

PATENT SPECIFICATION

(11) 1 567 764

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- (21) Application No. 42319/76 (22) Filed 12 Oct. 1976
(31) Convention Application No. 622 031
(32) Filed 14 Oct. 1975 in
(33) United States of America (US)
(44) Complete Specification published 21 May 1980
(51) INT CL³ C07C 103/52; A61K 37/24; (A61K 37/24, 37/28)
(52) Index at acceptance
C3H 201 203 220 241 242 320 326 331 339 A3 H1 HX2
ASB 111 112 121 134 136 137 180 315 317 31Y 38Y 393 396
AA AC H
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(54) MODIFIED PROTEIN REPRODUCTIVE HORMONE ANTIGENS

(71) We, THE OHIO STATE UNIVERSITY, of 190 North Oval Drive, Columbus, Ohio 43210, USA a non profit making organisation under the laws of the State of Ohio, USA, and entitled under such laws to hold title to inventions and patents, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

Our earlier Specification No. 1,473,601 describes *inter alia* the chemical modification of natural endogenous polypeptides which influence the reproductive process to provide antigens capable of inducing formation of antibodies which neutralise not only the antigenically modified polypeptide but also its unmodified endogenous counterpart, thus providing a means for immunological contraception. The modified polypeptides are produced from the natural, endogenous polypeptides in the species involved or are immunologically equivalent to the modified polypeptides so produced. In practice, the polypeptides modified are derived from the species involved or from a closely related species.

More particularly, our earlier specification describes the chemical modification of protein reproductive hormones and fragments thereof, to provide antigens for use in immunological contraception. The fragments of the protein hormones must be large enough and sufficiently distinctive in chemical and physical character to enable them to be recognised as a specific part of the whole protein in question. They must also naturally possess the chemical nature, i.e. the requisite amino acid make-up, to enable them to be chemically modified in the manner desired.

The term "protein reproductive hormone" used herein includes those hormones essential to the normal events of the reproductive process.

For the purposes of the present invention, the term "fragment" with reference to a protein reproductive hormone embraces both amino acid chains forming part of the structure of the hormone and amino acid chains which although not identical with chains forming part of the structure of the hormone are sufficiently similar for them to be immunologically equivalent, i.e. to provoke essentially the same antibody response upon administration.

Our earlier specification specifically discloses the chemical modification of protein reproductive hormones, such as Follicle Stimulating Hormone (FSH), Leutinisising Hormone (LH), Human Placental Lactogen (HPL), Human Prolactin and Human Chorionic Gonadotropin (HCG), and fragments thereof.

Specific fragments which may be modified in accordance with our earlier specification include the β -subunit of FSH and specific unique fragments of natural HPL or Human Prolactin, which fragments may bear little resemblance to analogous portions of other protein hormones. Preferred fragments include the β -subunit of HCG which, according to the two authoritative views, has either structure I or II as follows (* indicates site locations of carbohydrate moieties):—

(11)

Ser - Lys - Glu - Pro - Leu - Arg - Pro - Arg - Cys - Arg - Pro - Ile - Asn - Ala -
20
Thr - Leu - Ala - Val - Glu - Lys - Glu - Gly - Cys - Pro - Val - Cys - Ile - Thr -
40
Val - Asn - Thr - Thr - Ile - Cys - Ala - Gly - Try - Cys - Pro - Thr - Met - Thr -
50
Arg - Val - Leu - Gln - Gly - Val - Leu - Pro - Ala - Leu - Pro - Gln - Val - Val -
60
Cys - Asn - Try - Arg - Asp - Val - Arg - Phe - Glu - Ser - Ile - Arg - Leu - Pro -
80
Gly - Cys - Pro - Arg - Gly - Val - Asn - Pro - Val - Val - Ser - Tyr - Ala - Val -
90
Ala - Leu - Ser - Cys - Gln - Cys - Ala - Leu - Cys - Arg - Arg - Ser - Thr - Thr -
100
Asp - Cys - Gly - Gly - Pro - Lys - Asp - His - Pro - Leu - Thr - Cys - Asp - Asp -
110
Pro - Arg - Phe - Gln - Asp - Ser - Ser - Ser - Ser - Lys - Ala - Pro - Pro - Pro -
120
* 130 * * 140
10 Ser - Leu - Pro - Ser - Pro - Ser - Arg - Leu - Pro - Gly - Pro - Ser - Asp - Thr -
10
Pro - Ile - Ieu - Pro - Gln

(Structure I)

Ser - Lys - Gln - Pro - Leu - Arg - Pro - Arg - Cys - Arg - Pro - Ile - Asn - Ala -
20
Thr - Leu - Ala - Val - Glu - Lys - Glu - Gly - Cys - Pro - Val - Cys - Ile - Thr -
29 * 40
15 Val - Asn - Thr - Thr - Ile - Cys - Ala - Gly - Tyr - Cys - Pro - Thr - Met - Thr -
50
Arg - Val - Leu - Gln - Gly - Val - Leu - Pro - Ala - Leu - Pro - Gln - Leu - Val -
60
Cys - Asn - Tyr - Arg - Asp - Val - Arg - Phe - Glu - Ser - Ile - Arg - Leu - Pro -
80
Gly - Cys - Pro - Arg - Gly - Val - Asn - Pro - Val - Val - Ser - Tyr - Ala - Val -
90
Ala - Leu - Ser - Cys - Gln - Cys - Ala - Leu - Cys - Arg - (Arg) - Ser - Thr -
100
20 Thr - Asp - Cys - Gly - Gly - Pro - Lys - Asp - His - Pro - Leu - Thr - Cys - Asp -
110
Asp - Pro - Arg - Phe - Gln - Asp - Ser - Ser - Ser - Lys - Ala - Pro - Pro - Pro -
120
* 130 * 140
Ser - Leu - Pro - Ser - Pro - Ser - Arg - Leu - Pro - Gly - Pro - Asp - Thr - Pro -
147
Ile - Leu - Pro - Gln - Ser - Leu - Pro

(Structure II)

25 As disclosed in our earlier specification, for specificity of antibody action, it is
desirable that polypeptides be modified which comprise molecular structures completely
or substantially completely different to those of other protein hormones. In this con-
30 nection, the β -subunit of HCG possesses a chain or chains of amino acids which differ
greatly from those of LH and such chains may also be modified in accordance with
the invention. Such chains include 20-30 or 30-39 amino acid peptides consisting
of the C-terminal residues of the β -subunit of HCG. More particularly, such chains
include those of the following formulae III and IV (C-terminal portions of structure
I, above) and V and VI (C-terminal portions of Structure II, above):

Asp - Asp - Pro - Arg - Phe - Gln - Asp - Ser - Ser - Ser - Ser - Lys - Ala - Pro -
35
Pro - Pro - Ser - Leu - Pro - Ser - Pro - Ser - Arg - Leu - Pro - Gly - Pro - Ser -
Asp - Thr - Pro - Ile - Leu - Pro - Gln

(Structure III)

Gln - Asp - Ser - Ser - Ser - Ser - Lys - Ala - Pro - Pro - Pro - Ser - Leu - Pro -
Ser - Pro - Ser - Arg - Leu - Pro - Gly - Pro - Ser - Asp - Thr - Pro - Ile - Leu -
Pro - Gln.

(Structure IV)

5 Thr - Cys - Asp - Asp - Pro - Arg - Phe - Gln - Asp - Ser - Ser - Ser - Lys - Ala -
Pro - Pro - Pro - Ser - Leu - Pro - Ser - Pro - Ser - Arg - Leu - Pro - Gly - Pro -
Pro - Asp - Thr - Pro - Ile - Leu - Pro - Gln - Ser - Leu - Pro.

(Structure V)

10 Phe - Gln - Asp - Ser - Ser - Ser - Lys - Ala - Pro - Pro - Pro - Ser - Leu - Pro -
Ser - Pro - Ser - Arg - Leu - Pro - Gly - Pro - Pro - Asp - Thr - Pro - Ile - Leu -
Pro - Gln - Ser - Leu - Pro.

(Structure VI)

15 Such structures may be obtained by purely synthetic methods or by enzymatic
degradation of the parent polypeptide [Carlson et. al., J. Biological Chemistry, 284
(19), 6810 (1973).

20 In one aspect, the present invention provides further polypeptide chains which
may be chemically modified to provide antigens for use in immunological contracep-
tion. In particular the present invention relates to the chemical modification of the
polypeptide chains of the following formulae VII, VIII, IX and X. By virtue of their
substantial resemblance to the chemical structure and configuration of HCG, and the
immunological response provided by them when chemically modified, these fragments
are considered to be fragments of HCG.

25 Phe - Gln - Asp - Ser - Ser - Ser - Ser - Lys - Ala - Pro - Pro - Pro - Ser - Leu -
Pro - Ser - Pro - Ser - Arg - Leu - Pro - Gly - Pro - Ser - Asp - Thr - Pro - Ile -
Leu - Pro - Gln

(Structure VII)

Asp - Asp - Pro - Arg - Phe - Gln - Asp - Ser - Ser - Ser - Lys - Ala - Pro - Pro -
Pro - Ser - Leu - Pro - Ser - Gly

(Structure VIII)

30 Asp - Asp - Pro - Arg - Phe - Gln - Asp - Ser - Ser - Ser - Ser - Lys - Ala - Pro -
Pro - Pro - Ser - Leu - Pro - Ser - Pro - Ser - Arg - Leu - Pro - Gly - Pro - Pro -
Asp - Thr - Pro - Ile - Leu - Pro - Gln - Ser - Leu - Pro

(Structure IX)

35 Asp - His - Pro - Leu - Thr - Cys - Asp - Asp - Pro - Arg - Phe - Gln - Asp - Ser -
Ser - Ser - Ser - Lys - Ala - Pro - Pro - Pro - Ser - Leu - Pro - Ser - Pro - Ser -
Arg - Leu - Pro - Gly - Pro - Pro - Asp - Thr - Pro - Ile - Leu - Pro - Gln - Ser -
Leu - Pro

(Structure X)

40 Again, such structures may suitably be produced by known synthetic methods.

40 In one aspect, therefore, the present invention provides (i)a) an antigen for the
immunological control of fertility which comprises a chemically modified polypeptide,
the unmodified polypeptide being of structure VII, VIII, IX or X, stated above.

45 As disclosed in our earlier specification, the degree of chemical modification of the
polypeptide is such that the resulting antigens will induce generation of antibodies
which will neutralise not only the antigens but also some, at least, of their natural
counterpart, i.e. HCG, and this as well as the type of modification will depend on such
factors as the nature of the polypeptide involved. If too little modification is effected,
the body may not recognise the modified polypeptide as a foreign intruder and may

therefore not generate antibodies against it. If, on the other hand, too much modification is effected, the body will generate antibodies specific to the injected antigen which will not neutralise the natural endogenous protein counterpart, i.e. HCG.

In general, the chemical modification may involve attachment of foreign modifying groups to the polypeptide involved. The number of foreign modifying groups to be added will, of course, vary depending on the circumstances but, generally, it is preferred that 1 to 40, suitably 2 to 40, in particular 5 to 30, for example 10 to 26 modifying groups per molecule of polypeptide be attached. Given modifying groups will attach to particular amino acid sites in the peptide molecule, so that the maximum possible number of a given modifying group may be selected accordingly. It is also possible that several modifying groups may attach to each other and then attach to a single amino acid moiety but, for the purposes of this invention, such a substitution is regarded as attachment of a single modifying group. The chemical modification may alternatively comprise the attachment of 2 or more polypeptide molecules to a modifying group.

For the purposes of the present invention, it is to be understood that where reference is made to attachment of modifying groups to the polypeptides (or *vice versa*) the attachment may be direct or via linking groups. The polypeptide or modifier may, for example, be activated with a modifying group for conjugation.

The modifying groups may as indicated vary in chemistry depending on the circumstances. As disclosed in our earlier application, suitable modifying groups comprise diazo groups. These may suitably be introduced by reaction with the appropriate number of moles of diazosulfanilic acid. Introduction of diazo groups into proteins is a well-known technique and may, for example, be effected as described, by Cinader et al., J. Am. Chem. Soc. 78, 746 (1955); by Phillips et al., J. Biol. Chem. 244, 575 (1969); by Tabachnick et al., J. Biol. Chem. 234 (7), 1726 (1959) or by Crampton et al., Proc. Soc. Ex. Biol. & Med. 80, 448 (1952). In general, the methods of Cinader et al. and Phillips et al. are preferred.

Additional modifying groups include those derived from a dinitrophenol, a trinitrophenol, S-acetomercaptosuccinic anhydride, polytyrosines or polyalanines (in either straight or branched chains), biodegradable polydextran or, rather less preferably, natural proteins such as thyroglobulin. Generally, synthetic modifiers are preferred to natural modifiers.

The above reactions, as well as many other suitable hapten coupling reactions are well known in protein chemistry. The following references may, for example, be cited in this connection:—

1. Klotz et al., Arch. Biochem. & Biophys. 96, 605—612 (1966);
2. Khorana, Chem. Rev. 53, 145 (1953);
3. Sela et al., Biochem. J. 85, 223 (1962);
4. Eisen et al., J. Am. Chem. Soc. 75, 4583 (1953);
5. Certano et al., Fed. Proc. (ABSTR.) 25, 729 (1966);
6. Sokolowski et al., J. Am. Chem. Soc. 86, 1212 (1964);
7. Goodfriend et al., Science 144, 1344 (1964);
8. Sela et al., J. Am. Chem. Soc. 78, 746 (1955);
- and 9. Bahl, J. Biol. Chem. 244, 575 (1969).

As disclosed in our earlier specification, the chemical modification may alternatively or additionally comprise removal of moieties from the polypeptides. Thus, for example where certain of the natural proteins have carbohydrate moieties, these may be removed in conventional manner, using, for example, N-acetylneuraminidase or N-acetylglucosidase, materials useful for removing specific carbohydrate moieties.

In a further aspect, the present invention relates to the use of additional modifying groups. More particularly, in this aspect, the invention provides (i) b) an antigen for the immunological control of fertility, comprising a chemically modified polypeptide, the unmodified polypeptide being a protein reproductive hormone or a fragment thereof, and the chemical modification comprising the attachment to the unmodified polypeptide of one or more modifying groups derived from polymerised sugars, serum proteins, keyhole limpet hemocyanin, marine gastropod mollusc, viruses, or bovine gamma globulin, or the attachment of two or more unmodified polypeptide molecules to such a modifying group.

Suitable polymerised sugars for modifying the polypeptides include copolymers of sucrose with epichlorohydrin, such as Ficoll (RTM) 70 or Ficoll (RTM) 400 [Pharmacia Fine Chemicals, Pharmacia Laboratories Inc., 800 Centennial Avenue, Piscataway, N.J. 08854] or a polyglucose such as Dextran T70 [synthesised micro-biologically by the action of leuconostoc maseuteroides (a strain in NRRL B—512) on

sucrose. Glucan containing alpha-1,6-glucosidic bonds. Av. mol. wt. approx. 70,000]. Suitable serum proteins include homologous serum albumin or bovine serum albumin and suitable viruses include influenza virus (Type A, B or C) or poliomyelitis virus, live or killed (Type 1, 2 or 3).

Any of the protein reproductive hormones or fragments thereof may be modified with these additional modifying groups in accordance with the present invention. In particular, the protein reproductive hormones and fragments thereof described above in connection with our earlier specification may be so modified as may the further polypeptide claims of formulae VII, VIII, IX and X, stated above. It will be appreciated that such modification may comprise attachment of the modifying groups to the polypeptide via linking groups and that this may be accomplished by reaction of the polypeptide, the modifier and a linker (activator) in any order or simultaneously, with or without protection and subsequent disprotection of one or more reactive groups in the polypeptide or modifier. Quite generally, such modification may be carried out in known manner, for example as described in the literature cited above.

In a further aspect, the present invention provides processes for the production of antigens for the immunological control of fertility comprising chemically modifying polypeptide which is a protein reproductive hormone or a fragment thereof.

- a) by attaching to the unmodified polypeptide modifying groups derived from modifiers having free amino groups, by activation of the modifier or polypeptide to be modified by reaction with tolylene diisocyanate and reaction of the resulting activated product with the polypeptide to be modified or modifier, respectively; or
- b) by attaching to the unmodified polypeptide modifying groups derived from modifiers having free amino group or being polymerised sugars, by conjugation of the unmodified polypeptide with the modifier employing a water-soluble carbodiimide as activating agent; or
- c) by attaching to the unmodified polypeptide modifying groups derived from modifiers having free amino groups by reaction of the modifier with glutaric dialdehyde and reaction of the resulting product with the polypeptide to be modified, in the presence of an alkali metal borohydride; or
- d) by attaching to the unmodified polypeptide modifying groups derived from polymerised sugars by reaction of the polymerised sugar with a cyanuric halide, and reaction of the resulting dihalotriazinyl adduct with the polypeptide to be modified; or
- e) by attaching to the unmodified polypeptide modifying groups derived from polymerised sugars by treatment of the sugar with an alkali metal periodate and reaction of the resulting product with the polypeptide to be modified.

Process a), which, as indicated, may be employed for modification with modifiers having free amino groups, such as natural protein, e.g. keyhole limpet hemocyanin, serum proteins, e.g. homologous serum albumin or bovine serum albumin, polytyrosine or polyalanine, bovine gamma globulin or thyroglobulin, may suitably be effected as described by Singer and Schick, J. Biophys. Biochem. Cytology 9, 519 (1961). In particular the modifier is conveniently taken up in a buffer solution such as phosphate buffer, in sodium chloride solution at a pH of, e.g. 6—8, and a solution of tolylene diisocyanate (TDIC) is then suitably added to this solution. The TDIC is suitably added diluted from 1:10 to 40 (by volume) in dioxane and the amount of TDIC added may suitably range from 0.075 to 1000 molar equivalents. The reaction may, for example, be effected at a temperature of from -5° to +10°C, preferably 0° to 4°C and the reaction time may, for example vary from about ½ to 2 hours. Any excess TDIC is then suitably removed by centrifugation and the precipitate washed with the above phosphate buffer and the supernatants combined. The activated modifier may then suitably be added to a buffered solution of the polypeptide to be modified, preferably in the same phosphate buffer (5—30 mg/ml), the quantities of modifier and polypeptide being in accordance with the molar ratio desired in the resulting modified polypeptide. The combined solution is suitably reacted at 30° to 50°C, conveniently 35—40°C, suitably for 3 to 6 hours. In an alternative embodiment, the first step may comprise addition of the TDIC solution to the buffered polypeptide, followed by addition of buffered modifier to the resulting activated polypeptide, the conditions for the first and second steps being as described above.

The modifiers for use in process b), which is generally described by Moore and Kashland, J. Biol. Chem. 242, 2447 (1967), include natural proteins such as keyhole limpet hemocyanin, serum proteins such as homologous or bovine serum albumin, polymerised sugars, such as Ficolls (70 or 40) or dextrans (Dextran T70), and polyalanine or polytyrosine. The free amino groups of the polypeptide to be modified are suitably

protected initially, e.g. by acetylation, and the acetylated polypeptide is then conveniently conjugated to the modifier, preferably in the presence of a guanidine, e.g. guanidine hydrochloride, and in the presence, preferably of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide as the water-soluble carbodiimide activator. Where a polymerised sugar, such as Ficoll, is the modifier it is preferred that this first be treated with ethylene diamine to render the final coupling more efficient and this treatment is conveniently effected in a solvent, such as saline and dioxane, and suitably at about room temperature and a pH of about 9 to 12, preferably 10—11. The reaction time may, for example, vary from $\frac{1}{2}$ to 2 hours. The subsequent conjugation of the protected polypeptide and the modifier may conveniently be carried out in a solvent, such as glycine methyl ester, and at a pH of for example, 4 to 5, preferably 4.5 to 4.8. The reaction temperature is suitably room temperature and the reaction time may, for example, vary from 2 to 8 hours, usually about 5 hours.

Process c), which may be used with natural protein modifiers, such as serum proteins, e.g. homologous serum albumin, is based on a theory of Richards and Knowles [J. Mol. Biol. 37, 231 (1968)] that commercial glutaric dialdehyde contains virtually no free glutaric dialdehyde, but rather consists of a complex mixture of polymers rich in α,β -unsaturated aldehydes. Upon reaction with natural protein modifiers, these polymers form a stable bond through the free amino group, leaving aldehyde groups free. This intermediate product may then be reacted with the unmodified polypeptide in the presence of an alkali metal borohydride, such as sodium borohydride. The intermediate is suitably formed at pH 7—10, preferably 8—9 and at room temperature. The subsequent conjugation is then conveniently effected at about room temperature and for a period of, for example, $\frac{1}{2}$ to 2 hours.

Process d), which may be employed with polymerised sugar modifiers such as sucrose copolymers with epichlorohydrin, e.g. Ficolls, e.g. Ficoll 70 or 400, or polyglucoses, e.g. Dextran T70, is effected by reaction of the modifier with a cyanuric halide, e.g. cyanuric chloride, e.g. at a temperature of 0° to 20°C, suitably in an organic medium such as dimethyl formamide and conveniently for $\frac{1}{2}$ to 4 hours. The resulting dihalotriazinyl intermediate is conveniently dialysed until essentially halide ion free, and lyophilised and treated with the polypeptide, suitably at pH 8—11, preferably pH 9—10, and at room temperature, and conveniently for $\frac{1}{2}$ to 12 hours.

Process e), which is also applicable for polymerised sugar modifiers, e.g. copolymers of sucrose with epichlorohydrin, e.g. Ficolls, e.g. Ficoll 70 or 400, or polyglucoses, e.g. Dextran T70, may conveniently be effected by treatment of the modifier with an alkali metal periodate, e.g. sodium periodate, at a temperature of 30° to 60°C and a pH of 3 to 6. The resulting intermediate is suitably reacted with the polypeptide at a pH of about 7—11, preferably 8—10, and conveniently at a temperature of from 15° to 80°C, preferably 20° to 60°C. The reaction time may, for example, vary from $\frac{1}{2}$ to 4 hours. The resulting product is suitably reduced with an alkali metal borohydride, e.g. sodium borohydride.

The resulting modified polypeptides may be isolated and purified using conventional techniques, such as gel filtration, column chromatography, or dialysis and lyophilisation. Prior to conjugation (modification) picogram amounts of I^{125} labeled polypeptide may be added as a tracer to the reaction mixture. The quantity of polypeptide conjugated to modifying groups (molar ratio) may then be determined from the amount of radio-activity recovered.

It will be appreciated that the methods a) to e), described above may be employed for modification of any protein reproductive hormones or fragments thereof, in particular those described above, to produce antigens for use in immunological contraception.

As described above, the production of antigens for use in fertility control in accordance with our earlier application and the present invention can be accomplished by attaching one or more modifying groups to the selected polypeptide or attaching two or more polypeptide molecules to a modifying group.

A further aspect of the present invention provides antigens for the immunological control of fertility comprising a chemically modified polypeptide, the unmodified polypeptide being a protein reproductive hormone or a fragment thereof, and the chemical modification comprising

- (ii) polymerisation of the unmodified polypeptide via an organic bifunctional agent,
- or (iii) dimerisation of the unmodified polypeptide via a sulphur-sulphur bridge formed from the thiol group of a cysteine residue present in the unmodified

polypeptide or added to the unmodified polypeptide where this does not contain a cysteine residue.

The antigens of embodiment (ii) may be formed by polymerisation of the unmodified polypeptide with an appropriate bifunctional reagent, such as a bifunctional imidoester, e.g. dimethyl adipimidate, dimethyl suberimidate, or diethyl malonimidate, in conventional manner [e.g. Hartmann and Wold, Biochem. 6, 2439 (1967)]. The polymerisation may thus be effected at room temperature in aqueous solvent and suitably at a pH of 9—12, preferably 10—11, and conveniently for a period of $\frac{1}{2}$ to 2 hours.

The antigens of embodiment (iii) may be formed by oxidation of the thiol group in a cysteine residue, for example using iodobenzoic acid, in known manner, e.g. room temperature reaction for 10 to 40 minutes. Where the polypeptide to be modified possesses no cysteine residue, this may first be joined to the polypeptide in conventional manner.

It will be appreciated that the chemical modification of embodiments (ii) and (iii) above may be applied to any protein reproductive hormone or fragment thereof, in particular, however, those described above, for the purpose of producing antigens for use in immunological fertility control.

The antigens of the present invention may suitably be admixed with a pharmaceutically acceptable liquid carrier and preferably administered parenterally. The dosage to be administered will of course, vary depending on various factors. However, in general, unit doses of 0.1 to 50 mg are indicated, suitably administered one to five times at intervals of one to three weeks.

The following Examples illustrate the invention.

EXAMPLE 1.

Structure X — Keyhole limpet hemocyanine [Process a)]

Hemocyanin from Keyhole limpet (KLH) solution (7 mg/ml) in 0.05 M sodium phosphate buffer in 0.2 M NaCl, pH 7.5, is prepared. Insoluble particles are removed by centrifugation. To 1 ml of this solution, tolylene diisocyanate (TDIC) reagent is added (20 μ l) diluted to 1/30 with dioxane, the amount being essentially the equivalent of the moles of lysyl residues in the KLH molecules. After 40 minutes at 0°C, the TDIC activated KLH solution is combined with 0.5 mg of synthetic β -HCG peptide identified hereinabove as Structure X which is first dissolved in 25 μ l of 0.05 M sodium phosphate buffer in 0.2 M NaCl, pH 7.5. The mixture is incubated at 37°C for four hours. The resulting product is purified by gel filtration.

EXAMPLE 2.

Structure III — Bovine gamma globulin [process a)]

To an ice water bath cooled and vigorously stirred, 0.23 ml of bovine gamma globulin (10 mg/ml) in 0.05 M phosphate buffer with normal saline (PBS) pH 7.5, 50 μ l of 1/10 TDIC in dioxane is added. After 40 minutes, the excess TDIC is removed by centrifugation (0°C, 10 minutes, 10,000 g) and the precipitate is washed twice with 0.1 ml of PBS. The combined supernatants are added to 7.7 g of the peptide of structure III dissolved in 0.8 ml of PBS, pH 7.5. The mixture is stirred at room temperature for 10 minutes, then incubated at 37°C for 4 hours. The conjugate product is purified by dialysis.

EXAMPLE 3.

Structure VI — Bovine serum albumin [process a)]

Bovine serum albumin (10 mg/ml) in PBS solution (0.25 ml) is treated with 50 μ l of 1/10 TDIC dioxane solution and conjugated to 7.5 mg of synthetic β -HCG peptide of structure VI in 0.8 ml of PBS (pH 7.5) as in Example 2 to obtain the product.

EXAMPLE 4.

Structure VI — Poly(DL-Lys-Ala) [process a)]

To an ice water bath cooled and vigorously stirred 0.6 ml of β -HCG peptide of structure VI (10 mg/ml) in phosphate buffered saline, pH 7.5, is added 30 μ l of 1/10 TDIC in dioxane. After 40 minutes, the excess TDIC is removed by centrifugation (10,000 g, 0°C, 10 minutes) and the precipitate is washed twice with 0.1 ml PBS. The mixture is incubated at 37°C for 4 hours. The product is then dialysed and lyophilised.

EXAMPLE 5.

Structure III — Keyhole limpet hemocyanin [process b)]

Two mg of the compound of Structure III containing picogram amount of I^{125} labeled adduct and KLH (1.6 mg) is dissolved in 1 ml of 1.0M glycine methyl ester in 5 M guanidine hydrochloride. 1-Ethyl-3-(3-dimethyl aminopropyl)carbodiimide (EDC) 19.1 mg is added to this solution. The acidity is adjusted to and maintained at pH 4.75 with 1 N HCl at room temperature for 5 hours. The KLH-peptide conjugate is purified by passing it through a Bio-Gel p—60 2.2 × 28 cm column equilibrated with 0.2 M NaCl.

Ficoll 70 — Ethylenediamine [Starting material — process b)]

One g of Ficoll 70 is dissolved in 1 ml each of normal saline and 2 M ethylene diamine (adjusted to pH 10 with hydrochloric acid) solution. The solution is kept at room temperature in a water bath and stirred with a magnetic stirrer. Cyanogen bromide 4 g, dissolved in 8 ml of dioxane, is added to the Ficoll 70 solution. The acidity of the mixture is maintained at pH 10—10.5 for 8 minutes by adding drops of 2 N sodium hydroxide solution. An additional 2 ml of 2 M ethylene diamine, pH 10, solution is added, and stirring at room temperature is continued for 30 more minutes. The product is purified by passing it through a Bio-Gel p—60 column. This material can be used to couple Ficoll 70 to polypeptides in accordance with process b).

EXAMPLE 6.

Structure IX — Homologous Serum Albumin [process c)]

To a 20 mg/ml solution of homologous serum albumin in 0.1 M borate buffer, pH 8.5, 1000% mole excess of 25% aqueous solution of glutaric dialdehyde is added at room temperature. The excess dialdehyde is removed by gel filtration in water using Bio-Gel p—2. The material collected at the void volume is lyophilised, and the dried product is redissolved in 0.1 M borate buffer, pH 8.5 (20 mg/ml), mixed with the required amount of polypeptide of Structure IX (20 mg/ml) in the same buffer at room temperature. Twenty minutes later, sodium borohydride in 250 percent molar excess of polypeptide-IX is added. The reaction is terminated after one hour. The conjugated product is purified by gel filtration on Bio-Gel p—60 column, dialysed free of salt and lyophilised.

EXAMPLE 7.

Structure X — Ficoll 70 [process d)]

Ficoll 70 1 g, NaHCO_3 500 mg, cyanuric chloride 3 g, H_2O 20 ml, and dimethylformamide 80 ml, are stirred at a temperature below 16°C for 2 hours. The product is dialysed against distilled water until Cl-free, then lyophilised. A polypeptide of Structure X (2 mg) containing a minute quantity of I^{125} -labeled analogue is incubated with 1 mg of this product in 0.25 ml of 0.2 M sodium borate buffer, pH 9.5, for one hour at 20°C, and the product is recovered from a Bio-Gel p—60 2.2 × 28 cm column.

When the above procedure is carried out and Dextran T70 is used in place of Ficoll 70, the corresponding modified polypeptide is obtained.

EXAMPLE 8.

Structure X — Ficoll 70 [process e)]

Ficoll 70 1 g, NaIO_4 1.2 g, and KCl 0.42 g are dissolved in 1.5 ml of 1 M sodium acetate buffer, pH 4.5, and incubated at 37°C for 1 hour.

Two mg (= 588 μ moles) of polypeptide of Structure X above mixed with a minute quantity of I^{125} -labeled analogue is incubated with 2 mg of the product obtained above in 0.3 ml of 0.2 M borate buffer, pH 9.5 at 55°C for 1 hour. The reaction mixture is then chilled in an ice water bath and NaBH_4 1 mg is then added into this solution. The reduction reaction is terminated by passing the product through a Bio-Gel p—60 2.2 × 28 cm column equilibrated and eluted with 0.2 M NaCl.

EXAMPLE 9.

Structure III — Polymer

Solid bifunctional imidoester dihydrochloride (3 mole) is added in 2 mg portions at 5 minute intervals to a constantly stirred solution of 1 mole of polypeptide of structure III (1—20 mg/ml) in 0.1 M sodium phosphate, pH 10.5, at room temperature. Sodium hydroxide 0.1 N is added to maintain the acidity at pH 10.5. One hour after the addition of the diimidoester has been completed, a polymerised product according to this invention is obtained.

EXAMPLE 10.

Structure V or X dimer

5 Iodosobenzoic acid dissolved in a slight excess of 1 N potassium hydroxide in 10% molar excess is added to the peptide structure V or X in phosphate buffer with normal saline at pH of 7.0. After thirty minutes at room temperature, the product polypeptide dimer is purified by gel filtration. 5

EXAMPLE 11.

Test Results

10 Numerous rabbits are immunised with a variety of synthetic peptides conjugated to different modifying groups. Following two or three immunisations at 3—5 week intervals, sera from animals are assessed by determining their ability to bind *in vitro* to radiolabeled HCG. The specificity of this binding is studied by reacting the same sera against similarly labeled other protein hormones, particularly, pituitary LH. Sera are further assessed by determining their ability to inhibit the biological action of exo- 15 geneously administered HCG in bioassay animals. Thus, the increase in uterine weight of the immature female rat in response to a prescribed dose of HCG is noted. The dose of HCG is administered subcutaneously in saline in five injections over a three day period and the animal is sacrificed for removal of the uterus on the fourth day. The weight of the uterus increases in dose response fashion to the hormone injections. 20 When assessing the effects of antisera in this response, varying quantities of test serum are administered intraperitoneally separately from the subcutaneous injection of hormone during the assay. This procedure permits the antiserum to be adsorbed rapidly into the rat's blood-stream and will permit interaction of it with hormone when the latter likewise enters this fluid. If the antiserum is capable of reacting with the hormone in a manner preventing stimulation of the uterus, the antiserum is considered to be effective for biological inhibition of hormone action. 25

The frequency of animals showing a positive response to immunological binding and neutralisation of biological activity is presented in the following Table.

30 The results set out in the following Table provide further evidence of the broad applicability of this invention as indicated previously in this specification.

Using standard methods of testing in rabbits, both immunological binding response and neutralisation of biological activity were established for the modified polypeptides indicated with the result as set out below:

(19)

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Frequency of Positive Antibody Responses to Various HCG Peptide - Conjugates

Peptide	Carrier	Immunized	Number of Rabbits	
			Immunological Binding Responses	Neutralization of Biological Activity
35 amino acid 111-145 <i>Morgan et al</i> Peptide III	Bovine Gamma Globulin Keyhole Limpet Hemocyanin	10 10	10 5	6 *
31 amino acid 115-145 <i>Morgan et al</i> Peptide VI	Poly-DL-Alanine Bovine Serum Albumin	10 12	9 12	5 6
44 amino acid 105-148 Peptide X	Keyhole Limpet Hemocyanin	10	8	*
Natural 109-145 Keutman Peptide	Keyhole Limpet Hemocyanin	10	10	*

* additional time needed for assessment

WHAT WE CLAIM IS:—

1. An antigen for the immunological control of fertility which comprises a chemically modified polypeptide, the unmodified polypeptide being a protein reproductive hormone (wherein defined) or a fragment (as herein defined) thereof, and the chemical modification comprising

- 5 (i) the attachment of one or more modifying groups to the unmodified polypeptide, or the attachment of two or more unmodified polypeptide molecules to a modifying group, provided that the modifying groups are derived from polymerised sugars, serum proteins, keyhole limpet hemocyanin, marine gastropod mollusc, viruses or bovine gamma globulin, when the unmodified polypeptide is other than of structure VII, VIII, IX or X, 10

Phe - Gln - Asp - Ser - Ser - Ser - Ser - Lys - Ala - Pro - Pro - Pro - Ser - Leu - Pro - Ser - Pro - Ser - Arg - Leu - Pro - Gly - Pro - Ser - Asp - Thr - Pro - Ile - Leu - Pro - Gln

15 (Structure VII) 15

Asp - Asp - Pro - Arg - Phe - Gln - Asp - Ser - Ser - Ser - Lys - Ala - Pro - Pro - Pro - Ser - Leu - Pro - Ser - Gly

(Structure VIII)

20 Asp - Asp - Pro - Arg - Phe - Gln - Asp - Ser - Ser - Ser - Ser - Lys - Ala - Pro - Pro - Pro - Ser - Leu - Pro - Ser - Pro - Ser - Arg - Leu - Pro - Gly - Pro - Pro - Asp - Thr - Pro - Ile - Leu - Pro - Gln - Ser - Leu - Pro 20

(Structure IX)

25 Asp - His - Pro - Leu - Thr - Cys - Asp - Asp - Pro - Arg - Phe - Gln - Asp - Ser - Ser - Ser - Ser - Lys - Ala - Pro - Pro - Pro - Ser - Leu - Pro - Ser - Pro - Ser - Arg - Leu - Pro - Gly - Pro - Pro - Asp - Thr - Pro - Ile - Leu - Pro - Gln - Ser - Leu - Pro 25

(Structure X)

- or (ii) polymerisation of the unmodified polypeptide via an organic bifunctional reagent;
30 or (iii) dimerisation of the unmodified polypeptide via a sulphur-sulphur bridge formed from the thiol group a cysteine residue present in unmodified polypeptide or added to the unmodified polypeptide if it does not contain a cysteine residue. 30

2. An antigen for the immunological control of fertility which comprises a chemically modified polypeptide, the unmodified polypeptide being a protein reproductive hormone (as herein defined) or a fragment (as herein defined) thereof, and the chemical modification comprising the attachment of one or more modifying groups to the unmodified polypeptide, or the attachment of two or more unmodified polypeptide molecules to a modifying group, provided that the modifying groups are derived from polymerised sugars, serum proteins, keyhole limpet hemocyanin, marine gastropod mollusc, viruses or bovine gamma globulin, when the unmodified polypeptide has a structure other than of formula VII, VIII, IX or X, stated in Claim 1. 35 40

3. An antigen according to Claim 2, in which 1 to 40 modifying groups are attached per molecule of polypeptide.

45 4. An antigen according to Claim 2 or 3, in which 2 to 40 modifying groups are attached per molecule of polypeptide. 45

5. An antigen according to any one of Claims 2 to 4, in which 5 to 30 modifying groups are attached per molecule of polypeptide.

50 6. An antigen according to Claim 2, in which 2 or more polypeptide molecules are attached to the modifying group. 50

7. An antigen according to any one of Claims 2 to 6, in which the unmodified polypeptide is of structure VII, VIII, IX or X, stated in Claim 1.

8. An antigen according to Claim 7, in which the modifying groups are diazo groups.

55 9. An antigen according to Claim 8, in which the modifying groups are introduced by reaction with diazosulfanilic acid. 55

10. An antigen according to Claim 7, in which the modifying groups are derived from a dinitrophenol, a trinitrophenol, S-acetomercaptosuccinic anhydride, a polytyrosine, a polyalanine, biodegradable polydextran or thyroglobulin.

**WHAT WE CLAIM IS:—**

1. An antigen for the immunological control of fertility which comprises a chemically modified polypeptide, the unmodified polypeptide being a protein reproductive hormone (wherein defined) or a fragment (as herein defined) thereof, and the chemical modification comprising

- 5 (i) the attachment of one or more modifying groups to the unmodified polypeptide, or the attachment of two or more unmodified polypeptide molecules to a modifying group, provided that the modifying groups are derived from polymerised sugars, serum proteins, keyhole limpet hemocyanin, marine gastropod mollusc, viruses or bovine gamma globulin, when the unmodified polypeptide is other than of structure VII, VIII, IX or X, 10

Phe - Gln - Asp - Ser - Ser - Ser - Ser - Lys - Ala - Pro - Pro - Pro - Ser - Leu - Pro - Ser - Pro - Ser - Arg - Leu - Pro - Gly - Pro - Ser - Asp - Thr - Pro - Ile - Leu - Pro - Gln

15 (Structure VII) 15

Asp - Asp - Pro - Arg - Phe - Gln - Asp - Ser - Ser - Ser - Lys - Ala - Pro - Pro - Pro - Ser - Leu - Pro - Ser - Gly

(Structure VIII)

20 Asp - Asp - Pro - Arg - Phe - Gln - Asp - Ser - Ser - Ser - Ser - Lys - Ala - Pro - Pro - Pro - Ser - Leu - Pro - Ser - Pro - Ser - Arg - Leu - Pro - Gly - Pro - Pro - Asp - Thr - Pro - Ile - Leu - Pro - Gln - Ser - Leu - Pro 20

(Structure IX)

25 Asp - His - Pro - Leu - Thr - Cys - Asp - Asp - Pro - Arg - Phe - Gln - Asp - Ser - Ser - Ser - Ser - Lys - Ala - Pro - Pro - Pro - Ser - Leu - Pro - Ser - Pro - Ser - Arg - Leu - Pro - Gly - Pro - Pro - Asp - Thr - Pro - Ile - Leu - Pro - Gln - Ser - Leu - Pro 25

(Structure X)

- or (ii) polymerisation of the unmodified polypeptide via an organic bifunctional reagent; 30
or (iii) dimerisation of the unmodified polypeptide via a sulphur-sulphur bridge formed from the thiol group a cysteine residue present in unmodified polypeptide or added to the unmodified polypeptide if it does not contain a cysteine residue. 30

2. An antigen for the immunological control of fertility which comprises a chemically modified polypeptide, the unmodified polypeptide being a protein reproductive hormone (as herein defined) or a fragment (as herein defined) thereof, and the chemical modification comprising the attachment of one or more modifying groups to the unmodified polypeptide, or the attachment of two or more unmodified polypeptide molecules to a modifying group, provided that the modifying groups are derived from polymerised sugars, serum proteins, keyhole limpet hemocyanin, marine gastropod mollusc, viruses or bovine gamma globulin, when the unmodified polypeptide has a structure other than of formula VII, VIII, IX or X, stated in Claim 1. 35 40

3. An antigen according to Claim 2, in which 1 to 40 modifying groups are attached per molecule of polypeptide. 45

4. An antigen according to Claim 2 or 3, in which 2 to 40 modifying groups are attached per molecule of polypeptide. 45

5. An antigen according to any one of Claims 2 to 4, in which 5 to 30 modifying groups are attached per molecule of polypeptide. 50

6. An antigen according to Claim 2, in which 2 or more polypeptide molecules are attached to the modifying group. 50

7. An antigen according to any one of Claims 2 to 6, in which the unmodified polypeptide is of structure VII, VIII, IX or X, stated in Claim 1. 55

8. An antigen according to Claim 7, in which the modifying groups are diazo groups. 55

9. An antigen according to Claim 8, in which the modifying groups are introduced by reaction with diazosulfanilic acid. 55

10. An antigen according to Claim 7, in which the modifying groups are derived from a dinitrophenol, a trinitrophenol, S-acetomercaptosuccinic anhydride, a polytyrosine, a polyalanine, biodegradable polydextran or thyroglobulin.



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11. An antigen according to any one of Claims 2 to 6, in which the modifying groups are derived from polymerised sugars, serum proteins, keyhole limpet hemocyanin, marine gastropod mollusc, viruses, or bovine gamma globulin.

12. An antigen according to Claim 11, in which the polymerised sugar is a copolymer of sucrose with epichlorohydrin, or a polyglucose, the serum protein is homologous serum albumin or bovine serum albumin, and the virus is influenza virus (Type A, B or C), or poliomyelitis virus, live or killed, (Type 1, 2 or 3).

13. An antigen according to Claim 11 or 12, in which the modifying groups are derived from a copolymer of sucrose with epichlorohydrin, homologous serum albumin or bovine serum albumin or keyhole limpet hemocyanin.

14. An antigen according to Claim 13, in which the modifying groups are derived from a copolymer of sucrose with epichlorohydrin.

15. An antigen according to any one of Claims 11 to 14, in which the unmodified polypeptide is Follicle Stimulating Hormone, Leutinsing Hormone, Human Placental Lactogen, Human Prolactin, Human Chorionic Gonadotropin or a fragment of any of these.

16. An antigen according to Claim 15, in which the unmodified polypeptide is Human Chorionic Gonadotropin or a fragment thereof.

17. An antigen according to Claim 16, in which the unmodified polypeptide is Human Chorionic Gonadotropin.

18. An antigen according to Claim 16, in which the unmodified polypeptide is the β -subunit of Human Chorionic Gonadotropin.

19. An antigen according to Claim 16, in which the unmodified polypeptide is a 20 to 30 amino-acid C-terminal fragment of the β -subunit of Human Chorionic Gonadotropin.

20. An antigen according to Claim 16, in which the unmodified polypeptide is a 30 to 39 amino acid C-terminal fragment of the β -subunit of Human Chorionic Gonadotropin.

21. An antigen according to Claim 16, in which the unmodified polypeptide is of structure VII, VIII, IX or X, stated in Claim 1, or of structure III, IV, V or VI (the asterisks indicating site locations of carbohydrate moieties),

Asp - Asp - Pro - Arg - Phe - Gln - Asp - Ser - Ser - Ser - * - Lys - Ala - Pro -
Pro - Pro - Ser - Leu - Pro - Ser - Pro - Ser - Arg - Leu - Pro - Gly - Pro - Ser -
Asp - Thr - Pro - Ile - Leu - Pro - Gln

(Structure III)

Gln - Asp - Ser - Ser - Ser - Ser - * - Lys - Ala - Pro - Pro - Pro - * - Leu - Pro -
Ser - Pro - * - Arg - Leu - Pro - Gly - Pro - * - Asp - Thr - Pro - Ile - Leu -
Pro - Gln.

-(Structure IV)

Thr - Cys - Asp - Asp - Pro - Arg - Phe - Gln - Asp - * - Ser - Ser - Ser - Lys - Ala -
Pro - Pro - Pro - Ser - Leu - Pro - * - Pro - * - Arg - Leu - Pro - Gly - Pro -
Pro - Asp - Thr - Pro - Ile - Leu - Pro - Gln - Ser - Leu - Pro.

(Structure V)

Phe - Gln - Asp - * - Ser - Ser - Ser - Lys - Ala - Pro - Pro - Pro - Ser - Leu - Pro -
* - Pro - * - Arg - Leu - Pro - Gly - Pro - Pro - Asp - Thr - Pro - Ile - Leu -
Pro - Gln - Ser - Leu - Pro.

(Structure VI)



22. An antigen according to Claim 21, in which the unmodified polypeptide is of structure IX or X, stated in Claim 1, or III or VI, stated in Claim 21.

23. An antigen according to Claim 22, in which the unmodified polypeptide is of structure III, stated in Claim 21.

24. An antigen for the immunological control of fertility which comprises a polypeptide of structure X, stated in Claim 1, chemically modified by the attachment of one or more modifying groups derived from keyhole limpet hemocyanin.

25. An antigen for the immunological control of fertility which comprises a polypeptide of structure III, stated in Claim 21, conjugated to keyhole limpet hemocyanin.

26. An antigen for the immunological control of fertility which comprises a polypeptide of structure IX, stated in Claim 1, conjugated to homologous serum albumin.

27. An antigen for the immunological control of fertility which comprises a polypeptide of structure X stated in Claim 1, conjugated to a copolymer of sucrose with epichlorhydrin or a polyglucose.

28. An antigen for the immunological control of fertility comprising a polypeptide of structure VI, stated in Claim 21, conjugated to bovine serum albumin.

29. A process for the production of an antigen for the immunological control of fertility comprising chemically modifying a polypeptide which is a protein reproductive hormone (as herein defined) or a fragment (as herein defined) thereof,

a) by attaching to the unmodified polypeptide modifying group(s) derived from modifiers having free amino groups, by activation of the modifier or polypeptide to be modified by reaction with tolylene diisocyanate and reaction of the resulting activated product with the polypeptide to be modified, or modifier, respectively; or

b) by attaching to the unmodified polypeptide modifying group(s) derived from modifiers having free amino groups or being polymerised sugars by conjugation of the unmodified polypeptide with the modifier employing a water-soluble carbodiimide as activating agent; or

c) by attaching to the unmodified polypeptide modifying group(s) derived from modifiers having free amino groups by reaction of the modifier with glutaric dialdehyde and reaction of the resulting product with the polypeptide to be modified, in the presence of an alkali metal borohydride; or

d) by attaching to the unmodified polypeptide modifying group(s) derived from polymerised sugars by reaction of the polymerised sugar with a cyanuric halide, and reaction of the resulting dihalotriazinyl adduct with the polypeptide to be modified; or

e) by attaching to the unmodified polypeptide modifying groups derived from polymerised sugars by treatment of the sugar with an alkali metal periodate and reaction of the resulting product with the polypeptide to be modified.

30. A process for the production of an antigen for the immunological control of fertility comprising chemically modifying a polypeptide which is a protein reproductive hormone (as herein defined) or a fragment (as herein defined) thereof by attaching to the unmodified polypeptide modifying group(s) derived from modifiers having free amino groups, by activation of the modifier or polypeptide to be modified by reaction with tolylene diisocyanate and reaction of the resulting activated product with the polypeptide to be modified or modifier, respectively.

31. A process according to Claim 30, in which a solution of tolylene diisocyanate is added to a buffered solution of the modifier, the mixture allowed to react at -5° to $+10^{\circ}\text{C}$, and the resulting activated modifier is added to a buffered solution of the polypeptide to be modified and the mixture allowed to react at 30° to 50°C .

32. A process according to Claim 30 or 31, in which the modifier is a serum protein, keyhole limpet hemocyanin or a polytyrosine, polyalanine, bovine gamma globulin or thyroglobulin.

33. A process according to any one of Claims 30 to 32, in which the polypeptide is as stated in any one of Claims 15 to 23.

34. A process according to any one of Claims 30 to 33, in which the modifier is keyhole limpet hemocyanin and the polypeptide is of structure X, stated in Claim 1.

35. A process according to any one of Claims 30 to 33, in which the modifier is bovine gamma globulin and the polypeptide is of structure III, stated in Claim 21.

36. A process according to any one of Claims 30 to 33, in which the modifier is bovine serum albumin and the polypeptide is of structure VI, stated in Claim 21.

37. A process according to any one of Claims 30 to 33, in which the modifier is poly (DL-Lys-Ala) and the polypeptide is of structure VI, stated in Claim 21.

38. A process according to any one of Claims 30 to 37, substantially as herein described with reference to any one of Examples 1 to 4.

39. An antigen whenever produced by a process according to any one of Claims 30 to 39.

40. An antigen for the immunological control of fertility comprising a polypeptide of structure III, stated in Claim 21, conjugated to bovine gamma globulin.

41. An antigen for the immunological control of fertility comprising a polypeptide of structure VI, stated in Claim 21, conjugated to bovine serum albumin.

42. An antigen for the immunological control of fertility comprising a polypeptide of structure VI, stated in Claim 21, conjugated to poly(DL-Lys-Ala).

43. A process for the production of an antigen for the immunological control of fertility comprising chemically modifying a polypeptide which is a protein reproductive hormone (as herein defined) or a fragment (as herein defined) thereof, by attaching to the unmodified polypeptide modifying group(s) derived from modifiers having free amino groups or being polymerised sugars by conjugation of the unmodified polypeptide with the modifier employing a water-soluble carbodiimide as activating agent.

44. A process according to Claim 43, in which the free amino groups of the polypeptide to be modified are protected prior to conjugation with the modifier and the protecting groups are removed subsequent to conjugation.

45. A process according to Claim 43 or 44, in which, when the modifier is a polymerised sugar, said sugar is treated with ethylene diamine prior to conjugation with the polypeptide to be modified.

46. A process according to any one of Claims 43 to 45, in which the water-soluble carbodiimide is 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.

47. A process according to any one of Claims 43 to 46, in which the conjugation is effected in the presence of a guanidine and at a pH of from 4 to 5.

48. A process according to any one of Claims 43 to 47, in which the modifier is keyhole limpet hemocyanin, a serum protein, a polymerised sugar, or a polyalanine or polytyrosine.

49. A process according to Claim 48, in which the serum protein is homologous serum albumin, and the polymerised sugar is a copolymer of sucrose with epichlorohydrin or a polyglucose.

50. A process according to any one of Claims 43 to 49, in which the polypeptide is as stated in any one of Claims 15 to 23.

51. A process according to any one of Claims 43 to 50, in which the modifier is keyhole limpet hemocyanine and polypeptide is of structure III, stated in Claim 21.

52. A process according to Claim 43, substantially as herein described with reference to Example 5.

53. An antigen whenever produced by a process according to any one of Claims 43 to 52.

54. A process for the production of an antigen for immunological control of fertility comprising chemically modifying a polypeptide which is a protein reproductive hormone (as herein defined) or a fragment (as herein defined) thereof by attaching to the unmodified polypeptide modifying group(s) derived from modifiers having free amino groups by reaction of the modifier with glutaric dialdehyde and reaction of the resulting product with the polypeptide to be modified, in the presence of an alkali metal borohydride.

55. A process according to Claim 54, in which the reaction of the modifier with glutaric dialdehyde is effected at a pH of 7 to 10, and at room temperature and the conjugation of the resulting product with the polypeptide is effected in the presence of sodium borohydride.

56. A process according to Claim 54 or 55, in which the modifier is a serum protein.

57. A process according to any one of Claims 54 to 56, in which the polypeptide is as stated in any one of Claims 15 to 23.

58. A process according to any one of Claims 54 to 57, in which the modifier is homologous serum albumin and the polypeptide is of structure X, stated in Claim 1.

59. A process according to Claim 54, substantially as herein described with reference to Example 6.

60. An antigen whenever produced by a process according to any one of Claims 54 to 59.

61. A process for the production of an antigen for the immunological control of fertility comprising chemically modifying a polypeptide which is a protein reproductive hormone (as herein defined) or a fragment (as herein defined) thereof by attaching to



21

the unmodified polypeptide modifying group(s) derived from polymerised sugars by reaction of the polymerised sugar with a cyanuric halide, and reaction of the resulting dihalotriazinyl adduct with the polypeptide to be modified.

5 62. A process according to Claim 61, in which the reaction of the modifier with a cyanuric halide is effected at a temperature of 0° to 20°C in an organic medium, and the resulting adduct is reacted with the polypeptide at pH 8 to 11. 5

63. A process according to Claim 61 or 62, in which the cyanuric halide is a cyanuric chloride.

10 64. A process according to any one of Claims 61 to 63, in which the modifier is a copolymer of sucrose with epichlorhydrin or a polyglucose. 10

65. A process according to any one of Claims 61 to 64, in which the modifier is a copolymer of sucrose with epichlorhydrin.

15 66. A process according to any one of Claims 61 to 65, in which the polypeptide is as stated in any one of claims 15 to 23. 15

67. A process according to any one of Claims 61 to 66, in which the modifier is a copolymer of sucrose with epichlorhydrin and the polypeptide is of structure X, stated in Claim 1. 15

68. A process according to any one of Claims 61 to 66, in which the modifier is a polyglucose and the polypeptide is of structure X, stated in Claim 1.

20 69. A process according to Claim 61, substantially as herein described with reference to Example 7. 20

70. An antigen whenever produced by a process according to any one of Claims 61 to 69.

25 71. A process for the production of an antigen for the immunological control of fertility comprising chemically modifying a polypeptide which is a protein reproductive hormone (as herein defined) or a fragment (as herein defined) thereof by attaching to the unmodified polypeptide modifying group(s) derived from polymerised sugars by treatment of the sugar with an alkali metal periodate and reaction of the resulting product with the polypeptide to be modified. 25

30 72. A process according to Claim 71, in which the modifier is treated with the periodate at a temperature of from 30° to 60°C and a pH of 3 to 6 and the resulting product is reacted with the polypeptide at a pH of 7 to 11 and a temperature of 15° to 80°C. 30

35 73. A process according to Claim 70 or 71, in which the periodate is sodium periodate. 35

74. A process according to any one of Claims 71 to 73, in which the modifier is a copolymer of sucrose with epichlorohydrin.

40 75. A process according to any one of Claims 71 to 74, in which the modifier is a polyglucose. 40

76. A process according to any one of Claims 71 to 75, in which the polypeptide is as stated in any one of Claims 15 to 23.

45 77. A process according to any one of Claims 71 to 76, in which the modifier is a copolymer of sucrose with epichlorhydrin and the polypeptide is of structure X, stated in Claim 1. 45

78. A process according to Claim 71, substantially as herein described with reference to Example 8. 45

79. An antigen whenever produced by a process according to any one of Claims 71 to 78.

50 80. An antigen for the immunological control of fertility which comprises a chemically modified polypeptide, the unmodified polypeptide being a protein reproductive hormone (as herein defined) or fragment (as herein defined) thereof, and the chemical modification comprising polymerisation of the unmodified polypeptide via an organic bifunctional reagent. 50

55 81. An antigen according to Claim 80, in which the bifunctional reagent is a bifunctional imidoester. 55

82. An antigen according to Claim 80 or 81, in which the bifunctional reagent is dimethyl adipimide, dimethyl suberimide or diethyl malonimide.

60 83. An antigen according to any one of Claims 80 to 82, in which the unmodified polypeptide is as stated in any one of Claims 15 to 23. 60

84. An antigen according to any one of Claims 80 to 83, in which the unmodified polypeptide is of structure III, stated in Claim 21. 60

85. A process for the production of an antigen according to any one of Claims 80 to 84, comprising reacting the unmodified polypeptide with the bifunctional reagent.

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16

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16

86. A process according to Claim 85, in which the reaction is effected at a pH of from 9 to 12.

87. A process according to Claim 85, substantially as herein described with reference to Example 9.

5 88. An antigen, whenever produced by a process according to any one of Claims 85 to 87.

10 89. An antigen for the immunological control of fertility which comprises a chemically modified polypeptide, the unmodified polypeptide being a protein reproductive hormone (as herein defined) or a fragment (as herein defined) thereof, and the chemical modification comprising dimerisation of the unmodified polypeptide via a sulphur-sulphur bridge formed from the thiol a cysteine residue present in the unmodified polypeptide or added to the unmodified polypeptide if it does not contain a cysteine residue.

15 90. An antigen according to Claim 89, in which the unmodified polypeptide is as stated in any one of Claims 15 to 23.

91. An antigen according to Claim 89 or 90, in which the unmodified polypeptide is of structure V, stated in Claim 21, or X, stated in Claim 1.

20 92. A process for the production of an antigen according to any one of Claims 89 to 91, comprising oxidising the thiol group in a cysteine residue present in or added to the unmodified polypeptide.

93. A process according to Claim 92, in which the oxidation is effected with iodo-benzoic acid.

94. A process according to Claim 92, substantially as herein described with reference to Example 10.

25 95. An antigen, whenever produced by a process according to any one of Claims 92 to 94.

96. A pharmaceutical composition comprising an antigen according to any one of Claims 1 to 28, 39 to 42, 53, 60, 70, 79 to 84, 88, 91 or 95, in association with a liquid pharmaceutically acceptable carrier.

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Printed for Her Majesty's Stationery Office by the Courier Press, Leamington Spa, 1980.
Published by the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from
which copies may be obtained.