OBTAINING ANTISERA AGAINST GROWTH HORMONE BY IMMUNIZING RABBITS WITH MICRODOSES OF THE ANTIGEN

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After a prolonged period of failures [5], antisera were obtained [2,6,7,8,9,13] against growth hormone (somatotropic hormone, STH), secreted from the hypophyses of man and animals. This fact is linked to the beginning of intensive development of the immunochemistry of protein hormones in general, and in particular, of the growth hormone. The greatest application of immunochemical agents was found in investigations of the species specificity of the hormone [10,11], and especially in developing methods for its determination in the serum of man [7,13,14], which is practically impossible to accomplish by any other physico-chemical or biological methods.

In order to increase the results of growth hormone immunization, it was used in very high doses, mixed with the non-specific stimulator for antibody formation—Feind's adjuvant [3]. The total expenditure of bovine growth hormone for immunization of one rabbit reached 80 mg [9], and of human growth hormone, 5 mg [7]. In addition to antibodies against the growth hormone, the obtained antisera usually contain additional antibodies against proteins, ofter unidentified impurites, which can lead to erroneous results when they are used.

In this report, we describe a method for obtaining highly specific antisera, which, practically speaking, contain antibodies against only the growth hormone. This is achieved by immunizing the rabbits with microdoses of the antigen, in which the negligibly small adulterants to the hormone are no longer in a state where they can realize their potential capacity for the formation of antibodies. Using microdoses of growth hormone, we started with the premise of its high antigenicity. Actually, it would be difficult to imagine that, stimulating the immunogenesis of any other antigen [1], the growth hormone would remain indifferent to synthesis of its own antibodies.

EXPERIMENTAL METHOD

We immunized female rabbits of different breeds, weighing 2.5 kg. As the antigens, we used highly purified preparations of growth hormone, obtained according to the method of Raben [12]. The immunization schema was worked out as applicable to any species of growth hormone, with the exception of swine hormone, for which the method of immunization in microdoses of antigen is unsuitable. Depending on the species, the antigen contained a varying amount of hormone: 1 mg of bovine or sheep STH, and 0.1 mg of human STH.

The hormone, dissolved in 0.2 ml of physiological saline with a pH of 8.0-8.2, was carefully mixed with 0.2 ml of a solution of 0.05-0.1 mg of BCG vaccine and 0.6 ml of pure, vaseline oil before forming a stable emulsion. This antigen composition is the optimal one, as compared with all other investigated complex antigens. We injected 0.25 ml of the obtained antigen emulsion subcutaneously into the area of the axillary and inguinal lymph nodes of the rabbits' paws. After 2 weeks, the antigen injection was repeated in the same manner. After another 1.5 weeks, blood was drawn from the ear vein of the rabbit, taking 40-50 ml three times a day. The titer of antiserum against the human hormone corresponded to its weakest concentration, equal to 0.004 mg/ml in the reaction of microprecipitation on agar gel, and 2 micrograms/ml using the phenomenon of hemagglutination inhibition. To obtain antisera against growth hormone of animal origin, an additional, 3rd antigen injection was carried out.

After an interval pause (5-6 weeks), the rabbits were revaccinated with one multiple antigen injection, containing 0.01 mg of human STH or 0.1 mg of STH of animal origin.

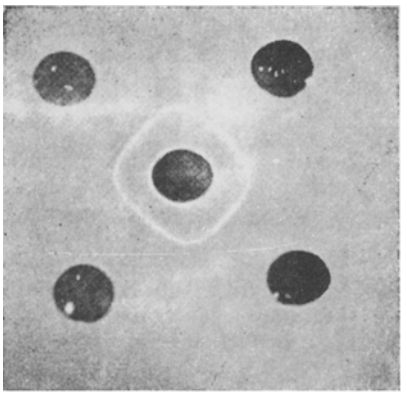

Fig. 1. Precipitation of growth hormone with antiserum. In the center of the agar plate is a hole containing 0.02 ml of untreated antiserum against ox growth hormone. The peripheral holes contain 0.2 ml of hormone solution with a concentration of 0.05%. Duration of the precipitation was 16 h at 37°. Magnification 3×.

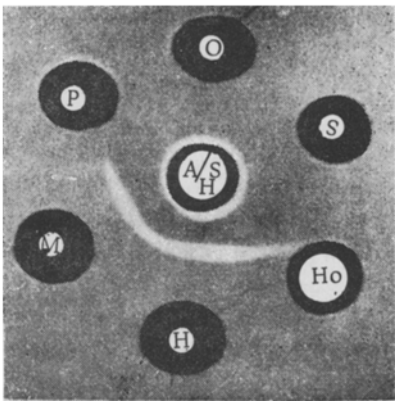

Fig. 2. Species specificity of the antiserum against human growth hormone. In the center of the agar plate is a hole containing 0.02 ml of the antiserum (A/S) against human growth hormone. The peripheral holes contain 0.02 ml of a solution of growth hormone, with a concentration of 0.012%, from the following species: human (H), monkey (M), sheep (S), ox (O), pig (P), horse (Ho). Duration of the precipitation was 24 h. Magnification 3.5×.

The obtained antisera were warmed for 30 min at 56°, preserved in mertiolatom 1:5000, and poured into test tubes and maintained at −20°. For work with tissue cultures, we used freshly prepared antisera without preservative.

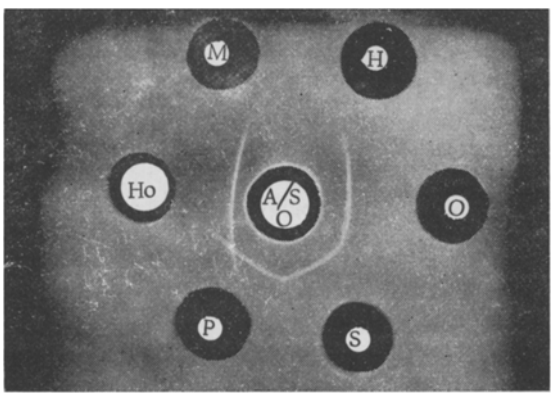

Fig. 3. Species specificity of the antiserum against ox growth hormone. In the center of the agar plate is a hole containing 0.02 ml of antiserum (A/S) against ox growth hormone. The peripheral holes contain 0.02 ml of a solution of growth hormone from ox (O) with a concentration of 0.002 mg/ml, from sheep (S) with a concentration of 0.004 mg/ml, from pig (P) with a concentration of 0.125 mg/ml, from horse (Ho) with a concentration of 2 mg/ml, from monkey (M) with a concentration of 2 mg/ml, and from a human (H) with a concentration of 2 mg/ml. Duration of the precipitation was 24 h. Magnification 3.5×.

For controlling the rise of the antibody titer in the rabbit serum in the process of immunization, and for performing other immunochemical investigations on the growth hormone, it was more convenient to use a somewhat modified version of the reaction of microprecipitation on agar gel in glass plates [4]. For this, 4.5 ml of a 2% agar solution, prepared in the M/60 phosphate buffer at pH 7.8, were placed on a clean glass slide, measuring 7.5×2.5 cm. After solidifying, the agar was punctured with a stamp, forming 5 holes measuring 2.5 mm in diameter. In the central hole, we placed 0.02 ml of antiserum, and in the peripheral ones, 0.02 ml of the hormone solution in the same buffer, in multiple, decreasing concentrations (beginning with 1 mg/ml). The formation of a precipitation line occurred between the 3rd and 12th hours of incubating the plates in a moist chamber at 37°.

EXPERIMENTAL RESULTS

The obtained antisera reacted with the STH to form one line of precipitation (Fig. 1), in contrast to the polyvalent antisera, which, with high concentrations of the hormone, yield two and even three lines. These sera are formed as a result of overdosage of the antigen (0.4 mg of human STH or more, and 2-3 mg of bovine STH). Still higher doses lead to inhibition of immunogenesis, and prolongations of the intervals of immunization.

The antisera obtained according to our method did not precipitate with the possible additional antigens: normal serum, albumin, γ-globulin, BCG, ACTH, gonadotropins, and prolactin. They possessed a clearly manifested species specificity. The antiserum against human growth hormone did not, under any conditions, precipitate with the hormones of animal origin, with the exception of monkey hormone (Fig. 2). Conversely, the antiserum against animal hormone did not precipitate with either human or monkey growth hormone. However, cross immunochemical reactions did arise between the growth hormones of animal origin. As shown in Fig. 3, their species characteristics were characterized by a difference in the concentration of antigen; the antiserum against bovine growth hormone precipitated with bovine and sheep hormones in extreme dilutions—0.002 and 0.004 mg/ml respectively, with swine hormone—in a concentration greater than 0.125 mg/ml, and with horse hormone—in a concentration of 2 mg/ml or more. Details of an immunochemical investigation on the growth hormones of different origin will be reported later.

SUMMARY

The authors describe a method for obtaining antisera in response to a highly purified preparation of growth hormone, free of additional antibody impurities. A high antiserum titer is obtained as a result of two-three subcutaneous injections of the antigen (microdoses) into all the rabbit extremities, in the area of the axillary and inguinal lymph nodes.

The hormone was administered in conjunction with a nonspecific stimulant of antibody production, containing BCG vaccine and vaseline oil. The antisera, examined by microprecipitation on agar gel, exhibited high species and hormone specificity.

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