conformational changes in the progesterone binding globulin-progesterone complex. *Biochemistry.* **14,** 3296–3300.

Squire P. G. (1964) A relationship between the molecular weights of macromolecules and their elution volumes based on a model for Sephadex gel filtration. *Arch. Biochem. Biophys.* **107,** 471–478.

Van Aarde R. J. and Potgieter H. C. (1986) Circulating progesterone, progesterone-binding proteins and oestradiol-17β concentrations in the pregnant Cape porcupine, *Hystrix africaeaustralis. Reprod. Fert.* **76,** 545–552.

Van Wyk V. (1991) Plasma progesterone-binding proteins in the Cape porcupine, *Hystrix africaeaustralis.* MSc thesis, University of Pretoria, Pretoria.

Westphal U., Stroupe S. D. and Cheng S.-L. (1977) Progesterone binding to serum proteins. *Ann. N.Y. Acad. Sci.* **286,** 10–27.