The Purification and Characterization of Rabbit Placental Lactogen

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Rabbit placental lactogen, a polypeptide hormone functionally related to the growth hormone/prolactin family, was isolated from placenta by $(\text{NH}_4)_2\text{SO}_4$ precipitation, gel filtration and ion-exchange chromatography on DEAE- and CM-cellulose. The hormone was purified to more than 90% homogeneity, as determined by end-group analysis. On disc gel electrophoresis at pH 9.0 it migrates as a pair of closely spaced bands with mobilities of 0.489 (minor band) and 0.511 (major band), and its isoelectric point is 6.1. Its mol.wt. is 20600, as determined by sedimentation-equilibrium centrifugation, and 24200, as estimated by gel electrophoresis in sodium dodecyl sulphate. Its amino acid composition resembles that of rabbit growth hormone and rat prolactin, except for a lower glutamic acid and leucine content. Like the prolactins, rabbit placental lactogen has two tryptophan and six cysteine residues, and its *N*-terminus, valine, is identical with that for human placental lactogen. By radioimmunoassay, it does not cross-react with antisera to either rat growth hormone or rat prolactin; in addition, it does not cross-react with antisera to bovine placental lactogen by double immunodiffusion. The similarity of the biochemical characteristics of rabbit placental lactogen to the other non-primate placental lactogens lends further support to the hypothesis that these molecules occupy a more central position in the growth hormone/prolactin 'tree' than do their primate counterparts.

Previous studies in the rabbit have yielded conflicting, but generally negative, evidence about the existence of a placental lactogen. Starling (1905) found that the injection of a rabbit placental emulsion into rabbits for 10 days had no effect on mammary development. Others have reported that rabbit placental extracts were inactive in stimulating the development of mammary-gland explants from mid-pregnant mice *in vitro* (Talmantes, 1975); and serum from pregnant rabbits did not displace labelled prolactin from mammary-gland plasma membranes (Kelly *et al.*, 1973). However, Gusdon *et al.* (1970) showed that rabbit placental extracts did inhibit haemagglutination by antisera to human placental lactogen. The purpose of the present study was to determine if a lactogen was present in rabbit placenta and, if its existence was confirmed, to isolate and characterize this hormone.

Experimental

Materials

Hormones. Bovine growth hormone (NIH-BGH-B17) and bovine prolactin (NIH-P-B3) were kindly provided by the Hormone Distribution Program,

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National Institute of Arthritis, Metabolism and Digestive Diseases, and were further purified on a column of Sephadex G-150 as previously described (Fellows & Rogol, 1969). Rat growth hormone (NIAMD-Rat GH-I-2 and NIAMD-Rat GH-RP-1), rat prolactin (NIAMD-Rat Prolactin-I-2 and NIAMD-Rat Prolactin-RP-1), rabbit growth hormone (AFP-684-C) and rabbit prolactin (AFP-808-P) were generously supplied by the Rat Pituitary Hormone Distribution Program, National Institute of Arthritis, Metabolism and Digestive Diseases, and were used without further purification.

Reagents. Sodium dodecyl sulphate (Sequal grade) was purchased from Pierce Chemical Co. (Rockford, IL, U.S.A.), HCl (Ultrex) was from J. T. Baker Chemical Co., Phillipsburg, NJ, U.S.A.), and $(\text{NH}_4)_2\text{SO}_4$ (Ultra Pure) was from Schwarz/Mann (Orangeburg, NY, U.S.A.). Acrylamide and *NN'*-methylenebisacrylamide (Practical grade) were obtained from Eastman Kodak (Rochester, NY, U.S.A.) and were recrystallized from hot chloroform (70g/litre) and hot acetone (10g/litre) respectively. Phenylmethanesulphonyl fluoride was from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and ATP was from Calbiochem (San Diego, CA, U.S.A.). Sephadex G-150 (fine) was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden); Ultrogel AcA 34 was from LKB Instruments (Rockville, MD, U.S.A.);

DEAE-cellulose (DE-52) and CM-cellulose (CM-52) were from Whatman (Maidstone, Kent, U.K.). Iodo[^{14}C]acetic acid (lot no. 861-029) was obtained from New England Nuclear (Boston, MA, U.S.A.) and had a specific radioactivity of 13.3 Ci/mol. All other reagents were of the highest purity available from commercial sources.

Animals. Pregnant rabbits (New Zealand albino) were purchased from a local rabbitry, and the placentas were surgically delivered on day 28–30 of gestation.

Methods

Radio-receptor assays. The purification of rabbit placental lactogen was monitored by the lactogenic radio-receptor assay developed by Shiu *et al.* (1973). Bovine prolactin was used for both the tracer and the standards during the isolation procedure, but for the recovery assays, rat prolactin was used as the tracer and both rat and rabbit prolactin were used as standards. The activity of purified rabbit placental lactogen was also measured in a modified growth-hormone radio-receptor assay (Tsushima & Friesen, 1973) by using rat growth hormone as the tracer and both rat and rabbit growth hormone as the standards. All hormones were iodinated by the lactoperoxidase method of Miyachi *et al.* (1972) as modified by Bolander & Fellows (1975) and had specific radioactivities between 66.2 and 97.9 $\mu\text{Ci}/\mu\text{g}$.

Purification protocol. All steps of this purification procedure were done at 4°C unless otherwise stated, and protein concentrations were determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Rabbit placentas were stored at -20°C until the day of processing, when they were partially thawed under running water. After removal of attached membranes, the cotyledons were rinsed in water, weighed, cut into 5 g pieces and homogenized in 3 vol. of ethanol. After centrifuging at 9000g for 30 min, the precipitate was air-dried on a Buchner funnel for 1–2 h and dried under a high vacuum (<65 kPa) overnight. The resulting powder was approx. 10–15% of the original wet weight. The placental powder was then extracted for 36 h with 12 vol. of 0.1 M-NH₄HCO₃, pH 9.0, containing 1 mM-phenylmethanesulphonyl fluoride, 0.1 mM-ATP and 0.1 mM-MgCl₂. After centrifuging at 27000g for 30 min, the supernatant was 40% saturated with (NH₄)₂SO₄ by the slow addition of the solid salt. Precipitation was complete after 45–60 min, as indicated by light-scattering at 320 nm, and the precipitate was removed by centrifugation at 27000g for 30 min. The supernatant was then made 70% saturated with (NH₄)₂SO₄, equilibrated for 1 h and centrifuged.

The precipitate from the 70%-satd.-(NH₄)₂SO₄ step was dialysed for 24 h against water, freeze-dried, redissolved in 0.1 M-NH₄HCO₃, pH 9.5, and applied

to a column (2.5 cm × 90 cm) of Ultrogel AcA 34 equilibrated and eluted with 0.1 M-NH₄HCO₃ (Fig. 1). Fractions active in the lactogenic radio-receptor assay were pooled, dialysed against water, freeze-dried, redissolved in 0.01 M-Tris/HCl, pH 9.0, and applied to a column (2.5 cm × 40 cm) of DEAE-cellulose equilibrated with the same buffer. A 1000 ml gradient was run from 0.01 M-Tris/HCl, pH 9.0, without NaCl to 0.01 M-Tris/HCl, pH 9.0, containing 0.1 M-NaCl (Fig. 2).

The active peak was pooled, dialysed against water, freeze-dried, redissolved in 0.01 M-ammonium acetate, pH 5.5, and applied to a column (2.5 cm × 20 cm) of CM-cellulose equilibrated in the same buffer. A 200 ml gradient was run from 0.01 M-ammonium acetate, pH 5.5, without NaCl to 0.01 M-ammonium acetate, pH 5.5, containing 0.1 M-NaCl (Fig. 3). The active material was pooled, dialysed against water and freeze-dried. All subsequent studies were conducted with material obtained from CM-cellulose chromatography.

Polyacrylamide-gel electrophoresis. Disc electrophoresis was performed at pH 9.0 by the method of Ornstein (1964) and Davis (1964). Electrophoresis in sodium dodecyl sulphate was carried out as described by Weber & Osborn (1969) by using a 10% resolving gel. Both methods were performed at 2–3 mA/gel on a model 1200 Canaco disc electrophoresis apparatus

Fig. 1. Chromatogram of rabbit placental lactogen on Ultrogel AcA 34

The sample [1723 mg of the 40–70%-satd.-(NH₄)₂SO₄ precipitate] was applied to a column (2.5 cm × 90 cm) of Ultrogel AcA 34 equilibrated and eluted with 0.1 M-NH₄HCO₃. The solid bar indicates the fractions pooled for further purification. The column was eluted at 25 ml/h and the fraction size was 5 ml. —, Protein concentration (E_{280}); ····, haemoglobin concentration (E_{410}); ----, rabbit placental lactogen.

Fig. 2. Chromatogram of rabbit placental lactogen on DEAE-cellulose

The sample (1090mg of the active fraction from the Ultrogel column) was applied to a column (2.5 cm $\times$ 40 cm), equilibrated with 0.01 M-Tris/HCl, pH 9.0, and a 1000 ml gradient was run from 0.01 M-Tris/HCl without NaCl (A) to 0.01 M-Tris/HCl with 0.1 M-NaCl (B). The eluting buffer was then changed to 1.0 M-NaCl (C). The solid bar indicates the fractions pooled for further purification. The column was pumped at 100 ml/h, and the fraction size was 15.5 ml. —, Protein concentration (E_{235}); ----, rabbit placental lactogen.

Fig. 3. Chromatogram of rabbit placental lactogen on CM-cellulose

The sample purified by DEAE-cellulose chromatography (85 mg) was applied to a column (2.5 cm $\times$ 20 cm), equilibrated with 0.01 M-ammonium acetate, pH 5.5, and a 200 ml gradient was run from 0.01 M-ammonium acetate without NaCl (A) to 0.01 M-ammonium acetate with 0.1 M-NaCl (B). The eluting buffer was then changed to 0.2 M-NaCl (C) and finally to 1.0 M-NaCl (D). The solid bar indicates the fractions pooled for study. The column was pumped at 100 ml/h and the fraction size was 6.5 ml. —, Protein concentration (E_{235}); ----, rabbit placental lactogen.

at 23°C. All gels were fixed in 10% (w/v) trichloroacetic acid and stained with Coomassie Blue.

Isoelectric point. The isoelectric point of rabbit placental lactogen was determined on a model 1200 Canalco disc electrophoresis apparatus by using pH 5–7 ampholytes (Bio-Lytes; Bio-Rad Laboratories, Richmond, CA, U.S.A.). pH was determined on extracts of the gel slices and confirmed by electrophoresis of standard proteins with known isoelectric points.

Molecular weight. The molecular weight was determined by sedimentation-equilibrium centrifugation in a Beckman model E analytical ultracentrifuge by using the meniscus-depletion method of Yphantis (1964). The samples were dissolved in 0.05 M-NaHCO₃, pH 9.0, containing 0.15 M-NaCl and 6.5 mM-dithiothreitol and were loaded into a double-sector synthetic-boundary cell. The partial specific volume of the hormone was calculated from its amino acid composition as described by Schachman (1957). The molecular weight was also estimated from the molecular size indicated by polyacrylamide-gel electrophoresis in sodium dodecyl sulphate, as described above. The standards used were bovine serum albumin (mol.wt. 69000), aldolase (mol.wt. 40000), pepsin, (mol.wt. 35000), chymotrypsinogen (mol.wt. 25000), bovine prolactin (mol.wt. 23000), bovine growth hormone (mol.wt. 21800) and ribonuclease (mol.wt. 13700). The bovine serum albumin, chymotrypsinogen and ribonuclease were from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and the aldolase and pepsin were from Worthington Biochemical Corp. (Freehold, NJ, U.S.A.).

Amino acid composition. Rabbit placental lactogen was hydrolysed in 6 M-HCl at 110°C under reduced pressure for 24, 48, 72 and 96 h. The hydrolysates were then subjected to amino acid analysis on a Beckman 121M microcolumn amino acid analyser by using single-column methodology. The cysteine content was determined as cysteic acid after performic acid oxidation (Hirs, 1967), and by scintillation counting after reduction and alkylation with iodo[¹⁴C]₁acetic acid (Crestfield *et al.*, 1963). Tryptophan was measured by the spectrophotometric method of Edelhoch (1967), and total amide content was determined by Conway microdiffusion (Wilcox, 1967).

N-Terminus. Rabbit placental lactogen was reduced and carboxymethylated as described above (Crestfield *et al.*, 1963) and 150 nmol was subjected to automated Edman degradations on an updated Beckman model 890B sequencer by using a Quadrol program. After conversion, the amino acid phenylthiohydantoin derivatives were analysed by g.l.c. and t.l.c. (Niall, 1973).

Immunocharacterization. Double immunodiffusion was performed as described by Ouchterlony (1949) on ether-washed slides coated with 1% Noble agar (Difco Laboratories, Detroit, MI, U.S.A.) in 0.05 M-

Tris/HCl, pH9.0. The immunological properties of rabbit placental lactogen were also evaluated in radio-immunoassays for rat growth hormone and rat prolactin by using both reagents and the instructions provided by the Rat Pituitary Hormone Distribution Program, National Institute of Arthritis, Metabolism and Digestive Diseases, except that the hormones were iodinated by the lactoperoxidase method (Bolander & Fellows, 1975) rather than with chloramine-T. The preparation of antisera to bovine placental lactogen was described by Bolander & Fellows (1976).

Results

Hormone purification

Rabbit placental lactogen was eluted from Ultrogel ACA 34 as a single peak ($K_{av} = 0.73$), with an ascending shoulder similar to that seen with bovine placental lactogen on gel filtration (Bolander & Fellows, 1976). The hormone was co-eluted with bovine growth hormone and chromatographed slightly ahead of bovine prolactin ($K_{av} = 0.78$). Because of the limited availability of rabbit growth hormone and prolactin, the properties of rabbit placental lactogen were often compared with those for the bovine or rat hormones. Unlike bovine or monkey placental lactogen (Shome & Friesen, 1971), only one active peak was seen on DEAE-cellulose chromatography and it was eluted from the column between salt concentrations of approx. 0.05M- and 0.075M-NaCl. On CM-cellulose, rabbit placental lactogen was eluted as a single symmetrical peak after a 200ml gradient to 0.1M-NaCl.

A summary of the purification protocol is given in Table 1. The data are from a preparation which began with 196.4g of semi-thawed placentas from five pregnant does.

Polyacrylamide-gel electrophoresis

Disc gel electrophoresis of rabbit placental lactogen reveals a single major band having a mobility of 0.511 and a minor band having a mobility of 0.489

(Plate 1). Bovine growth hormone is more retarded (mobilities of 0.242, 0.330 and 0.403), whereas bovine prolactin runs faster (mobilities of 0.667 and 0.749). Occasionally a diffuse band is seen at the top of the disc gel and is thought to represent an aggregate. In a similar system, rabbit growth hormone migrates more slowly than rabbit placental lactogen (mobilities of 0.198, 0.264 and 0.308; Ellis *et al.*, 1968); no data are available from the literature on the mobility of rabbit prolactin on alkaline disc gel electrophoresis.

Molecular weight

In the presence of 6.5mM-dithiothreitol, rabbit placental lactogen has a mol.wt. of 20600 by sedimentation equilibrium (Fig. 4), and this agrees well

Fig. 4. Molecular-weight determination of rabbit placental lactogen by sedimentation-equilibrium centrifugation

The hormone was dissolved in 0.05M-NaHCO₃, pH9.0, containing 0.15M-NaCl and 6.5mM-dithiothreitol and was loaded into a double-sector synthetic-boundary cell. The same was centrifuged at 40000 rev./min for 48 h at 20°C in a Beckman model E analytical ultracentrifuge. y_0 , Radius (cm) from the centre of rotation to the top of the meniscus; y_r , radius (cm) from the centre of rotation to the point of measurement.

Table 1. Purification of rabbit placental lactogen

The data given below were from a preparation which began with 196.4g of semi-thawed cotyledons from surgically obtained placentas from five does. Protein concentrations were determined by the method of Lowry *et al.* (1951), and activity was measured in the lactogenic radio-receptor assay (Shiu *et al.*, 1973) by using rat prolactin as the tracer and both rat and rabbit prolactin as the standards.

Purification step	Total protein (mg)	Total activity (μ g)	Specific activity (ng/mg)	Successive yields (%)	Cumulative yields (%)
1. Extraction	3795	53	14	—	100
2. Precipitation	1723	45	26	85	85
3. Gel filtration	1090	42	39	93	79
4. DEAE-cellulose	85	22	263	52	42
5. CM-cellulose	19	11	579	50	21

EXPLANATION OF PLATE I

Disc gel electrophoresis of rabbit placental lactogen

Rabbit placental lactogen (*b*) is compared with bovine growth hormone (*a*) and bovine prolactin (*c*). Electrophoresis was performed at pH 9.0 in a 7.5% polyacrylamide gel at 2–3 mA/gel. The anode is at the bottom.

with the minimum mol.wt. of 22100 obtained from the amino acid composition. However, this hormone gives rise to four bands on polyacrylamide-gel electrophoresis in sodium dodecyl sulphate; these bands correspond to proteins having mol.wts. of 24200, 43500, 60600 and 78600. In view of the aggregation problems typically encountered in this family of hormones (Dellacha *et al.*, 1968), it is noteworthy that these molecular weights are approximate multiples of that of the rabbit placental lactogen monomer.

Isoelectric point

When rabbit placental lactogen was subjected to isoelectric focusing on disc gels with narrow-range ampholytes (pH 5–7), only one band was seen. It had an isoelectric point of 6.13.

Table 2. Amino acid composition of rabbit placental lactogen

Values for amino acids are expressed as residues per molecule, based on a molecular weight of 22000 and the nearest integral residues are given in parentheses. The amino acid composition of rabbit placental lactogen is compared with that of rabbit growth hormone (Ellis *et al.*, 1968) and rat prolactin (Parlow & Shome, 1976).

Amino acid	Rabbit placental lactogen	Rabbit growth hormone	Rat prolactin
Lysine	15.2 (15)	12	13
Histidine	6.0 (6)	3	6
Arginine	8.1 (8)	14	8
Aspartic acid	21.1 (21)	17	23
Threonine*	11.2 (11)	9	6
Serine*	15.1 (15)	14	12
Glutamic acid	22.1 (22)	26	28
Proline	11.8 (12)	7	12
Glycine	14.9 (15)	11	6
Alanine	15.2 (15)	16	10
‡ Cystine†	6.1 (6)	3	6
Valine	15.0 (15)	8	12
Methionine‡	3.1 (3)	3	2
Isoleucine	4.1 (4)	5	14
Leucine	12.9 (13)	25	23
Tyrosine	7.1 (7)	6	7
Phenylalanine	8.0 (8)	12	7
Tryptophan§	2.2 (2)	1	2
Amides	13.9 (14)	—	—
No. of residues	198	192	197

* Extrapolated to zero time of hydrolysis.

† Determined by scintillation counting after reduction and alkylation with iodo[¹⁴C]₁acetic acid and as cysteic acid after performic acid oxidation.

‡ Determined as methionine sulphone after performic acid oxidation.

§ Determined spectrophotometrically (Edelhoc, 1967).

|| Determined by Conway microdiffusion (Wilcox, 1967).

Amino acid composition

The amino acid compositions of rabbit placental lactogen, rabbit growth hormone and rat prolactin are given in Table 2. The numbers of residues are remarkably similar; however, like bovine (Bolander & Fellows, 1976) and sheep placental lactogen (Hurley *et al.*, 1975), rabbit placental lactogen has a much lower leucine content (13 residues/molecule; cf. 25 for rabbit growth hormone and 23 for rat prolactin). The number of glutamic acid residues is also lower (22 residues/molecule; cf. 26 for rabbit growth hormone and 28 for rat prolactin), and glycine is slightly increased (15 residues/molecule; cf. 11 for rabbit growth hormone and 6 for rat prolactin).

After performic acid oxidation and acid hydrolysis, rabbit placental lactogen yielded 6.05 residues of cysteic acid per molecule, and reduction and alkylation with iodo[¹⁴C]₁acetic acid revealed 6.07 residues of carboxymethylcysteine per molecule. Amino acid analysis of the reduced and carboxymethylated hormone demonstrated quantitative conversion of the cysteine and complete recovery of the methionine residues. There were 2.18 residues of tryptophan per molecule, as determined spectrophotometrically (Edelhoc, 1967).

N-Terminus

When the carboxymethylated rabbit placental lactogen was subjected to automated Edman degradation, the phenylthiohydantoin of valine was identified in the first cycle by t.l.c. and g.l.c. Yields estimated by the latter were 90.7% of the expected.

Fig. 5. Lactogenic radio-receptor assay of rabbit placental lactogen

¹²⁵I-labelled rat prolactin was bound to a membrane fraction from rabbit mammary tissue and was displaced by increasing concentrations of rat prolactin (●), rabbit prolactin (■) or rabbit placental lactogen (▲).

The phenylthiohydantoin of alanine (5.3%) and leucine (3.3%) were the only other amino acids identified.

Immunocharacterization

By radioimmunoassay, rabbit placental lactogen (50 μ g) does not bind to antisera to rat growth hormone or rat prolactin, although as little as 0.5 ng of the rat hormones could be detected; standard curves for the rabbit growth hormone and rabbit prolactin in these assays were essentially identical with those for the rat hormones. By double immuno-

diffusion, rabbit placental lactogen (5mg/ml) does not form a precipitin line against undiluted antisera to bovine placental lactogen.

Radio-receptor assays

The displacement curves for rabbit placental lactogen in both the lactogenic and growth-hormone radio-receptor assays were parallel to those of the standards (Figs. 5 and 6), indicating that rabbit placental lactogen is binding to the same receptor sites as prolactin and growth hormone respectively. However, its activity was low in the lactogenic radio-receptor assay, since 1 mg of the pure hormone had the displacing potency of only 580 ng of rat prolactin. Similarly, it possessed a low specific radioactivity in the growth-hormone radio-receptor assay, where 1 mg was equivalent to only 670 ng of rat growth hormone.

Discussion

The low activity of rabbit placental lactogen in the lactogenic and growth-hormone radio-receptor assays, which closely resembles the activity of the bovine placental lactogen in the same assays (Bolander & Fellows, 1976), explains why it could not be detected by investigators using biological assays. Although most of the placental lactogens have low somatotrophic activity, they are usually potent lactogens; rat placental lactogen has 40% of the activity of sheep prolactin in the lactogenic radio-receptor assay (Robertson & Friesen, 1975), and sheep placental lactogen is as potent as ovine prolactin in this assay system (Handwerger *et al.*, 1974). The significance of these low activities in rabbit and bovine placental lactogen is unknown.

Fig. 6. Growth-hormone radio-receptor assay of rabbit placental lactogen

125 I-labelled rat growth hormone was bound to a plasma-membrane fraction from rabbit liver and was displaced by increasing concentrations of rat growth hormone (●), rabbit growth hormone (■) or rabbit placental lactogen (▲).

Table 3. Comparison of the physical and immunological properties of rabbit growth hormone, rabbit placental lactogen and rat prolactin

Average hydrophobicity ($H\Phi_{av}$) was calculated by the method of Bigelow (1967).

Property	Rabbit growth hormone*	Rabbit placental lactogen	Rat prolactin†
Molecular weight	21 800	22 300	22 600
Isoelectric point	5.8‡	6.1	5.46
Tryptophan content (residues/molecule)	1	2	2
Cysteine content (residues/molecule)	3	6	6
N-Terminus	Phe	Val	Leu
$H\Phi_{av}$ (J/residue)	261	239	287
Cross-reaction to:			
Anti-(rat growth hormone)	+	0	0
Anti-(rat prolactin)	0	0	+
Anti-(bovine placental lactogen)	0	0	0

* From Ellis *et al.* (1968).

† From Ellis *et al.* (1969).

‡ From Wilhelmi (1974).

Rabbit placental lactogen also resembles the other non-primate placental lactogens in several other properties. The similarity in their charge is reflected in their isoelectric points (6.1 for the rabbit molecule, 5.9 for the bovine molecule and 6.7 for the sheep molecule), their mobilities on disc electrophoresis and their elution profiles on ion-exchange chromatography. Further, it shares many characteristics with the rodent growth hormones and prolactins (Table 3). They have a monomer mol.wt. of approx. 22000 with a strong tendency to polymerize; for rabbit placental lactogen this tendency is readily apparent in the ascending shoulder on gel filtration (Fig. 1), the polymeric bands on polyacrylamide-gel electrophoresis in sodium dodecyl sulphate, and the small diffuse band which is retarded on disc electrophoresis (Plate 1). The oligomers of human (Belleville *et al.*, 1975), bovine (Bolander & Fellows, 1976) and sheep placental lactogen (Fellows *et al.*, 1976) produce a similar pattern on disc electrophoresis. The amino acid composition of rabbit placental lactogen closely resembles that of the rodent hormones, especially prolactin which possesses the same number of tryptophan residues and disulphide bonds as rabbit placental lactogen. In view of these similarities, it is surprising that it does not cross-react with antisera to either rat growth hormone or prolactin, since both human (Josimovich & McLaren, 1962) and bovine (Bolander & Fellows, 1976) do cross-react with antisera to the growth hormones from their respective species. However, sheep placental lactogen does not cross-react with sheep growth-hormone antisera, although the latter growth hormone does cross-react with antisera to sheep placental lactogen (Handwerger *et al.*, 1974).

The primate placental lactogens exhibit a strong structural similarity to their growth hormones: human placental lactogen possesses a 92% homology in amino acid sequence with human growth hormone (Niall *et al.*, 1973), whereas monkey placental lactogen shares 11 of 42 tryptic peptides with human growth hormone (Shome & Friesen, 1971). However, the biochemical characteristics of rabbit, bovine (Bolander & Fellows, 1976) and sheep placental lactogen (Hurley *et al.*, 1975) suggest that these non-primate molecules may be more directly derived from the ancestral-stem hormone, which gave rise to the growth hormones and the prolactins.

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