CHROM. 3401

DISC AND CELLULOSE ACETATE ELECTROPHORESIS OF HUMAN PLACENTAL PROTEINS

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(First received December 13th, 1967; modified January 19th, 1968)

SUMMARY

A protein-rich concentrate, PLSR, has been isolated from acetone-dried human term placenta by steps involving extraction with water, dialysis and treatment of the dialysis residue with 95 % ethanol and the composition analyzed by electrophoresis on cellulose acetate and polyacrylamide gel. The latter criterion showed the presence of two pre-albumin bands and which could be further enriched by subjecting PLSR to COHN fractionation procedures. Pre-albumins occurred in only trace amounts in concentrates isolated from late pregnancy and cord sera. As based on electrophoresis on cellulose acetate, splitting of the γ-globulin was observed with several of the fractions. Of a number of media tested, water was quite effective in the extraction of the acetone-dried powder although PLSR obtained by way of a borate buffer of pH 9.0 contained appreciable pre-albumin. Disc electrophoresis of human chorionic gonadotrophin applied as a solution containing 39,000 IU/ml revealed bands analogous to those of PLSR but in contrast to the latter, the zones were of low intensity.

INTRODUCTION

The composition of placental proteins has been studied by several investigators and the plasma proteins identified by means of fluorescent antibodies by BARDAWIL *et al.*[1] and isolated by others[2–4]. MUZHNAI[5] extracted fresh washed minced placenta with barbital buffer and on agar gel electrophoresis, noted the presence of a weak albumin, a strong β_2-globulin and a zone in the α-globulin when staining was carried out without recourse to an immune developer. However, by use of the latter, a total of 7 arcs appeared consisting of a weak pre-albumin (PA), an albumin, an α_1- and α_2-globulin and two in the γ-globulin region. In view of the availability of placenta, commercial schemes based on COHN fractionation procedures have been instituted for the isolation of plasma proteins, notably albumin and γ-globulin[6–8]. Although complete removal of blood proteins from placenta is virtually an impossibility, yet definite placental protein components have been described and tested. Thus, the placenta is reported to produce, among others, the purified protein, PPP[9,10], a shock-producing factor[11], a contractile protein related to or identical with actomyosin[12], a lactogen[13], a lipid-mobilizing entity[14] and a hormone simulating human

somatotrophin[15–17]. The latter has been extracted from a placental by-product, Fraction VII, in γ-globulin isolation[17]. A protein, TPP, is purported to occur in γ-globulin isolated by the COHN procedures from frozen placenta but only in minimal amounts in products from the fresh unfrozen tissue; it could not be detected in human blood[18].

In the present study, placenta was dried with acetone and an aqueous extract submitted to fractionation by COHN methods, emphasis being directed toward the concentration of PA and the relative distribution followed by electrophoresis on cellulose acetate and polyacrylamide gel. In this respect, DEUTSCH AND GOODLOE[19] using a modified Tiselius apparatus, noted the presence of a component which moved ahead of albumin on electrophoresis of blood plasma of a variety of mammalian and avian species and recently, GERSHBEIN AND SPENCER[20] demonstrated PA levels up to 7 % in mink serum. The occurrence of PA in cerebrospinal fluid is well known and the distribution of such components in this fluid under various pathological conditions continues to occupy the attention of many workers. Although it occurs in small quantity in human serum, PA has been isolated from this source and purified by SCHULTZE *et al.*[21] and GOT AND BOURRILLON[22]. PA plays a role in biological phenomena, especially in relation to its binding with thyroxine[23, 24], in which a greater affinity is displayed than in the combination of the hormone with thyroxine-binding protein, TBP[25]. The latter has been shown to exist electrophoretically in the α_1- and α_2-globulin region[26]. TATA[27] observed the formation of a complex between PA and α_1- and α_2-globulin based on recovery experiments in which serum containing added PA was electrophoresed. He concluded that the thyroxine binding power of TBP was due mainly to the PA present in the α-globulin–PA complex. A high concentration of serum ^{131}I–trichloroacetic acid-soluble radioactivity has been shown to occur in the PA fraction[28].

EXPERIMENTAL

Cellulose acetate strips (S&S) originated from Serometrics and measured 12 × 2.5 cm with a thickness of 150–200 μ and porosity of 1–3 μ. For paper electrophoresis, an Owen buffer of pH 8.6 was employed (composition: 5.0 g sodium barbital, 1.9 g anhydrous sodium acetate, 0.38 g calcium lactate and 34.2 ml of 0.1 *N* HCl per liter[29]). The concentration of the latter was altered occassionally so as to enable the voltage across the system to be maintained at 160 V. The dye solution for staining the strips contained 1.0 g Buffalo Black 10B, 20 ml glacial acetic acid and 180 ml methanol. The latter dye in addition to Amido Schwarz and bromphenol blue applied to the polyacrylamide gels was obtained from the National Aniline Corporation. For disc electrophoresis, a kit was purchased from Canal Industrial Corporation and in later experiments, a Canalco Model 6 apparatus was used. The combination of gels produced a 7.5 % polyacrylamide gel of pH 9.4; a Tris buffer of 8.4 was employed in the disc electrophoresis. Human chorionic gonadotrophin (HCG) isolated from pregnancy urine and containing 1300 IU/mg was secured from Glogau and Company, Chicago.

Placental extracts

Drying of placenta. Human term placenta, refrigerated at 4°, was processed up to 24 h following delivery. The tissues were trimmed of cord and membranes, washed free of blood clots with portions of water (total: 1 l/placenta), cut into pieces and

blended with acetone in a Waring blender. The blend was transferred to an 18 l wide mouth jar and covered with excess solvent; 12 placentas were employed per bottle. The mixture was allowed to stand at 25° up to 7 days with frequent daily stirring, filtered under suction, washed with copious amounts of distilled acetone and the cake air-dried. Connective tissue fragments were removed and the product stored at 4°, a temperature used throughout in conjunction with aqueous solutions, unless otherwise stated. The weight of the placental parenchyme averaged 300 g and the dry powder amounted to 71 g or about 24 % based on the wet tissue.

Fractionation of acetone-dried placental powder. The dried powder was blended with water (10 ml/g), centrifuged for 10 min at 3000 r.p.m. and the supernatant portion passed through four thicknesses of cheesecloth and recentrifuged. The volume of the filtrate represented 60–70 % of the water employed, the difference being involved in the wetting of the powder. The fluid was shell-frozen and lyophilized, the dry solid being stored at 4°. In several runs where large volumes were handled, final filtration was affected in a Sharples centrifuge and the fluid preserved with merthiolate (25–50 mg/l) prior to freezing and concentration. The resulting product was blended with 95 % ethanol and the supernatant fluid decanted. Extraction with fresh portions was continued until the washings were colorless and generally, 50 ml alcohol was required per g powder over a period of 48 h. The air-dried solid was taken up in water (10 ml/g) and the mixture allowed to steep for 1 h at 4° after which time it was centrifuged in the cold for 20 min at 15,000 r.p.m. The supernatant liquid was dialyzed in Visking cellophane casing (size 36–100) against water for 48 h with frequent changes at 4°. The freeze-dried dialysis residue is designated PLSR as depicted in the flow diagram, Fig. 1.

PLSR types were also isolated by extraction of 100 g portions of one batch of acetone-dried powder with Sorensen phosphate buffer of pH 7.1, acetate buffer

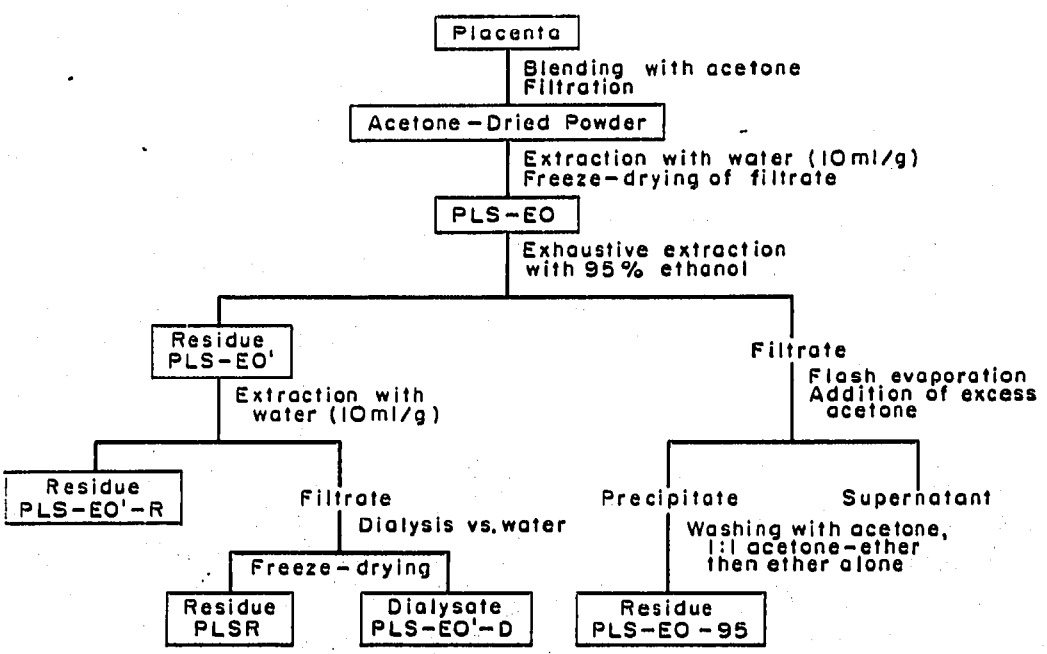

Fig. 1. Flow diagram depicting steps in the isolation of PLSR and allied concentrates from human placenta.

TABLE I

PLACENTAL CONCENTRATES FROM EXTRACTION OF THE ACETONE-DRIED TISSUE

Batch number[a]	*Acetone-dried tissue powder*	*Volume of filtrate*	*PLS-EO*[b]		*PLS-EO'*[b]	*Solid removed by alcohol*	*PLSR recovery*		
								Based on PLS-EO	*Based on dry powder*
	(*kg*)	(*l*)	(*g*)	(%)	(*g*)	(%)	(*g*)	(%)	(%)
1	2.3	15	108	4.7	76	30.0	14.0	13.0	0.61
2	12.0	72	560	4.7	433	22.0	106.0	18.9	0.88
3	8.0	58	314	3.9	231	31.7	60.0	19.1	0.75
4	10.0	70	498	5.0	436	30.5	70.6	14.2	0.71
5 (Saline)	0.100		5.12	5.1			0.33	6.4	
5 (Phosphate buffer; pH 7.1)	0.100		6.92	6.9			0.68	9.8	
5 (Acetate buffer; pH 3.5)	0.100		3.50	3.5			0.04	1.1	
5 (Borate buffer; pH 9.0)	0.100		3.93	3.9			0.68	9.8	

[a] Unless otherwise stated, water was employed in the extraction of the acetone-dried powder.
[b] For description, see Fig. 1.

(Walpole; pH 3.5), borate buffer (1.3 g boric acid and 8.4 ml 1 *N* NaOH per l; pH 9.0) and 0.90 % sodium chloride. The recoveries of fractions from 5 batches of acetone-dried powder, including the smaller runs with special buffer systems are presented in Table I. The subcutaneous injection of a saline solution containing 25 mg PLSR into the virgin rabbit did not affect the tubes or ovaries over a period of 48 h. The test for chorionic gonadotrophin was positive in both the rabbit and amphibian, *Xenopus laevis*, when the dosage was increased to 150 mg. The protein content of PLSR based on nitrogen (biuret) was 70.9, 76.5 and 78.1 % for batches 1–3, inclusive and ranged lower with the products extracted from the acetone-dried powder with buffers. Thus, the values were 44.9 and 58.5 % for PLSR isolated by means of the acetate and borate buffers, respectively.

Resolution of PLSR by COHN *procedures.* 'Method 6' of COHN *et al.*[30], originally designed for plasma protein fractionation by variations in alcohol concentration, ionic strength and pH at low temperatures was applied to 6 % solutions of PLSR. Adjustment was attempted to cope with the fact that the solution had a pH of 6.5–6.7, in contrast to plasma with a pH of 7.4–7.5. However, as too large a volume of buffer was required to raise the pH to the plasma value, this step was abandoned. A second COHN method[31] was also employed for the fractionation of PLSR. The resulting fractions were dissolved in water and freeze-dried. The final filtrate was flash-evaporated to remove alcohol, lyophilized, the solid redissolved in water, dialyzed against water (12 v/v solution) for 48 h and finally relyophilized (PLSR VI). The conditions and weight percentage yields of the various fractions derived from PLSR are shown in Table II. The weights of PLSR of batches 1–3 inclusive submitted to resolution by 'Method 6' were 16.0, 50.0 and 18.0 g, respectively, and of batch 4 employed in the second COHN method, amounted to 3.0 g. Unless otherwise indicated, the Roman numeral follows the conventional COHN fraction designation.

Human blood extracts

Cord serum. A pool of cord serum (49 ml) collected from 12 cases was treated with excess acetone and the resulting powder (2.87 g) blended with 50 ml water. The filtrate was freeze-dried and the solid (680 mg; recovery: 24 %) extracted with ethanol in an amount of 70 ml. The residue was taken up in 40 ml water and dialyzed against 800 ml water for 48 h. Processing of the residue yielded the PLSR-type concentrate in amounts of 378 mg or a recovery of 56 %.

Prenatal serum. Serum (70 ml) pooled from 10 women in the last trimester of pregnancy yielded 5.14 g powder on drying with acetone. The lyophilized aqueous extract (1.56 g; 30 % recovery) gave rise to 745 mg or a 48 % yield of the PLSR product.

Cellulose acetate electrophoresis

The cellulose acetate was soaked in Owen buffer, blotted to remove excess fluid and the 6–8 % test solution applied in amounts of 5–10 μl. The runs were carried out in a Shandon electrophoresis unit at 1.0–1.2 mA/strip and at 140–160 V; duration: 80–90 min. The strips were then dried at 90° for 15 min and stained with Buffalo Black 10B solution for 10 min, the excess dye being rinsed with methanol. The strips were dipped in 5 % acetic acid prior to drying between filter paper, cleared with mineral oil and scanned in a Varicord microdensitometer.

TABLE II

CONDITIONS AND RECOVERIES OF PLACENTAL FRACTIONS BY THE COHN PROCEDURES

Placental batch	COHN *fraction*	*pH at which fraction was removed*	*T* (°C)	*Alcohol concentration* (%)	*Recovery* (g %)
	'Method 6'[30]				
1	I	6.9	−2.5	8	2.0
	II + III	5.8	−5	25	6.7
	IV-1	5.1	−5	18	44.3
	IV-4	5.8	−5	40	2.4
	V	4.8	−6	40	8.4
	VI				22.0
2	I	6.6	−2.5	8	0.7
	II + III	6.5	−5	25	0.9
	IV-1	5.4	−5	18	42.6
	IV-4	6.0	−5	40	8.5
	V	4.8	−6	40	13.3
	VI				22.8
3	I	6.2	−2	8	1.3
	V	4.8	−6	40	3.7
	VI				15.3
	Later COHN *procedure*[31]				
4	I + III-3	—	0	—	2.7
	II	5.6	0	14	25.1
	III-0	6.4	0	15	17.0
	III-1,2	6.8	0	15	4.5
	IV-1	—	0	—	1.9
	IV-6 + 7	6.3	0	15	1.3
	V	5.5	0	15	1.0
	VI	5.8	0	18	24.8

Electrophoretic analyses for PLSR and subfractions isolated by 'Method 6' of the COHN group appear in Table III and tracings for PLSR as such and the derived fraction VI of batch 1 are shown in Fig. 2. The splitting of β-globulin did not occur consistently and accordingly, was calculated as one band. The splitting of γ-globulin as illustrated in Fig. 2 was noted in PLSR VI of batches 1–3 inclusive as well as in some of the other fractions and where splitting was absent, the values are expressed as total γ-globulin. PA was very distinct in PLSR VI of each batch in which it occurred at a high level. However, in most fractions, PA diffused into the albumin portion so that a line of demarcation was required. The unresolved protein, U.P., signifies an amount which did not migrate but remained at the site of application.

Disc electrophoresis[32]

With the pH of the buffer at 8.4 and at a constant current of 5 mA per sample gel, the corresponding voltage was 180–200. A solution of bromphenol blue was added to the buffer as tracking dye and in amount sufficient to give a slight tinge of blue in a 7.5 cm path. The sample gel was prepared by mixing 5 μl of the 6–8 % test solution into 0.25 ml of dilute upper gel and applying 0.15 ml. Electrophoresis was carried out for 30–40 min at which time the migration front was about 3.5 cm into the lower

TABLE III

WEIGHT PERCENTAGE DISTRIBUTION OF PLACENTAL COMPONENTS AS BASED ON PAPER ELECTROPHORESIS[a]

Batch	*Fraction*	*PA*	*Albumin*	*α-Globulin*	*β-Globulin*	*U.P.*[b]	*γ_1-Globulin*	*γ_2-Globulin*	*Total γ-globulin*
1	PLSR[c]	2.5	62.3	5.5	9.4	14.2			6.0
	PLSR I	1.8	20.6	12.1	14.9	44.6			6.0
	PLSR II + III	1.0	14.7	10.3	30.7	34.3			9.0
	PLSR IV-1	2.3	61.8	8.1	7.3	15.9			4.6
	PLSR IV-4	1.2	23.2	27.4	23.6	16.6			7.9
	PLSR V	1.0	71.1	6.5	7.0	8.5			5.9
	PLSR VI	10.2	28.1	6.7	24.3		16.1	14.6	30.7
2	PLSR	1.7	47.4	10.9	10.9	15.1	5.9	8.1	14.0
	PLSR I	1.5	48.6	20.6	22.3				7.0
	PLSR II + III	1.7	26.4	18.0	29.9	17.5			6.5
	PLSR IV-1	1.2	57.5	20.6	12.8		5.3	2.5	7.8
	PLSR IV-4	1.8	75.5	12.6	7.2	0.7			2.2
	PLSR V	1.8	86.0	8.1	2.0	1.5			0.6
	PLSR VI	11.1	3.9	14.5	19.8		33.8	16.9	50.7
3	PLSR	0.7	67.0	14.7	8.4	5.9			3.3
	PLSR I	2.2	57.3	17.0	16.3		5.3	1.9	7.2
	PLSR V	3.4	65.5	14.7	11.5				4.9
	PLSR VI	13.4	3.6	19.6	20.5		29.0	13.9	42.9

[a] Fractionation by 'Method 6' of COHN *et al.* [30].

[b] Unresolved protein or the portion remaining at the site of application.

[c] Tracing appears in Fig. 2.

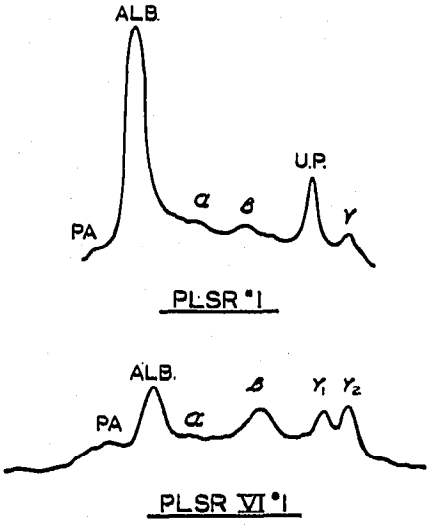

Fig. 2. Tracings of PLSR of placental batch 1 and fraction VI, the processed supernatant portion from the COHN procedure, obtained by cellulose acetate electrophoresis. U.P. denotes the unresolved protein at the site of application.

TABLE IV

WEIGHT PERCENTAGE DISTRIBUTION OF PLACENTAL FRACTIONS DETERMINED BY DISC ELECTROPHORESIS[a]

Batch	*Fraction*	*PA_1*	*PA_2*	*Albumin*	*Post albumin*	*β-Globulin*	*γ-Globulin region*
1	PLSR	3.4	4.1	58.0	14.2	13.6	6.7
	PLSR I	14.2	9.2	54.8	5.2	10.0	6.6
	PLSR II + III	4.3	2.8	36.2	22.7	20.0	14.0
	PLSR IV-1	12.4	10.0	50.8	14.8	8.0	4.0
	PLSR IV-4	4.0	1.6	46.4	11.9	21.2	14.9
	PLSR V	4.1	3.4	59.0	9.1	11.8	12.6
	PLSR VI	17.0	10.0	26.4	19.0	12.0	15.0
2[b]	PLSR	8.1	11.8	55.6	7.1	10.4	7.0
	PLSR I	14.3	7.8	49.2	7.0	8.7	13.0
	PLSR II + III	18.3	9.4	44.8	4.9	10.9	11.7
	PLSR IV-1	15.3	19.7	41.2	5.2	11.2	7.4
	PLSR IV-4	5.2	9.9	51.0	6.5	13.6	13.8
	PLSR V	4.7	6.1	62.9	8.0	13.9	4.4
	PLSR VI	11.3	32.5	2.2	5.3	31.2	17.5
3	PLSR	8.6	16.5	41.8	9.5	10.6	13.0
	PLSR I	14.7	8.4	60.0	3.9	7.9	5.1
	PLSR V	3.7	7.8	64.2	4.6	9.3	10.4
	PLSR VI	6.4	11.0	3.1	48.3	18.8	12.4
4	PLSR II	0.0	0.0	64.5	16.0	11.7	7.8
	PLSR III-0	0.0	0.0	61.3	14.7	17.5	6.5
	PLSR III-1,2	3.1	7.0	42.5	16.6	17.5	13.3
	PLSR V	51.6	16.8	7.9	5.5	13.3	4.9
5	PLSR[c]	20.0	10.6	26.3	15.2	15.2	12.6

[a] Subfractions were obtained by 'Method 6' of the COHN group[30] except for batch 4, which employed a later procedure[31].

[b] Tracings and electrophoretograms appear in Figs. 3 and 4.

[c] From extraction of the acetone-dried placental powder with borate buffer of pH 9.0

gel. The gel was stained with Amido Schwarz solution for 16 h and destaining was performed in 7.5 % acetic acid. The destained gel was preserved in 7.5 % acetic acid and an integrated tracing obtained in a Canalco Model E microdensitometer.

Disc electrophoretic data are presented in Table IV for PLSR and COHN subfractions and in which the β-globulin is calculated as a single peak. Although visual observation indicated two sharp bands lying close together, the β-globulin appeared as one peak on scanning. The region between the β-globulin and the spacer gel constituted the slow moving globulins and is denoted as the γ-globulin area, whereas, the region between the albumin and β-globulin is labeled the 'post albumin'. Tracings for COHN fractions from PLSR of batch 2 depicting the relevant areas and electrophoretograms for two of these samples appear in Figs. 3 and 4, respectively. Some of the sub-

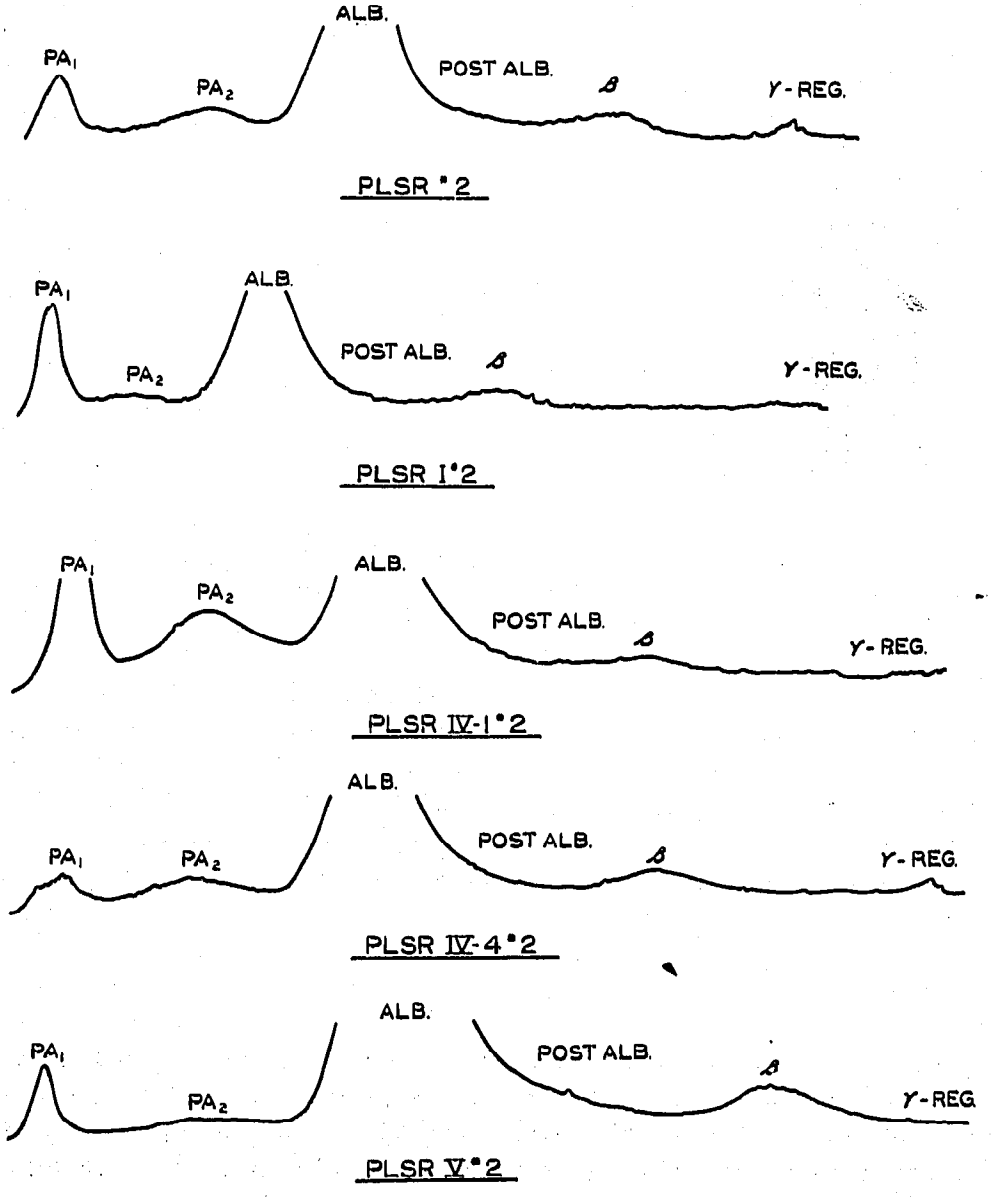

Fig. 3. Disc electrophoretic tracings of PLSR of batch 2 and derived COHN fractions showing off-the-paper scan of albumin and one PA_1 band.

fractions from batch 4 isolated by way of the second COHN method[31] displayed a few measurable peaks. Thus, fractions I + III-3, IV-6 + 7 and VI presented two faint bands in the β-globulin region and in common with fraction IV-1, only one faint PA_1 band. Although PLSR prepared by extraction of the dried placental powder with borate buffer contained a high level of PA (30.6 %; Table IV), the analogous products obtained by use of saline and phosphate and acetate buffers did not present measurable or organized patterns. The acetate-extracted PLSR displayed faint PA_1, PA_2 and albumin and two sharp bands in the β-globulin region; each of the bands of PLSR isolated by way of the phosphate buffer was faint at concentrations of protein comparable to those employed with the conventional concentrate.

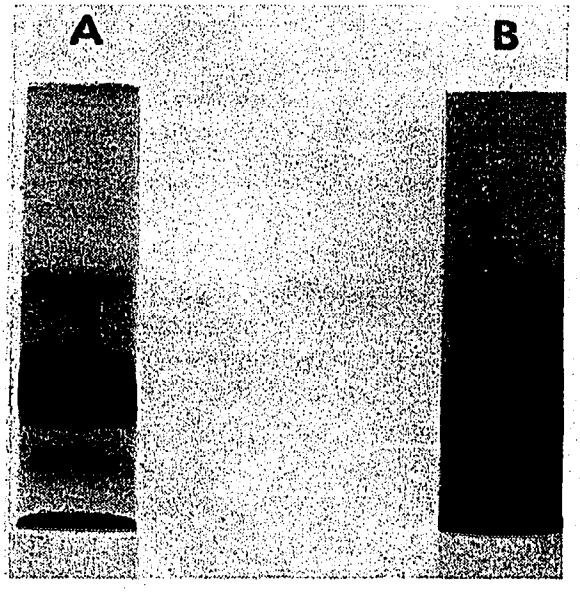

Fig. 4. Polyacrylamide gel electrophoretograms of (A) PLSR and (B) PLSR IV-1 derived from batch 2 and for which tracings and analyses are presented in Fig. 3 and Table IV, respectively. Increasing electrophoretic mobility is toward the bottom as represented.

PLSR-type products isolated from prenatal and cord sera showed disc electrophoretic patterns similar to an analogous concentrate from a large adult male serum pool. PA was observed as faint bands, two occurring in cord and pregnancy PLSR and up to three, in the male serum product but these could not be recorded by the microdensitometer. On disc electrophoresing a solution of HCG containing 39,000 IU/ml, 7 bands were obtained, each of which was far lower in intensity as compared to those of placental PLSR.

DISCUSSION

The present report advances information on various protein concentrates from washed human term placenta which was converted to a powder of good storage properties on drying with acetone. The powder was extracted with water and the resulting freeze-dried filtrate treated with 95 % ethanol, whereby a total of about 30 % of the solids was removed (Table I). When an aqueous solution was dialyzed against water, the nondialyzable dried product, PLSR. was obtained with a recovery

of just under 1 % as based on the acetone-dried powder. Similar PLSR products were isolated from late pregnancy, cord and adult male sera but only 4 % by weight was extracted by ethanol. The present treatment brings about the dissociation of lipoproteins and other components. Thus, the yellowish solid obtained on addition of excess acetone to the alcoholic extract from PLS-EO (Fig. 1) whitened on washing with ether and the solubility became less in water but increased in chloroform.

Such preliminary processing yielded a suitable product toward the concentration of PA, a component which was essentially absent in placental protein concentrates reported in the past, except possibly for the study of MUZHNAI[5]. PLSR lent itself to further fractionation by COHN procedures for plasma proteins, 'Method 6'[30] and a later one designed for smaller amounts of material[31]. The basic difference between the two is that the latter procedure deals with the extraction of fractions from precipitates, whereas, the first one involves the removal of subfractions by precipitation. However, it must be emphasized that the pH of PLSR solutions (6.5–6.7) was not raised to that of plasma, a step which would have required relatively large amounts of buffer and no attempt was made to employ alkali in order to avoid artifacts. As based on paper electrophoresis, with few exceptions, the resulting PLSR subfractions possessed the same type of components as in the case of serum as such, but varied in their respective distribution from fraction to fraction. PLSR and allied products are not simply mixtures of blood proteins but are unique in containing a variety of protein factors and hormones as reviewed above.

In relation to paper electrophoresis, 'Method 6' gave rise to fractions containing a PA peak, the latter being especially prominent in PLSR VI, the supernatant remaining after removal of fractions I–V, inclusive. Disc electrophoresis revealed two distinct PA bands, one of which occurred in the tracking dye region. That PA_1 is not tracking dye is evidenced by the disappearance of the band from human serum but not from PLSR and derived COHN fractions when many samples were electrophoresed simultaneously in a larger apparatus. No significant level of PA occurred in PLSR-type concentrates derived from late pregnancy and cord sera but the two faint bands observed in the gels might be related to those of placenta. COHN subfractions from serum PLSR were likewise low in PA. It is quite probable that by the enrichment procedures, liberation of PA might have resulted from the dissociation of PA-globulin complexes, not unlike those described for TBP by TATA[27]. Although the presence of calcium lactate in the Owen buffer enhances the resolution of β-globulin into two components, the splitting was not observed with all fractions. However, splitting of γ-globulin was frequent (Table III).

By disc electrophoretic criteria, PA was further concentrated in PLSR IV-1 and occurred in even greater amount in PLSR VI. The highest percentage was encountered in PLSR V of batch 4 fractionated by way of the later COHN procedure[31]. On comparing the PA levels by paper and disc electrophoresis, it is noted that the values by the latter approach are much higher; extensive shouldering of the peak was evident with cellulose acetate. In this respect, as disc electrophoretic criteria have much better resolving power, such PA data are probably much closer to the true values. The splitting of γ-globulin in PLSR VI and other fractions observed with cellulose acetate could not be followed too closely with the gels due to the fact that γ-globulin was not as pronounced as such areas as the albumin region. The extraction of the acetone-dried powder with the other aqueous media led to PLSR products of lower PA content

or in which the patterns were not as consistent in make-up as compared to the usual concentrate, except for the borate-extracted material in which a similar or even higher level resulted (Table IV).

The presence of chorionic gonadotrophin in PLSR was demonstrated by bioassay, dosages of 150 mg but not 25 mg, being effective on subcutaneous injection into the virgin rabbit doe. CAWLEY *et al.*[33] applied polyacrylamide gel electrophoresis to a commercial HCG preparation from human pregnancy urine and reported the presence of 7 bands consisting of two PA, one albumin, one post albumin, two in the transferrin region and one in the slow moving γ-globulin or slow α_2-globulin; the relative concentration or zonal intensity of each was not given. Immunodiffusion electrophoresis revealed that the transferrin region was the center of hormonal activity. In corroboration of such findings, a similar distribution obtained with a sample of HCG applied at a level of 39,000 IU/ml and which paralleled the PLSR gel bands but the latter were far more intense. Presumably, the presence of gonadotrophin contributes to the observed gel patterns of the placental products. A profitable approach to this problem might be a model investigation involving the concentration of human pregnancy urine, isolation of the protein portion and extensive disc electrophoretic analysis of components, experiments of which are not included in the current study. In this connection, according to a recent report[34], some differences were noted between amniotic fluid and maternal and cord sera by disc electrophoresis but no PA component was described. In yet another account[35], fluids obtained from women by amniocentesis were concentrated, dialyzed and submitted to gel electrophoresis. A constant feature of the fluids was the presence of β-globulin and four PA bands to the virtual exclusion of γ-globulin.

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