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VACCINE PRODUCTS AND METHOD OF
PREPARING SAME

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This invention relates to vaccine products and to methods of preparing the same. More particularly, in invention relates to improved vaccine products capable of evoking higher and/or more durable serum antibody levels than can be obtained with presently available vaccines.

The vaccines in most common use at the present time have been termed "fluid vaccines." The term "fluid vaccine" is used herein to mean a solution of an immunogenic or desensitizing agent in water or in a medium comprising a single, aqueous, liquid phase. Such vaccines are subject to the criticism that neither sufficiently high serum antibody levels nor serum antibody levels of sufficient duration can be obtained with one or two vaccine injections having a reasonable close volume. Many fluid vaccines must therefore be administered in a number of injections over a period of a few weeks or more if the desired protection is to be obtained. Additionally, combination of a number of antigens in a single vaccine with a reasonable dose volume is not easily possible with normal fluid vaccines. Attempts have been made to overcome these difficulties by emulsification of fluid vaccines with mineral oils to potentiate the activity of the fluid vaccines. Such mineral oil adjuvant vaccines have not been fully accepted because of inherent disadvantages in their use. Mineral oils in general have very complex and largely unknown compositions, even those which comply with all National Formulary (N.F.) and United States Pharmacopeia (U.S.P.) requirements may vary considerably in their chemical composition from batch to batch. This variability in composition may lead to undesirable inconsistency in adjuvant activity. Further, because of their variable and largely undefined nature, mineral oils may contain potentially deleterious components. Many vaccines are dried to increase their antigenic stability during storage and shipping. Before use, however, the dried preparations must be reconstituted with distilled water or other proper aqueous diluents, thereby giving rise to normal fluid vaccines with all of the attending limitations discussed earlier. Therefore, there is a need for vaccines which have none of the above-discussed limitations and which are capable of evoking better antibody response levels than can be obtained with presently available vaccines.

An object of the present invention is to provide vaccines which are capable of evoking higher and more durable serum antibody levels upon inoculation of human or animal subjects than can be obtained with fluid vaccines.

A further object of this invention is to provide vaccines wherein a number of antigens can be easily combined in a single vaccine having a reasonable dose volume.

Another object of this invention is to provide vaccines which consistently and predictably give rise to high, durable serum antibody levels.

Still another object of this invention is to provide vac-

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cines with the precise vaccine potentiating characteristics necessary to obtain any desired antibody response pattern.

Yet another object of the present invention is to provide, as a stable vaccine, a non-aqueous suspension of dried antigenic material which will not need reconstitution before administration, but rather, may be used as such.

The above objects, as well as other objects which will appear hereinafter, are realized by providing vaccine products comprising an intimate mixture of at least one antigen and a substantially chemically pure, branched chain, saturated, non-cyclic hydrocarbon containing 23 to 30 carbon atoms inclusive and having a melting point not greater than 4° C., a viscosity of not less than 2 and not greater than 30 centipoises at 22–24° C. and a guinea pig toxicity index not greater than 0.3±0.2 unit. The terms "antigen" and "antigenic material" which are used interchangeably herein and in the claims include any non-viable immunogenic or desensitizing (anti-allergic) agent of bacterial, viral or other origin. The antigen component of the products of the invention may consist of a dried powder, an aqueous solution, an aqueous suspension and the like, including mixtures of the same, containing a non-viable immunogenic or desensitizing agent or agents.

The vaccine products of the invention which are suspensions of the solid or aqueous antigenic material in a hydrocarbon produce in both human and animal subjects a much higher and more prolonged serum antibody level than can be obtained by the administration of the same amount of antigen in the form of an aqueous vaccine. The antibody titers obtained with the products of the invention are, in general, higher and/or persist at a higher level than those obtained with the suspensions of the antigen in mineral oil. Moreover, the vaccine products of the invention, due to their reproducibly consistent composition, give an antibody response which is more uniform and predictable than that obtainable with mineral oil vaccine products which may vary greatly when different mineral oils or different batches of the same mineral oil are used in their preparation. Additionally, the products of the invention are free from the potentially deleterious or toxic substances which may be present in mineral oil vaccine products due to the unknown and variable composition of mineral oils.

The products of the invention can be prepared by mixing an antigen with a substantially chemically pure branched chain, saturated, non-cyclic hydrocarbon containing 23 to 30 carbon atoms inclusive and having a melting point of 4° C. or less, a viscosity of not less than 2 and not greater than 30 centipoises at 22–24° C. and a guinea pig toxicity index not greater than 0.3±0.2 unit. When the antigen component consists of dry material, the products are prepared simply by suspending the dry antigen in the hydrocarbon. The relative proportions of the antigen and hydrocarbon components in such preparations are not particularly critical. The vaccine product must, however, be sufficiently fluid to be administered hypodermically and contain sufficient antigen to produce a protective antibody response. In general, such products should contain less than 50% by volume of the dry antigen and preferably less than about 25% by volume. When the antigen component consists of aqueous material, the products are prepared by emulsifying the aqueous antigen component with the hydrocarbon component to produce a dispersion of the aqueous antigen

in the hydrocarbon component. Such products can contain between 25% and 75% by volume of the hydrocarbon component, said hydrocarbon component being composed of a 23 to 30 carbon atom hydrocarbon of the type described above plus an emulsifier. When higher percentages of the hydrocarbon component are used phase separation takes place quite rapidly. If less than 25% by volume of the hydrocarbon component is used it is difficult to produce a suitable suspension which is sufficiently fluid for administration. The preferred products containing an aqueous antigen component are those wherein the hydrocarbon component comprises between about 45 and 55% by volume of the final vaccine product. The nature of the emulsifying agent is not particularly critical but it must be an antigen-compatible and pharmaceutically-acceptable emulsifier; that is, an emulsifier which does not have a deleterious effect on the particular antigen or antigens in the preparation, and which does not produce toxic effects when administered parenterally in the amounts and at the concentrations in which it is present in the products of the invention. Some examples of emulsifiers which have been found to be suitable for use in the products of the invention are glyceryl fatty acid esters and long chain fatty acid esters of hexitol anhydrides, such as sorbitan, sorbitide, mannitan, and mannide. Mannide monooleate is a preferred emulsifying agent. The concentration of the emulsifier in the final vaccine product can vary from 0.5 to 15% by volume. The preferred concentration of emulsifier is between 4.5 and 8.0% by volume.

Some examples of the many antigens which can be used in the practice of the invention include non-viable virus antigens such as those of poliomyelitis, rabies, smallpox, influenza, adenovirus, other viral respiratory infections and measles; bacterial toxoids and other non-viable bacterial antigens such as those of diphtheria, tetanus and pertussis; and non-viable desensitizing agents such as house dust, ragweed pollen, rose pollen, goldenrod pollen, dog dander and grass pollen.

Some specific examples of the hydrocarbons which can be used in the preparation of the products of the invention are the following:

7-n-hexyloctadecane
7-n-hexylheptadecane
11-n-butylheneicosane
9-n-octyleicosane
2,6,10,15,19,23-hexamethyltetracosane

The hydrocarbons which contain 24 carbon atoms are preferred and, in particular, 7-n-hexyloctadecane. The concentration of the 23 to 30 carbon atom hydrocarbon in the vaccine products prepared from aqueous antigenic materials can range from 10 to 74.5% by volume, the preferred concentration being between 25 and 50% by volume. If desired, a simple mixture, that is a mixture of two or three of the aforementioned 23 to 30 carbon atom hydrocarbons can be used in the preparation of the products of the invention. However, since no particular advantage is attained by so doing it is preferable to use only a single 23 to 30 carbon atom hydrocarbon.

In many instances the potency of the vaccine products of the invention, that is, the antibody titers attainable with the products, can be increased by incorporating a substantially chemically pure, branched chain, saturated, non-cyclic hydrocarbon containing 16 to 22 carbon atoms inclusive with the aforementioned 23 to 30 carbon atom hydrocarbon. When such mixtures of hydrocarbons are used the mixture must meet the same standards as the single 23 to 30 carbon atom hydrocarbons. That is, the mixture must have a melting point of 4° C. or less, a viscosity of not less than 2 and not greater than 30 centipoises at 22–24° C. and a guinea pig toxicity index not greater than 0.3±0.2 unit. The preferred branched chain, saturated, non-cyclic hydrocarbons for incorporation with the 23 to 30 carbon atom hydrocarbons are

those which contain 16 to 19 carbon atoms inclusive, and particularly those which contain 16 or 17 carbon atoms.

The hydrocarbons used in the practice of the invention are either known substances prepared by chemical synthesis from simpler organic chemicals or substances which can be prepared from other known chemicals by the known methods for synthesis of high molecular weight hydrocarbons. As stated above, these hydrocarbons are substantially chemically pure, that is, they have a purity of 99% or greater as determined by organic microanalysis, infrared spectrophotometry and gas chromatography. The guinea pig toxicity index of the hydrocarbons and hydrocarbon mixtures used in the preparation of the vaccine products of the invention is determined as described below. Four white adult guinea pigs each weighing between 200 and 250 grams are inoculated intradermally with 0.1 ml. portions of the hydrocarbon or hydrocarbon mixture being tested. Each animal is given a single injection of the test sample at a different site (upper left ventral, upper right ventral, lower left ventral, and lower right ventral). For control purposes each of the test animals is inoculated intradermally with 0.1 ml. of physiological saline at a different site. The animals are held for 14 days and examined daily for local reactions of inflammation and induration. Reactions are rated as follows:

0—No reaction
1—1 mm. zone of induration or inflammation
2—5 mm. zone of induration or inflammation
3—10 mm. zone of induration or inflammation
4—Necrosis

The guinea pig toxicity index is calculated by adding the daily scores of the four test animals in the group and dividing the sum by 56, which is the number of days in the test (14) multiplied by the number of test animals (4). Thus the highest possible index is 4 while the lowest is 0.

The vaccine products of the invention are prepared under aseptic conditions using previously sterilized components. Sterility on storage is insured by incorporation of a very small amount of an antigen-compatible germicidal substance such as thimerosal or benzethonium chloride.

The invention is illustrated but not limited by the following examples:

Example 1

8.5 parts by volume of 7-n-hexyloctadecane is mixed with 1.5 parts by volume of mannide monooleate (an emulsifier) and the mixture sterilized by filtration through an 03 Sela bacteria retaining filter. 4 ml. of the 7-n-hexyloctadecane-mannide monooleate mixture is drawn into a 10 ml. hypodermic syringe and 4 ml. of fluid non-viable Type 1 poliomyelitis vaccine [prepared as described by McLean and Taylor in Progress in Medical Virology, 1, 122–164 (1958) and conforming to the specifications and regulations of the Division of Biologics Standards, United States National Institutes of Health] is drawn into a second 10 ml. hypodermic syringe. The syringes are joined to one another by means of an 18 gauge double-hubbed syringe needle. The fluids are alternately forced into each of the syringes by hand pressure on the plungers to mix the vaccine with the hydrocarbon. Emulsification is accomplished by forcing the vaccine-hydrocarbon mixture back and forth between the two syringes through the syringe needle at the rate of 7 passes per minute by means of air pressure on the syringe plunger or by other mechanical means. After 30 minutes, the 18 gauge needle is replaced with a 22 gauge, double-hubbed needle and the reciprocal mixing continued for 10 passes. The 22 gauge needle is then replaced with a 25 gauge double-hubbed hypodermic needle and mixing continued for two passes. Small samples of the emulsion are removed for bacterial sterility tests (in thioglycollate broth) and for a determination of the completeness of

emulsification (by microscopic examination and centrifugation). The remainder of the vaccine product is left in the joined syringes and stored under refrigeration (+4° C.) until use. Before inoculation the product is re-emulsified by seven passes through a 22 gauge hypodermic needle. The final vaccine product so produced contains 42.5% by volume of 7-n-hexyloctadecane and 7.5% by volume of mannide monooleate.

Using the same procedure and quantities set forth above, Type 1 poliomyelitis vaccine products containing 42.5% by volume of each of the following hydrocarbons are prepared:

7-n-hexylheptadecane
11-n-butylheneicosane
9-n-octyleicosane
2,6,10,15,19,23-hexamethyltetracosane

By using 7.65 parts by volume of 7-n-hexyloctadecane, 0.85 part by volume of 7-n-propyltridecane and 1.5 parts by volume of mannide monooleate in the above procedure, a Type 1 poliomyelitis vaccine product is prepared which contains 38.25% by volume 7-n-hexyloctadecane, 4.25% by volume of 7-n-propyltridecane and 7.5% by volume of emulsifier. In a similar manner other Type I poliomyelitis vaccine products are prepared, each of which contains mixtures of hydrocarbons as follows:

34% 7-n-hexyloctadecane—8.5% 7-n-propyltridecane
34% 7-n-hexyloctadecane—8.5% 2-methyl-3-isopropyltridecane
29.75% 7-n-hexyloctadecane—12.75% 2-methyl-3-isopropyltridecane

ly injected with 0.5 ml. of the fluid control vaccine. Blood samples are also collected at 2, 3, 4 and 8-week intervals after the first inoculation and the serum fractions from each blood sample stored individually at -20° C. until titrated for Type 1 poliomyelitis antibody.

The test sera are diluted 1 to 4 with 0.5% LAH (Lactalbumin-Hydrolyzate) in Hank's balanced salt solution and heated at 56° C. for 30 minutes. The resulting sera are serially diluted and the dilutions combined with equal volumes of the virus challenge diluted to contain 100 TCID₅₀ per 0.1 ml. The resulting mixtures are incubated at 37° C. for 3 hours and refrigerated until plated. The plastic panel method of Richtsel et al. [J. Immunol., 76, 464 (1956)] is used for plating. Triplicate cups are used for each serum dilution and each cup contains 0.2 ml. of the serum-virus mixture. To each of the cups is added 0.1 ml. of a suspension of monkey kidney cells containing 1.5% chicken serum and 24,000 cells. The cups are sealed with transparent tape and incubated at 37° C. for six days. At the end of the incubation period the cups are examined microscopically for appearance of polio virus cytopathology. The highest dilution of serum required for complete neutralization of the virus challenge is determined for each triplicate cup series and the titer recorded as the reciprocal of the mean end point dilution level. The following table shows the results obtained with vaccine products prepared as described above. The antibody values set forth in the table are the geometric means of the serum titers for each group of three rabbits and hence represent the result of nine determinations.

Hydrocarbon used ¹	Hydrocarbon viscosity, cps. at 23.5-24° C.	Guinea pig toxicity index of hydrocarbon	Type 1 polio antibody titers					
			Time in weeks after first inoculation					
			0	1	2	3	4	8
100% 7-n-hexylheptadecane.....	8.81	0.50	<4	74	708	594	891	363
100% 7-n-hexyloctadecane.....	10.32	0.22	<4	20	594	508	891	389
100% 11-n-butylheneicosane.....	10.50	0.05	<6	87	471	640	1,190	1,202
100% 9-n-octyleicosane.....	15.30	0.14	<4	40	143	113	226	339
100% 2,6,10,15,19,23-hexamethyltetracosane.....	29.96	0.14	<4	46	594	275	149	389
90% 7-n-hexyloctadecane, 10% 7-n-propyltridecane.....	8.78	0.04	<4	403	2,630	1,016	776	501
80% 7-n-hexyloctadecane, 20% 7-n-propyltridecane.....	7.81	0.32	<4	137	748	869	912	1,445
80% 7-n-hexyloctadecane, 20% 2-methyl-3-isopropyltridecane.....	8.36	0.05	<4	32	344	438	575	457
60% 7-n-hexyloctadecane, 40% 2-methyl-3-isopropyltridecane.....	6.83	0.27	<6	27	374	347	457	490
100% mineral oil.....	12.05	0.33	<4	41	589	318	201	170
Fluid control.....			<4	23	109	43	47	76

¹ All samples, except fluid control, contain 42.5% by volume hydrocarbon, 7.5% by volume emulsifier, and 50% by volume aqueous Type 1 poliomyelitis vaccine.

For comparison purposes a Type 1 poliomyelitis vaccine product is prepared exactly as described above using 8.5 parts by volume of a highly purified commercially available mineral oil (Drakeol-6 VR-Pennsylvania Refining Company). The viscosity of the particular batch used in these tests was 12.05 centipoises and the guinea pig toxicity index was 0.33 unit.

The potency of each of the above Type 1 poliomyelitis vaccine products and the potency of the original fluid Type 1 poliomyelitis vaccine used in their preparation is determined by subcutaneous inoculation of rabbits weighing three to six kilograms. Three rabbits are used for each of the preparations being tested. The fluid vaccine is diluted with an equal volume of Medium No. 199 to obtain a fluid control vaccine having the same volume and amount of antigen as the preparations being tested. Each of the rabbits in the various groups is subcutaneously inoculated with a single 0.5 ml. dose of the preparation being tested. Seven days later, after withdrawal of the 1-week blood sample, all of the rabbits are subcutaneous-

Example 2

Fluid influenza virus vaccine (PR-8 strain) is prepared as follows:

The allantoic cavities of 11-day-old embryonated eggs are inoculated with the PR-8 strain of influenza virus and the eggs incubated at 35° C. for 48 hours. The eggs are refrigerated at 4° C. overnight and then the fluid aseptically withdrawn from the allantoic cavity. The fluids are pooled (volume about 11 liters) and the liquid centrifuged to concentrate the virus. The virus concentrate is collected and ball-milled with 60 to 75 ml. of citrate buffered saline. 0.6 ml. of 10% formalin is added to the virus suspension, the suspension diluted with buffered saline to 720 ml. and the suspension angle centrifuged twice to remove egg debris. The purified virus supernatant is irradiated with ultraviolet light (13.5 watts) to inactivate the virus and then thimerosal added to a concentration of 1 to 10,000. Samples are tested for

sterility and viral CCA content, and the suspension is diluted with sterile buffered saline to produce a vaccine containing 100 CCA units per ml.

8.5 parts by volume of 7-n-hexyloctadecane is added to 1.5 parts by volume of redistilled mannide monooleate and the mixture sterilized by filtration through an 03 Sela bacteria retaining filter. 4 ml. of the hydrocarbon-emulsifier mixture is emulsified, as described in Example 1, with 4 ml. of the fluid influenza virus vaccine (PR-8 strain) prepared above to obtain a final vaccine product containing 50 CCA units per ml., 42.5% by volume of 7-n-hexyloctadecane and 7.5% by volume of emulsifier.

By using 9 parts of 7-n-hexyloctadecane and 1 part of mannide monooleate in the above procedure one obtains a vaccine product containing 45% by volume of 7-n-hexyloctadecane and 5% by volume of emulsifier.

Example 3

Fluid tetanus toxoid concentrate [prepared by the general procedure of Mueller and Miller, J. Immunol., 50, 377-384 (1945), and J. Immunol., 56, 143-147 (1947), and conforming to the Minimum Requirements for Tetanus Toxoid, 4th revision (1952) of the United States National Institutes of Health] is diluted with sterile 0.9% saline and with 10% aqueous thimerosal solution to produce a vaccine containing 20 Lf. units of tetanus toxoid per ml. and thimerosal in a concentration of 1 to 10,000.

7.65 parts by volume of 7-n-hexyloctadecane, is mixed with 0.85 part by volume of 7-n-propyltridecane and 1.5 parts by volume of freshly distilled mannide monooleate. The mixture is sterilized by filtration through a Sela bacteria retaining filter. 4 ml. of the sterile hydrocarbon mixture is emulsified, as described in Example 1, with 4 ml. of the tetanus vaccine to obtain a vaccine product which contains 10 Lf. units of tetanus toxoid per ml. The vaccine product also contains 38.25% by volume of 7-n-hexyloctadecane, 4.25% by volume of 7-n-propyltridecane, and 7.5% by volume of mannide monooleate.

Example 4

Fluid diphtheria toxoid concentrate [prepared by the method of Holt, Developments in Diphtheria Prophylaxis, William Heineman Ltd., London, England, 1950, and conforming to the Minimum Requirements for Diphtheria Toxoid, 4th revision (1947), Amendment No. 1 (1954) of the United States National Institutes of Health] is diluted with sterile 0.9% saline and with 10% aqueous thimerosal solution to produce a vaccine containing 60 Lf. units of diphtheria toxoid per ml. and thimerosal in a concentration of 1 to 10,000.

A mixture consisting of 7.65 parts by volume of 7-n-hexyloctadecane, 0.85 part by volume of 7-n-propyltridecane, and 1.5 parts by volume of freshly distilled mannide monooleate is sterilized by filtration through a Sela filter. 4 ml. of the sterile hydrocarbon mixture is emulsified with 4 ml. of the diphtheria toxoid to produce a vaccine product containing 30 Lf. units of diphtheria toxoid per ml., 38.25% by volume of 7-n-hexyloctadecane, 4.25% by volume of 7-n-propyltridecane, and 7.5% by volume of emulsifier.

By substituting a diphtheria toxoid containing 200 Lf. units per ml. for the 60 Lf. diphtheria toxoid used in the above procedure, one obtains a vaccine product containing 100 Lf. units of diphtheria toxoid per ml.

Example 5

Phase-I cultures of *H. pertussis* are prepared in a modified Cohen-Wheeler liquid medium [American Journal of Public Health, 36, 371-376 (1946)]. Formalin is added to the cultures to produce a concentration of 1 to 1,000, the cultures are incubated at 37° C. and finally

centrifugated to obtain the detoxified *H. pertussis* cells. The cells are suspended in sufficient sterile physiological saline (0.9%) to produce a suspension containing 80-billion cells per ml. and thimerosal is added to the cell suspension in a concentration of 1 to 10,000.

A mixture consisting of 7.65 parts by volume of 7-n-hexyloctadecane, 0.85 part by volume of 7-n-propyltridecane, and 1.5 parts by volume of mannide monooleate is sterilized by filtration through a Sela bacterial filter. 4 ml. of the hydrocarbon mixture is emulsified with 4 ml. of the *H. pertussis* cell suspension, as described in Example 1, to produce a vaccine product containing 40-billion *H. pertussis* cells per ml., 38.25% by volume of 7-n-hexyloctadecane, 4.25% by volume of 7-n-propyltridecane, and 7.5% by volume of emulsifier.

Example 6

25 grams of ether de-fatted mixed grass pollens are added to 200 ml. of Coca's solution [J. Immunol. 7, 163 (1922)] and extracted for 48 hours at 4° C. The extract is filtered and then sterilized by filtration through a Seitz No. 6 filter. The extract is tested for sterility by the thioglycollate method and the protein nitrogen content is determined colorimetrically by the phosphotungstic acid or thiochloroacetic acid method. The extract is diluted with sterile physiological 0.9% saline to produce a solution containing 0.2 mg. of protein nitrogen per ml.

A mixture consisting of 7.65 parts by volume of 7-n-hexyloctadecane, 0.85 part by volume of 7-n-propyltridecane, and 1.5 parts by volume of mannide monooleate is sterilized by filtration through a Sela bacteria-retaining filter. 4 ml. of the hydrocarbon mixture is emulsified, as described in Example 1, with 4 ml. of the grass pollen extract to produce a vaccine product containing 0.1 mg. of protein nitrogen per ml., 38.25% by volume of 7-n-hexyloctadecane, 4.25% by volume of 7-n-propyltridecane, and 7.5% by volume of emulsifier.

Example 7

Six-day-old 32-ounce bottle cultures of trypsin dispersed, bovine embryo skin cells are inoculated with a 1:1000 dilution of calf lymph vaccinia virus seed in medium No. 199. The cultures are incubated at 37° C. for 3 days, at the end of which time the infected cells have separated from the glass surface. Infected culture fluid and cells are harvested from the bottles and frozen at -70° C. to disrupt intact cells. 1% Formalin in Hank's balanced salt solution is added to the vaccinia virus culture fluid suspension to produce a concentration of 1:4000, and the fluid is incubated at 37° C. for 24 hours to inactivate the virus. Inactivity of the virus is confirmed by a tissue-culture safety test consisting of inoculation and incubation of bovine embryo skin and monkey kidney cell bottle and tube cultures. 1% benzethonium chloride solution is added in a concentration of 1:40,000 as preservative and the sterility of the vaccine confirmed by the thioglycollate broth method.

A mixture consisting of 7.65 parts by volume of 7-n-hexyloctadecane, 0.85 part by volume of 7-n-propyltridecane, and 1.5 parts by volume of mannide monooleate is sterilized by filtration through a Sela bacteria-retaining filter. 4 ml. of the hydrocarbon mixture is emulsified, as described in Example 1, with 4 ml. of the smallpox vaccine to produce a final smallpox vaccine product containing 38.25% by volume of 7-n-hexyloctadecane, 4.25% by volume of 7-n-propyltridecane, and 7.5% by volume of emulsifier.

Example 8

The contents of one ampoule of lyophilized Standard Reference Rabies Vaccine supplied by the United States National Institutes of Health (Lot No. 167) is suspended

in 8 ml. of a hydrocarbon mixture containing 10% 7-n-propyltridecane in 7-n-hexyloctadecane. The resulting non-aqueous vaccine is considered to be a 10% brain suspension.

One ampoule lyophilized Standard Reference Rabies Vaccine supplied by the United States National Institutes of Health (Lot No. 167) is reconstituted with 4 ml. distilled water containing 0.25% phenol and 0.01% thimerosal to make a fluid rabies vaccine of 20% brain suspension. 4 ml. of this fluid vaccine is emulsified, as in Example 1, with an equal volume of 10% 7-n-propyltridecane in 7-n-hexyloctadecane to which has been added 15% mannide monooleate emulsifier. The final vaccine contains 10% brain suspension in a 50% aqueous phase volume, 7.5% emulsifier, 4.25% 7-n-propyltridecane and 38.25% 7-n-hexyloctadecane.

Example 9

In a 22-liter flask fitted with a mechanical stirrer, reflux condenser, and 1.5 liter dropping funnel is placed 320 g. of purified magnesium metal. The magnesium metal is then covered with one liter of dry ether. (Both the reflux condenser and the dropping funnel are equipped with drying tubes.) A few crystals of iodine are added to the flask along with 10 ml. of pure n-hexylbromide. The reaction flask is then heated in warm water for about 15 minutes to start Grignard formation. An additional two liters of anhydrous ether are added. n-Hexylbromide (2268 g.) is diluted with three liters of anhydrous ether and this solution is added to the reaction flask dropwise through the dropping funnel at a rate sufficient to maintain a continuous reaction. The addition of n-hexylbromide takes about 3.5 hours. The reaction mixture is then refluxed for 1.5 hours and stirred at room temperature overnight. Ethylbutyrate (696 g.) is diluted to 2.5 liters with anhydrous ether and the resulting solution added dropwise to the Grignard reagent over a period of seven hours. The reaction mixture is subsequently stirred overnight and then decomposed cautiously with 1400 ml. of saturated aqueous ammonium chloride solution over a period of three hours. The ether is removed by decantation and the salts are washed twice with ether. The ether extracts are then concentrated on the steam bath and the resulting residue is diluted with 1000 ml. of reagent grade benzene. One ml. of concentrated sulfuric acid is added and the solution refluxed over a water trap for 17 hours. A total of 87 ml. of water is collected (out of a theoretical total of 108 ml.), the benzene is stripped off under reduced pressure and the residue distilled to give 7-n-propyltridecene-6, B.P. 130–132° C. at 10 to 12 mm. A solution of 702 g. of 7-n-propyltridecene-6 in one liter of glacial acetic acid is catalytically reduced at a pressure of 50 pounds of hydrogen using 1 g. of platinum oxide as catalyst. The acetic acid is removed by distillation and the resulting residue is distilled under reduced pressure. The resulting 7-n-propyltridecane has B.P. 87–88° C. at 0.7 to 0.75 mm., $n_D^{25}=1.4339$. Infrared absorption indicated that the reduction was complete and gas chromatography showed the presence of only a single substance.

A Grignard reagent is prepared as described earlier in this example using 198 g. purified magnesium metal, 1413 g. n-hexylbromide and 3500 ml. anhydrous ether. To the Grignard reagent is added 856.4 g. ethyl laurate diluted with 900 ml. of anhydrous ether. The addition is dropwise over a four-hour period. The reaction mixture is refluxed for three hours, stirred overnight, and then carefully decomposed with 1300 ml. of a saturated aqueous ammonium chloride solution. The ether solution is separated and concentrated to an oil. The oil is combined with 1500 ml. of reagent grade benzene, 2 ml. of concentrated sulfuric acid is added and the mixture is refluxed over a water trap until a total of 53 ml. of water is collected (theoretical quantity of water is 67

ml.). The benzene is then removed under reduced pressure to leave, as a crude oil, 7-n-hexyloctadecene-6. This entire quantity of 7-n-hexyloctadecene-6 is dissolved in 1200 ml. of glacial acetic acid and reduced catalytically at a hydrogen pressure of 50 pounds using 2.0 g. of platinum oxide as catalyst. The glacial acetic acid is removed in vacuo and the residue distilled under reduced pressure to give 7-n-hexyloctadecane, B.P. 150–151° C. at 50–60 microns, $n_D^{25}=1.4439$. An infrared curve indicated complete reduction and gas chromatography indicated an impurity of less than one-tenth of 1% based on relative areas.

We claim:

1. A biological composition comprising a suspension of an antigen in a substantially chemically pure, branched chain, saturated, non-cyclic hydrocarbon containing 23 to 30 carbon atoms inclusive and having a melting point not greater than 4° C., a viscosity of not less than 2 and not greater than 30 centipoises at 22 to 24° C. and a guinea pig toxicity index not greater than 0.3 ± 0.2 unit.

2. A biological composition comprising a suspension of an antigen in a substantially chemically pure, branched chain, saturated, non-cyclic hydrocarbon containing 24 carbon atoms and having a melting point not greater than 4° C., a viscosity of not less than 2 and not greater than 30 centipoises at 22 to 24° C. and a guinea pig toxicity index not greater than 0.3 ± 0.2 unit.

3. A non-aqueous biological composition comprising a suspension of a dry, non-viable, immunogenic, viral agent in a substantially chemically pure, branched chain, saturated, non-cyclic hydrocarbon containing 23 to 30 carbon atoms inclusive and having a melting point not greater than 4° C., a viscosity of not less than 2 and not greater than 30 centipoises at 22 to 24° C. and a guinea pig toxicity index not greater than 0.3 ± 0.2 unit.

4. A biological composition comprising an emulsion of an aqueous, non-viable, immunogenic agent in a substantially chemically pure, branched chain, saturated, non-cyclic hydrocarbon containing 23 to 30 carbon atoms inclusive and having a melting point not greater than 4° C., a viscosity of not less than 2 and not greater than 30 centipoises at 22 to 24° C. and a guinea pig toxicity index not greater than 0.3 ± 0.2 unit; said emulsion containing from 0.5 to 15% by volume of an antigen-compatible, pharmaceutically-acceptable emulsifier.

5. A biological composition comprising an emulsion of an aqueous, non-viable, immunogenic agent in a mixture of hydrocarbons composed of one substantially chemically pure, branched chain, saturated, non-cyclic hydrocarbon containing 23 to 30 carbon atoms inclusive and one substantially chemically pure, branched chain, saturated, non-cyclic hydrocarbon containing 16 to 17 carbon atoms inclusive, said mixture of hydrocarbons and said hydrocarbon containing 23 to 30 carbon atoms inclusive both having a melting point not greater than 4° C., a viscosity of not less than 2 and not greater than 30 centipoises at 22 to 24° C. and a guinea pig toxicity index not greater than 0.3 ± 0.2 unit; said emulsion containing from 0.5 to 15% by volume of an antigen-compatible, pharmaceutically-acceptable emulsifier and between 10 and 74.5% by volume of said mixture of hydrocarbons.

6. A biological composition comprising an emulsion of an aqueous, non-viable, poliomyelitis vaccine in a substantially chemically pure, branched chain, saturated, non-cyclic hydrocarbon containing 23 to 30 carbon atoms inclusive and having a melting point not greater than 4° C., a viscosity of not less than 2 and not greater than 30 centipoises at 22 to 24° C. and a guinea pig toxicity index not greater than 0.3 ± 0.2 unit; said emulsion containing from 0.5 to 15% by volume of an antigen-compatible, pharmaceutically-acceptable emulsifier.

7. A biological composition comprising an emulsion of an aqueous, non-viable, poliomyelitis vaccine in a mixture of hydrocarbons composed of one substantially chemically pure, branched chain, saturated, non-cyclic hydro-

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carbon containing 24 carbon atoms and one substantially chemically pure, branched chain, saturated, non-cyclic hydrocarbon containing 16 carbon atoms, said mixture of hydrocarbons and said hydrocarbon containing 24 carbon atoms both having a melting point not greater than 4° C., a viscosity of not less than 2 and not greater than 30 centipoises at 22 to 24° C. and a guinea pig toxicity index not greater than 0.3 ± 0.2 unit; said emulsion containing from 0.5 to 15% by volume of an antigen-compatible,

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pharmaceutically-acceptable emulsifier and between 10 and 74.5% by volume of said mixture of hydrocarbons.

References Cited in the file of this patent

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