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PROCESS FOR THE MANUFACTURE OF THE CORTICOTROPIC HORMONE

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1 Claim. (Cl. 167—74)

This invention relates to a process for the manufacture of the corticotropic hormone.

The anterior pituitary lobe contains a hormone which is capable of rectifying changes caused in the suprarenal cortex on removal of the pituitary, as for example the occurrence of a zone containing less fat (sudanophobe) (Collip and others, Lancet, 1933 page 347; Nirehours Archiv vol. 290, page 23, 1933). For production of this hormone there has been employed ultra-filtration (Hoffmann and Anselmino Archiv f. Gynäkologie, vol. 157, page 86, 1934) or separation by alcohol (Dutch Patent 77,332). These prior methods, however, as we have ascertained by testing them, only yield the hormone in a very impure state.

In accordance with the present invention the hormone is obtained in a pure state and in good yield by the application of a method involving a combination of precipitation steps. In the method of the invention the aqueous extract of the anterior pituitary lobe produced by extraction with weakly acid or alkaline media at elevated temperature, suitably at boiling temperature in known manner, is freed from albuminous substances by means of a precipitating agent, such as sulpho-salicylic acid, or by any other albumen-precipitating agent whose capacity for precipitating albumens while leaving the corticotropic hormone in solution has been previously established by test experiments, preferably at strong acid reaction for example pH=1 to 2. From the albumen-free extract the hormone is then precipitated by means of a precipitating agent of the type disclosed hereinbelow, or by other agents capable of precipitating the hormone without destroying its therapeutic activity, as can be determined by simple experiment. The precipitate obtained is freed in known manner from precipitating agent, suitably by dissolving in dilute alkali and precipitating by means of alcohol or acetone or any suitable water-soluble solvent.

As albumen precipitating agent sulpho-salicylic acid has proved to be particularly suitable, while for precipitation of the hormone, precipitating agents such as picric acid, picrolonic acid, flavianic acid, phosphotungstic acid and others can be employed. Sulphosalicylic acid and trichloracetic acid are unsuitable for the step of precipitating the hormone.

It is obvious that other precipitating agents than those specifically disclosed in the foregoing may be used to obtain the corticotropic hormone. The suitability of such precipitating agents must, of course, be determined beforehand by tests as to their precipitating action and by biological tests of the products obtained. The biological tests consist in determining the histological changes produced in the suprarenal cortex of hypophysectomized rats upon administration of the products. These biological tests are described in detail in Bomskov "Methoden der Hormonforschung," vol. 2 (1939), pages 965–971, and in Abderhalden's "Handbuch der biologischen Arbeitsmethoden," sec. V, part 3B, 2nd half, pages 1107–1108, 1938.

The pure hormone is a white, water-soluble powder of good activity which is free from glandular tissue, from the naturally accompanying albuminous substances, and from other hormones of the anterior pituitary lobe.

The following example illustrates the invention:

Examples

1 kg. of the de-fatted dry powder from acetone treated anterior pituitary lobes of cattle is extracted at boiling temperature with stirring for 30 minutes with 20 litres of $^1/_{100}$ N HCl. The centrifuged or pressed off extract is acidified with HCl to pH 2 and treated with the necessary quantity, determined in a preliminary experiment, of a 20% aqueous solution of sulpho-salicylic acid. The quantity of sulpho-salicylic acid is calculated on the basis that in the test filtrate on further addition no more turbidity is produced. The separated precipitate is brought into solution in 5 litres of water with the necessary quantity of concentrated ammonia and again precipitated by further addition of sulpho-salicylic acid. The filtrate therefrom is combined with that from the first sulphosalicylic acid precipitate and after addition of sufficient caustic soda solution to reduce the mixture to a weakly acid reaction to Congo red, the hormone is precipitated by saturation with picric acid. The picric acid precipitate, centrifuged or filtered off is treated in 500 cc. of water with the necessary quantity of ammonia to a condition neutral to litmus, whereby the precipitate for the most part passes into solution. From this solution the hormone is precipitated by addition of 5–10 times the volume of alcohol or acetone and dried with ether. Yield 8.0–10.0 grams which contain 70–80% of the total activity of the starting material. If a unit is taken as that quantity to be injected daily, which on 7 days continuous daily injection into hypophysectomised rats, is just capable of causing the sudanophobe zone in the suprarenal cortex to dis-

appear, then the preparation obtained according to the invention contains per gram of starting material 1 million to 1.2 millions units.

The therapeutic usefulness of corticotropic hormone is already known. Thus Grüneberg, "Archiv für Dermakologie und Syphilis" (1937), vol. 174, page 638, reports good results in the treatment of psoriasis with corticotropic hormone. The treatment is given intramuscularly or subcutaneously over a period of two to three weeks in daily doses of 1–4 ccs. of a solution corresponding to a hormone content of .3–1.2 grams of fresh gland.

Having now particularly described and ascertained the nature of my said invention and in what manner the same is to be performed, I declare that what I claim is:

A process for the production of the corticotropic hormone, which comprises extracting defatted anterior pituitary lobes with dilute hydrochloric acid, clarifying the extract, adding hydrochloric acid to a pH of about 2, adding an aqueous solution of sulphosalicylic acid to precipitate albumens, removing the precipitate, treating the solution with alkali to render the same weakly acid to Congo red, then treating the resulting solution with a member of the group consisting of picric, picrolonic, flavianic, and phosphotungstic acids, dissolving the hormone precipitate with such an amount of alkali as to yield a solution which is neutral to litmus, and precipitating the hormone by the addition of a water-miscible organic solvent.

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