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(54) DERIVATIVES OF THE PREGNANCY-SPECIFIC β_1-GLYCOPROTEIN, AND PROCESS FOR THEIR MANUFACTURE

(71) We, BEHRINGWERKE AKTIENGESELLSCHAFT, a body corporate organised according to the laws of the Federal Republic of Germany, of Marburg/Lahn, Federal Republic of Germany, 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:-

The present invention relates to derivatives of the pregnancy-specific β_1-glycoprotein, to a process for their manufacture and to immunising agents which contain as an essential constituent a derivative of the pregnancy-specific β_1-glycoprotein.

British Patent Specification No. 1 410 338 described and claims a pregnancy-specific β_1-glycoprotein and a process for its isolation. The starting materials for the isolation are human organs or body fluids. Upon immunisation of vertebrate animals with human pregnancy-specific β_1-glycoprotein an antibody against the pregnancy-specific β_1-glycoprotein is found in the plasma of the immunised animals, which antibody can undergo a specific reaction with the original antigen.

The pregnancy-specific β_1-glycoprotein (SP 1) is characterised by the following parameters:

a) an electrophoretic motility in agar gel in the β_1-globulin range of the human plasma proteins,
b) a sedimentation constant in the ultracentrifuge of 4.6 ± 0.5 S,
c) a molecular weight of 100,000 ± 15,000 determined by gel filtration
d) An extinction coefficient of $E^{1\%}_{1\ cm} = 11.6 \pm 0.5$

(determined at 278 mu in 1/15 M of phosphate buffer of pH 7)
e) a carbohydrate content of 28.05 ± 1.55.

As a result of tests which demonstrated that contraception or induction of miscarriages in primates can be achieved using antibodies against the pregnancy-specific β_1-glycoprotein, German Patent Application P 23 45 953.2 has proposed the use of the pregnancy-specific β_1-glycoprotein for active immunisation of primates or to achieve contraception or induction of miscarriages by passively administered antibodies. The passive transmission of antibodies has been found to give satisfactory results, but the active immunisation has proved to be less satisfactory because often the reduced amounts of antibodies formed in response to the administration of the homologuous pregnancy-specific β_1-glycoprotein are insufficient for reliable contraception. (A homologuous pregnancy-specific β_1-glycoprotein is that derived from the same species as that to which it is to be administered.)

It is known that a glycoprotein corresponding to the human pregnancy-specific β_1-glycoprotein can be isolated from monkeys, and it might be expected that upon its administration to a human a sufficient amount of antibodies would form in the blood, which antibodies would be capable of reacting with the human pregnancy-specific β_1-glycoprotein. The starting material for the preparation of the pregnancy-specific β_1-glycoprotein isolated from monkeys is available to a restricted extent only, whereas human pregnancy-specific β_1-glycoprotein can be prepared in satisfactory amounts from lyophilised human placentae or from the serum of pregnant women, as described in the above Patent Specification.

The present invention is based on the observation that any one of a series of processes for the chemical modification of pregnancy-specific β_1-glycoprotein also leads to a modification of the immunological behaviour in the resulting derivatives, so that they are able to induce

antibodies to a satisfactory extent in the homologous system.

The present invention provides derivatives of the pregnancy-specific β_1-glycoprotein which derivatives have been prepared by reaction of the glycoprotein with a compound capable of chemically modifying the protein.

Reactions of this type fall into two basic categories, which both result in the chemical modification of the native structure and thus in the denaturation of the protein:

1. Processes for chemical modification of the pregnancy-specific β_1-glycoprotein using reactants which lead to a modification of chemical groups present therein.
2. Processes for chemical modification of the pregnancy-specific β_1-glycoprotein by the introduction of further groups into the molecule or by the linkage of the molecule to other compounds.

The processes mentioned in group 1 may modify one or more of the following groups in the glycoprotein: amino, guanidyl, imidazole, indole, aliphatic hydroxyl, amide, thioether, disulphide, sulphydryl, phenol and carboxyl groups. In the literature, a large number of such reactions are described, of which the following are given by way of example only:

1.1. Oxidation with iodosobenzoate, porphyrindine, ferrocyanide, or iodine, in which case generally the concentration of the oxidising agent is 0.001 - 0.01 M, the pH is 7, the temperature is within the range of from 0 to 25°C and the reaction time is 5 - 30 minutes. Oxidation with iodine is generally carried out in the presence of iodine in high concentration at pH 7. Oxidation can alternatively be carried out using hydrogen peroxide, in which case the preferred conditions are concentration of the oxidising agent about 0.005 M, pH value about 6.6, temperature about 25°C, and reaction time 0.5 to 40 hours.

1.2. Reduction with cystein, thioglycolic acid, thioglycol, cyanide, or sulphide, preferably under the following conditions: concentration of the reducing agent 0.001 - 0.1 M, pH value 7 - 8, room temperature of about 25°C and reaction time 0.5 to 4 hours.

The reaction conditions in each case are described in detail in the literature. They are reported, for example by H.S. Olcott and H. Fraenkel-Conrat, Chem. Rev. *41*, page 151 *et seq.* (1947). Furthermore, some of the reactants and the reaction conditions are described in H.E. Schultze and J.F. Heremans, Molecular Biology of Human Proteins (1966), pages 40 - 41, particularly the references made on page 58 *et seq.*.

Many of the further groups which may be introduced into the pregnancy-specific β_1-glycoprotein are called from the immunological point of view "haptens". A "hapten" is defined as a protein depleted substance which can react with an antibody specifically acting against its configuration, but which is itself not capable of forming identifiable amounts of antibodies. A hapten is immunifacient only in combination with a carrier protein. Most haptens are low-molecular weight compounds having a molecular weight of less than 1,000, but certain macromolecules, for example the pneumococcus polysaccharides, can also be considered as haptens.

A hapten-protein compound generally leads to the induction of two types of antibodies, one being specific against the hapten grouping and the other against the protein carrier. A pregnancy-specific β_1-glycoprotein-hapten compound therefore induces the production of antibodies against the glycoprotein in the homologous system.

Haptens according to the invention are the chemical groups and compounds described as such in the literature. These are especially aromatic ring systems, steroids, peptides, purines, pyrimidines, penicillin and its derivatives, and also small molecules, for example iodine. Moreover, within the scope of this invention compounds having peptide or protein or carbohydrate character and which themselves have antigenic properties, can be bound to the pregnancy-specific β_1-glycoprotein.

The processes for the preparation of the derivatives of the pregnancy-specific β_1-glycoprotein according to the above-mentioned principle (2) using haptens are processes known *per se* for the introduction of protein-modifying groups and of haptens into proteins with the formation of covalent bonds between the substances introduced and the protein. The reactions mentioned are especially those which are described for the modification of individual groups in protein molecules. These reactions, too, are described by Olcott and Fraenkel-Conrat and by Schultze-Heremans. Examples of those processes are

2.1	The alkylation with iodoacetate, iodoacetamide	(0.05-0.1 M, pH 7-8, 0-25°C, 0.5-2 hours) or
	dinitrofluorobenzene:	(0.17 M, pH 7-8, 25°, 2 hours).
2.2	The acylation with ketene	(pH 5-8, 0.25°C, 5-30 min)
	acetic anhydride	(pH 7-8, 0°, 30 min)
	carbon suboxide	(pH 5-8, 0-25°, 5-30 min)
	azides, benzoyl-, carbobenzoxy- or benzenesulfonyl chloride	(pH 7-9, 0-25°, 0.5-2 hours)
	nitric acid	(1 M, pH 4, 30 min)
	iodine	(pH 5-11, -5° to 25°, 0.5-3 hours: in contradistinction to the above oxidation the amount of iodine is smaller and the concentration of iodide reduced),
	formaldehyde	(1-2 M, 25°, at pH 7-8, 1 hour at pH 11, 10 min)
	epoxides	(1-2 M, pH 5-6, 1-4 days)
	mustard oil	(pH 5-6, 25°, 0.5-4 hours)
	acid/alcohol	mineral acid in absolute alcohol), 0.01-0.1 M, 0-25°, 1-2 days)
	methyl diazoacetate, diazoacetamide	(pH 5 (ester), pH 6 (amide) 0°)
	p-chlormercuribenzoate	(10^{-5}–10^{-2} M, pH 7, 25°, 5-30 min)
	diazonium compounds	(pH 7-9, 25°, 30 min) or
	o-methylisourea	(0.5 M, pH 10.5, 0°, 3 days).

The following is a detailed description of processes with references to some of the above groups:

a) *Iodization* (literature: Kabat and Mayer's Experimental Immuno-chemistry, sec. edition 1961, 816-818)
Iodine is reacted as iodinate with tyrosine groups of a protein, in which reaction one or two iodine atoms are introduced into the tyrosine and the iodinated protein is obtained. In this manner, iodine derivatives of the pregnancy-specific β_1-glycoprotein may be prepared.

b) *Diazotization and coupling:* (Literature: Kabat and Mayer's Experimental Immunochemistry, second edition 1961, 798-799).
A compound containing an aromatic amino group, for example, arsanilic acid or sulfanilic acid is diazotized and the diazonium compound reacted with a protein. In this reaction, mainly tyrosine groups, but also histidine and lysine groups of the protein are bound to the aromatic component via a covalent bond. According to this process, for example the diazo-arsanilic acid derivative or the diazobenzene-sulfonic acid derivative of the pregnancy-specific β_1-glycoprotein can be prepared.

c) Reaction with a vinylsulfonyl compounds (in analogy to the reaction of a reactive dyestuff which contains the vinylsubfonyl group as the reactive component, with cellulose or wool). An aliphatic or aromatic vinylsulfonyl derivative or a sulfuric acid semi ester of a β-hydroxyethylsulfone can be reacted with the amino groups and/or hydroxyl groups of a protein. In this way, for example, a hapten-containing derivative of the pregnancy-specific β_1-glycoprotein can be obtained using 1-aminobenzene 4β-hydroxyethylsulfon-sulfuric acid ester.

d) *Reaction with isocyanate and isothiocyanate compounds*
(Literature: Kabat and Mayer's Experimental Immunochemistry, sec. edition 1961, 809-811). This reaction which occurs via the free amino groups of a protein allows permits the introduction of a hapten having the appropriate chemical grouping into the pregnancy-specific β_1-glycoprotein.

e) *Dinitrophenylation:* (Literature: Carsten, M.E.; Eisen, H.N., J. Am. Chem. Soc. 77, 1273 (1955). This reaction, which is generally carried out with dinitrobenzene sulfonate or dinitrofluorobenzene occurs with the free amino groups of a protein to form dinitrophenyl derivatives. In this manner, a dinitrophenylated pregnancy-specific β_1-glycoprotein may be obtained.

f) *Reaction with mixed anhydrides:* (Literature: Kabat and Mayer's Experimental Immunochemistry, sec. edition 1961, 813-815). In this process, a series of different haptens which have first to be converted by acylation into a mixed anhydride, can be

linked to amino groups of a protein. Moreover, the direct reaction of a protein with an anydride allows the preparation of an acid amide of the general formula R-CO-NH-CH_2-protein, in which R can be any hapten. This reaction allows the preparation for example of an R-CO-N4-CH_2-pregnancy-specific β_1-glycoprotein compound in which R represents a benzyl group.

g) Reaction with carbodiimides(Literature: Makino, T. et al., Contraception *8* (2), 133 (1973). The carbodimides of the general formula R-N=C=N-R (R= any organic radical) are suitable for binding haptens containing carboxyl groups to the amino groups of proteins. The reaction product thereby obtained is the compound R-CO-NH-protein, in which case R may be any hapten with contains a carboxyl group. This reaction allows one to obtain for example the hapten compound of the pregnancy-specific β_1-glycoprotein with cyclohexanecarboxylic acid as hapten.

The immunological behaviour of the pregnancy-specific β_1-glycoprotein can be modified according to the invention by binding the glycoprotein with a peptide or protein, either by covalent bonding or by complex formation. Reactants are peptides, for example, those having a biological function of their own, for example decapeptides, for example, the luteinizing hormone release factor (LRF). LRF may be bound to the pregnancy-specific β_1-glycoprotein in the same manner as described for the linkage with albumin by T. Makino et al. in Contraception *8* 2 (1973), pages 3 *et seq.*, The reaction on which it is based is the activation of the carboxyl groups by carbodiimide.

A further possibility for modifying the immunological behaviour of the pregnancy-specific β_1-glycoprotein is the linkage of the protein to a second protein substance in the way described for example for the linkage of enzymes to proteins, in which case the pregnancy-specific β_1-glycoprotein may replace one of the two reactants. The linkage of the two proteins to each other can be effected according to any of a series of known methods. In these methods, individual groups of activated substances, for example carbodimides, cyanurice chloride, *p,p*-difluoro-*m,m*-dinitrophenylsulphone or glutardialdehyde are used. The linkage of the pregnancy-specific β_1-glycoprotein can be effected, for example using glutardialdehyde in a reaction analogous to that described by S. Avrameas: Immunochemistry *6*, 1969, 43-52, for the coupling of gammaglobulin and peroxidase, by means of a series of proteins. Preferably, the pregnancy-specific β_1-glycoprotein provided as immunising agent is bound to a protein substance which acts itself as vaccine component, for example tetanus toxoid.

The various methods mentioned above are merely to show by way of example that any of the reactions described in the literature as being suitable for preparing derivatives of proteins can be used for the preparation of the products according to the invention.

The invention therefore provides a process for the manufacture of a derivative of the pregnancy-specific β_1-glycoprotein which comprises reacting the pregnancy-specific β_1-glycoprotein with a compound capable of chemically modifying the protein, especially by oxidation, reduction, alkylation or acylation, or by the formation of a complex between the protein and a hapten or a macromolecule.

The invention accordingly provides a vaccine suitable for parenteral administration, which comprises a derivative of the pregnancy-specific β_1-glycoprotein of the invention in admixture with a pharmaceutically suitable carrier. Stabilising agents and/or inorganic or organic adjuvants may also be present.

Stabilising adjuvants are substances which improve the durability of the preparations, for example especially carbohydrates, proteins or their derivatives, for example, decomposed and re-cross-linked collagen, for example, the gelatin product known under the Trade Mark Haemaccel. Further additives, for example, amino acid salts, for example, sodium glutamate may also be used. The additives are advantageously added in amounts of from 0.5 to 5 %, sometimes up to 10 %. The immunising agents may be made available in liquid, dry, or advantageously lyophilised form. The lyophilised product will have to be dissolved before administration in water or in a physiologically tolerable medium.

The vaccines preferably comprise from 0.1 to 1 mg of the derivative per ml, advantageously 0.2 mg per ml, and may be in unit dosage form.

The invention further provides a method of contraception which comprises administering to a female human a vaccine of the invention, preferably in an amount of 1 mg of the derivative. It is preferable to administer more than one dose of the vaccine, for example, a course of immunisation comprises a series of four injections of a vaccine of the invention, each dose comprising 1 mg of the glycoprotein derivative, at intervals of two weeks.

The following tests carried out with the products of Examples 1 and 2 illustrate the active immunisation of monkeys with the derivatives of the pregnancy-specific β_1-glycoprotein according to the invention and the results achieved thereby.

Test description:

Sexually mature female monkeys of the species Cynomolgus were immunised alternately by the intravenous and subcutaneous routes with a total of 3 mg of a derivative of the pregnancy-specific β_1-glycoprotein in isotonic sodium chloride solution containing finely dispersed aluminium hydroxide as adjuvant. All the animals developed antibodies against the pregnancy-specific β_1-glycoprotein, which antibodies were determined qualitatively in the gel diffusion test using pregnancy-specific β_1-glycoprotein and were determined quantitatively by means of radial immune diffusion. An average titre of 0.09 mg of antibody/ml was obtained.

After immunisation, a female animal and a male animal capable of fertilisation were brought together at the date of ovulation for 3 days for the purpose of copulation and the result of the copulation was examined. Half of the sample of animals were given booster injections (0.4 mg of protein) monthly between two copulatons or during pregnancy.

Compared to untreated control animals the female animals immunised against the pregnancy-specific β_1-glycoprotein were strongly inhibited in their reproductiveness. Table 1 shows the result obtained from immunised animals. In the case of some monkeys, repeated copulation did not lead to pregnancy, while others became pregnant only after the third or fourth copulation. Most of the monkeys that became pregnant upon the first or one of the following copulations had an abortion in the first or in the second third of the pregnancy. Only one of the monkeys that had become pregnant upon the first copulation had a normal pregnancy resulting in a normal birth. Another monkey carried its pregnancy normally to full term upon the third copulation.

Table 1

Antigen	Monkey No.	Antibody titre mg/ml	Copulation & Success of Conception	+ positive − negative	A = Aborted () = Days of Gestation
Derivative of the pregnancy-specific β_1-glycoprotein of Example 2.	2	0.10	- - -	+ A (35)	
	3	0.05	- - -	- - -	- - - -
	4	0.09	- - -	- -	
	5	0.05	+A +A (40-135)		
	6	0.15	- - +A (34-45)	+A (29-36)	
	7	0.29	+		Birth
	10	0.05	+A (110)		
	12	0.09	+A (104)		
	13	0.05	- -		
Derivative of the pregnancy-specific β_1-glycoprotein of Example 1	14	0.05	+A +A (20-34) (21-29)		
	15	0.05	+A (92)		
	16	0.05	- - +		Birth

Control tests carried out for comparison:
Of 39 monkeys 31, i.e. 80 % were pregnant after 3 copulations at the latest. Only one of these pregnancies was terminated by a spontaneous abortion, the others had a normal course.

The following Examples illustrate the invention:

Example 1:
Modification of pregnancy-specific β_1-glycoprotein with diazotized sulphanilic acid

a) Preparation of the diazonium salt .....

1 g of sulfanilic acid is dissolved at room temperature in 100 ml of 0.1 N HCl, the solution is placed in ice-water and cooled to 0°C while stirring. The sulfanilic acid precipitates as its salt. 50 ml of a cold 1 % solution of sodium nitrite in water is slowly added dropwise to the suspension while stirring (in the course of 1 hour); the end of the reaction is indicated by the blue coloration of sodium iodide starch paper.

b) Preparation of the hapten-compound

The conjugation of pregnancy-specific β_1-glycoprotein with diazotized sulfanilic acid takes place at slightly alkaline pH. 100 mg of pregnancy-specific β_1-glycoprotein are dissolved in 10 ml of 0.2 M sodium phosphate buffer of pH 8.2 and the solution is cooled to 4°C. 0.57 ml of the diazonium salt solution is added (the molar ratio of pregnancy-specific β_1-protein to diazotized sulfanilic acid in the reaction mixture is in this case 1:20) and the solution is stirred at 4°C for 4 hours. The pH value which shall not fall below 8 is adjusted by the addition of 0.2 M of NaOH. Then, the solution is allowed to stand at 4°C during 15 hours. The modified protein is then thoroughly dialysed against water for several days and finaly lyophilised. In the reaction of the pregnancy-specific β_1-glycoprotein with the diazotized sulfanilic acid the protein is dyed orange. Moreover, the introduction of acid groups modifies the electrophoretic mobility. The electrophoresis in agar or in polyacrylamide-(PAA) gel shows that the modified protein migrates more rapidly towards the anode than the native pregnancy-specific β_1-glycoprotein.

A dose of the immunising agent has the following composition:
0.2 mg of the derivative of the pregnancy-specific β_1-glycoprotein dissolved in 3 ml of isotonic sodium chloride solution containing 0.05 % of $Al(OH)_3$, 5 % of Haemaccel and 0.15 mg of sodium timerfonate as preserving agent.

Example 2:
Preparation of the hapten compound using 1-aminobenzene-4β-hydroxyethylsulfonyl-sulfuric acid ester

The starting substance is 1-aminobenzene-4β-hydroxyethylsulfonyl-sulfuric acid ester which splits off sulfuric acid in the alkaline pH range (pH 9-10) yielding a reactive aromatic vinylsulfone compound the so-called "parabase" which reacts with the amino or the hydroxyl groups in the protein.

100 mg of the pregnancy-specific β_1-glycoprotein are dissolved in 10 ml of a 2.5 % solution of Na_2PHO_4 (pH 9.25). To this solution, 3.1 ml of a 1 % sulution of the "parabase" ester in water (molar ratio: pregnancy-specific β_1-glycoprotein to "parabase" = 1:100) is added at room temperature while stirring. The solution is stirred during 4 hours at room temperature and the pH is kept constant at 9.2 by adding 0.1 N of NaOH. Then, the solution is allowed to stand at 4°C during 15 hours, it is thoroughly dialysed against H_20 and the modified protein is lyophilised.

A dose of the immunising agent has the following composition:
1 mg of the pregnancy-specific β_1-glycoprotein-hapten compound dissolved in 5 ml of isotonic sodium chloride solution.

Example 3:
Coupling of the pregnancy-specific β_1-glycoprotein with tetanus toxoid.

19 mg of pregnancy-specific β_1-glycoprotein and 54 mg of tetanus toxoid (tetanus toxin treated with formalin) are dissolved in 5.4 ml of water, the solution is adjusted to pH 5 by adding 1/10 of hydrochloric acid to which 6.3 mg of N-ethyl-N' (3-dimethylaminopropyl)-carbodiimide-HCl are added while stirring. The mixture is stirred during 3 hours at about 20°C and 15 hours at +4°C, neutralised and dialysed against isotonic sodium chloride solution.

When monkeys (of the cynomolgen species) are immunised with this coupling product in the manner described in the test, the reproducibility of these animals is inhibited.

A dose of the immunising agent has the following composition:
0.5 mg of protein in 3 ml of isotonic sodium chloride solution and 1.5 mg of γ-$Al(OH)_3$.

WHAT WE CLAIM IS:-

1. A derivative of the pregnancy-specific β_1-glycoprotein obtained by reacting the

β_1-glycoprotein characterised by the following parameters:
a) electrophoretic motility in the agar gel in the β_1-globulin range of the human plasma protein
b) a sedimentation constant in the ultracentrifuge of 4.6± 0.5 S
c) a molecular weight of 100,000 ± 15,000 determined by gel filtration
d) an extinction coefficient of $E^{1\%}_{1\ cm}$ = 11.6 ± 0.5 (determined at 278 mu in 1/15 M of phosphate buffer of pH 7)
e) a carbohydrate content of 28.05 ± 1.55, with a compound capable of chemically modifying the protein.

2. A derivative as claimed in claim 1, wherein one or more groups of the glycoprotein have been oxidised or reduced.

3. A derivative as claimed in claim 1, wherein a hapten or a compound having antigenic activity *per se* has been introduced into the glycoprotein.

4. A derivative as claimed in claim 3, wherein the hapten is an alkyl or acyl group, an iodine atom, a diazo group, a vinylsulphonyl or sulphuric acid semi ester of a *p*-hydroxyethylsulphone, an isocyanate group or an isocyanate derivative, or a dinitrophenyl group.

5. A derivative as claimed in claim 3, wherein the hapten and the protein are joined via an amide group.

6. A derivative as claimed in claim 3, wherein the compound having antigenic activity *per se* is a protein or a peptide which is covalently linked or held in the form of a complex by non-covalent bonds.

7. A derivative as claimed in claim 1, and which is substantially as described in any one of the Examples herein.

8. A process for the manufacture of a compound as claimed in claim 1, which comprises reacting the pregnancy-specific β_1-glycoprotein defined in claim 1 with a compound capable of chemically modifying it.

9. A process as claimed in claim 8, wherein one or more groups present in the glycoprotein is or are chemically modified.

10. A process as claimed in claim 9, wherein the group or groups is or are modified by oxidation or by reduction.

11. A process as claimed in claim 10, wherein the oxidation is carried out with iodosobenzoate, porphyridine, ferrocyanide, iodine or hydrogen peroxide.

12. A process as claimed in claim 10, wherein the reduction is carried out with cystein, thioglycollic acid, thioglycol, cyanide, or a sulphide.

13. A process as claimed in claim 8, wherein the glycoprotein is modified by the introduction of a further group.

14. A process as claimed in claim 13, wherein the group is a hapten or a compound having antigenic activity *per se*.

15. A process as claimed in claim 14, wherein the compound having antigenic activity is a protein or a peptide.

16. A process as claimed in claim 15, wherein the protein or peptide has a biological function.

17. A process as claimed in claim 14, wherein a hapten is introduced by alkylation, acylation, iodization, diazotisation and coupling, by reaction with isocyanate or an isocyanate compound, by dinitrophenylation or by reaction with a mixed anhydride or a carbodiimide.

18. A process as claimed in claim 8, carried out substantially as described in any one of the Examples herein.

19. A derivative as claimed in claim 1, whenever prepared by a process as claimed in any one of claims 8 to 18.

20. A vaccine which comprises a derivative as claimed in any one of claims 1 to 7 or claim 19 in admixture with a pharmaceutically suitable carrier.

21. A vaccine as claimed in claim 20, which also comprises an organic or inorganic adjuvant.

22. A vaccine as claimed in claim 20 or claim 21, which also comprises a stabilizing agent.

23. A vaccine as claimed in any one of claims 20 to 22, which comprises from 0.1 to 1 mg of the derivative per ml.

24. A vaccine as claimed in claim 23, which comprises 0.2 mg of the derivative per ml.

25. A vaccine as claimed in any one of claims 20 to 24, in lyophilised form.

26. A vaccine as claimed in any one of claims 20 to 25 in unit dosage form.

27. A vaccine as claimed in claim 20 and which is substantially as described in any one of the Examples herein.

28. A method of contraception, which comprises administering to a female human a

vaccine as claimed in any one of claims 20 to 27.

29. A method as claimed in claim 28, wherein there is administered 1 mg of the glycoprotein.

30. A method as claimed in claim 29, wherein there is administered 4 doses of the vaccine, each dose containing 1 mg of the glycoprotein derivative, the doses being administered at fortnightly intervals.

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