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OBTAINING GROWTH HORMONE FROM CALF BLOOD

L.A. Kalchev, K.K. Ralchev, and I.T. Nikolov
Department of Animal Physiology

Growth hormone (somatotropin) has an important place in the single nervous-endocrine system, achieving humoral regulation in the organism. It is found in the pituitaries of vertebrates. It is formed in eosinophile α_1 cells from the anterior pituitary portion (Kracht, 1957) and is spread through the blood to all the cells in the organism, its anabolitic or catabolitic action thus being assured. In all probability, the beginning of its action is indirect, through its effect on the activity of different enzymes in the system. /175

A number of components in the blood are altered in one direction or another depending on the level of the hormone in the blood. Increasing its quantity (through venous injection) leads to a decrease in potassium and amino acids in the blood and to an increase in free fatty acids Satio 1967 and albumin [Ulrich et al., 1945]. What the transported form is or the natural state of the somatotropin hormone is is difficult to say. There is research which shows that it is associated with α-macroglobulin [Hadden and Prout, 1956].

Of interest is the amount of somatotropin in the blood, that which has theoretical as well as practical significance. Immunochemical and radioimmunological methods for its determination give similar data, which are on the order of 10 to 30 μg/ml of human serum [Dominguez et al., 1962; Lazarev et al., 1968]. Earlier data, however, are higher [Segalofiet et al., 1955, according to Berzin, 1964]. These are reported in serum from normal people to be from 0 to 40 μg/ml and after agglomeration from 41 to 61 μg/ml. Immunochemical methods for the quantitative determination of growth hormone are based on the use of the phenomenon of "inhibition of the hemoagglutination of sheep erythrocytes sensitized with growth hormone" [Read and Stone, 1958], and they have known deficiencies. It has been established that in human serum, there are substances which nonspecifically inhibit hemoagglutination and stimulate growth hormone [Ehrlich and Randle, 1961, according to Lazarev, 1968]. Dominguez et al. [Dominguez and Pearson, 1962] developed a method for the extraction of /176
somatotropin from blood serum in humans for the purpose of removing nonspecific inhibitors. But for both immunochemical and radioimmunological methods for obtaining immune antisera, native preparations from gorwth hormone are used, i.e. intact molecules. Consequently, the possibility is not precluded of partially degraded molecules; their biological activity is preserved [Li, 1961] and they do not inhibit hemoagglutination in erythrocytes. For such reasons, the actual amount of somatotropin permissible in blood plasma is elevated from 30 μg/100 ml. Since data are lacking on growth

hormone in the blood plasma of calves, the aim of the present experiment is, using the extraction method of Dominguez, to obtain a preparation of somatotropin and to study its biological activity and some of its chemical properties.

Procedure

Calf's blood is taken from the slaughterhouse. This is left to be (unknown) at room temperature, after which the serum is separated out. Removal of erythrocytes occurring in the serum is done by centrifuging in a cold centrifuge (+5°C) at 6000 rpm for 25 min. The serum rapidly obtained is treated further according to the Dominguez method [Dominguez and Pearson, 1962], with some changes. Instead of a phthalic acid - sodium base buffer (pH 6.1), a butyric acid - sodium base buffer is used (pH 6.1). The protein preparation obtained through extraction with 0.0075M sodium chloride is lyophilized. All further studies were done with the lyophilized preparation. The biological activity was traced through an increase in free fatty acids in rat plasma after injection with the preparation. For this purpose, Mosinger's method was used [Mosinger, 1965] in the modification of Goss and Leine [1967]. Nonvarietal female rats were used with a weight of 180-200 g, which were injected after 18 hours without food with 1 mg of protein solution in 1 ml 0.1N KOH. After 6 hours, blood was taken from the heart and treated according to the method mentioned.

The homogeneity of the preparation is traced through paper electrophoresis and gel filtration in Sephadex G-100.

The electrophoresis was done in a Veronal buffer, pH 8.6 and ion strength 0.04, current voltage 180v, current strength 0.3 ma/cm for 18 hours [Pavlova, 1963]. 20 mg of the preparation was dissolved in 0.1 ml of 0.1N sodium base and 0.2 ml distilled water. The preparation from the solution was applied on the electrophoregram every 0.2 ml. Filtrak No. 1 filter paper was used, first soaked with buffer and slightly dried. Serum from rats was also applied for comparison. All the procedures were done at room temperature.

The gel filtration was done in a glass column (2.5 X 95 cm), completed with Sephadex G-100, equilibrated, and elutriated with carbonate-bicarbonate buffer, pH 9.4. The preparation was dissolved in this buffer in a concentration close to 20 mg/ml and was applied to the column. Flow rate was 40 ml/hr. The fractions were collected every 5 ml with an automatic LKB collector equipped with a meter for wavelength 2573 Å and a recording adjustment. The width of the quartz dish was 0.4 cm. /177

The amount of protein was determined by the method of Lowry et al. [1951].

The data were processed by variant statistics.

Results

The method described for obtaining growth hormone from human serum was applied with known changes for the preparative extraction of somatotropin from calf serum. The results from the experiment carried out are reflected in Table 1.

Table 1

Добиви на растежен хормон, получен от серум на телета
Yields of Growth Hormone Prepared from Calf Serum

№ на опита Experiment №	Серум в мл Serum ml	Соматотропин в мг GH mg	Соматотропин в мкг мл серум GH μg /ml serum
1	200	12,08	60,4
2	200	7,36	36,8
3	200	12,48	62,4
4	200	12,36	61,8
5	200	7,64	38,2
6	200	7,60	38,0

In using 200 ml of serum, the yields varied on the average from 36.8 to 62.4 μg/ml. These data are many times greater than the amount of somatotropin in human serum. The possibility must be kept in mind of loss in the process of obtaining the hormone, not precluding the numbers obtained being smaller than the actual ones.

The studies of biological activity in the total lyophilized preparation, by tracing the quantitative changes in free fatty acids in the rat serum, show that 6 hours after the introduction of the hormone into the experimental animal, it increased reliably by 36.4% (Table 2).

The rats exhibited a continuous response reaction to somatotropin, which, without being specific, was proof of the presence of the growth hormone.

The electrophoretic studies (Fig. 1) show that the preparation obtained under the conditions described for separation is a one-component one. Its mobility is low and, in comparison with the data obtained from a parallel electrophoretic separation of serum from white rats, coincides with the mobility of α- or β-globulin. /178

Gel filtration on Sephadex G-100 showed the presence of two fractions, A and B (Fig. 2). The elutriated volume of

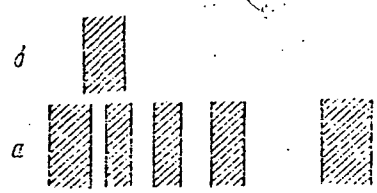
Table 2

Влияние на изолирания от телешки серум соматотропен хормон (СТХ) върху свободните мастни киселини в кръвта на бели плъхове

Influence of the isolated from calf serum growth hormone (GH) on the free fat acids in the blood of rats

Опити Experiment	Свободни мастни киселини в плазмата (μ екв мл) Free fat acids in the plasma (μ equv ml)	Достоверност (Fp) True potency of test preparation lat ve to standard	% на увеличението на свободните мастни к-ни Increase of the free fatacids per cent
Контрола Control	0,55 ± 0,045 (6)	7	36,4
Опит Experiment	0,75 ± 0,028 (12)		

Note: In parentheses, numbers of experimental animals.



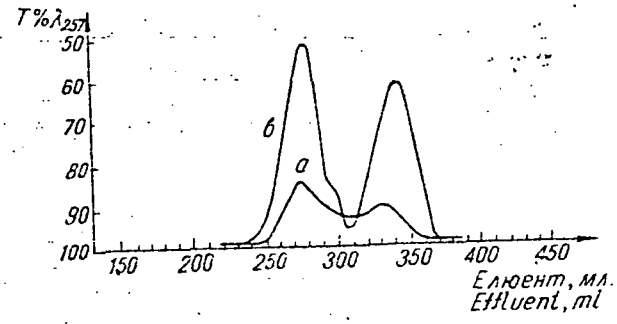
Фиг. 1. Електрофореза на серумни белтъци (а) и телешки соматотропин (б)

Electrophoresis of serum protein of rat (a) and calf growth hormone from blood (b)

fraction A is equal to the elutriated volume of the corresponding fraction upon separation of calf somatotropin obtained from the pituitary glands (Fig. 2). This also corresponds to fraction B. The amount of fraction A is about 80% of the amount of protein application, which is also characteristic for calf hormone obtained from the pituitary and separated under these conditions.

Discussion

Application of the Dominguez method [Dominguez and Pearson, 1962] for obtaining growth hormone from human serum to obtain somatotropin from calf serum gives significantly elevated yiedds. It is possible that human serum contains less growth hormone, although the immunochemical method of determination is not precluded in /179
order to report only the amount of intact molecules. If this is actually so, obtaining the preparation of the hormone is probably included and those relatively hydrolyzed from serum protease molecules preserve their biological activity. Variations in yield from a milliliter of serum could be explained by differences in initial material. The serum for each experiment was obtained from the blood of various animals.



Фиг. 2. Гелна филтрация на растежен хормон от кръв (*а*) и телешки хипофизи (*b*)

Gel filtration of growth hormone from blood (*a*) and from calf pituitary glands (*b*)

Changes are also possible which arise under the conditions of obtaining the preparation: pH, temperature, degree of drying with ammonium sulfate, and so on. An increase in the amount of free fatty acids in the serum of white rats shows that the preparation obtained is somatotropin. The percentage of increase obtained for free fatty acids (36.4%) is most characteristic of growth hormone.

The data from paper electrophoresis, indicating the presence of one peak with the mobility of serum globulins, is one more way of confirming the identity of the preparation obtained as the hormone somatotropin. Column chromatography in Sephadex G-100 is characteristic with the presence of two peaks. The chromatographic profiles of the hormone obtained from calf serum are completely similar to those obtained from calf pituitaries, according to the Wilhelm method in our modification [Kalchev, Ralchev, and Nikolov, 1968]. It must be kept in mind that column chromatography of two preparations is done under the same conditions and facts, that the elutriated volumes are the same, and one may consider that the two preparations do not noticeably correpond in molecular weight.

Conclusions /180

1. The Dominguez method was used for obtaining a preparation of growth hormone from calf serum. Instead of a phthalic acid - sodium base buffer with pH 6.1 for removing the mixture from the protein containing somatotropin, a butyric acid - sodium base buffer was used with a pH of 6.1.

2. Growth hormone was obtained in amounts of 36.8 to 62.4 μg/ml of serum. The preparation was biologically active. An increase was observed in free fatty acid after injection of the hormone. This increase was 36.4% relative to the control animals.

3. Electrophoretic study of the lyophilized hormone in Veronal buffer, pH 8.6, indicates its one-component nature.

4. Column chromatography in Sephadex G-100 shows a pro-

file which is identical with the profile of growth hormone obtained from the anterior hypophyseal portions of calves.

5. The question arises of whether immunobiological quantitative determinations of growth hormone in blood reflect the whole amount of it or only that in intact protein molecules.

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16. Abstract

The method of Dominguez for the preparation of a growth hormone from human serum was used for the isolation of the hormone from calf serum. The method was somewhat modified with respect to the buffer solutions used. The yield of the growth hormone varied from 36.8 μg/ml serum to 61.8 μg/ml. The preparation is biologically active - it increases the quantity of the free fatty acids released in rat plasma by 36.4 per cent. Electrophoresis in Veronal buffer, pH 8.6, showed the presence of a single fraction having mobility intermediate between that of α- and β-globulins. Gel filtration through Sephadex G-100 showed an elutriation curve identical to that obtained by the growth hormone prepared from pituitary glands.

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