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(54) ANTIGENIC MODIFICATION OF POLYPEPTIDES

(71) We, THE OHIO STATE UNIVERSITY, of 190 North Oval Drive, Columbus, Ohio 43210, United States of America, 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:—

This invention relates to the chemical modification of polypeptides to provide antigens which may be used in active immunisation for the purposes of the control or treatment of various physiological processes, particularly reproduction.

It is well known that polypeptides, particularly, proteins, are responsible for, contribute to or influence many physiological processes. For example, certain protein hormones and non-hormonal proteins are known to be essential for the normal events of the reproductive process. Unusual excesses of certain polypeptides, such as gastrin, angiotension II or somatomedin, are also known to cause or influence various disease states and maladies.

The present invention is concerned with the control or treatment of such physiological processes by active immunisation (i.e. administration of antigens) causing formation of antibodies which neutralise (render biologically ineffectual) the endogenous polypeptides which are responsible for, contribute to or influence the physiological process in question.

Immunology, as a means of controlling reproduction, has been the subject of much recent investigation. R. G. Edwards, in a paper entitled "Immunology of Conception and Pregnancy (British Medical Journal, 26, 72—78 [1970]), surveys the literature on this topic, for example that concerning the production and use of antibodies against testes or sperm. Hormone antibodies have also been studied for many years and the effects of specific antisera have been recorded. Most of the past approaches to contraception by immunological means have, however, used passive immunisation, that is injection of antibodies produced elsewhere. There are serious limitations to the use of passive immunisation for human therapy since the repeated injection of animal antibodies into humans is known to produce undesirable reaction in many individuals.

It has now been found that by chemically modifying natural, endogenous polypeptides which play a rôle in the physiological process to be treated or controlled, antigens are obtained which are capable of inducing formation of antibodies which neutralise not only the antigenically modified polypeptide but also its unmodified endogenous counterpart. Thus, immunisation apparently takes place because of the inability of the antibodies produced to distinguish between the antigenically modified polypeptides and their unmodified endogenous counterparts. 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 to be modified are derived from the species involved or from a closely related species.

As used herein, the term "fragment" in relation to an endogenous polypeptide means a sequence of amino acids in the complete amino acid chain of the endogenous polypeptide. These fragments must be sufficiently large and distinctive in chemical and physical character to enable them to be recognised as a specific part of the whole. As used herein, the term "polypeptide which is immunologically equivalent to" in relation to an endogenous polypeptide or fragment thereof, means a polypeptide which, although not identical to the polypeptide or fragment in question, elicits, when modified in

accordance with the invention, essentially the same antibody response as the endogenous polypeptide or fragment concerned, when modified.

The invention accordingly provides an antigen for use in active immunisation for the control or treatment of a physiological process in a particular species, comprising a chemically modified polypeptide, the unmodified polypeptide being

- a) an endogenous polypeptide which influences such physiological process in that species and selected from proteins reproductive hormones, non-hormonal protein antigens, isolated from placental tissue, gastrin, Angiotensin II, growth hormone, somatomedin, parathyroid hormone, glucagon, thyroid stimulating hormone or secretin;
- b) a fragment (as herein defined) of such endogenous polypeptide; or
- c) a polypeptide which is immunologically equivalent (as herein defined) to such endogenous polypeptide or fragment thereof, and the chemical modification being such as to permit the chemically modified polypeptide to induce, in that species, formation of antibodies which biologically neutralise the endogenous polypeptide as well as the chemically modified polypeptide, and comprising (a) the attachment of one or more foreign modifying groups to the unmodified polypeptide or (b) the removal of one or more moieties from the unmodified polypeptide.

The invention also provides a method of controlling or influencing a physiological process in a species comprising injecting into that species an immunologically effective amount of the antigens or antibodies of the invention. The invention particularly provides such a method of contraception. The term "animal" as used herein includes humans.

Protein reproductive hormones which may be chemically modified to provide the antigens of the invention include Follicle Stimulating Hormone (FSH), Leutinizising Hormone (LH), Human Placental Lactogen (HPL), Human Prolactin and Human Chorionic Gonadotropin, and fragments thereof.

Specific fragments of protein reproductive hormones which may be modified in accordance with the invention include the β -subunit of FSH and specific unique fragments of natural HPL of 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):

Ser - Lys - Glu - Pro - Leu - Arg - Pro - Arg - Cys - ¹⁰Arg - Pro - Ile - Asn - Ala -
 Thr - Leu - Ala - Val - Glu - ²⁰Lys - Glu - Gly - Cys - Pro - Val - Cys - Ile - Thr -
 Val - Asn - Thr - Thr - Ile - Cys - Ala - Gly - Tyr - Cys - Pro - Thr - Met - Thr -
 Arg - Val - Leu - Gln - Gly - Val - Leu - ⁵⁰Pro - Ala - Leu - Pro - Gln - Val - Val -
 Cys - Asn - Try - ⁶⁰Arg - Asp - Val - Arg - Phe - Glu - Ser - Ile - Arg - Leu - Pro -
 Gly - Cys - Pro - Arg - Gly - Val - Asn - Pro - Val - ⁸⁰Val - Ser - Tyr - Ala - Val -
 Ala - Leu - Ser - Cys - Gln - ⁹⁰Cys - Ala - Leu - Cys - Arg - Arg - Ser - Thr - Thr -
 Asp - ¹⁰⁰Cys - Gly - Gly - Pro - Lys - Asp - His - Pro - Leu - Thr - ¹¹⁰Cys - Asp - Asp -
 Pro - Arg - Phe - Gln - Asp - Ser - Ser - ¹²⁰Ser - Ser - Lys - Ala - Pro - Pro - Pro -
 Ser - Leu - Pro - ¹³⁰Ser - Pro - Ser - Arg - Leu - Pro - Gly - Pro - Ser - Asp - Thr -
 Pro - Ile - Leu - Pro - Gln (Structure I)

Ser - Lys - Gln - Pro - Leu - Arg - Pro - Arg - Cys - ¹⁰Arg - Pro - Ile - Asn - Ala -
 Thr - Leu - Ala - Val - Glu - ²⁰Lys - Glu - Gly - Cys - Pro - Val - Cys - Ile - Thr - Val -
 Asn - Thr - Thr - Ile - Cys - Ala - Gly - Tyr - Cys - Pro - Thr - Met - Thr - Arg -

Val - Leu - Gln - Gly - Val - Leu - Pro - Ala - Leu - Pro - Glx - Leu - Val - Cys -
 Asn - Tyr - ⁶⁰Arg - Asp - Val - Arg - Phe - Glu - Ser - Ile - Arg - Leu - ⁷⁰Pro - Gly -
 Cys - Pro - Arg - Gly - Val - Asn - Pro - Val - ⁸⁰Val - Ser - Tyr - Ala - Val - Ala -
 Leu - Ser - Cys - Gln - ⁹⁰Cys - Ala - Leu - Cys - Arg - (Arg) - Ser - Thr - Thr -
 5 Asp - ¹⁰⁰Cys - Gly - Gly - Pro - Lys - Asp - His - Pro - Leu - Thr - ¹¹⁰Cys - Asp - Asp -
 Pro - Arg - Phe - Gln - Asp - ^{*}Ser - Ser - ¹²⁰Ser - Lys - Ala - Pro - Pro - Pro - Ser -
 Leu - Pro - ^{*}Ser - ¹³⁰Pro - ^{*}Ser - Arg - Leu - Pro - Gly - Pro - Pro - Asx - Thr - ¹⁴⁰Pro - Ile -
 Leu - Pro - Gln - Ser - Leu - ¹⁴⁷Pro

(Structure II)

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 connection, 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, suitable 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 -
 20 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 - ^{*}Ser - Leu -
 25 Pro - Ser - Pro - ^{*}Ser - Arg - Leu - Pro - Gly - Pro - ^{*}Ser - 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 - ^{*}Ser - Pro - ^{*}Ser - Arg - Leu - Pro - Gly -
 Pro - Pro - Asx - Thr - Pro - Ile - Leu - Pro - Gln - Ser - Leu - Pro.

(Structure V)

Phe - Gln - Asp - ^{*}Ser - Ser - Ser - Lys - Ala - Pro - Pro - Pro - Ser - Leu -
 30 Pro - ^{*}Ser - Pro - ^{*}Ser - Arg - Leu - Pro - Gly - Pro - Pro - Asx - Thr - Pro - Ile -
 Leu - Pro - Gln - Ser - Leu - Pro.

(Structure VI)

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)]. A polytyrosine chain may also be added to the structures III to VI and the resulting polypeptide modified in accordance with the invention.

Another group of polypeptides which may be modified according to the invention are non-hormonal protein antigens isolated from placental tissue.

Other proteins which may be modified according to the invention and used for active immunisation in the treatment of various disease states and maladies, are gastrin, the hormonal polypeptide known as Angiotensin II, growth hormone, Somatomedin, parathyroid hormone, glucagon, thyroid stimulating hormone (TSH) and secretin.

The degree of chemical modification of the polypeptides, in accordance with the invention, is, as indicated, 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 endogenous counterparts and this as well as the type of modification will depend on the specific problem being treated as well 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 involved.

The chemical modification may involve addition of foreign modifying groups to the polypeptide involved (hapten-coupling). 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, preferably 2 to 40, more preferably 5 to 30, and most suitably 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 which will attach to a given polypeptide can readily be calculated. The nature of the 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 modifying groups may as indicated vary in chemistry depending on the circumstances. Suitable modifying groups include 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 introduced by reaction of the polypeptides with dinitrophenol, trinitrophenol, S-acetomercaptosuccinic anhydride, polytyrosine 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).

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.

As indicated, the modified polypeptides of the invention are indicated for use as antigens in active immunisation for the purposes of control or treatment of various physiological processes.

More particularly, the modified protein reproductive hormones or fragments thereof, of the invention are indicated for use as antigens in active immunisation for the purposes of controlling reproduction. Thus, these materials induce formation of antibodies which neutralise not only the antigens but also the natural endogenous protein reproductive hormones which are essential for the normal events of the reproductive process, thus disrupting the natural processes of conception and/or gestation. These antigens are therefore indicated for use in contraception and for terminating pregnancies soon after conception. As used herein, the term "contraception" does not include abortion. The specific non-hormonal protein antigens isolated from the placental tissue and modified according to this invention are also indicated for such use. There is direct evidence that inhibition of substances which are specific to the

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placental tissue and do not have similar antigenic properties to antigens from other organs, can result in disruption of pregnancies by passive immunisation. Such specific placental substances when modified in accordance with the invention can be injected into the body of the same species as a fertility control means by active immunisation. The advantage of these substances is that placental antigens are foreign to the non-pregnant female humans and are therefore unlikely to cause any cross-reaction or disruption of normal body functions in the non-pregnant female.

It will be understood while the methods of controlling reproduction described are mainly applicable to females, certain antigens, in particular FSH, its β -subunit and fragments thereof, when modified in accordance with the invention, may be applicable to males.

Gastrin, when modified in accordance with the invention is indicated for use in the treatment by active immunisation of the digestive disorder known as the Zollinger-Ellison Syndrome. This state is generally described as a condition in which there is hyper-secretion of the polypeptide gastrin, which is produced in the pancreas and brings about a state of hyperacidity in the stomach resulting in a chronic digestive disorder. Injections of modified gastrin will enhance formation of antibodies against the hypersecretion of gastrin and thereby alleviate the disease without the need for the surgical treatment which is the only effective treatment presently known.

Angiotensin II, when modified in accordance with the invention, is an antigen for use in the treatment of hypertension. In general terms, the state of hypertension is the abnormal level or fluctuation of ones blood pressure. The blood pressure in an individual is controlled by many physiological processes in the body. However, one major substance affecting the regulation of such pressure is the hormonal polypeptide known as Angiotension II. In certain states of high blood pressure (hypertension) it is difficult to medically control the secretion and therefore the level of Angiotension II in the circulatory system. By the appropriate modification of this hormone and subsequent immunisation with the resulting antigen, it is possible to reduce the secretion of angiotension II in patients with chronically elevated hormone levels. The predictable and controlled reduction of this substance is beneficial to certain patients with chronic problems of hypertension.

Growth Hormone and Somatomedin, when modified according to the invention are indicated for use as antigens in immunological treatment of diabetes and associated micro and macro-vascular disorders. Currently, the treatment of diabetes is limited to dietary and/or drug treatment to regulate blood glucose levels. Recent scientific data support the concept that Growth Hormone and Somatomedin (both polypeptides) are intimately involved in the disease syndrome.

Antigens of the invention are suitable admixed with a pharmaceutically acceptable liquid carrier and administered parenterally. The dosage to be administered will, of course, vary depending on various factors, including the condition being treated and its severity. 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.

As indicated, the theory leading to the present invention was that the chemical modification of a protein essential for reproduction or influencing a particular disease state would render it antigenic such that it would cause formation of antibodies which would, at least partially, neutralise the endogenous protein in addition to the antigen. With this in mind, reproductive hormones of various species were modified and tested in baboons as described in Example 1, hereinafter. The results demonstrated that modified hormones of unrelated species do not produce the desired result whereas modified hormones of the same species or closely related species do. The remaining Examples 2 to 9 also illustrate the invention.

Example I

Adult female baboons were studied for at least one menstrual cycle for patterns of urinary estrogens, plasma, progesterin, and in some cases urinary LH. Only those animals displaying normal patterns of these hormones were immunized. The criteria for normality and the procedures for housing animals are well known and will not be described.

Gonadotropin Preparations

Human Luteinizing Hormone (HLH)—partially purified preparation from human pituitaries with a biological potency of 2.5 units per mg. (NIH-LH-SI).

Human Follicle Stimulating Hormone (HFSH)—a partially purified preparation from human pituitaries with a biological potency of 86 units per mg. (NIH-FSH-SI).

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Human Chorionic Gonadotropin (HCG)—a highly purified preparation from human pregnancy urine with biological potency of 13,200 IU/mg. (2nd IRP-HCG).

Monkey Luteinizing Hormone (MLH)—a crude preparation from rhesus monkey pituitaries with a biological potency of 0.75 units per mg. (NIH-LH-SI).

Ovine Luteinizing Hormone (OLH) (NIH-LH-S5).

Baboon Luteinizing Hormone (BLH)—partially purified baboon pituitary preparation with a biological potency of 1.1 units per mg. (NIH-LH-S1).

All preparations, excepting the OLH, were prepared in the inventor's laboratory. LH and HCG biological activity was determined by the ovarian ascorbic acid depletion test and the FSH preparation assayed by the ovarian augmentation assay.

Hormones were altered as antigens by coupling with a hapten in varying ratios of hapten to hormone as described by Cinader et al., supra. For convenience, the Cinader process is discussed herein although Phillips, supra, may provide a more stable bond under certain circumstances. In this procedure, the protein hormone serves as a carrier and the hapten is coupled to it by diazo bonds. Although a variety of hapten groups were coupled to different hormones, the same basic procedure was used for any combination. Fifteen to thirty-five haptenic groups per hormone molecule were found most useful for preparing immunizing antigens. The basic reaction consisted of diazotizing the hapten (sulfanilic acid) by adding it to a solution of 0.11 N HCl and then slowly adding this solution dropwise to a 1 percent solution of NaNO₂ with constant stirring at 4°C. Diazotization was considered complete when free HNO₂ was detected in the reaction mixture. Although the above reaction was accomplished at 4°C., optimum temperatures for the reaction normally are about 0–6°C., although 4°C. is preferred.

The hapten-protein coupling was performed by dissolving the protein hormone in an alkaline buffer, pH 8.0. The diazotized hapten was added slowly to the hormone solution with continuous stirring at 4°C. The pH of the reaction was constantly monitored and kept near 8.0. After all the hapten was added, the pH was finally adjusted to 8.0, stirred for 1–2 hours and allowed to stand at 4° overnight. The mixture was thoroughly dialyzed for 6–8 days against distilled water to remove unreacted hapten.

Although the number of diazo groups per hormone molecule could be regulated by the number of moles of hapten and hormone reacted, a parallel control experiment with S³⁵ labelled sulfanilic acid to evaluate the precise composition of the hapten-protein samples was performed with each diazotization. The same hormone preparation to be used for immunization was used in the control experiment. After the reaction was completed, an aliquot was taken from the reaction mixture and the remainder thoroughly dialyzed. Equal volumes of the dialyzed and undialyzed solutions were counted by liquid scintillation. By comparing the counts of the dialyzed and undialyzed samples, the moles of hapten coupled to each mole of hormone was calculated since the unreacted hapten was removed by dialysis. For this calculation, a molecular weight of 30,000 was assumed for all gonadotropin preparations.

Following dialysis, hapten-hormones were lyophilized and stored at 4°C. Diazo-HCG (35 groups/molecule) and HLH (26 groups/molecule) were bioassayed by the ovarian ascorbic acid depletion method and found to retain 62 and 85 percent respectively of the activity of the unaltered hormones from which they were derived. None of the other hormones were assayed for biological activity.

Immunization Procedures

Female baboons received their initial immunization on days 3–5 of the menstrual cycle and the second and third injections one week apart. The fourth injection was given 2–3 weeks after the third. A few animals received a fifth injection at 70–80 days after the first injections. All antigens were administered subcutaneously in a suspension of mannide manoleate or peanut oil. Doses of antigens for each injection varied between 3 and 5 mg. Injection sites were inspected daily for 5 days after each immunization for local reactions.

Monitoring Effects of Immunization

Daily 24 hour urine specimens and frequent serum samples were collected during at least one menstrual cycle prior to immunizations and following immunizations until the effects of treatment were assessed. Urinary LH, urinary estrogens and plasma progestins were measured. Antibodies were detected in post-immunization serum samples by reacting 0.2 ml. of a 1:1000 dilution of serum in phosphate-buffered saline (pH 7.4) 0.5 percent normal baboon serum with 250 pg of ¹²⁵I labelled hormone. Sera were reacted with both the unaltered immunizing hormone and unaltered baboon

LH for antibody detection. A purified baboon LH preparation (1.9×NIH-LH-S1) was used as a tracer antigen. Antigen-antibody complexes were precipitated with ovine anti-baboon gamma globulin after a 24 hr. incubation at 4°C. Antibody levels were expressed as pg of labelled hormone bound. Significant antibody levels were considered to be those that would bind 5.0 pg or more of the ¹²⁵I labelled antigen.

Antisera were fractionated by gel filtration of Sephadex G-200 according to the procedure of Fahey and Terry (at p. 36, Experimental Immunology, F. A. Davis Co., Philadelphia, Pa., 1967, incorporated by reference to the extent necessary to understand the invention) to determine the proportion of IgM and IgG antibodies in the baboon sera. Since the IgG fraction in this procedure contained a portion of IgA and IgD antibodies, only IgM and total titers were determined. The IgM fraction from the column was reacted with ¹²⁵I hormones and the binding capacity determined. The volumes of the fractionated sera were adjusted so that antibody levels would be comparable to those of whole serum.

Antibody Production

No significant reactions were observed at the site of injection following any immunization. On 4 occasions, a slight induration (2—3 cm in diameter) was seen when mannide manoleate was used as a vehicle but the redness and swelling disappeared within 4—5 days. Antibodies were detected against the immunizing antigen within 3—5 weeks in all animals. The extent, duration and cross reactivity of these antibodies is recorded. Generally speaking, higher levels were observed to heterologous gonadotropin immunization than to homologous ones.

The cross-reactivity of induced antibodies with baboon LH was studied on each animal. Cross-reactivity of antisera at peak levels was recorded. Although relatively high antibody activity against human LH and HCG were seen, relatively little reaction with baboon LH occurred. An intermediate cross-reaction was noted with anti-ovine LH and a high degree of cross-reactivity was seen with anti-monkey LH. Diazo-human FSH was weakly antigenic in the baboon. The duration of antibody production was generally longer with the human and sheep gonadotropin immunization than with those of monkey or baboon origin.

Peak antibody levels usually occurred at the time when the antibodies had shifted to principally the IgG type. Early antibodies had a larger proportion of IgM type and were generally more cross-reactive with baboon LH. The change in the proportion of the total antibody population that was IgM was recorded from the time antibodies were first detected. Significant cross-reactivity to baboon LH was observed in anti-human gonadotropins when IgM was abundant but dropped sharply as the antisera shifted to nearly all IgG. This drop in cross-reactivity did not occur with monkey and baboon immunizations. Again, the ovine LH immunizations produced an intermediate change in reactivity with the shift from IgM to IgG.

Effects on the Menstrual Cycle

The effects of immunization upon the events of the menstrual cycle were determined by observing changes in sex skin turgescence and levels of pituitary and/or ovarian hormones. Based on these parameters, the delay or retardation of ovulation from the expected time, as judged by the control cycle was calculated. One animal immunized with HCG had no interruption in ovulation and another immunized with HFSH was delayed for only one cycle. Two animals injected with HLH and two injected with HCG had ovulation delays equivalent to two menstrual cycles. A third animal immunized with HLH was delayed a calculated 86 days. Ovine LH immunizations produced an 88 day delay in ovulation.

Immunizations with diazo-monkey or baboon LH resulted in longer disruption of the menstrual cycle. Calculated delays in ovulation for the two animals receiving monkey LH was 146 and 122 days whereas the animals receiving altered baboon LH were retarded from ovulation 224 and 210 days.

Effects on specific hormone patterns following immunization with HLH in one animal were recorded. The interval between menses was considered to represent a "cycle". Urinary estrogens and plasma progestin patterns indicated that no ovulation occurred during the cycle of immunization which was 85 days in duration. Urinary estrogens were elevated during treatment but did not reflect a typical pattern. Plasma progestins were not elevated until about day 19 of the first post-treatment cycle. Patterns of both estrogens and progestins were within normal limits during the second post-treatment cycle. Antibody levels were elevated from about day 35 of the treatment cycle until 289 days from the first detection of antibodies. An LH assay was not

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available when this animal was studied and no data on plasma or urinary levels of this hormone was obtained.

Hormonal patterns following an immunization with diazo-baboon LH were recorded. In this animal, antibody levels were lower and persisted, in general, for a shorter period than did immunizations with human gonadotropins. During the treatment cycle, levels of urinary estrogens and plasma progestins followed a normal pattern but were quantitatively lower than normal. Urinary LH patterns fluctuated markedly due to the injections of diazo-LH during this period. No conclusive evidence of ovulation was obtained for the treatment cycle. The first post-treatment cycle lasted 246 days. During this cycle urinary LH and estrogens were elevated on days 35-41 but there was no subsequent elevation in plasma progestins that would indicate ovulation had occurred. Following day 42 of this cycle, there was no significant elevation in any of the three hormone levels until day 231 when significant elevations of urinary estrogens and LH occurred. These rises were followed 3 days later by an elevation in plasma progestins indicating the presence of a functioning corpus luteum. A second post-treatment menstrual cycle was of normal duration and the endocrine patterns were normal.

Antibodies to unaltered baboon LH attained maximum levels by about day 70 of the post-treatment cycle and remained relatively constant until day 190 when a steady decline was observed. By day 215 of this cycle, antibody levels were barely detectable. Approximately 16 days after this time, a peak of LH commensurate with a normal midcycle elevation was observed. From this point the animal appeared to have the normal function of the pituitary-ovarian axis. Hormonal patterns in animals with other heterologous gonadotropin immunizations were similar to animal receiving HLH and other animals receiving monkey or baboon LH were similar in response to animal receiving baboon LH.

These results in baboons indicated that the modification of a reproductive hormone by the procedures outlined, did render it antigenic and the antibodies thus formed did neutralize natural endogenous hormones if the natural hormone was obtained from the species receiving the immunizations with modified hormone.

EXAMPLE 2

HCG is a hormone naturally present only in pregnant women. HCG is also commercially available. LH hormone is immunologically and biologically identical to HCG hormone, even though there are chemical differences. Since they are biologically identical and HCG is readily available from commercial sources it was presumed that the effectiveness of this immunological procedure could be evaluated by injecting modified HCG into non-pregnant women and monitoring the blood levels of LH. Antibodies formed will neutralize both the LH and the modified HCG.

Women have a pattern of LH levels; the level is substantially constant until the middle period between menstrual cycles, immediately prior to ovulation; at that point the LH level rises greatly and helps induce the ovulation. Monitoring the LH level and the antibody level will show that the procedure used did or did not cause the production of antibodies capable of neutralizing the endogenous reproductive hormone, namely LH.

A woman aged 27 years was selected for study. Hormone was obtained, purified and modified. The modified human hormone (HCG) was injected into the subject. It is well known that antibodies to HCG react identically to LH as well as HCG. The effect of the immunization was elevated, principally by monitoring blood levels of LH hormone. Finally the results were evaluated.

Preparation of Hormone

Clinical grade HCG derived from pregnancy urine was obtained from the Vit-American Corp., Little Falls, New Jersey. This material has an immunological potency of 2600 IU/mg. Contaminants were detected in this preparation. Purification consisted of chromatography and elution. Fractions were dialyzed and lyophilized. The most potent fraction contained approximately 7600 IU/mg.; however, it was heterogenous on polyacrylamide gel electrophoresis.

The fraction was further purified by gel filtration. The elution profile revealed two major protein peaks. The most potent HCG was found in the first peak and had an immunological potency of 13,670 IU per mg. This fraction was subjected to polyacrylamide gel electrophoresis. Further purification by gel filtration showed no evidence of heterogeneity of the HCG at this stage. Consequently, materials for study were processed according to the above procedure.

The contamination of this purified HCG was tested with I^{131} used for identifica-

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tion and a sample was reacted with antisera against several proteins offering potential contamination. Those proteins were follicle stimulating hormone, human growth hormone, whole human serum, human albumin, transferrin, alpha one globulin, alpha two globulin and orosomucoid. No detectable binding of the purified HCG was observed with any antisera at a dilution of 1:50 of each. These negative results, calculated against potential binding of the respective proteins, indicated that contamination with any was less than 0.005 percent.

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Alteration of Hormone

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Hormone was altered by coupling with a hapten (sulfanilazo). This method couples the hapten molecules to the protein via the amino group of the aliphatic or aromatic portion of the hapten. The number of hapten molecules coupled to each HCG molecule (Ha-HCG) can be regulated and for this study, forty haptenic groups per HCG molecule were used for preparing the immunizing antigen.

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Following the hapten-coupling process, the Ha-HCG was sterilized and tested.

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Subject

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The subject was multiparous and had terminated her reproductive capabilities by prior elective bilateral salpingectomy. She was in good health and had regular cyclic menstruation. She underwent complete history, physical examination and laboratory evaluation including blood count, urinalysis, latex fixation and Papanicolaou smear. She had no history of allergy.

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To demonstrate normal functioning of the pituitary-ovarian axis prior to immunization, blood samples were obtained every other day from the first day of menses for 10 days, then daily for 10 days and finally, every other day until the next menses. Serum determinations of FSH, LH, estrone, estradiol and progesterone were performed. These studies indicated an ovulatory pattern.

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Immunization Procedures

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Ten mg. of the Ha-HCG antigen was dissolved in 1.0 ml. of saline and emulsified with an equal volume of oil. Prior to injection, scratch tests to antigen and vehicle were performed. Immunizations were begun in the luteal phase of the treatment cycle to prevent superovulation from the administered HCG. Four injections at two week intervals were given to the subject. The first two of these were administered in oil subcutaneously (1.0 ml. in each upper arm); the final two injections were given in saline only via the intradermal route. Following each injection, blood pressure readings were taken and the subject observed for allergic reactions.

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Monitoring Effects of Immunizations

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Blood samples were collected at weekly intervals beginning two weeks after the initial injection to test for the presence of humoral and cellular antibodies. Following completion of the immunization schedule, blood samples were collected in the same manner as in the control cycle to assess effects of immunization on hormonal patterns of the menstrual cycle. Since antibodies to HCG react identically to LH as with HCG, LH was monitored as an index of effectiveness of the procedure. A third cycle was similarly studied six months after initial immunization. Upon completion of the study, physical and pelvic examinations and laboratory evaluations were repeated.

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Serum samples from the control and post-treatment cycles were assayed for FSH, LH, estrone, estradiol and progesterone.

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The subject was tested for delayed hypersensitivity before immunization and at two week intervals until the injection schedule was completed by an *in vitro* lymphocyte transformation test.

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Results

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Temporal relationships of serum pituitary and gonadal hormones in the control cycles of the subject were recorded. Antibody titers to HCG were detected in the subject after two injections. Menses occurred at regular intervals during the immunizations.

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Following the initial injection in mannide manoleate, some itching and swelling at the injection site occurred. Subsequent intradermal injections in saline produced no reactions and it was concluded that the local reactions were induced by the mannide manoleate. Lymphocyte transformation tests on plasma samples were negative.

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In the post-treatment cycle, baseline follicular and luteal phase LH levels were not noticeably changed in the subject. Very small midcycle elevations in LH levels were observed as compared to the normal large increases. FSH patterns in the post-

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treatment cycle were normal. This indicated that the antibodies were neutralizing the action of endogenous LH.

The subject showed an ovulatory progesterone pattern but attained relatively high antibody titers to LH and HCG after only two injections of Ha-HCG.

The subject was studied during another cycle approximately six months from the first immunization. Significant antibody titers were found. LH patterns indicated a small midcycle elevation. FSH patterns were essentially normal. Thus, the specificity of anti-HCG antibodies to LH was shown but not to FSH.

EXAMPLE 3

Another woman aged 29 years was selected for further study. Hormone was obtained, purified, and modified as in Example 2. This modified hormone was injected into this subject in the same way as in Example 2. The subject was monitored and tested as in Example 2.

The results were similar to the results found in Example 2 except that (1) the levels of estrone and estradiol was substantially normal, (2) the subject acquired significant antibody titers late in the post-immunization cycle, and (3) in the cycle studied after six months this subject showed no significant midcycle elevation in LH patterns.

EXAMPLE 4

Another woman aged 29 years was selected for further study. Hormone was obtained and purified and modified as in Example 2. This modified hormone was injected into this subject in the same way as in Example 2. The subject was monitored and tested as in Example 2.

The results were similar to the results found in Example 2 except that (1) baseline follicular and luteal phase LH levels were noticeably depressed in the post-treatment cycle, (2) no midcycle elevations were observed in LH, (3) estrone levels were elevated during the follicular phase of the post-immunization cycle, and (4) during the six months study there was no significant midcycle elevation in LH patterns.

EXAMPLE 5

Another woman aged 35 years was selected for further study. Hormone was obtained, purified, and modified as in Example 2. This modified hormone was injected into this subject in the same way as in Example 2. The subject was monitored and tested as in Example 2.

The results were similar to the results found in Example 2 except that (1) baseline follicular and luteal phase LH levels were noticeably depressed in the post-treatment cycle, (2) a very small midcycle elevations of LH were observed, (3) levels of FSH patterns in the post-treatment cycle were depressed, and (4) levels of both estrone and estradiol were reduced, during the follicular phase of the post-immunization.

EXAMPLE 6

Another woman aged 28 years was selected for further study. Hormone was obtained, purified, and modified as in Example 2. This modified hormone was injected into this subject in the same way as in Example 2. The subject was monitored and tested as in Example 2.

The results were similar to results found in Example 2 except that (1) baseline follicular and luteal phase LH levels were depressed in the post-treatment cycle, (2) no peaks were observed in midcycle levels of LH, (3) estrone levels appeared elevated in the follicular phase of the post immunization cycle, and (4) LH patterns indicated no significant midcycle elevation in the six month post-immunization cycle.

EXAMPLE 7

Another woman aged 28 was selected for further study. Hormone was obtained, purified, and modified as in Example 2. This modified hormone was injected into this subject in the same way as in Example 2. The subject was monitored and tested as in Example 2.

The results were similar to results found in Example 2 except that (1) antibody titers to HCG were not detected until after three injections, (2) baseline follicular and luteal phase LH levels were depressed in the post-treatment cycle, (3) no peaks nor midcycle elevation in the LH were observed, (4) estrone levels were elevated during the follicular phase, and (5) no significant antibody titers were found in the six month cycle.

All the above examples show the practicality of injecting modified hormones for

the purpose of neutralizing an endogenous reproductive hormone and thereby offering a procedure for the prevention of conception or the disruption of gestation.

EXAMPLE 8

Data obtained in earlier experiments and discussed in Examples 1 to 7 showed that a modified natural reproductive hormone, when injected into an animal of species from which it was derived, would produce antibodies that would neutralize the action of the unmodified endogenous natural hormone in the body of the animal. Hormones used in experiments 1 to 7 were FSH, LH and HCG. New experiments were performed, based on this knowledge, to identify another reproductive hormone (placental lactogen) that could be used in a similar fashion.

Preparation of Hormone

A purified preparation of placental lactogen was prepared from placentae of baboons since it was intended to use modified placental lactogen to immunize baboons. Placentae were extracted and purified on column chromatograph according to previously published procedures. The purity was tested by polyacrylamide gel, electrophoresis and by radio-immunoassay. The material obtained showed a high degree of purity on electrophoresis and radioimmunoassay showed no contamination with other placental hormones.

Hormone Modification and Immunizations

The baboon placental lactogen (BPL) was altered by coupling with the diazonium salt of sulfanilic acid as outlined for other hormones in Example I. The number of diazo molecules per BPL molecule in this instance was 15. Immunization procedures were also similar to those described in Example I for other hormones.

Results

Within 4—6 weeks after the first injection of diazo-BPL, antibody levels to natural unmodified BPL *in vitro* were detected in 6 female baboons. Levels rose to a plateau within 8—10 weeks and remained there for several months. Hormonal measurements indicated that there were no effects on the normal events of the menstrual cycle due to the immunizations. Since BPL is normally secreted only in pregnancy, this was not a surprising observation.

All six females were mated with a male of proven fertility three times (once each in three different cycles during the fertile period). Pregnancy diagnosis by hormonal measurement was performed after each mating. From the 18 matings, there were 13 conceptions as judged by pregnancy tests. The animals that were pregnant had menstrual bleeding 7—12 days later than was expected for their normal menstrual cycles. Subsequent hormonal measurements confirmed that these 13 pregnancies were terminated by abortions approximately one week after the time of expected menses.

These findings suggest that the antibodies formed in the animals body after immunization had no effect on the non-pregnant menstrual cycle but when pregnancy was established, they neutralized the baboon placental lactogen in the baboon placenta and the result was abortion very early after conception.

When in Examples 1 to 8 above, structures 1 to 7 are modified by use of diazo-sulfanilic acid, dinitrophenol, or S-aceto mercaptosuccinic anhydride or structures III—VI modified by addition of polytyrosine or polyalanine, are used, similar results may be obtained.

Similarly, when FSH, somatomedin, growth hormone or angiotension II modified by use of diazosulfanilic acid or trinitrophenol, the results obtained upon administration of the purified modified polypeptide into a male or female human or animal indicate the stimulation of antibodies which neutralise all or some of the modified polypeptide as well as corresponding endogenous polypeptide.

Example 9

The subjects used in the studies reported in the example are female baboons. All baboons were adults of reproductive age. A description of subjects and the conditions of experimentation have been described in Example 1. The animals were studied using highly purified beta-subunits of HCG using a preparation with a biological activity of less than 1.0 IU/mg. Animals were immunized with 14—26 moles/mole of polypeptide, of diazasulfanilic acid coupled subunits in mannide manoleate.

Antibody levels were assessed by determining the binding of serum distillations with I^{125} labelled antigens. Cross-reactivity of antisera was measured by direct binding of labelled antigens and by displacement radioimmunoassays. Antifertility effects in

actively immunized animals were tested by mating females with males of proven fertility. Effects in pregnant baboons passively immunized with either sheep or baboon anti- β -HCG were determined by monitoring serum levels of gonadotropins and sex steroid hormones before and after immunizations.

Eight female baboons were immunized with the modified beta subunit of HCG. Significant antibody levels were attained in all animals.

Baboon immunizations with modified beta subunit of HCG resulted in high antibody levels reacting to HCG, human LH and baboon CG but not to baboon LH. All animals remained ovulatory, however, no pregnancies resulted from numerous matings with males of proven fertility. Passive immunization of non-immunized pregnant baboons with sheep anti- β -HCG serum produced abortions within 36—44 hours.

Example 2 of United Kingdom Patent 1,237,268 describes the chemical modification of human chorionic gonadotropin by reaction with pyrrole-2-carboxylic acid azide and its further modification by reaction of the resulting product with the reaction product of red blood cells (erythrocytes) and bis-diazotised benzidine or bis-diazotized dianisidine. The resulting products are stated to be useful as indicators in immunological tests.

We make no claim herein to human chorionic gonadotropin when chemically modified as described in Example 2 of U.K. Patent 1,237,268.

Subject to the foregoing disclaimer

WHAT WE CLAIM IS:—

1. An antigen for use in active immunisation for the control or treatment of a physiological process in a particular species, comprising a chemically modified polypeptide, the unmodified polypeptide being

- a) an endogenous polypeptide which influences such physiological process in that species and selected from protein reproductive hormones, non-hormonal protein antigens isolated from placental tissue, gastrin, Angiotensin II, growth hormone, somatomedin, parathyroid hormone, glucagon, thyroid stimulating hormone or secretin;
- b) a fragment (as herein defined) of such endogenous polypeptide; or
- c) a polypeptide which is immunologically equivalent (as herein defined) to such endogenous polypeptide or fragment thereof,

and the chemical modification being such as to permit the chemically modified polypeptide to induce, in that species, formation of antibodies which biologically neutralise the endogenous polypeptide as well as the chemically modified polypeptide, and comprising

- a) the attachment of one or more foreign modifying groups to the the unmodified polypeptide; or
- b) the removal of one or more moieties from the unmodified polypeptide.

2. An antigen according to Claim 1, for use in active immunisation for the control of fertility, in which the unmodified polypeptide is

- a) a protein reproductive hormone;
- b) a fragment (as herein defined) thereof; or
- c) a polypeptide which is immunologically equivalent (as herein defined) to such protein reproductive hormone or fragment thereof.

3. An antigen according to Claim 2, in which the protein reproductive hormone is Follicle Stimulating Hormone, Leutinsing Hormone, Human Placental Lactogen, Human Prolactin or Human Chorionic Gonadotropin.

4. An antigen according to Claim 3, in which the protein reproductive hormone is Follicle Stimulating Hormone.

5. An antigen according to Claim 4, in which the unmodified polypeptide is Follicle Stimulating Hormone or the β -subunit thereof.

6. An antigen according to Claim 3, in which the protein reproductive hormone is Human Placental Lactogen or Human Prolactin.

7. An antigen according to Claim 3, in which the protein reproductive hormone is Human Chorionic Gonadotropin.

8. An antigen according to Claim 7, in which the unmodified polypeptide is a Human Chorionic Gonadotropin.

9. An antigen according to Claim 7, in which the unmodified polypeptide is a fragment (as herein described) of Human Chorionic Gonadotropin.

10. An antigen according to Claim 7, in which the unmodified polypeptide is the β -subunit of Human Chorionic Gonadotropin.

11. An antigen according to Claim 7, in which the unmodified polypeptide is a



actively immunized animals were tested by mating females with males of proven fertility. Effects in pregnant baboons passively immunized with either sheep or baboon anti- β -HCG were determined by monitoring serum levels of gonadotropins and sex steroid hormones before and after immunizations.

5 Eight female baboons were immunized with the modified beta subunit of HCG. Significant antibody levels were attained in all animals. 5

10 Baboon immunizations with modified beta subunit of HCG resulted in high antibody levels reacting to HCG, human LH and baboon CG but not to baboon LH. All animals remained ovulatory, however, no pregnancies resulted from numerous matings with males of proven fertility. Passive immunization of non-immunized pregnant baboons with sheep anti- β -HCG serum produced abortions within 36—44 hours. 10

15 Example 2 of United Kingdom Patent 1,237,268 describes the chemical modification of human chorionic gonadotropin by reaction with pyrrole-2-carboxylic acid azide and its further modification by reaction of the resulting product with the reaction product of red blood cells (erythrocytes) and bis-diazotised benzidine or bis-diazotized dianisidine. The resulting products are stated to be useful as indicators in immunological tests. 15

We make no claim herein to human chorionic gonadotropin when chemically modified as described in Example 2 of U.K. Patent 1,237,268.

20 Subject to the foregoing disclaimer
WHAT WE CLAIM IS:— 20

1. An antigen for use in active immunisation for the control or treatment of a physiological process in a particular species, comprising a chemically modified polypeptide, the unmodified polypeptide being 25

25 a) an endogenous polypeptide which influences such physiological process in that species and selected from protein reproductive hormones, non-hormonal protein antigens isolated from placental tissue, gastrin, Angiotensin II, growth hormone, somatomedin, parathyroid hormone, glucagon, thyroid stimulating hormone or secretin; 25

30 b) a fragment (as herein defined) of such endogenous polypeptide; or 30

30 c) a polypeptide which is immunologically equivalent (as herein defined) to such endogenous polypeptide or fragment thereof, 30

35 and the chemical modification being such as to permit the chemically modified polypeptide to induce, in that species, formation of antibodies which biologically neutralise the endogenous polypeptide as well as the chemically modified polypeptide, and comprising 35

35 a) the attachment of one or more foreign modifying groups to the the unmodified polypeptide; or

40 b) the removal of one or more moieties from the unmodified polypeptide. 40

40 2. An antigen according to Claim 1, for use in active immunisation for the control of fertility, in which the unmodified polypeptide is 40

40 a) a protein reproductive hormone;

40 b) a fragment (as herein defined) thereof; or

45 c) a polypeptide which is immunologically equivalent (as herein defined) to such protein reproductive hormone or fragment thereof. 45

45 3. An antigen according to Claim 2, in which the protein reproductive hormone is Follicle Stimulating Hormone, Leutinising Hormone, Human Placental Lactogen, Human Prolactin or Human Chorionic Gonadotropin.

50 4. An antigen according to Claim 3, in which the protein reproductive hormone is Follicle Stimulating Hormone. 50

50 5. An antigen according to Claim 4, in which the unmodified polypeptide is Follicle Stimulating Hormone or the β -subunit thereof.

55 6. An antigen according to Claim 3, in which the protein reproductive hormone is Human Placental Lactogen or Human Prolactin.

55 7. An antigen according to Claim 3, in which the protein reproductive hormone is Human Chorionic Gonadotropin. 55

60 8. An antigen according to Claim 7, in which the unmodified polypeptide is a Human Chorionic Gonadotropin.

60 9. An antigen according to Claim 7, in which the unmodified polypeptide is a fragment (as herein described) of Human Chorionic Gonadotropin. 60

60 10. An antigen according to Claim 7, in which the unmodified polypeptide is the β -subunit of Human Chorionic Gonadotropin.

11. An antigen according to Claim 7, in which the unmodified polypeptide is a



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20 to 30 amino acid C-terminal fragment of the β -subunit of Human Chorionic Gonadotropin.

12. An antigen according to Claim 7, in which the unmodified polypeptide is a 30 to 39 amino acid C-terminal fragment of the β -subunit of Human Chorionic Gonadotropin.

13. An antigen according to Claim 7, in which the unmodified polypeptide is of structure III

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 -
Ser - Asp - Thr - Pro - Ile - Leu - Pro - Gln.

14. An antigen according to Claim 7, in which the unmodified polypeptide is of structure IV

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. An antigen according to Claim 7, in which the unmodified polypeptide is of structure V

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 - Asx - Thr - Pro - Ile - Leu - Pro - Gln - Ser - Leu - Pro.

16. An antigen according to Claim 7, in which the unmodified polypeptide is of structure VI

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

17. An antigen according to Claim 1, in which the unmodified polypeptide is

- a) a non-hormonal protein antigen isolated from placental tissue;
- b) a fragment (as herein described) thereof; or
- c) a polypeptide which is immunologically equivalent (as herein defined) to such antigen or fragment thereof.

18. An antigen according to Claim 1, for use in active immunisation for the treatment of the Zollinger-Ellison syndrome, in which the unmodified polypeptide is

- a) gastrin;
- b) a fragment (as herein defined) thereof; or
- c) a polypeptide which is immunologically equivalent (as herein defined) to such gastrin or fragment thereof.

19. An antigen according to Claim 1, for use in active immunisation for the treatment of hypertension, in which the unmodified polypeptide is

- a) Angiotensin II;
- b) a fragment (as herein defined) thereof; or
- c) a polypeptide which is immunologically equivalent (as herein defined) to such Angiotensin II or fragment thereof.

20. An antigen according to Claim 1, for use in active immunisation for the treatment of diabetes and associated micro- and macro-vascular disorders, in which the unmodified polypeptide is

- a) Growth Hormone or Somatomedin;
- b) a fragment (as herein defined) of such Growth Hormone or Somatomedin; or
- c) a polypeptide which is immunologically equivalent (as herein defined) to such Growth Hormone or Somatomedin or fragment thereof.

21. An antigen according to any one of the preceding Claims, in which the chemical modification comprises the attachment of one or more foreign modifying groups to the unmodified polypeptide.

22. An antigen according to Claim 21, in which 1 to 40 modifying groups are attached per mole of the unmodified polypeptide.

23. An antigen according to Claim 22, in which 2 to 40 modifying groups are attached per mole of the unmodified polypeptide.

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24. An antigen according to Claim 23, in which 5 to 30 modifying groups are attached per mole of the unmodified polypeptide.
25. An antigen according to Claim 24, in which 10 to 26 modifying groups are attached per mole of the unmodified polypeptide.
26. An antigen according to any one of Claims 2 to 25, in which the foreign modifying groups are diazo groups.
27. An antigen according to Claim 26, in which the diazo groups are introduced by reaction of the polypeptide with diazo sulfanilic acid.
28. An antigen according to any one of Claims 2 to 25, in which the foreign modifying groups are derived from dinitrophenol, trinitrophenol, S-acetomercapto-succinic anhydride, a polytyrosine, a polyalanine, biodegradable polydextran or thyroglobulin.
29. Human Chorionic Gonadotropin modified by introduction of 35 diazo groups per molecule, the diazo groups being derived from diazotised sulfanilic acid.
30. Human Leutinising Hormone modified by introduction of 26 diazo groups per molecule, the diazo groups being derived from diazotised sulfanilic acid.
31. Human Chorionic Gonadotropin modified by introduction of 40 diazo groups per molecule, the diazo groups being derived from diazotised sulfanilic acid.
32. Baboon Placental Lactogen modified by introduction of 15 diazo groups per molecule, the diazo groups being derived from diazotised sulfanilic acid.
33. The β -subunit of Human Chorionic Gonadotropin modified by introduction of 14 to 26 diazo groups per molecule, the diazo groups being derived from diazotised sulfanilic acid.
34. An antigen according to Claim 1, substantially as herein described with reference to any one of the Examples.
35. A pharmaceutical composition comprising an antigen according to any one of Claims 1 to 33, in association with a pharmaceutically acceptable liquid carrier, and in a form suitable for parenteral administration.
36. A pharmaceutical composition according to Claim 35, in which the liquid carrier is mannide monooleate, saline or an oil.
37. A pharmaceutical composition according to Claim 35 or 36 in unit dosage form and containing 0.1 to 50 mg of the antigen.
38. A pharmaceutical composition according to Claim 35, substantially as herein described with reference to any one of the Examples.
39. A method of contraception comprising injecting into an animal an immunologically effective amount of an antigen according to any one of Claims 2 to 17, 21 to 28 (as dependent on Claims 2 to 17) and 29 to 33.
40. A method according to Claim 39, comprising injection of 0.1 mg to 50 mg of the antigen.
41. A method according to Claim 39 or 40, in which the antigen is injected in the form of a solution or suspension in a pharmaceutically acceptable liquid carrier.
42. A method according to Claim 41, in which the liquid carrier is mannide monooleate, saline or an oil.
43. A method according to Claim 39, substantially as herein described with reference to any one of the Examples.

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