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PATENT SPECIFICATION

NO DRAWINGS

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COMPLETE SPECIFICATION

Vaccine and Its Preparation

We, MERCK & CO., INC., a corporation duly organised and existing under the laws of the State of New Jersey, United States of America, or Rahway, New Jersey, 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 vaccines. More particularly it concerns an attenuated, live mumps virus which is to be injected in human beings to protect them against mumps. The invention also includes the development of the attenuated virus strain.

Owing to its attenuated nature, the virus causes little if any clinical reaction. It does, however, evoke a significant level of antibody against the virus in man and this antibody is essential to protection against infection with the virus and disease.

Mumps, like measles, is usually regarded as one of the minor illnesses of childhood. In the ordinary clinical infection, this is true. In the extraordinary case, however, the clinical consequence of viral infection may be severe and the consequences may be as devastating as in measles.

In the usual case of mumps, the acute febrile episode follows an incubation period of 18 to 21 days. The principal clinical manifestations are fever with unilateral or bilateral parotid swelling, sometimes with involvement also of the submaxillary and sublingual glands.

Viremia commonly accompanies mumps virus infection and involvement of a variety of organs or organ systems may take place. This may include the epididymis, prostate, ovary, liver, pancreas, spleen, thyroid, kidneys, labyrinth, eye, thymus, myocardium, mammary glands, and central nervous system. Most prominent disease manifestations are encephalitis, encephalomyelitis, neuritis of facial, trigeminal or optic nerves; aseptic meningitis (0.5—10% of cases), soleritis; pancreatitis sometimes leading to diabetes; and orchitis or ovaritis sometimes leading to atrophy and sterility. Serious complications are least frequent amount children. The more serious aspect attends occurrence of the infection in adults who have escaped the illness in childhood.

Uncomplicated mumps in children is debilitating, distressing to parents, and results in sizable time loss from school. Complicated mumps, either in childhood or in adulthood, may range from mild disease with total apparent recovery to destruction or incapacitation of essential organ systems which may lead to life-long crippling or death.

In accordance with the present invention there is provided the preparation of a live mumps virus useful as an antigen in a vaccine that will evoke in man an antibody response against a virulent mumps virus without causing the severe clinical manifestations of the disease, by growing the virus in at least ten successive passage in embyonated hens eggs followed by propagation in chick embryo tissue culture containing tissue of 9 to 11 day old chick embryo at 30—38°C. Preferably at least ten passages are made in the chick embryo tissue culture. Successive passages are preferably carried out at 30 to 34°C (optimal 32°C) or 35 to 38°C optimal 36°C).

The virulent mumps virus used in the process of the invention is obtained by isolating the virulent virus in any variety of cells in culture or embryonated hens' eggs, and adapting it to chick embryo tissue culture.

The isolation and adaptation of the mumps virus can be accomplished in chick embryo tissue culture using clinical material (e.g. throat swab) or virus previously incubated in

[*Price* 4*s.* 6*d.*]

embryonated hens' eggs. Representative isolation procedures are the following:

Procedure 1. Mumps virus is isolated in embryonated hens' eggs from clinical material, i.e. from persons known to have mumps.

Procedure 2. Mumps virus is isolated in chick embryo tissue culture from clinical material. The process for the preparation of a live non-pathogenic and antigenic mumps virus is preferably carried out as follows:

The virus which has been established to be mumps virus, is added to glass bottles containing chick embryo tissue cultures prepared from minced and trypsinized nine-to-eleven day-old chick embryos. The culture medium may be any of those which support cell growth and this may, for example, be the known medium 199 to which calf serum has been added. After the addition of the virus, the infected cell cultures are incubated in successive passages at 30—38°C for variable time periods during which time the virus is replicated and becomes attenuated. The virus-containing fluids are then harvested and stored frozen or at other low temperature so as to preserve the infectiousness of the agent. The above passages are performed using diluted inoculum and multiple harvests are collected at various intervals. Infestivity titrations are performed in HeLa, grivet monkey kidney tissue or chick embryo tissue cultures.

The mumps virus obtained by the process of the invention may be used to prepare a vaccine.

This mumps virus is nonpathogenic for monkeys and rodents, and causes little if any clinical reaction in human recipients, and evokes a sigificant level to antibody. The virus infectivity is stabilized by a suitable stabilizer such as sucrose, human albumin, glutamine, phosphate or mixtures thereof. After titration to establish it potency, the virus pool is subdivided and filled into ampules for use. The product can be stored and dispensed frozen or preferably dried from the frozen state and kept free of moisture.

The following examples, in which "Difco" is a trademark, illustrate the invention.

EXAMPLE I

The inoculum is that obtained from Procedure 1 described above.

Nine - to - eleven - day - old chick embryos, after removal of the head and extremities, are finely minced under aseptic conditions and the minced tissue washed in several changes of Hanks' BSS. The washed tissue is trypsinized at 36°C using 0.25 trypsin (Difco 1:250) in tris saline buffer for two to three hours. The trypsin-cell suspension is harvested through two thicknesses of sterile cheese cloth and centrifused at 1500 rpm for five minutes. Packed cells are resuspended in growth medium for counting. Growth medium consisted of medium 100 containing 2% agamma calf serum (heated 56°C—30 minutes) and 50 mcg/ml Neomycin. Bottle cultures are planted at a concentration of 350,000 viable cells per millilitre. Following incubation at 36°C for 48 to 72 hours, bottle cultures can be used for serial passage or vaccine preparation.

Chick embryo tissue cultures are prepared in glass bottles using medium 199 containing 2% inactivated calf serum as growth medium. Three to four days post-planting, the growth medium is drawn off aseptically and the bottle cultures are washed four times with Hanks' BSS, 100 millilitres per wash, and each bottle inoculated with 2.5 ml. of diluted mumps virus seed that has previously undergone ten serial passages through embryonated hens' eggs at 30—38°C. 70 ml. of medium 199 containing 10% of suitable viral infectivity stabilizer is added to each bottle culture and the bottles incubated at 30—38°C. Neomycin at a concentration of 50 mcg/ml was incorporated in the growth and maintenance medium. Multiple harvests are collected at 2—4 day intervals and the bottle cultures are re-fed with fresh maintenance medium containing the above-mentioned stabilizer. Ten successive passages are performed, all at the approximate temperature of 32°C. Infectivity titrations of each harvest are performed in HeLa or grivet monkey kidney tissue cultures. Each harvest is collected aseptically into a sterile container, samples are removed for microbial sterility testing and the remainder is shell frozen in a dry ice-alcohol bath. The virus-containing fluids are stored at −70°C in an electrically operated freezing unit prior to selection of a harvest or harvests for preparation of the vaccine.

Appropriate harvest or harvests are selected following completion of infectivity titrations. The selected material is removed from the freezer and thawed. A sample is removed for control and safety testing. The remaining fluid is clarified and a sample removed for monkey safety testing. Appropriate additional stabilizer is added to the remaining fluid. The fluids are distributed into individual vials and dried. Following the drying procedure, the vials are capped, sealed and retained for reconstitution as a vaccine by the addition of sterile water.

Tests in man.

Approximately 150 children without prior history of mumps infection received a dose of attenuated mumps virus vaccine by the parenteral route. Essentially all the children responded with a significant antibody titer within a month after vaccination. The children developed little, if any, clinical manifestation depending on the strain of mumps virus and the passage level in chick embryo tissue culture and in embryonated hens' eggs.

EXAMPLE II

The procedure of Example I is carried out but twenty serial passages through embryonated hens' eggs at 30—38°C and two serial passages through chick embryo tissue are carried out.

The invention has been described with particular reference to ten serial passages through CET to assure attenuation of the virus, but it is to be understood that a fewer number may suffice. Thus, the invention contemplates a single incubation in CET to achieve the desideratum of a virus which will evoke a mumps antibody response in humans with a reduction in the otherwise severe symptoms of the disease. Successive passage after the first growth in CET can be expected to result in increased attenuation.

WHAT WE CLAIM IS:—

1. The preparation of a live mumps virus useful as an antigen in a vaccine that will evoke in man an antibody response against a virulent mumps virus without causing the severe clinical manifestations of the disease, by growing the virus in at least ten successive passages in embryonated hens' eggs followed by propagation in chick embryo tissue culture containing tissue of 9 to 11 day old chick embryo at 30—38°C.

2. A process according to claim 1, in which at least ten passages are made through the chick embryo tissue culture.

3. A process as claimed in claim 1 or 2, including the step of stabilizing the product with one or more of the following: sucrose, human albumin, glutamine and phosphate, to produce a vaccine.

4. A process for the preparation of mumps vaccine substantially as hereinbefore described in Example I or II.

5. A mumps vaccine when prepared by a process according to claim 3 or 4.

6. A live non-pathogenic and antigenic mumps virus when prepared by a process according to claim 1 or 2.

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1. The preparation of a live mumps virus useful as an antigen in a vaccine that will evoke in man an antibody response against a virulent mumps virus without causing the severe clinical manifestations of the disease, by growing the virus in at least ten successive passages in embryonated hens' eggs followed by propagation in chick embryo tissue culture containing tissue of 9 to 11 day old chick embryo at 30—38°C.

2. A process according to claim 1, in which at least ten passages are made through the chick embryo tissue culture.

3. A process as claimed in claim 1 or 2, including the step of stabilizing the product with one or more of the following: sucrose, human albumin, glutamine and phosphate, to produce a vaccine.

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