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ATTORNEYS
ADRENOCORTICOTROPIC HORMONE COMPOSITION

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Application January 12, 1954, Serial No. 403,548

Claims priority, application Netherlands January 17, 1953

7 Claims. (Cl. 167—74)

This invention relates to pharmaceutical preparations and more particularly to pharmaceutical preparations containing the adrenocorticotropic hormone of the anterior lobe of the pituitary gland and to a process of making same.

For a number of years attempts have been made to supply the medical profession with a preparation containing said adrenocorticotropic hormone, having a prolonged activity. Preparations of said adrenocorticotropic hormone have successfully been administered in the treatment of collagen or connective tissue diseases, such as rheumatoid arthritis, rheumatic fever, many acute inflammatory diseases of the eye, in cases of extensive and severe burns, asthma and many diseases due to adrenal cortical hypofunction.

Heretofore, such preparations, having a prolonged activity, due to retarded absorption of the adrenocorticotropic hormone by the body, contained a combination of the hormone with one or more vehicles, such as gelatin and polyvinyl pyrolidone. The hormone, in such preparations, is present in colloidal or suspended form and the preparations are viscous dispersions. On account of the viscosity of such preparations it is necessary to liquefy the same by heating in order to permit administration by injection. Some of the known preparations have the drawback that they, after injection, give rise to a local irritation.

It is one object of this invention to provide adrenocorticotropic hormone preparations having not only a considerably prolonged activity but also an effectiveness that is considerably greater than may be expected on the ground of the hormone content, determined according to the Sayers test (see Endocrinology 42, 379 (1948)), said preparations being fluid at room temperature and, thus, being readily administered by intramuscular injection without causing pain to the patient and local irritation and without having the drawbacks attaching to the known long-acting preparations.

Another object of this invention is to provide a simple and effective process of producing such long-acting preparations of adrenocorticotropic hormone.

Other objects of this invention and advantageous features thereof will become apparent as the description proceeds.

In principle, such long-acting preparations of adrenocorticotropic hormone are produced by combining said hormone in aqueous solution with one or more salts, hydroxides or oxides of metals as hereinafter set forth, having a retarding effect on the resorption of proteinic hormones by the body fluids, said compounds being capable of forming a sparingly soluble complex compound with said hormone at the pH of blood serum. Preferably the preparation is adjusted before the injection—e.g. by means of an alkali hydroxide solution or a buffer—to said pH value whereby the sparingly soluble complex compound is formed, or the hormone and the metal compound are comprised at that pH. It is also possible, however, to prepare preparations not themselves containing sparingly soluble ACTH-complex but in which the complex is only formed after the injection by the buffering action of the tissue fluid. These preparations are prepared by combining a soluble metal compound and ACTH in acid solution.

While the invention is not intended to be limited to any particular theory it is believed that this ACTH-containing complex is composed of a carrier consisting of a sparingly soluble salt, hydroxide or oxide of one or more of the metals zinc, nickel, cobalt, copper and iron, and the ACTH adsorbed on this carrier as an active constituent. Such a complex may be designated by the name "adsorption complex." The sparingly soluble salt of these metals that may act as a carrier, is formed when adjusting to the desired pH by the addition of a buffer to the mixture of normal ACTH and a soluble metal compound in a solvent, or is as such combined with the ACTH. The sparingly soluble metal hydroxide is formed when adding an alkali hydroxide to the said mixture for adjustment of the pH to a value about equal to that of the tissue fluid, or it is combined as such—viz. in the form of a suspension—with the ACTH preparation.

The preferred metal compounds which are suitable for the purpose of this invention are zinc compounds. There may also be used, although less advantageously, nickel, cobalt, copper, iron which have a retarding effect on the resorption of proteinic hormones and are free from any tendency to cause local irritation. Addition of said metal compounds in an amount corresponding to 0.2 mg. to 60.0 mg. and preferably between about 5 mg. and about 15 mg. of the metal component of said complex per 100 units of adrenocorticotropic hormone has proved to be sufficient. The U. S. P. unit for adrenocorticotropic hormone as used herein is as described by Sayers (see Endocrinology 42, 379 (1948)) accepted as the original Sayers method of intravenous assay.

The resulting adrenocorticotropic hormone-metal complex compounds obtained according to the present invention are only sparingly soluble under the conditions prevalent in the body tissue. In contrast hereto the adrenocorticotropic hormone itself is more readily soluble in the body fluids at their pH-value of about 7.0 and, therefore, is more readily distributed therein and more rapidly decomposed.

The prolonged activity of preparations obtained according to the present invention can be demonstrated by determining the number of eosinophilic leucocytes in blood at definite intervals after injection. A decrease in number of said eosinophilic leucocytes is a sensitive standard for increased activity of the adrenal gland and, thus, for the action of the adrenocorticotropic hormone. Said test of counting the eosinophils is especially adapted to determine the prolonged activity of an adrenocorticotropic hormone preparation. This test is based on the observation that injection of adrenocorticotropic hormone decreases the number of eosinophils in blood.

The test is carried out in the following manner: Eosinophil counts are made in the manner described by Zollkoefl in a number of fasted patients at 9 a.m. each day. In cases where the values are relatively constant during a control period from 5 to 10 days, a curve of the eosinophil count of one day is drawn thereby counting every four hours. If this curve shows only minor variations, the patient is given an injection of the adrenocorticotropic hormone preparation to be tested and, again, eosinophil counts are made at least every four hours. On carrying out such tests it was found that, when injecting an aqueous solution of 20 units of adrenocorticotropic hormone, the maximum of decrease in eosinophils was reached after about 4 hours. The initial eosinophil count before injection was again attained about 8 hours after injection, i.e., the adrenocorticotropic hormone has
practically lost its activity after such a period of time. When injecting 80 units of said hormone, the maximum of decrease in eosinophils was reached after about 6 hours and the initial value was again reached after about 13 hours. Fig. 1 of the attached drawings shows four curves of such an eosinophil count. Patients with rheumatoid arthritis were chosen for this test who had a constant level of eosinophils in the capillary blood for a considerable period of time.

Curve I—II shows the eosinophil count of injection of 20 units of an aqueous solution of adrenocorticotropic hormone. Curve II—III shows the eosinophil count on injection of 20 units of a long-acting adrenocorticotropic hormone preparation according to the present invention, as prepared according to the hereinafter given Example 9. Curve III—IV shows the eosinophil count on injection of 80 units of an aqueous solution of adrenocorticotropic hormone.

Curve IV—V shows the eosinophil count on injecting four times 20 units each of an aqueous solution of adrenocorticotropic hormone every four hours. Curve V—VI shows that injection of 80 units of hormone results in a more pronounced and prolonged decrease of the number of eosinophils than that illustrated by curve II—III and obtained with only 20 units of said hormone. However, it is evident that the prolonged effect of the preparations according to this invention as illustrated by curve II—III with only 20 units of hormone is not obtained even when injecting four times the amount of the hormone itself (cf. curve III—IV). Only when injecting 80 units of said hormone subdivided in four doses of 20 units each, as illustrated by curve IV—V, is it possible to approximately obtain the same results. Fig. 2 of the attached drawings illustrates the results of 22 combined observations obtained from studies of 18 patients with respect to their eosinophil count on injecting 20 units of a preparation according to Example 9 given hereinafter (curve V—VI). In contrast to the effect of administrations of an aqueous solution of the hormone itself, a preparation according to the present invention exhibits maximum effects between 8 and 16 hours and becomes ineffective after about 40—48 hours.

The clinical results achieved with 25 patients suffering from rheumatoid arthritis by injection of long-acting preparations according to the present invention did not differ from those achieved by injecting the heretofore used aqueous hormone solutions; for, with a daily dose of 20 units of adrenocorticotropic hormone in the form of a preparation according to this invention, approximately the same clinical result was achieved as with four daily injections of 20 units each of said hormone in aqueous solution every six hours. The maintenance dose is as low as two injections of 20 units of a preparation according to this invention per week, even in rather serious forms of rheumatoid arthritis. At no instance was there observed any local irritation, allergic reactions, symptoms of resistance to therapy, or other undesirable symptoms, even when administering such preparations over a period of 5 to 7 months.

Sterile aqueous solutions containing a preservative are preferably used as solvent for the adrenocorticotropic hormone and the metal compound. Phenol has proven to be especially suitable as preservative although other preservative agents may also be employed. The aqueous solutions preferably contains also a substance rendering the preparation isotonic. Such substances are, for instance, glycerol and sodium chloride in proper concentration. It is advisable that the preparation has buffering capacity in order to maintain the desired pH-value. Suitable buffer substances are, for instance, phosphate buffer, citrate buffer or acetate buffer. It is, of course, understood that any substance added to the hormone solution must be compatible to the human body in the concentration used and must not cause any irritating or toxic effects. The preferred solvent used is an aqueous solution containing about 0.25—0.5% of phenol, 2.0—2.5% of glycerol, and sufficient amounts of trisodium phosphate resp. sodium hydroxide solution to keep the pH of the preparation at about 6.0 to 8.0.

The order in which the adrenocorticotropic hormone and the metal compound and the solvent are mixed with each other is of no particular importance. Usually the hormone is dissolved in an aqueous solution containing phenol and glycerol and the resulting solution is mixed with an aqueous solution of the metal salt or with a suspension of the metal hydroxide or oxide. Thereafter the mixture is adjusted to the desired pH-value by the addition of a buffer solution or of an alkali hydroxide solution. The resulting suspension is then, if necessary, diluted to yield preparations containing definite predetermined quantities of adrenocorticotropic hormones, for instance, 10 units or 20 units per cc, phenol and glycerol. Preparations according to this invention are tested for their activity by three test methods to be discussed in detail hereinafter.

1. Hepatic glycogen test.—This test is carried out on starving hypophysectomized rats. During the experimental period of about 8 hours injections of glucose solution are administered to all animals. One group of said animals receives simultaneously an injection of the hormone used in order to eliminate errors caused by the solvent. A second group receives a single injection of a certain quantity of an aqueous solution of the adrenocorticotropic hormone, usually 1 unit. A third group receives the same quantity of the hormone, but subdivided into 8 doses of 1/8 of the total amount and administered in hourly intervals. A fourth group of the experimental animals receives one single injection of the same quantity of hormone as administered to the second and third groups but in the form of the long-acting preparation according to this invention. After 8 hours, the animals are killed and the glycogen content of the liver is determined.

The glycogen content found in the second group of animals is indicated by a, that found in the third group by b, and that found in the fourth group by c. The index of prolonged activity p may be expressed by the following equation:

\[ \log c - \log a = \frac{p}{100} \log b - \log a \]

When \( p = 0 \), there is no prolonged effect; when \( p = 100 \) or more, the effect of the preparation is equal to that or is even more prolonged than that of the same dose of adrenocorticotropic hormone in aqueous solution subdivided in 8 doses administered in intervals of one hour each. This test readily permits to determine not only whether a preparation has a prolonged effect but also quantitatively the extent of said prolonged effect.

2. Eosinophil count test.—This test is carried out in an analogous manner as described hereinbefore on normally fed animals and especially on dogs. One group of said animals receives a subcutaneous injection of an aqueous solution of adrenocorticotropic hormone and another group an injection of the same amount of the long-acting preparation to be tested. At regular intervals, for instance, every 3 hours, a count of the number of eosinophilic leucocytes in the blood is made. Decrease in eosinophil count and restoration of the blood picture to that found at the beginning of the experiment allow to determine the prolonged activity of the preparation tested.

3. Thomas involution test.—Said test is described by H. M. Bruce, A. S. Parkes, and W. L. M. Perry in "Lancet" vol. 262, p. 790 (1952). Nestling rats are used as experimental animals. Intramuscular injection of a certain quantity of adrenocorticotropic hormone in aqueous solution causes a decrease in the height of the newborn. In addition, the adrenals of the treated animals are weighed and their increase in weight is also determined.
acting preparations according to the present invention exhibit an activity at least 10 to 20 times stronger than that of the corresponding quantity of an aqueous solution of adrenocorticotropic hormone. The number "n" indicates how many times more of such an aqueous solution of the hormone must be administered in order to obtain a decrease in thymus weight comparable to that produced by a given dose of the long-acting preparation.

It is very interesting to note that the adrenocorticotropic hormone-metal complex compound is much less rapidly destroyed by serum enzymes than the hormone alone. The following experiments clearly demonstrate this surprising property of the complex according to the present invention. 8 units of adrenocorticotropic hormone are mixed with 1 cc. of rat serum and the mixture is allowed to stand overnight at room temperature. The adrenocorticotropic activity is then determined by means of the ascorbic acid depletion test developed by M. A. Sayers, O. Sayers, and L. A. Woodbury, and described in "Endocrinology" vol. 42, page 379 (1948), and is compared with that of a freshly prepared mixture. The same determinations were made with long-acting preparations obtained according to the present invention and containing the adrenocorticotropic hormone-zinc complex compound. The average decrease, in mg. of ascorbic acid per 100 mg. of adrenal gland, is given in the following table:

<table>
<thead>
<tr>
<th></th>
<th>ACTH-serum</th>
<th>ACTH-zinc-serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats</td>
<td>Adrenocorticotropic hormone ascorbic acid depletion</td>
<td>Adrenocorticotropic hormone ascorbic acid depletion</td>
</tr>
<tr>
<td>1st experiment:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not incubated</td>
<td>9</td>
<td>17.4</td>
</tr>
<tr>
<td>Incubated</td>
<td>10</td>
<td>19.4</td>
</tr>
<tr>
<td>Statistical data</td>
<td></td>
<td>P&gt;0.01</td>
</tr>
<tr>
<td>2nd experiment:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not incubated</td>
<td>12</td>
<td>20.4</td>
</tr>
<tr>
<td>Incubated</td>
<td>10</td>
<td>19.4</td>
</tr>
<tr>
<td>Statistical data</td>
<td></td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

It is clearly evident that adrenocorticotropic hormone as such is readily destroyed by rat serum on standing overnight while the long-acting preparations obtained according to the present invention exhibit a surprising stability in the presence of serum.

Exhaustive experiments on the composition of the liquid phase of the suspensions in question showed that in the adsorption complexes with zinc phosphate and zinc hydroxide as carriers, at a pH of about 7, 99% of the total activity of the preparation are precipitated, in other words that some 99% of the ACTH are present in sparingly soluble form.

**Example 1**

73.5 mg. of ACTH from hog pituitaries, with an activity of 1.36 U. S. P. u./mg., are dissolved in 8 cc. of water containing 0.5 percent phenol and 2.5 percent glycerol. To this are added 0.7 cc. of a zinc sulphate solution with a concentration of 10 mg. of zinc/cc, in the same solvent, and finally this is mixed with 0.54 cc. of NaPO₄-solution (40 mg./cc.) in the solvent. Then the pH has reached a value of 7.02. After diluting the resulting suspension with the solvent to 10 cc., a preparation is obtained, containing 10 u. of ACTH/cc. This preparation is highly active both in the thymus involution test and in the liver glycogen test.

**Example 2**

41.6 mg. of ACTH obtained from hog pituitaries with an activity of 2.4 U. S. P. u./mg., are dissolved in 4 cc. of the solvent mentioned in Example 1. To this are added 0.8 cc. of a cobalt sulphate solution with a concentration of 10 mg. of cobalt/cc, in the same solvent. The mixture is diluted to 9 cc. and subsequently the pH is adjusted to 6.0 with the aid of NaPO₄-solution containing 40 mg. of this salt/cc. Finally the volume of the preparation is adjusted to 10 cc. so that it contains 10 u. of ACTH/ml. In the eosinophils test this preparation is active at least 40 hours.

**Example 3**

83.2 mg. of ACTH containing 2.4 U. S. P. u./mg., are dissolved in 8 cc. of the solvent mentioned before. To this are added 0.32 cc. of a solution of zinc chloride, with a concentration of 37.5 mg. of zinc/cc. To this mixture 0.5 cc. of NaPO₄-solution containing 40 mg. of this salt/cc. is added and finally 0.5 cc. of 0.23 N sodium hydroxide solution, as a result of which the pH reaches a value of 7.0. After completing the volume to 10 cc. this preparation contains 20 u. of ACTH/ml. In the thymus test this preparation is about 16 times as active as the normal ACTH.

**Example 4**

83.2 mg. of ACTH, with an activity of 2.4 U. S. P. u./mg., are dissolved in 12 cc. of solvent. To this are added 1.8 cc. of copper sulphate solution containing 10 mg. of copper/cc. after which the volume is completed to 17.5 cc. using solvent. With the aid of NaPO₄-solution (40 mg. of this salt/cc.) the pH is adjusted to 6.0. The resulting preparation is diluted to 20 cc. so that it contains 10 u. of ACTH/ml. In the liver glycogen test this preparation is highly active.

In a similar way as described before one prepares the following preparations.

**Example 5**

The composition of the preparation is as follows:

<table>
<thead>
<tr>
<th></th>
<th>ACTH</th>
<th>NISO₄</th>
<th>NaPO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg. (=10 U. S. P. u.)</td>
<td>7.35</td>
<td>3.00</td>
<td>Until pH=7.</td>
</tr>
<tr>
<td>cc.</td>
<td></td>
<td>mg. of nickel/cc.</td>
<td></td>
</tr>
<tr>
<td>Solvent</td>
<td>Water containing 0.5 percent phenol and 0.75 percent NaCl.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Example 6**

The composition of the preparation is as follows:

<table>
<thead>
<tr>
<th></th>
<th>ACTH</th>
<th>CoSO₄</th>
<th>HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg. (=10 U. S. P. u.)</td>
<td>7.35</td>
<td>1.50</td>
<td>Until pH=3.0.</td>
</tr>
<tr>
<td>cc.</td>
<td></td>
<td>mg. of cobalt/cc.</td>
<td></td>
</tr>
<tr>
<td>Solvent</td>
<td>Water containing 0.26 percent phenol and 2.0 percent glycerol.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Example 7**

The composition of the preparation is as follows:

<table>
<thead>
<tr>
<th></th>
<th>ACTH</th>
<th>ZnSO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg. (=10 U. S. P. u.)</td>
<td>7.35</td>
<td>1.00</td>
</tr>
<tr>
<td>cc.</td>
<td></td>
<td>mg. of zinc/cc.</td>
</tr>
<tr>
<td>Solvent</td>
<td>Acetate buffer</td>
<td>Until pH=5.3.</td>
</tr>
</tbody>
</table>

**Example 8**

The composition of the preparation is as follows:

<table>
<thead>
<tr>
<th></th>
<th>ACTH</th>
<th>ZnCl₂</th>
<th>NaPO₄</th>
<th>NaOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg. (=10 U. S. P. u.)</td>
<td>2.77</td>
<td>1.00</td>
<td>Until pH=7.7.</td>
<td></td>
</tr>
<tr>
<td>cc.</td>
<td></td>
<td>mg. of zinc/cc.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solvent</td>
<td>Water containing 0.26 percent phenol and 2.0 percent glycerol.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Example 9**

The composition of the preparation is as follows:

<table>
<thead>
<tr>
<th></th>
<th>ACTH</th>
<th>ZnCl₂</th>
<th>NaPO₄</th>
<th>NaOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg. (=20 U. S. P. u.)</td>
<td>7.70</td>
<td>1.50</td>
<td>Until pH=6.0.</td>
<td></td>
</tr>
<tr>
<td>cc.</td>
<td></td>
<td>mg. of zinc/cc.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solvent</td>
<td>Water containing 0.26 percent phenol and 2.0 percent glycerol.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Example 10

77.1 mg. of ACTH, with an activity of 2.59 U. S. P. u./mg., are dissolved in 6 cc. of solvent (see Example 1). To this are added 1.5 cc. of ZnCl₂-solution containing 10 mg. of zinc/cc. in the same solvent. Finally to this solution is added 0.5 N sodium hydroxide solution, so that the pH reaches a value of 7.5 and the volume is made up to 10 cc. by means of solvent. The suspension obtained has prolonged, or enhanced action in the liver glycogen resp. in the thymus test. In this eosinophil test the number of eosinophil leucocytes has strongly fallen for a least 20 hours.

Example 11

55.5 mg. of ACTH with an activity of 36 U. S. P. u./mg., are dissolved in 60 cc. of the solvent mentioned in Example 1. To this are added 10 cc. of ZnCl₂-solution containing 10 mg. of zinc/cc. Then to this are added 0.5 N sodium hydroxide solution until the pH has reached a value of 7.5.

The volume is then completed to 100 cc. with the aid of solvent. This suspension has prolonged action in the liver glycogen test.

Example 12

77.1 mg. of ACTH from hog pituitaries, with an activity of 2.59 U. S. P. u./mg., are dissolved in 6 cc. of water containing 0.5% phenol and 2% glycerol. Then are added 2.46 cc. of a suspension of zinc hydroxide obtained by combining 1.5 cc. of zinc chloride solution containing 10 mg. of zinc/cc. and 0.96 cc. of 0.47 N sodium hydroxide solution. The pH of the finally obtained mixture is adjusted to 7.5 with NaOH. Subsequently the volume of the said mixture is completed to 10 cc. with the said solvent so that the ACTH-concentration becomes 20 U. S. P. u./cc. In the eosinophil test the preparation is active for more than 24 hours and highly active in the liver glycogen test. In the thymus involution test it is more than 16 times as active as the normal ACTH.

Example 13

55.4 mg. of ACTH, with an activity of 3.61 U. S. P. u./mg., are dissolved in 6 cc. of the solvent mentioned in Example 12. To this is added a suspension of zinc hydroxide obtained by mixing 2 cc. of zinc chloride solution containing 10 mg. of zinc/cc. with 1.25 cc. of 0.87 N NaOH. The pH is adjusted to 7.5 with NaOH. Eventually the volume obtained is diluted to 10 cc. This preparation is highly active in the liver glycogen test.

Example 14

73.5 mg. of ACTH, with an activity of 1.36 U. S. P. u./mg., are dissolved in 7.5 cc. of the said solvent. Then to this is added a zinc hydroxide-containing suspension obtained by mixing 1.06 cc. of zinc sulphate solution containing 10 mg. of zinc/cc. with 0.5 cc. of 1 N sodium hydroxide solution. The volume of the mixture obtained is adjusted to 10 cc.; the pH amounts to 7.4. The suspension contains 10 U. S. P. u./ml. In the liver glycogen test a single injection of this preparation exercises an effect equal to that exercised by a corresponding quantity of normal ACTH, spread over 8 equal injections administered every hour, and therefore possesses a distinctly prolonged action.

Example 15

73.5 mg. of ACTH, with an activity of 1.36 U. S. P. u./mg., are dissolved in 4 cc. of the said solvent. The pH of the solution is adjusted to 7.5 with a sodiurn hydroxide solution. Then are added 4 cc. of a suspension of nickel hydroxide with a pH of 7.5 totally containing 30 mg. of nickel. After making up the volume obtained to 10 cc., the suspension contains 10 U. S. P. u. of ACTH/ml. The preparation is readily active in the liver glycogen test.

Example 16

5.55 mg. of a highly purified ACTH preparation, with an activity of 36 U. S. P. u./mg., are dissolved in 8 cc. of a 0.5% solution of obtaining 0.5% of phenol and 0.9% of sodium chloride. To this are added 18.7 mg. of zinc oxide, after which the pH is adjusted to 7.5. The volume is made up to 10 cc. so that a preparation is obtained containing 20 U. S. P. u. of ACTH/ml. In the thymus involution test this preparation is highly active.

Example 17

83.2 mg. of ACTH, with an activity of 2.4 U. S. P. u./mg., are dissolved in 5.5 cc. of the solvent mentioned in Example 16, after which a suspension of hydroxide is added, obtained by mixing 2.5 cc. of cobalt sulphate solution containing 10 mg. of cobalt/cc. with a quantity of 0.5 N sodium hydroxide solution which is sufficient to adjust the pH of the total mixture to 7.5. The volume is then made up to 10 cc., so that a preparation is obtained containing 20 U. S. P. u. of ACTH/cc. An appreciable prolongation of activity is obtained in the liver glycogen test.

We claim:

1. An injectable adrenocorticotrophic hormone preparation comprising a sparingly soluble adrenocorticotrophic hormone-zinc phosphate complex compound, trisdium phosphate, sodium hydroxide, and an aqueous injectable vehicle, said complex compound being suspended in said vehicle in finely divided form, said trisdium phosphate being dissolved therein, the pH of said preparation being between about 6.0 and about 8.0, said preparation, on injection, producing a considerably prolonged and higher hormonal activity than produced on injecting an equal amount of the adrenocorticotropic hormone itself in aqueous solution, the zinc content of said preparation being between about 5 mg. and about 15 mg. per 100 U. S. P. units of said hormone, said aqueous vehicle containing about 0.25-0.5% of phenol as preservative agent and about 2.0-2.5% of glycerol and being substantially isotonic to blood serum.

2. An injectable adrenocorticotrophic hormone preparation comprising a sparingly soluble adrenocorticotrophic hormone-zinc hydroxide complex compound, sodium hydroxide and an aqueous injectable vehicle, said complex compound being suspended in said vehicle in finely divided form, said sodium hydroxide being present in said preparation in an amount sufficient to adjust its pH to about 6.0-8.0, said preparation, on injection, producing a considerably prolonged and higher hormonal activity than produced on injecting an equal amount of the adrenocorticotropic hormone itself in aqueous solution, the zinc content of said preparation being between about 5 mg. and about 15 mg. per 100 U. S. P. units of said hormone, said aqueous vehicle containing about 0.25-0.5% of phenol as preservative agent and about 2.0-2.5% of glycerol and being substantially isotonic to blood serum.

3. An injectable adrenocorticotrophic hormone preparation comprising adrenocorticotrophic hormone and zinc hydroxide, the adrenocorticotrophic hormone adsorbed on the zinc compound, the preparation having an inhibitory effect on resorption of proteinic hormones by tissue fluid, said preparation having a pH between 6.0 and about 8.0 and at such pH comprising an insoluble metal complex compound with said adrenocorticotrophic hormone, and an aqueous injectable vehicle, said complex compound being suspended in said vehicle in finely divided form, said preparation, on injection, providing a considerably prolonged hormonal activity than produced on injecting an equal amount of the adrenocorticotropic hormone itself in aqueous solution, the zinc content of said preparation being between about 5 mg. and about 15 mg. per 100 U. S. P. units of adrenocorticotrophic hormone.

4. An injectable adrenocorticotrophic hormone preparation comprising adrenocorticotrophic hormone and a sparingly soluble zinc compound, the zinc component having
an inhibitory effect on resorption of proteinic hormones by tissue fluids, said preparation having a pH between about 5.3 and 8.0, at said pH forming a sparingly soluble zinc complex compound with said adrenocorticotropic hormone, and an aqueous injectable vehicle, said complex compound being suspended in said vehicle in finely divided form with the adrenocorticotropic hormone adsorbed on said zinc compound, the zinc content of said preparation being about 5 mg. and about 25 mg. per 100 U. S. P. units of adrenocorticotropic hormone, said preparation, upon injection, producing a more prolonged activity than produced on injecting an equal amount of the adrenocorticotropic hormone itself in aqueous solution.

5. An injectable adrenocorticotropic hormone preparation comprising a sparingly soluble adrenocorticotropic hormone-zinc oxide complex compound, and an aqueous injectable vehicle, said compound being suspended in said vehicle in a finely divided form, the pH of said preparation being between 6.0 and about 8.0, the zinc content of said preparation being about 5 mg. and about 15 mg. per 100 U. S. P. units of said hormone, said aqueous vehicle containing about 0.25 to 0.5% of phenol as a preservative and about 2.0% to 2.5% of glycerol and being substantially isotonic to blood serum, said preparation, on injection, producing a considerably prolonged and higher hormonal activity than produced on injecting an equal amount of the adrenocorticotropic hormone itself in aqueous solution.

6. An injectable adrenocorticotropic hormone preparation comprising adrenocorticotropic hormone and a water-soluble zinc salt, the zinc component having an inhibitory effect on resorption of proteinic hormones by tissue fluid, causing no local irritation after injection and capable of forming at the pH of said tissue fluids a sparingly soluble zinc complex compound with the adrenocorticotropic hormone, and an aqueous injectable vehicle containing hydrochloric acid, in an amount sufficient to dissolve the hormone, the zinc metal content of said preparation being between 5 mg. and about 15 mg. per 100 U. S. P. units of adrenocorticotropic hormone, said preparation on injection producing a considerably prolonged activity than produced on injecting an equal amount of the hormone itself in aqueous solution.

7. An injectable adrenocorticotropic hormone preparation comprising adrenocorticotropic hormone and a sparingly soluble zinc compound, the zinc component having an inhibitory effect on resorption of proteinic hormones by tissue fluids, said preparation having a pH between about 5.3 and about 8.0, at said pH forming a sparingly soluble zinc complex compound with said adrenocorticotropic hormone, and an aqueous injectable vehicle, said complex compound being suspended in said vehicle in finely divided form with the adrenocorticotropic hormone adsorbed on said zinc compound, the zinc content of said preparation being between 0.2 mg. and 25 mg. per 100 U. S. P. units of adrenocorticotropic hormone, said preparation, upon injection, producing a more prolonged activity than produced on injecting an equal amount of the adrenocorticotropic hormone itself in aqueous solution.

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