FLOCCULATION ANALYSIS OF DISTRIBUTION OF GROWTH HORMONE AMONG FRACTIONS OF A RABEN EXTRACT OF HUMAN PITUITARIES

A. F. Lazarev

UDC 612.433'65-08

The flocculation reaction (a new immunochemical method of quantitative estimation of growth hormone) can be used as a control at each stage of obtaining hormone from human pituitaries by Raben's method. Flocculation analysis of the hormone content can be done rapidly in the course of the preparative work. Extraction of pituitaries with acetic acid was found to be highly efficient, and the method used for fractionating the Raben's extract is rational.

* * *

The efficiency of methods used for obtaining protein hormones has so far been assessed by the results of investigation of the final products. Because of the absence of an analytical procedure which could be used for control purposes in the course of the work at all stages of fractionation of the extract, considerable wastage of scarce raw material takes place and the development of new and improved preparative methods is restricted. Biological and physicochemical analysis are unsuitable for this purpose, for they are laborious and not sufficiently specific. The method of inhibition of hemagglutination is quicker, but still not sufficiently specific [4]. Radioimmunologic analysis is more specific, but takes too long and cannot therefore be used during preparative operations [7]. Only the analytical method based on the flocculation reaction in the system hormone–antihormonal horse serum, described by myself, satisfies all the demands of specificity and rapidity of performance [3]. In the present investigation the flocculation reaction was used to control each stage of production of growth hormone from human pituitaries by Raben's method [10, 11].

EXPERIMENTAL METHOD

The method of obtaining horse antiserum against human growth hormone and of carrying out the flocculation reaction was described previously [2, 3]. Native antiserum of batch No. 426, in a volume of 0.4 ml, was neutralized by 0.15 mg growth hormone (STH). The antiserum was titrated against a laboratory hormone standard (batch no. 89), which in a dose of 40 μg increased the width of the cartilage in the tibia test [8] from 158 ± 2.9 to 255 ± 1.9.

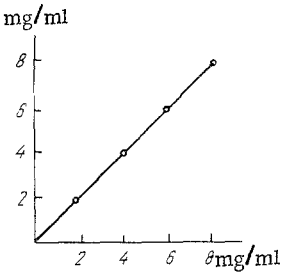

Fig. 1. Estimation of growth hormone added to extract of human pituitaries by flocculation analysis. Ordinate, quantity of STH added; abscissa, quantity of STH determined.

The method of obtaining the STH consisted of the following stages. Extraction of the pituitaries with 16 volumes glacial acetic acid at 70°. Precipitation of the first part of the ballast proteins by addition of $\frac{1}{2}$ volume of acetone to the extract. Isolation of crude STH from the supernatant by one volume of ether. Adsorption of a 2.5% solution of the raw product in 0.1M acetic acid with hydroxycellulose. Alkalification of the filtrate with 0.3M KOH solution and separation of the second and last part of the ballast proteins at pH 8.5. Preparation of STH from the filtrate at pH 8.5 by mixing it with an equal volume of cold ethanol.

To determine the STH content in the extract, 2.5 ml acetone was added to 0.2 ml of the sample, and the mixture was kept for 30 min in a refrigerator and centrifuged. The residue was washed with acetone and dried in a vacuum

P. A. Gertsen Moscow Oncologic Research Institute (Presented by Active Member of the Academy of Medical Sciences of the USSR P. F. Zdrodovskii). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 1, pp. 99-101, January, 1969. Original article submitted February 20, 1968.

TABLE 1. Yield of Growth Hormone at Stages of Its Preparation by Raben's Method (in percent)

1st Extract	Solution of crude STH	Adsorbed solution	Filtrate, pH 8.5	Final STH product
100	87.6 ± 5.7	88.4 ± 4.9	75.4 ± 7.5	50.3 ± 11.3

Content of STH in ballast fractions

1st Extract	2nd Extract	Acetone residue	Residue, pH 7.5	STH from "mother liquor"
100	10.6 ± 3.4	9.5 ± 2.4	6.7 ± 1.3	1.2 ± 0.6

TABLE 2. STH Content in Acetone Residue of Ballast Proteins

Acetone concentration (in %)	50	40	30
Weight of residue (in %)	100	92	78
STH content in residue (in % of extract)	7.7	6.2	5.2

exsiccator. The powder thus obtained was dissolved in 0.1 ml of 0.1N NaOH solution, 3.9 ml water was added, and the sample was then analyzed. This method is competent because, as Fig. 1 shows, the hormone added to the extract was determined quantitatively by flocculation analysis. Other intermediate products of STH preparation were analyzed directly after adjustment of the pH of their solution to 5.3-8.3 [4].

EXPERIMENTAL RESULTS

The results of successive analysis of all stages of production of human growth hormone by Raben's method are given in Table 1. Altogether more than 100 batches of Raben preparations were used. Stage-by-stage control was carried out on 27 batches, while for the rest the investigation was limited to analysis of the extract and the final product or analysis of stages in which deviations from the usual method were allowed.

Results of the analysis showed that practically all the growth hormone is extracted from the pituitaries in the course of one extraction. The mean yield of STH in the extract was 10.5-11.3% by weight of pituitary powder, and in the case of individual batches of pituitaries it reached 17%. The property of acetic acid to extract pituitary hormones quantitatively is unique. It was first established for ACTH [6], and now this property must be extended also to STH. In methods of production of STH in which other solvents are used for extraction, in order to increase the yield of STH it is necessary to repeat the extraction of the pituitaries twice [9, 13] or even four times [12, 16].

Loss of hormone was minimal during removal of the first part of the ballast proteins (acetone residue) from the extract. A further decrease in the quantity of acetone from 50 to 30%, as suggested by Vanderlaan and co-workers [14], is not rational because if this is done the yield of hormone is increased by only 2.5% and the content of ballast proteins not precipitated by acetone is increased by 22% (Table 2). These additional proteins are then separated from the supernatant by ether along with the STH, thus considerably increasing the percentage of impurities in the hormone.

During subsequent adsorption of ACTH for 48 h in two changes of hydroxycellulose, no loss of STH was found. In fact, the hormone content showed a slight but significant increase. It would be difficult to explain this fact without assuming that the acetic acid in which the adsorption was carried out is capable of regenerating protein hormone partially inactivated by acetone [17]. Whatever the case, the results of analysis indicate high stability of STH in 0.1N acetic acid.

The next stage, removal of the second and last part of the ballast proteins at pH 8.5, takes place without significant loss of STH. A trial reprecipitation of the residue at pH 8.5 did not increase the yield of STH.

The final stage of hormone production (isolation with ethanol) also took place quantitatively and practically no STH remained behind in the "mother liquor."

Using Raben's method as an example, it was thus shown that flocculation is a reliable and operative method of controlling STH production stage by stage. As a result of this control, the high efficiency of hormone extraction by acetic acid was established and all the other stages of fractionation of the extract by Raben's method were shown to be rational. In contrast to the preparative method of Wilhelmi [15, 16] or Reisfeld [12], the hormone is not distributed among many fractions, which increases the loss of hormone and complicates its purification. However, Raben's method must not be regarded as ideal. I have frequently observed disadvantages, and these apply both to extraction with hot glacial acetic acid, which causes modification of the hormone [5], and to the stage of isolation of the hormone with ethanol, when it undergoes partial denaturation [1]. Flocculation analysis also revealed another weak point of Raben's method, the possibility of incomplete removal of ballast proteins at pH 8.5.

LITERATURE CITED

1. A. F. Lazarev, in: Physiology and Pathology of the Endocrine System [in Russian], Khar'kov (1965), p. 250.
2. A. F. Lazarev, A. N. Kovchik, S. P. Belugina, et al., Byul. Éksperim. Biol. i Med., No. 8, 107 (1967).
3. A. F. Lazarev, Byul. Éksperim. Biol. i Med., No. 2, 120 (1968).
4. A. F. Lazarev and M. I. Balabolkin, Probl. Éndokrinol., No. 2, 54 (1968).
5. N. S. Fridman, A. F. Lazarev, E. E. Limanova, et al., Probl. Éndokrinol., No. 4, 73 (1965).
6. E. B. Astwood, M. S. Raben, and R. W. Payne, Recent Progr. Hormone Res., 7, 1 (1952).
7. W. E. Baum, W. F. Brown, and R. E. Crabtree, J. Pharm. Sci., 53, 738 (1964).
8. F. S. Greenspan, C. H. Li, M. E. Simpson, et al., Endocrinology, 45, 455 (1949).
9. C. H. Li, W. K. Zin, and J. S. Dixon, Arch. Biochem., Suppl. 1, 327 (1962).
10. M. S. Raben, Science, 125, 883 (1957).
11. M. S. Raben, Recent Progr. Hormone Res., 15, 71 (1959).
12. B. A. Reisfeld, U. I. Lewis, N. G. Brink, et al., Endocrinology, 71, 559 (1962).
13. P. Roos, H. R. Fevold, and C. A. Gemzell, Biochim. Biophys. Acta, 74, 525 (1963).
14. W. P. Vanderlaan, U. I. Lewis, A. E. Jones, et al., Calif. Med., 100, 175 (1964).
15. A. E. Wilhelmi, in: Ciba Foundation Colloquia on Endocrinology, London (1960), p. 25.
16. A. E. Wilhelmi, Canad. J. Biochem., 39, 1659 (1961).
17. D. Yamashiro, H. L. Aanning, and V. du Vigneaud, Proc. Nat. Acad. Sci. (Wash.), 54, 166 (1965).