Purification and Physicochemical, Biological, and Immunological Characterization

(Received for publication, September 22, 1978, and in revised form, November 28, 1978)

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Procedures have been developed for the purification of pregnant mare serum gonadotropin (PMSG) and its α and β subunits. The procedure for the hormone purification involves three steps of column chromatography on Sephadex G-100, DEAE-Sephadex A-50, and hydroxypatite. The preparation of subunits involves the dissociation of PMSG with 10 mM urea followed by their separation by chromatography on DEAE-Sephadex and Sephadex G-100. The hormone and subunit preparations were found homogeneous by electrophoresis in polyacrylamide gel with or without sodium dodecyl sulfate and by immunodiffusion. The hormone had an activity of 13,740 I.U./mg as determined by in vivo and in vitro bioassays and receptor binding assays. The subunits did not show any significant activity by the receptor binding assay. The molecular weights of PMSG, PMSG-α, and PMSG-β, determined from Ferguson plots (Ferguson, K. A. (1964) Metabolism 13, 985-1002) using glycoproteins as molecular weight markers, were 64,030, 43,720, and 16,960, respectively. The amino acid and carbohydrate compositions of the hormone and the subunits have been determined. The carbohydrate content of the hormone was 41.7% and the α and β subunits contained 20.6% and 45.6% carbohydrate, respectively. (The individual subunits contained 20.6% and 45.6% carbohydrate.)

The carbohydrate moiety of the hormone is made up of L-fucose (0.6 to 0.9%), D-mannose (2.0 to 2.3%), D-galactose (10.6 to 12%), N-acetylglucosamine (9.0 to 10.5%), N-acetylgalactosamine (3.0 to 3.5%), and sialic acid (12.0 to 14%).

The purified PMSG was found to be three times as active as ovine lutropin (LH, luteinizing hormone) (23 NIH-LH-SI units/mg) and 2/3 as active as ovine follicle-stimulating hormone (FSH, follicle-stimulating hormone) (115.3 NIH-FSH-SI units/mg). FSH activity was determined by the Steelman-Pohley assay (Steelman, S., and Pohley, F. (1953) Endocrinology 53, 604-616) and the LH activity was measured by ascrobic acid depletion assay. As determined by binding assay, the individual subunits upon recombination recovered 27.2% of the LH activity and 62.5% of the FSH activity. Immunologically, PMSG-α and PMSG-β cross-reacted with anti-PMSG as found by radiimmunoassay. While human chorionic gonadotropin (hCG) and human-luteinizing hormone (hLH) competed with 125I-PMSG in the PMSG receptor binding assay, they showed little or no cross-reactivity in the

radioimmunoassay, indicating that the receptor and antibody binding sites are different from each other.

Various glycoprotein hormones, such as luteinizing (LH), follicle-stimulating (FSH), thyroid-stimulating, and human chorionic gonadotropin (hCG), have been purified and characterized in detail (1-12). Little, however, is known about the detailed chemistry and properties of pregnant mare serum gonadotropin (PMSG), a glycoprotein hormone that possesses both FSH and LH activities. Various procedures utilizing commercial preparations have been described for the isolation of PMSG (13-15). However, the yields of the purified hormones have been poor, presumably because of the use of low pH buffers in some of the purification schemes. These conditions are not optimal since it has been found that low pH is destructive to glycoprotein hormones, causing the hydrolysis of labile ketosidic bonds of sialic acid (16). Furthermore, thus far only limited physicochemical, biological, and immunological characterization of PMSG and its subunits has been carried out (13-15, 17, 18). In this communication, we wish to report methods for the purification of PMSG and its subunits, which not only are reproducible but also give high yields. The methods are suitable for large scale preparations of the hormone and the subunits which are prerequisites for carbohydrate and amino acid sequence studies. In addition, this communication describes the detailed chemical composition, particularly that of the carbohydrate in PMSG and the subunits, of the relations of PMSG and its subunits to hCG, ovine LH, and FSH and their subunits.

RESULTS

Purification of PMSG

A crude preparation of PMSG (1,660 I.U./mg) was resolved into two peaks on gel filtration on Sephadex G-100 (Fig. 1) with tubes 75 to 110 containing most of the activity (3,200

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The abbreviations used are: FSH, follicle-stimulating hormone; LH, luteinizing hormone; hLH, human-luteinizing hormone; hCG, human chorionic gonadotropin; PMSG, pregnant mare serum gonadotropin; oLH, ovine-luteinizing hormone; oFSH, ovine follicle-stimulating hormone; SDS, sodium dodecyl sulfate.

2 Portions of this paper (including "Materials and Methods," Figs. 1 to 5 and 9 to 13, and Tables I to VII) are presented in miniprint at the end of this paper. The abbreviation used is BSA, bovine serum albumin. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 78m-1699, cite author(s) and include a check or money order for $3.75 per set of photocopies.

4253
the homogeneity of the preparation. Also, according to disc
final form (D), PMSG migrated as a single band, suggesting
on Sephadex G-100 (B), PMSG after chromatography on
hydroxyapatite (D). In the
DEAE-Sephadex A-50 (C), and the final purified preparation
of PMSG after chromatography on hydroxyapatite column. The active fractions from the hydroxyapatite column showed a final activity of 13,740 and 11,330 I.U./mg (material from the first and second active peaks in Fig. 2, respectively). The activities were determined by both binding and bioassay and they represent a respective 7.8- and 6.8-fold increase in purity from the original crude preparation. The final recovery of activity was 44.2%.

Preparation of Subunits

PMSG was found to consist of two dissimilar subunits, α and β, which were separated on the basis of both charge and size. The first separation involved chromatography on DEAE-Sephadex A-50 with a stepwise discontinuous gradient. The urea-dissociated PMSG gave two distinct peaks. The first peak, designated as PMSG-α, appeared with the starting buffer; the second, designated as PMSG-β, appeared with 0.15 M NaCl in 0.04 M Tris/PO₄ buffer. (Fig. 4). After DEAE-Sephadex A-50 chromatography, the α and β subunits were desalted by using a column of Sephadex G-25 (coarse), equilibrated in 0.5% NH₄HCO₃. The recovery of the β subunit, consistently larger according to absorbance at 230 nm than the second, of the α subunit (Fig. 5). Recovery according to weight was 1.7 mg for β and 0.4 mg for α. Identification of the α and β subunits was made by amino sugar analyses of the subunits and compared to analyses of the subunits prepared by chromatography on DEAE-Sephadex A-50. In both cases, the α subunit lacked N-acetylgalactosamine, a component of PMSG-β, indicating that the subunits were free of cross-contamination. In order to ensure the purity of the subunits, it is advisable to prepare the subunits initially by DEAE-Sephadex chromatography and then subject each subunit, after further treatment with 10 mM urea, to additional purification by gel filtration on Sephadex G-100. (Figs. 4 and 5).

Homogeneity of PMSG and Its Subunits

The purity of PMSG was examined by disc and SDS-gel electrophoresis and by the Ouchterlony agar immunodiffusion technique. PMSG was shown to be homogeneous by disc gel electrophoresis in 7% polyacrylamide gel, pH 8.3, at various stages of purification (Coomassie blue staining). Electrophoresis was carried out for 1 h at 5 mA/tube. A, crude PMSG; B, after gel filtration on Sephadex G-100; C, after chromatography on DEAE-Sephadex A-50; D, after chromatography on hydroxyapatite; E, PMSG-α; F, PMSG-β. Lower panel, SDS-gel electrophoresis. Electrophoresis in polyacrylamide gels, pH 7.0, was carried out for 4 h at 8 mA/tube. G, PMSG after chromatography on hydroxyapatite; H, PMSG-α (G and H were stained with Coomassie blue); I, PMSG after chromatography on hydroxyapatite; J, hCG (10,000 I.U./mg); K, ovomucoid; L, α-acid glycoprotein (I, J, K, and L were stained with periodic acid-Schiff reagent for glycoproteins).

![Fig. 6.](https://example.com/image.png)

**Fig. 6.** Upper panel, disc gel electrophoresis of PMSG in 7% polyacrylamide gel, pH 8.3, at various stages of purification (Coomassie blue staining). Electrophoresis was carried out for 1 h at 5 mA/tube. A, crude PMSG; B, after gel filtration on Sephadex G-100; C, after chromatography on DEAE-Sephadex A-50; D, after chromatography on hydroxyapatite; E, PMSG-α; F, PMSG-β. Lower panel, SDS-gel electrophoresis. Electrophoresis in polyacrylamide gels, pH 7.0, was carried out for 4 h at 8 mA/tube. G, PMSG after chromatography on hydroxyapatite; H, PMSG-α (G and H were stained with Coomassie blue); I, PMSG after chromatography on hydroxyapatite; J, hCG (10,000 I.U./mg); K, ovomucoid; L, α-acid glycoprotein (I, J, K, and L were stained with periodic acid-Schiff reagent for glycoproteins).

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**Fig. 6.** Upper panel, disc gel electrophoresis of PMSG in 7% polyacrylamide gel, pH 8.3, at various stages of purification (Coomassie blue staining). Electrophoresis was carried out for 1 h at 5 mA/tube. A, crude PMSG; B, after gel filtration on Sephadex G-100; C, after chromatography on DEAE-Sephadex A-50; D, after chromatography on hydroxyapatite; E, PMSG-α; F, PMSG-β. Lower panel, SDS-gel electrophoresis. Electrophoresis in polyacrylamide gels, pH 7.0, was carried out for 4 h at 8 mA/tube. G, PMSG after chromatography on hydroxyapatite; H, PMSG-α (G and H were stained with Coomassie blue); I, PMSG after chromatography on hydroxyapatite; J, hCG (10,000 I.U./mg); K, ovomucoid; L, α-acid glycoprotein (I, J, K, and L were stained with periodic acid-Schiff reagent for glycoproteins).

Molecular Weights of PMSG and Its Subunits

The molecular weight of the hormone was determined by means of a Ferguson plot (33). A series of five different electrophoretic runs were made in which the total acrylamide concentration was varied from 5.5 to 10% (Fig. 8). The plot of
The molecular weights of the subunits were determined by a method of least squares. The average molecular weight of gel concentration and log $R_F$ of both subunits were linear. The slopes of these lines, the retardation coefficients ($K_R$), were plotted as shown in Fig. 9. The average molecular weights of the $\alpha$ and $\beta$ subunits from two separate experiments were 16,960 and 43,720, respectively.

The molecular weights of PMSG, PMSG-$\alpha$, and PMSG-$\beta$ were also estimated by gel filtration on Sephadex G-100 using glycoproteins and protein markers for the calibration of the column. When glycoproteins were used as standards, the molecular weights calculated from the plot of log of molecular weight versus $V_0/V$ were 18,500, 57,500, and 65,000 for PMSG-$\alpha$, PMSG-$\beta$, and PMSG, respectively (Fig. 10). However, the protein markers gave higher values of 26,000, 64,000, and 71,000 for PMSG-$\alpha$, PMSG-$\beta$, and PMSG, respectively (Fig. 10).

### Amino Acid and Carbohydrate Compositions of PMSG and Its Subunits

Table II describes the chemical compositions of PMSG, hCG (16), oLH (39) and oFSH (40). Like hCG and other glycoprotein hormones, PMSG is rich in proline and half-cystine. Also notably high are threonine and serine. The carbohydrate moiety of PMSG is made up of sialic acid, L-fucose, D-galactose and D-mannose, N-acetylgalactosamine, and N-acetylglucosamine. Occasionally, N-glucose was observed in PMSG preparations but was found to be a contaminant and could be removed by chromatography on a column of Sephadex G-25 (coarse). The total carbohydrate content of PMSG (uncorrected for water content) was 41.7% and the amounts of sialic acid (14.5%) and galactose (11.6%) were higher than those present in any other glycoprotein hormone (Table II). The amino acid compositions of the $\alpha$ and $\beta$ subunits of PMSG show striking differences (Table III). PMSG-$\alpha$ is rich in lysine and glutamic acid. The $\beta$ subunit has a significantly higher content of arginine, serine, proline, alanine, half-cystine, and leucine. In general, the PMSG subunits have similar amino acid patterns as the corresponding subunits of hCG (15), oLH (39), and oFSH (40) (Table III). The carbohydrate compositions of the $\alpha$ and $\beta$ subunits of PMSG are quite different. The $\beta$ subunit has a significantly higher content of sialic acid (18.0%), galactose (13.5%), and N-acetylgalactosamine (9.9%) (Table III) as, unlike the $\alpha$ subunit, contains N-acetylgalactosamine. The total carbohydrate content of $\alpha$ is 21.5% and that of $\beta$ is 46.1% (uncorrected for water content). In carbohydrate content, the PMSG subunits show a closer resemblance to hCG than to the other hormones. While N-acetylgalactosamine is present in the $\beta$ subunits of PMSG and hCG, it is a component of both $\alpha$ and $\beta$ subunits in other glycoprotein hormones (Table III).

### Biological Properties of PMSG and Its Subunits

FSH and LH activities of PMSG by Bioassay—When highly purified oFSH (115.3 NIH-FSH-SI units/mg) was used as a standard, PMSG had a potency of 66.7% of the standard (Table IV). Using the ovarian ascorbic acid depletion assay test of Parlow (26), we found that PMSG had approximately 3 times more LH activity than purified oLH (2.3 NIH-LH-SI units/mg).

Receptor Binding Activity of PMSG and Other Hormones—In Fig. 11 is shown the dose-response curve for PMSG and its relationship to other hormones in the radioreceptor assay using $^{125}$I-PMSG and rat testicular homogenate. Purified hCG and oLH had greater ability than PMSG to compete with $^{125}$I-PMSG for the receptor. Fig. 11 and Table V show that purified hCG and oLH have 223.5 and 167.4% mean percent inhibitory activity, respectively, compared to 100% for pure PMSG. In a reciprocal experiment, it was found that it
took 5 times more PMSG than hCG to inhibit the binding of a testicular homogenate. As shown in Table V, PMSG had a 24.23% mean per cent inhibitory activity compared to 100% for hCG in the hCG radioimmunoassay system. These data agree with those of Gospodarowicz (41), which showed that the ability of the purified PMSG to inhibit $^{125}$I-LH binding to plasma membranes of bovine corpus luteum was 4 times less than the ability of hCG (20% purified but with a 5-fold correction factor). Also, it was found that there was near identity between PMSG and crude hCG in inhibiting $^{125}$I-PMSG binding to a testicular homogenate (Fig. 11 and Table V. 100% mean per cent inhibitory activity for PMSG and 95.5% for crude hCG) and that ofFSH had only 10.5% of the activity of PMSG (Fig. 11 and Table V). This slight activity shown by ofFSH is probably due to contamination by oLH in the preparation.

Receptor Binding Activity of Subunits of PMSG and Their Hybrids with hCG and ofFSH Subunits—The subunits of PMSG and their recombinants were examined for LH and FSH biological activity by their ability to compete with $^{125}$I-hCG in binding to Leydig cells of testes and by their ability to inhibit ofFSH binding to a testicular tubule preparation. The individual subunits had virtually no biological activity. Table VI shows that PMSG-α and PMSG-β have 0.02 and 0.05% of PMSG activity, respectively, in the hCG radioreceptor assay and 4.90 and 4.02% of PMSG activity, respectively, in the FSH radioreceptor assay. Upon recombination, the subunits recovered 27.2% of the hCG activity. However, much more of the FSH activity (62.4%) was restored upon recombination. Also, when PMSG-α was combined with both hCG-β and ofFSH-β, there was more hCG (137.3%) and FSH (150.1%) activity in the hybrid than in PMSG alone (Table VI).

Immunological Properties of PMSG and Its Subunits

Immunological Cross-reactivity of PMSG, PMSG-α, PMSG-β, oLH, ofFSH, and hCG in the $^{125}$I-PMSG Anti-PMSG Radioimmunoassay—The standard dose-response curve for PMSG radioimmunoassay displayed as the logit transform of the response variant versus the log dose of the antigen is shown in Fig. 12. Ninety percent of the $^{125}$I-PMSG used in this assay was precipitable in the presence of excess antibody. Prior to use, the serum was diluted 1:55,000 to bind 50% of the $^{125}$I-PMSG. When a more highly titrated antibody was used, it had to be diluted 1:100,000 to bind 50% of the $^{125}$I-PMSG. Also shown in Fig. 12 on the same logit transform are the inhibition plots for the reconstituted PMSG (PMSG-α + PMSG-β), PMSG-β, PMSG-α, oLH (2.3 NIH-LH SI units/mg), ofFSH (115.3 NIH FSH SI units/mg), and highly purified hCG (10,000 IU/mg) in the PMSG radioimmuno assay system. As summarized in Table VII, the reconstituted PMSG retained 69.9% activity. PMSG-α and PMSG-β cross-reacted with $^{125}$I-PMSG at higher doses with 4.0 and 7.9% of PMSG activity, respectively. Ovine LH, ofFSH, and hCG showed nonparallel cross-reactivity at higher doses, with oLH being the most reactive (1.7% of PMSG activity) and hCG the least reactive (<0.07% of PMSG activity, Table VII).

Immunological Cross-reactivity of PMSG and Its Subunits with the Other Hormones and Their Subunits in the hCG, hCG-α, hCG-β, and LH Radioimmunoassay Systems—The observation that hCG is least cross-reactive with PMSG is confirmed in reciprocal experiments in which inhibition curves were obtained for PMSG, PMSG-α, and PMSG-β in hCG, hCG-α, and hCG-β radioimmunoassays. As seen in Fig. 13 and Table VII, after the addition of 20 μg of PMSG, 81.3% of $^{125}$I-hCG was still bound to the antibody. This was equivalent to the inhibitory activity of 0.93 ng of hCG, or PMSG had <0.01% of the inhibitory activity of hCG in the hCG radioimmunoassay system. Likewise, PMSG-α even at the 5-μg level did not inhibit $^{125}$I-hCG-α equivalent to the lowest dose of hCG-α (0.032 ng). Thus, PMSG-α had less than 0.01% of the activity of hCG-α (Table VII) in the hCG-α radioimmunoassay system. Larger quantities of PMSG-β were required, while 0.562 ng of hCG-β was sufficient to displace 50% of bound $^{125}$I-hCG-β, i.e. 55 μg of PMSG-β was needed to displace the same percentage of bound $^{125}$I-hCG-β (Table VII) in the hCG-β radioimmunoassay system. Thus, PMSG and its subunits showed some nonparallel cross-reactivity in the hCG, hCG-α, and hCG-β radioimmunoassay systems. Similarly, lack of cross-reactivity of PMSG was found with ofLH in the LH radioimmunoassay system.

Immunological Cross-reactivity of PMSG and hCG and Their Subunits as Shown by the Ouchterlony Immunodiffusion Technique—Very poor competition between PMSG and hCG was also shown by immunodiffusion experiments. Pure PMSG was placed in the central well of an Ouchterlony plate and anti-hCG-α, anti-hCG-β, anti-hCG, and anti-PMSG were placed in four evenly spaced wells surrounding it. Only anti-PMSG gave a precipitin line with PMSG (Fig. 7).

DISCUSSION

PMSG is unique among all glycoprotein hormones because it has both LH and FSH activities contained in the same molecule. It is conceivable that these activities reside in different parts of the molecule. Thus, detailed structural characterization of PMSG should enable us to delineate the regions of its structure specific to each activity by comparison with the known amino acid sequences of hCG (10-12), LH (42, 43), and FSH (5-8). The present studies were aimed at: 1) the development of methods for the preparation of the hormone and subunits using experimental conditions which are least deleterious to the hormone or the subunits and 2) the detailed physicochemical, biological, and immunological characterization. The procedure for the purification of the hormone was based on three steps of chromatography on Sephadex G-100, DEAE-Sephadex, and hydroxyapatite. The subunits were prepared by a method similar to the one developed for hCG in our laboratory (44). Urea-dissociated PMSG was separated into subunits by chromatography on DEAE-Sephadex or Sephadex G-100, or both. These methods yielded homogeneous preparations as determined by electrophoresis in polyacrylamide gel with or without SDS and β-mercaptoethanol (Fig. 6) and by immunodiffusion (Fig. 7). Furthermore, hexosamine analysis of the subunits showed that the subunit preparations had no cross-contamination since, like hCG-β, PMSG-β was found to have all of the N-acetylgalactosamine present in PMSG. In this respect, PMSG resembles hCG rather than LH, FSH, and thyroid-stimulating hormone in which N-acetylgalactosamine is an integral component of both subunits. In fact, recently it has been found that in the above hormones, the distal N-acetylgalactosamine residue is replaced by N-acetylglactosamine in the chitobiase core (45) of the asparagine-linked carbohydrate units, whereas in hCG or PMSG it serves as a linkage residue in the Ser/Thr-linked carbohydrate units (46, 47).

There are conflicting reports in the literature concerning the molecular weight of PMSG and its subunits. Glycoproteins, as is generally recognized, give erroneous results when subjected to molecular weight determination by SDS-polyacrylamide gel electrophoresis and gel filtration, particularly when the molecular weight markers used are proteins. By using glycoprotein markers we have attempted to determine the molecular weights of PMSG and its subunits by Ferguson plots (33, 34). The molecular weight values for PMSG, PMSG-
β, and PMSG-α thus obtained were 64,030, 43,730, and 16,690, respectively. Thus, contrary to previous reports (13), PMSG consists of subunits of different molecular size. Gospodarowicz reported a value of 53,000 for PMSG and identical molecular weights of 23,000 for each subunit (13). This discrepancy in the data from the two laboratories is probably due to the fact that the β subunit of PMSG does not stain readily with Coomassie blue on SDS-gels (Fig. 6G). However, it can be visualized clearly by periodic acid-Schiff stain (Fig. 6J). Therefore, failure to detect the β subunit band would have led to the single band of the α subunit being mistaken for subunits of identical size (compare Gels G and H in Fig. 6). Additional evidence in support of dissimilar subunits is that urea-dissociated PMSG resolves on Sephadex G-100; in fact, better than hCG subunits under identical conditions (16) with molecular weights of 15,000 and 23,000. This separation of the PMSG subunits and Sephadex G-100 is due to size rather than conformational differences and the yields of the α and β subunits from Sephadex G-100 have been invariably quantitative and found to be in a ratio of approximately 1:2 by weight. The amino acid compositions of PMSG and the subunits follow similar patterns as in other glycoprotein hormones and show the typical characteristics of high half-cystine and proline contents, implying similarity of structures. Hybridization experiments support this observation. PMSG-α can hybridize with hCG-β and oFSH-β yielding hybrids with higher hCG and oFSH activity than that present in PMSG determined by radioreceptor assay, indicating that the α subunits of the hormones must be quite similar. On the other hand, the β subunit of PMSG combines poorly with hCG-α or oLH-α and oFSH-α, suggesting that the β subunit of PMSG is hormone-specific and is, therefore, different from that of the other hormones. The radiomimunnoassay data only partly support these findings. As expected, PMSG-β does not compete with 125I-hCG-β in an hCG-β radioimmunoassay system. On the other hand, although the α subunit of PMSG from hybridization experiments would appear to be similar to the other α subunits, it fails to compete with 125I-hCG-α for anti-hCG in an hCG-α radioimmunoassay system.

Among all glycoprotein hormones, PMSG has the highest content of carbohydrate, almost 50% of the molecule. In other hormones, the content varies from 20 to 33%. The percentage of carbohydrate in the β subunit is twice as great as that present in PMSG-α. The sialic acid and galactose content of PMSG or PMSG-β is unusually high. Based on a molecular weight of 60,000, PMSG appears to have 3 L-fucose, 35 D-galactose, 9 mannose, 25 N-acetyl-D-glucosamine, 9 N-acetyl-D-galactosamine, and 26 sialic acid residues. Two commonly occurring carbohydrate structural patterns present in animal glycoproteins are: asparagine-linked, complex, sialic acid-containing and serine-linked, N-acetylgalactosamine-containing. If one assumes PMSG has similar structures, the number of mannose residues seems to be less than required by such structures. Therefore, either the carbohydrate structures in PMSG are different or the mannose value as reported in this paper is low. The possibility of incomplete hydrolysis for neutral sugars determination was ruled out since the mannose value was found to remain unchanged on increasing the time of hydrolysis.

Biologically, the purified PMSG is ½ as active as highly purified oFSH (115.3 NIH-FSH-S1 units/mg) determined by Steelman-Pohley assay (25) and 3 times as active as purified oLH (2.3 NIH-LH-S1 units/mg) determined by the ascobic acid depletion assay. Using in vitro binding assays to determine biological activity of the subunits and of the reconstituted PMSG, it was found that the individual subunits have low biological activity. However, upon recombination there was a significant restoration of the hCG and FSH activity originally in the intact PMSG. Unlike hCG, full biological activity (binding activity) was not regained upon recombination, suggesting that either the optimal conditions for recombination of PMSG subunits have not been determined or possibly that PMSG subunits are less stable than hCG, or both. Like Papkoff (15), we found that PMSG-α can combine with hCG-β or FSH-β to yield significant amounts of hCG or FSH activity, respectively, suggesting that a similar α subunit is present in glycoprotein hormones and that the β subunit is the hormone-specific subunit. However, unlike Papkoff (15) (in vivo bioassay), we found that when PMSG-α is combined with both hCG-β and oFSH-β, there is greater hCG and FSH activity in the hybrid than in PMSG alone (Table VII).

Human chorionic gonadotropin was found to be least cross-reactive with PMSG in the PMSG radioimmunoassay system. Flux and Li (48), using antibody to a crude preparation of PMSG (2,500 I.U./mg), also found no cross-reactivity with hCG in a quantitative precipitin test. Similarly, Schams and Papkoff (14), using immunodiffusion and immunoelectrophoresis, found no cross-reactivity between hCG and antibody to purified PMSG. However, in the binding assay, hCG and LH were able to inhibit the binding of 125I-PMSG to a testicular homogenerate better than PMSG. This is, perhaps, due to greater affinity of hCG or oLH for the receptor than 125I-PMSG. PMSG. It seems as though the sites in PMSG which determine its biological activity (i.e. binding to the receptor) are similar to other gonadotropins (oLH and hCG) but that these sites are not the principal ones which are involved in binding to the antibody. In the PMSG radiomunnoassay, we also found nonparallel cross-reactivity of PMSG with oLH and oFSH, the former being more cross-reactive than the latter.

Briefly, the present investigations elucidate some of the biological and immunological relationships between PMSG and hCG, oLH and oFSH. It is firmly established that FSH and LH activities are integral components of PMSG molecule. While LH activity, like hCG, is predominant in PMSG, in contrast to hCG, the FSH activity of PMSG is quite substantial. Receptor binding sites in PMSG are similar to those in LH and hCG. The antibody binding or antigenic sites, however, are quite different. The present studies also throw light on the structural relationships between PMSG and hCG, oLH and oFSH. Based on the results of hybridization with hCG-β and oFSH-β, PMSG-α appears to have considerable homology with the α subunits of other hormones. On the other hand, from the inability of the PMSG-β to combine with the α subunits of other hormones and lack of immunological cross-reactivity with hCG-β, it appears that the β subunit is different. Therefore, it probably has lesser amino acid sequence homology with the other β subunits than PMSG-α. The size of PMSG-β is considerably larger (2 to 3 times) compared to the other β subunits, which indicates that the problem of structural elucidation is at a different order of magnitude than the other glycoprotein hormones. The carbohydrate which forms approximately 50% of PMSG-β, again points to the complexity of the structural problem merely because of the size alone. It is interesting to note, however, that all of N-acetylgalactosamine, like hCG, is present in PMSG-β and is involved in the linkage of the carbohydrate units to the serine and threonine residues of the polypeptide chain. Finally, it is expected that the present findings will facilitate studies on the complete structural determination of PMSG.

Acknowledgments—We wish to thank Mr. G. S. Bedi for molecular weight determinations by Ferguson plots, Professor B. B. Saxena for

3 S. Christakos and O. P. Bahl, unpublished work.
a generous gift of human LH, and the World Health Organization and the National Institutes of Health for various hormone standards.

REFERENCES

27. Ouchterlony, O. (1962) Prog. Allergy 6, 30-154
33. Ferguson, K. A. (1964) Metabolism 13, 985-1002

**Experimental Procedure**

Commercial preparations of PMSG were assayed between 1600 L to 26,000 mIU, i.e., purchased from Human Hormone Laboratories, and were used in the experiments. The hormone was further purified by column chromatography using Sephadex G-25 and Sephadex G-50 columns, and its purity was monitored by thin-layer chromatography (TLC) and by a specific assay based on the known molecular weight of PMSG. The purified PMSG was then used for the experiments described in this report.

**Results and Discussion**

The results obtained in this study indicated that PMSG purified by the described method was of high purity and was suitable for further experiments. The purification procedure was simple, rapid, and yielded a high percentage of recovery. The results were in agreement with previous reports in the literature.

**Conclusion**

In conclusion, the described method for the purification of PMSG is simple, rapid, and yields a high percentage of recovery. The purified hormone is of high purity and is suitable for further experiments.

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**References:**


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**Table 1:**

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<th>Method</th>
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<td>Sephadex G-50</td>
<td></td>
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</tbody>
</table>

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**Figure 1:**

A typical TLC profile of PMSG purified by the described method is shown. The purity of the hormone is indicated by the single spot at the origin on the TLC plate.

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**Figure 2:**

A typical Sephadex G-25 chromatogram of PMSG is shown. The purity of the hormone is indicated by the single peak at the origin on the chromatogram.
Pregnant mare serum gonadotropin. Purification and physicochemical, biological, and immunological characterization.
S Christakos and O P Bahl


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