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Chromatographic Separation of Growth Hormone and Other Peptides.* (22749)

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A chromatographic system was devised for the purification of growth hormone which appears, in addition, to be generally useful for the fractionation of polypeptides. The system may be adapted to either of two means of separation. The difference in maximal acetic acid concentration allowing adsorption on carboxymethylcellulose is sufficient to permit separation of some substances. Fractions which have similar adsorbability from acetic acid are separated by elution from carboxymethylcellulose with weak solutions of neutral salts in acetic acid. The resolving power of the salt elution appears to be particularly high when the acetic acid concentration is rather near the maximum strength permitting adsorption. The appropriate acetic acid concentration may be predetermined by small batch experiments, or by gradient elution from a column with the acid.

Results. Experiments with a purified growth hormone preparation indicated that adsorption occurred in acetic acid in strengths up to approximately 3.5 M. Gradient elution with acetic acid separated a minor component, but most of the material appeared with a sharp front at about 3.6 M but with extensive tailing and no further fractionation. Gradient elution with calcium chloride in 1.0 M acetic acid eliminated the tailing and yielded

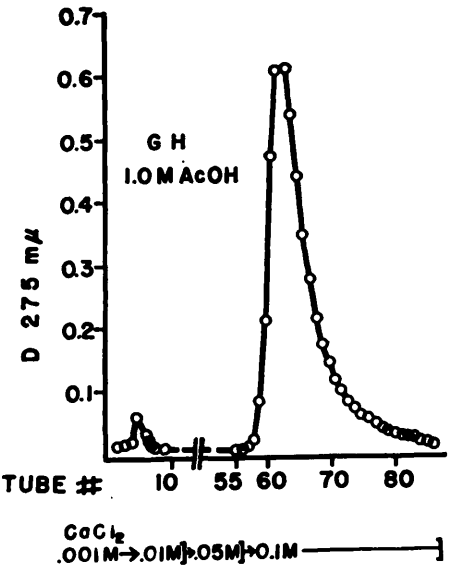

FIG. 1. 30 mg pig growth hormone preparation in 1.0 M acetic acid containing .001 M $CaCl_2$ on 3.5 g carboxymethylcellulose prewashed with same solution and packed under pressure. Column dimensions: 9 × 220 mm. Mixing flask for the gradient elution contained a constant vol of 120 cc. Flow rate, 7.4 ml/hr.

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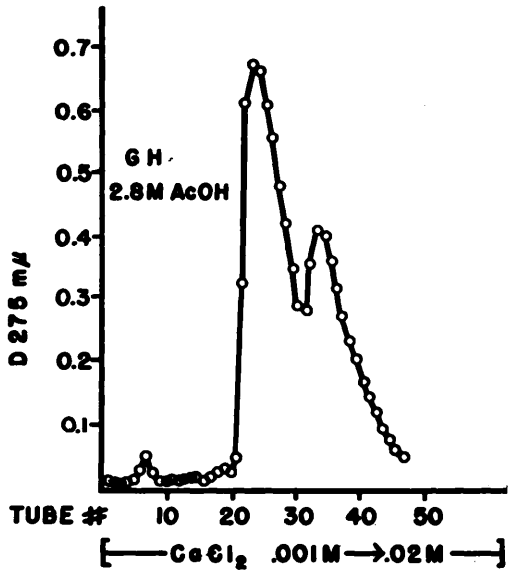

FIG. 2. Same as Fig. 1, except for changes in acetic acid and calcium chloride concentrations. Flow rate, 5.2 ml/hr.

one minor and one major fraction (Fig. 1); elution with calcium chloride in 2.0 M acetic acid separated the major fraction into two components, and the separation was somewhat better with 2.8 M acid (Fig. 2). The growth-promoting activity was concentrated in the faster running of the two components.

There was an inverse relation between acetic acid concentration and the concentration of salt required for elution. Elution with lanthanum chloride rather than calcium chloride decreased the required molar concentration of salt by about one-half. Gradient elution with calcium chloride in 0.3 M formic acid failed to separate the two major components, suggesting that the solvent character of the strong acetic acid solutions may contribute to the fractionating power of the system. However, the addition of .01 M calcium acetate to acetic acid prevented separation of the major components by gradient elution with calcium chloride, presumably due to the buffering effect. In general, resolution appeared to be improved by conditions favoring a weakened affinity of the adsorber for the peptide, and this may account for the impression obtained by Ellis and Simpson(1) that the resolving power of carboxymethylcellulose for growth hormone preparations was not particularly high under their conditions which were chosen to favor maximum adsorption.

Little, if any, growth hormone was bound irreversibly by the adsorbent, but loss of other protein material to the column may occur. The recovery from these columns was about 80%, but with another growth hormone preparation (Armour R285-128) slightly less than half the charge emerged under the same conditions. Application of a larger charge of the Armour material on a fresh column or reuse of the same column for the same charge yielded a major fraction of growth-inactive material appearing with the front and a growth-active fraction in the anticipated position.

Applying the information obtained from these studies, it was found possible to prepare growth hormone directly from a crude pituitary extract either by gradient elution, or by fractional elution with fixed concentrations of calcium chloride in 2.0 M acetic acid.

A purified corticotropin preparation was not adsorbed by carboxymethylcellulose from a 2.0 M solution acetic acid, but considerable fractionation was obtained by gradient elution with calcium chloride in a 1.0 M acetic acid solution (Fig. 3). Intermedin activity was concentrated in the third fraction and corticotropin activity in the unresolved fourth and fifth fractions. The small amount of spermine present in the preparation(2) was concentrated in the region of the second peak,

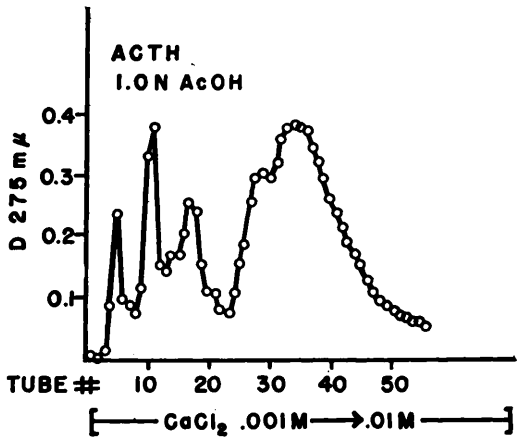

FIG. 3. 24 mg oxycel-purified corticotropin on column similar to those of Fig. 1 and 2. Flow rate, 6.4 ml/hr.

as judged by the formation of characteristic picrate crystals.

The carboxymethylcellulose used in these experiments was prepared from Solka-Floc BW according to the directions of Peterson and Sober(3) and contained approximately 0.75 milliequivalent carboxyl group per gram. The growth hormone was prepared from pig pituitaries as previously described(4), and the corticotropin preparation was purified by adsorption on oxidized cellulose as described by Astwood *et al.*(5).

It is of interest that carboxymethylcellulose adsorbed practically none of the corticotropin preparation from 2.0 M acetic acid but adsorbed 68 mg g of the growth hormone preparation. since it was previously noted that 10.4% carboxyl oxidized cellulose adsorbed. from 0.1 M acetic acid, 340 mg/g of the corticotropin and only 6 mg/g of growth hormone(4).

Summary. A chromatographic system of high resolving power was devised for the purification of growth hormone and was adapted for use in the separation of other peptides.

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Effect of Allergic Shock on Fate of Staphylococci in the Organs of Mice. (22750)

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As recently shown by several investigators (1,2,3) an anaphylactic type of sensitization can be elicited in mice by injecting intraperitoneally certain protein antigens in association with pertussis vaccine. The preliminary results here reported indicate that a mild allergic shock induced in animals so sensitized can modify their resistance to bacterial infections.

Experimental. Specific sensitization of mice to bovine serum. The ability of mice to develop an anaphylactic type of sensitization to bovine serum is illustrated in the following experiments with two different breeds of mice. Female mice of so-called Rockefeller Swiss strain were used when 7 weeks old; their average weight was 31 g. They were fed bread and milk throughout the experiment and sensitized as indicated in Table I. The bovine serum was a sterile filtrate distributed by Difco laboratories. The pertussis vaccine was a preparation distributed for human use by Lederle Laboratories and obtained through the kindness of Dr. Stanton M. Hardy. It contained approximately 60 billion bacterial cells ml. The animals were challenged in groups of 5 by intravenous injection of various amounts of serum. All deaths occurred within 30-60 minutes (Table I).

Irrespective of the amount of serum used for the challenge injection, there were no deaths among the mice pretreated with either physiological saline, or with pertussis vaccine alone (Table I). In contrast, many of the animals that had been pretreated with serum died shortly after the challenge injection, even when the challenge dose was smaller than 0.01 ml. There was evidence furthermore that the degree of sensitization had been increased by injecting pertussis vaccine in addition to bovine serum at the time of sensitization.

The following experiment carried out with another strain of mice provides evidence that