approximately 10^{-3} M solution of the reprecipitated albumin at pH 9.5 with 5×10^{-3} M iodoacetamide overnight at room temperature, followed by acidification and reprecipitation twice with ammonium sulfate, gave a product with a measured Δ 255 mμ following p-mercuribenzoate addition equivalent to only 0.07 -SH per mole. A control preparation similarly treated but without iodoacetamide additions gave a value of 0.89 -SH per mole. Reprecipitation of the iodoacetamide treated albumin was necessary because of formation in the reaction of substances which interfered in the spectrophotometric test. The nature of the impurity in the bovine albumin is not known.

ST. PAUL, MINN.

[CONTRIBUTION FROM THE HORMONE RESEARCH LABORATORY, UNIVERSITY OF CALIFORNIA]

Adsorption Chromatography of Hypophyseal Growth Hormone and Other Proteins on Hyflo Super-cel Columns

BY HUBERT CLAUSER[1] AND CHOH HAO LI

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The adsorption behavior of hypophyseal growth hormone (somatotropin), bovine serum albumin (BSA), methylated BSA, ovalbumin, ribonuclease and adrenocorticotropic hormone (ACTH) on a Hyflo Super-Cel column, has been investigated. It was found that BSA, methylated BSA and somatotropin can be adsorbed onto the column with buffers of a pH below the isoelectric point of these proteins, whereas ovalbumin, ribonuclease and ACTH could not be adsorbed at any pH. The adsorbed proteins could be eluted into separate peaks by increasing, either continuously or discontinuously, the pH of the developing solvent. No loss of biological activity was observed after somatotropin had been repeatedly subjected to this adsorption and elution procedure. It was further demonstrated that the hormone protein exhibits homogeneity according to this chomatographic method. The mechanism for the adsorption of proteins onto Hyflo Super-cel is discussed.

The adsorption and elution of proteins, and particularly of enzymes, have been subjects for study for many years.[2,3] While proteins of relatively low molecular weight and high isoelectric point have recently been chromatographed successfully,[4-8] proteins of high molecular weight have been submitted to chromatographic investigation with less striking success. Shepard and Tiselius[9] introduced the technique of "salting-out adsorption," whereby serum proteins were adsorbed on silica gel. Frontal analysis of hemoglobin, bovine plasma albumin and egg proteins on cation-exchange resin Dowex-50 has been reported by Sober, Kegeles and Gutter.[10] Although in the past Hyflo Super-Cel usually has been considered as a rather poor adsorbent,[11] celite products have been used in several instances to fractionate protein mixtures.[12-18]

In the present work, adsorption and elution analyses of hypophyseal growth hormone and several other proteins on Hyflo Super-Cel were performed. The separation of these proteins was carried out with both continuous and discontinuous pH gradient techniques.

Experimental

Protein Preparations.—The growth hormone preparations were isolated from ox anterior pituitary glands by the methods previously described[19-21]; they had been shown to behave as homogeneous substances according to electrophoretic, sedimentation and diffusion studies[19,21] and have recently been found to be essentially homogeneous by zone electrophoresis on starch[22] and end group analysis.[23] Growth-promoting activity of the hormone was estimated by the tibia test in hypophysectomized rats.[24]

The ACTH fraction employed was a peptic digest of a concentrate prepared from sheep glands, obtained by procedures previously described.[25] It possessed an activity of approximately 50 I.U. per mg. The crystalline proteins, bovine serum albumin, ribonuclease and ovalbumin were commercial products; the two former preparations were obtained from Armour and Co., the latter from the Worthington Biochemical Laboratories. The methyl ester of bovine serum albumin was kindly prepared for us by Dr. I. I. Geschwind by the acid methanol technique.[26]

Adsorbents.—Among the various celite products[27] investigated, Hyflo Super-Cel was found the most suitable for the present investigation. Before use, the adsorbent (200 g.) was pre-treated by washing twice in 6 hours with approximately 1 liter of 2 N HCl, filtered on a büchner funnel, washed with 1 liter of distilled water, suspended once more in 1 liter of distilled water and re-filtered twice. This procedure of washing with distilled water was repeated until the acidic reaction of the eluate disappeared, usually after 3 or 4 washings. Then the Hyflo Super-Cel was oven dried

(1) Rockefeller Foundation Fellow. Present address: Laboratoire de Chimie biologique de la Faculté des Sciences, Paris, France.

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(27) Johns-Manville Co., Philadelphia, Pa.

at 110°, pulverized with a stirring rod and kept in a stoppered bottle.

It was found that washing with acid was necessary in order to ensure an effective adsorptive capacity on the part of Hyflo Super-Cel for proteins. Once it has been acid-washed, the adsorbent remains active; on the other hand, if the Hyflo Super-Cel was pre-washed with a buffer of *p*H 5.3 instead of with 2 *N* HCl, there resulted an almost complete loss of adsorptive capacity for growth hormone. Pre-washing with 0.10 *M* Na_2CO_3 also had the effect of destroying the adsorptive capacity.

It should be pointed out that certain batches of Hyflo Super-Cel exhibited an adsorptive capacity for growth hormone which was less than others. In the case of the less adsorptive batches there occurred a leaking out of the protein with the "hold-up" volume of the column. This could not be prevented by any of the pre-treatments which were tried, such as shortening or lengthening the washing time, refluxing with 2 *N* HCl, changing the acid to 1 *N* HCl, drying at room temperature instead of oven-drying, etc. Decreasing the amount of protein put on the column proved to be equally ineffective in preventing leakage.

Buffers.—Buffers comprising a very extensive range of *p*H, from 3 to 11.2, were used. For proteins like growth hormone whose solubilities in buffers of low ionic strength are low, different amounts of $(NH_4)_2SO_4$ were added to these buffers. Arranged according to ascending *p*H the main buffers used can be listed as follows: (1) phosphate buffers of *p*H 3.0 and 4.0 made by mixing 0.6 *M* KH_2PO_4 with the required amount of 0.6 *M* H_3PO_4. (2) 150 ml. of 0.1 *M* KH_2PO_4 mixed with 20 ml. of SAS[28] and adjusted to either *p*H 3.0 or 4.0 with 0.1 *M* H_3PO_4. (3) 100 ml. of acetate buffer of *p*H 3.7, 0.1 ionic strength, mixed with 50 ml. of distilled water and 20 ml. of SAS, with a resulting *p*H of 3.6. (4) 100 ml. of citrate buffer at *p*H 5.5, 0.1 ionic strength, mixed with 50 ml. of distilled water and 20 ml. of SAS, with a resulting *p*H of 5.2. (5) 100 ml. of Na phosphate buffer at *p*H 5.5, 0.1 ionic strength, mixed with 50 ml. of distilled water and 20 ml. of SAS, with a resulting *p*H of 5.1. (6) 100 ml. of K phosphate buffer at *p*H 7.1, 0.1 ionic strength, mixed with 50 ml. of distilled water and 20 ml. of SAS, with a resulting *p*H of 6.6. (7) 100 ml. of borate buffer at *p*H 9.35, 0.1 ionic strength, mixed with 50 ml. of distilled water and 20 ml. of SAS, with a resulting *p*H of 8.1. (8) A mixture of 120 ml. of 0.2 *M* K_2HPO_4, 30 ml. of 0.1 *M* K_3PO_4, and 20 ml. of SAS, with a resulting *p*H of 8.0. (9) A mixture of 150 ml. of 0.1 *M* Na_2CO_3 and 20 ml. of SAS, with a resulting *p*H of 8.6.

Continuous Gradient Elution Device.—A device which facilitates adjustment of the buffers in continuously increasing *p*H for the elution procedure was employed. An erlenmeyer flask was provided with an elbow of glass tubing connecting it with the top of the column. This flask contains a measured amount of any buffer *a* with which the elution is to be started. A separatory funnel is fixed in a rubber stopper fitted into the top of the flask; the funnel contains the second buffer *b*, which by admixture with buffer *a* produces the *p*H gradient. In this way, the rate of flow from the funnel is controlled by the rate of flow of the column itself. Adequate stirring was ensured by a glass-coated magnetic stirrer moved by the usual magnetic stirring apparatus under the flask.

Preparation of the Column.—Columns of three sizes were employed: (1) A 25-ml. buret with standard ground stopcock. These columns were operated with 6 g. of adsorbent. (2) A glass tube, fitted with a sintered glass disk, with an internal diameter of 23 mm. and an average length of 35 cm. These columns were operated with 30 g. of adsorbent. (3) Same type of column, but with an internal diameter of 31 mm., and an average length of 35 cm. These columns operate with 60 g. of adsorbent.

After the desired amount of adsorbent had been weighed out, it was mixed with a small quantity of the buffer in which the adsorption was to take place, and thoroughly homogenized with a stirring rod. More buffer was added to form a slurry mixture before introduction into the column. When the buret was used, a glass wool plug was placed in the bottom of the column to prevent leaking of the adsorbent. The column was then washed with the same buffer for 5–6 hours. The columns were then put into the cold room where all experiments were run at a temperature of 2–3°.

(28) Saturated $(NH_4)_2SO_4$ will be designated throughout as SAS.

The proteins to be chromatographed were dissolved in 1–2 ml. of the initial buffer. With some proteins this is a relatively simple procedure, but with growth hormone, whose solubility is rather low, it proved necessary first to dissolve the protein, with the aid of one or two drops of 0.1 *M* NaOH, in a small amount of water, and then to add to this solution the more concentrated buffer in an amount sufficient to achieve the desired final concentration. The rate of free flow from the column was approximately 4 ml. per hour. The fractions, which were collected in a Misco[29] time-fraction collector, were read in a Beckman spectrophotometer at 275 mμ. The protein content of each fraction was estimated from a standard curve. In some cases, micro-Kjeldahl analysis for nitrogen was carried out on suitable samples after dialysis in the cold to remove any $(NH_4)_2SO_4$ which might be present.

Results

Chromatography of Growth Hormone.—When growth hormone was submitted to chromatography according to the procedures outlined above, the buffers used were usually either no. 4 citrate buffer, or no. 5 phosphate buffer; the capacity of the column was found to be about 2 mg. per g. of adsorbent. When the column was not overloaded, no leaking out of the hormone occurred and no protein could be eluted with the same buffer from which it had been adsorbed, even when the eluant was run up to 10 times the hold-up volume of the column. When a buffer of *p*H 3 is used, growth hormone is still completely adsorbed, but elution under the usual conditions is no longer possible. Elution, in this case, could be effected only at high *p*H's, for instance, with 0.1 *M* Na_2CO_3. The recovered protein no longer possessed growth-promoting activity, indicating that denaturation had taken place; this is to be expected, since it is well-known that growth hormone is denatured at low *p*H's.[30] Above *p*H 5.5, complete adsorption was no longer obtained, and at *p*H 6.4 almost all of the protein appeared with the hold-up volume.

When the hormone was adsorbed onto the column at *p*H 5.1–5.2, and the *p*H was shifted to values higher than the isoelectric point of the growth hormone (*p*H 6.85), the protein hormone was eluted and appeared as a single peak. It will be noted in Table I (exp. 4) that no significant change in biological activity occurred as compared with the starting material, when 8.5 mg. of the hormone in a column made up with 6 g. of Hyflo Super-Cel was eluted with No. 7 borate buffer. These results were duplicated in numerous experiments which yielded consistently the same pattern, provided that the *p*H of the final buffer and the approximate salt concentration were kept constant.

A characteristic example of an experiment in which continuous gradient elution was employed is presented in Fig. 1. A medium-sized column containing 30 g. of Hyflo Super-Cel was used; the starting buffer, as in the experiment described above with a discontinuous gradient, was no. 4, citrate buffer. After 47 mg. of the protein hormone had been introduced onto the column, the no. 4 buffer was then run for 6 hours. At the end of this time, the device for continuous gradient elution, as described above, was fitted onto the column. 150 ml. of the no. 4 buffer was put into the erlenmeyer flask; the second buffer was no. 9. As the *p*H gradually shifted, a sharp peak representing the protein appeared in the *p*H range between 6.8 and 8.1, reaching its maximum at 7.5.

The main peak was divided into 2 fractions and assayed in hypophysectomized rats. It may be seen in Table I (exp. 12) that 85% of the material appeared in the main peak, and that the activity of both shoulders was not significantly different from that of the starting material.[31] As in the case of the experiments performed with a discontinuous gradient, the nature of the adsorbing buffer was found to be unimportant provided that the *p*H and ionic strength were consistent. In all these experiments, no protein appeared in the effluent before the *p*H had reached the isoelectric point of the hormone. The maximal elution

(29) Manufactured by Microchemical Specialties, Berkeley, California.

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(31) Although in this particular case, the activity of the right side seemed to be slightly higher than that of the left, it should be emphasized that in other experiments the reverse was true. Hence, this slight difference must be attributed to the uncertainty of biological assay.

TABLE I

BIOASSAY OF SOMATOTROPIN BEFORE AND AFTER ADSORPTION CHROMATOGRAPHY

Experiment no.	Diameter of the column, cm.	Amount of hormone employed, mg.	Material recovered, %	Bioassay by the tibia test[a] Before chromatography No. of rats	Tibia width, μ	After recovery from column No. of rats	Tibia width, μ
4[b]	1	8.5	80	7	211.8 ± 2.9[c]	5	206.8 ± 4.3
5	1	6.6	91	7	216.1 ± 4.2	6	217.8 ± 2.7
7	1	5.4	88	7	225.2 ± 5.3	7	224.4 ± 1.7
12[d]	2.3	47.0	84	6	233.5 ± 2.6	I: 6	225.5 ± 3.7
						II: 5	242.5 ± 1.7
14–15	3.1	84.0	78	12	234 ± 2.0	15	237 ± 1.7
16	3.1	90.0	75	12	224 ± 3.5	11	226.3 ± 2.8
17	3.1	84.0	68	12	224 ± 3.5	12	223.5 ± 2.7
19	2.3	33.6[e]	81	6	230 ± 3.4	7	226 ± 5.8

[a] Animals given a total dose of 80 μg. in 4 days. Those receiving saline had an average tibia width of 155 μ. [b] This experiment was carried out with a discontinuous *p*H gradient; all others were conducted by the continuous gradient procedure. [c] Mean ± standard error. [d] In this experiment, the peak was divided into two fractions: I represents the left portion, and II, the right. Each fraction was assayed separately. [e] The hormone was recovered from experiment no. 17.

always took place between *p*H 7.5 and 7.7. When the biological activity of the fractions obtained was compared with that of the starting material, no significant difference could be detected (Table I).

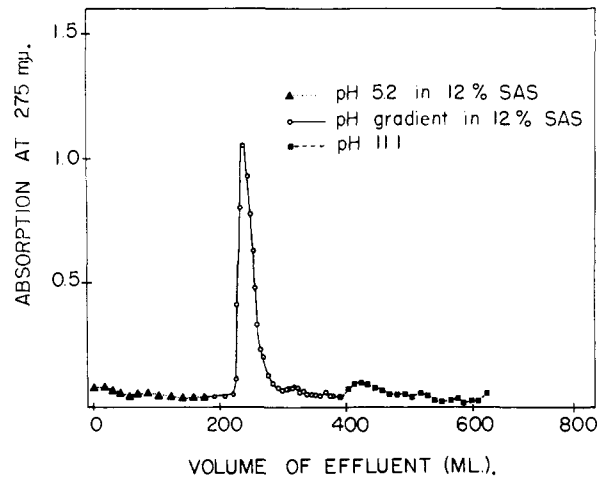

Fig. 1.—Adsorption and elution of growth hormone (47 mg.) on a 30-g. column, with a continuous *p*H gradient.

The salt concentration could not be varied greatly while still maintaining a satisfactory solubility of the hormone. The above experiments were carried out at a concentration of 12% SAS. No striking difference in adsorption and elution was noted when a similar experiment was carried out at a concentration of 5% SAS.

Several experiments were carried out in order to determine whether the hormone recovered from the column would manifest the same behavior if it were submitted to rechromatography. Figure 2 gives the pattern obtained with a 30-g. column. The main peak contained 81% of the initial material, and, as shown in Table I (exp. 19), possessed the same specific biological activity. It should be noted that a small ultraviolet-absorbing peak appeared with the hold-up volume of the column. This peak appeared consistently when growth hormone was rechromatographed. Its ultraviolet-absorption curve did not manifest the characteristics of a protein, indicating that this peak represented an impurity in the adsorbent, accumulated from the first column.

In all the experiments with growth hormone, it was found that the yield in the main peak was never quantitative, averaging 85%, with about 10% of the protein being eluted at a very high *p*H with 0.1 *M* Na_2CO_3. When this fraction was collected, dialyzed and assayed, it was found to be inactive. Nevertheless, it is very unlikely that it corresponds to some inert protein since it still appeared when the hormone was rechromatographed, and, if it were an impurity, it should have been removed in the course of the first chromatography. It appears reasonable to assume that this fraction represents the denatured hormone protein.

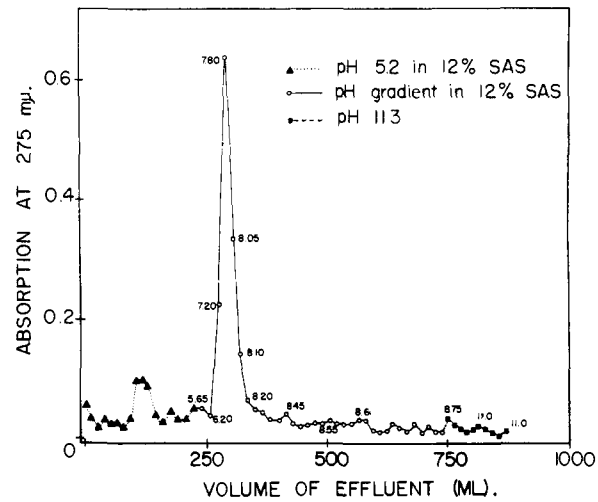

Fig. 2.—Rechromatography of growth hormone (33.6 mg.) on a 30-g. column with a continuous *p*H gradient. Figures along the curve represent the *p*H values of the effluent.

Chromatography of Other Proteins. 1. **Bovine Serum Albumin.**—The crystalline albumin (BSA) was adsorbed easily on Hyflo Super-Cel in buffers at a *p*H between 3 and 4. In a typical experiment a Hyflo Super-Cel column of 6 g. was equilibrated with a no. 2 phosphate buffer at *p*H 3.2 in 2% SAS. 8.6 mg. of BSA in 3.0 ml. of the buffer solution was introduced onto the column. No protein appeared until a *p*H gradient was instituted. As Fig. 3 indicates, the main peak began to appear at *p*H 4.5, reaching a maximum concentration at *p*H 5.6, and trailing off up to *p*H 7.15. After this point a small peak (10%) of irreversibly adsorbed material (denatured protein) could be eluted with 0.10 *M* sodium carbonate. The main peak, on the basis of ultraviolet absorption, corresponded to 81% of the protein placed on the column.

The elution of BSA tended to be less sharp than that of growth hormone, with the protein tending to distribute itself over a wider range of *p*H. Just as with growth hormone, the point in the *p*H gradient at which the albumin appeared coincided with its isoelectric point (*p*H 4.8). The nature of the buffer was not found to be important for adsorption of this protein as long as *p*H and ionic strength remained constant; however, it did play a part in determining the slope of the *p*H gradient and, as a consequence, the slope of the curve of the peak. The best results were obtained with the phosphate buffers, since their maximum buffering capacity is situated between *p*H 5 and 8, which made the slope of the gradient rather linear in this range.

In the series of experiments designed to test the effects of salt concentration on the adsorption and elution of BSA, the details of which are not reported here, it was found that when the *p*H was kept constant, varying the SAS concen-

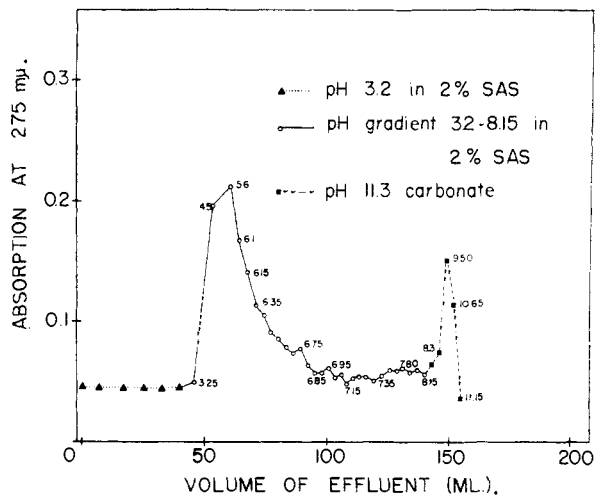

Fig. 3.—Adsorption and elution of bovine serum albumin on a 6-g. column with a continuous *p*H gradient.

tration from 0–25% had only minimal effects, such as a slight sharpening of the elution peak, but it did not improve or impair the adsorption at *p*H 3, nor did it affect the point at which the protein appeared in the eluate, or improve the total recovery of the protein.

2. **Esterified Bovine Serum Albumin.**—BSA esterified with methanol was completely adsorbed onto the column when a *p*H 3.7 acetate buffer was used. No elution of material could be obtained with either buffer no. 6 or buffer no. 7. This could be anticipated, as the isoelectric point of methylated BSA is considerably higher than *p*H 7.[32] However, complete elution of this protein was obtained with 0.1 *M* Na_2CO_3, the main peak yielding 99.3% of the original material.

3. **Ovalbumin and Other Proteins.**—No consistent adsorption of ovalbumin could be obtained under the conditions that were successful with BSA; most of the material came out in a slightly trailing peak with the hold-up volume. The yield of this trailing peak was not quantitative (70–75%) indicating that some of the protein had been either adsorbed or denatured. The residual material could be eluted with 0.1 *M* Na_2CO_3 and appeared to behave as a denatured protein.

No adsorption whatsoever of proteins having a molecular weight of less than 20,000 could be achieved under the conditions described in the above experiments. When adsorption of an ACTH preparation was attempted on a Hyflo Super-Cel column with no. 4 citrate buffer at *p*H 5.2, 90% of the material came out with the hold-up volume of the column. If this protein had behaved like the large proteins, it should have been adsorbed, since its isoelectric point was shown to be much higher than the *p*H of the buffer.[25] Similarly, when crystalline ribonuclease was introduced onto the column with a no. 1 phosphate buffer at *p*H 3.2, to which 5% SAS had been added, the protein came out completely with the hold-up volume of the column.

Separation of Artificial Mixtures of Proteins.—On the basis of the experiments outlined above, it seemed of interest to find out whether the separation of protein mixtures could be achieved on the column.

1. **Growth Hormone and ACTH.**—5.6 mg. of an ACTH preparation was dissolved in 3.4 ml. of citrate buffer no. 4, and 10.6 mg. of growth hormone was dissolved in 3.4 ml. of the same buffer. 3.2 ml. of ACTH solution and 1.6 ml. of growth hormone solution were mixed and transferred quantitatively to a previously equilibrated 6-g. Hyflo Super-Cel column. After the column had been run with about 40 ml. of citrate buffer, a discontinuous gradient was instituted. The results are shown in Fig. 4. The first peak which came out with the hold-up volume of the column represented unadsorbed ACTH in a yield of 90% of the original material on the basis of optical density. The second peak contained 96% of the growth hormone introduced onto the column. When these two peaks were assayed, no growth-promoting activity was found in the first peak, and scarcely detectable ACTH activity in the second peak. The ACTH activity of the first peak and the growth-promoting activity of the second peak, when compared with the activity of the original preparations, were unchanged.

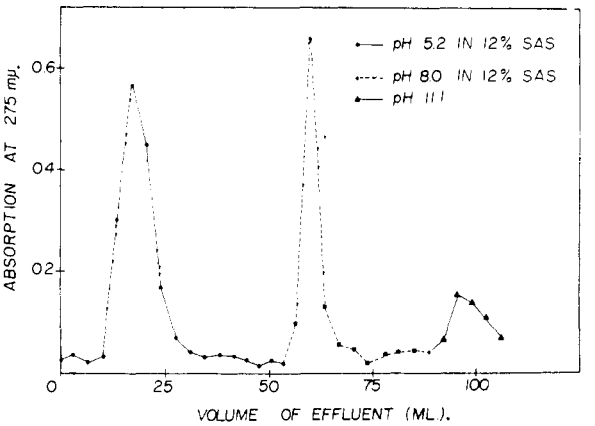

Fig. 4.—Separation of ACTH (5.3 mg.) and growth hormone (5.0 mg.) in a 6-g. column with a discontinuous *p*H gradient: the first peak ACTH; the second peak, growth hormone.

2. **BSA, Ovalbumin and Methylated BSA.**—It was found that separation of these three proteins could be accomplished easily from an initial *p*H of 3 or 4. Ovalbumin is not adsorbed, whereas BSA and methylated BSA are both adsorbed onto the column; BSA is subsequently eluted, when a *p*H gradient is applied, within the *p*H range of from 4.8 to 7; and methylated BSA is not eluted until 0.1 *M* Na_2CO_3 is applied. In a typical experiment, 4.3 mg. of ovalbumin, 4.2 mg. of methylated BSA and 4.3 mg. of BSA were dissolved in phosphate buffer no. 1 at *p*H 3.0 to which 5% SAS had been added, and transferred quantitatively to the column. After it had been run with about 50 ml. of the same buffer, a *p*H gradient was set up with phosphate buffer no. 8. Finally the column was washed with 0.1 *M* Na_2CO_3. The chromatographic pattern may be seen in Fig. 5. The first peak coincides with the location where the ovalbumin is found when it is chromatographed alone; the second appears at the point where BSA is usually eluted, and possesses its characteristic shape. The third peak is eluted at high *p*H's where methylated BSA generally appears. The yield of the first two peaks was not quantitative, as might have

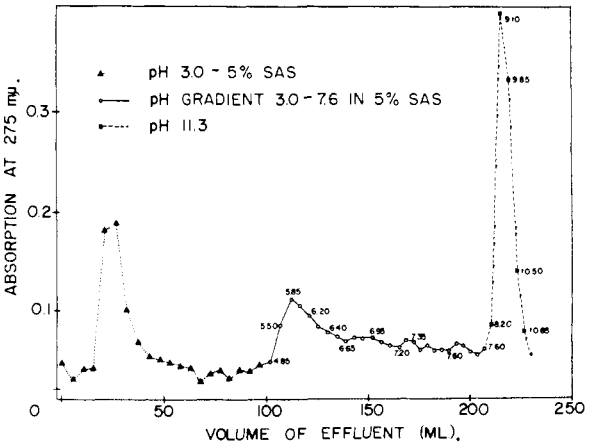

Fig. 5.—Separation of ovalbumin (4.3 mg.), BSA (4.3 mg.) and esterified BSA (4.2 mg.) in a 6-g. column with a discontinuous *p*H gradient; figures along the curve represent the *p*H values of the effluent; the first peak, ovalbumin; the second peak, BSA; the third peak, methylated BSA.

(32) H. A. Saroff, N. R. Rosenthal, E. R. Adamik, N. Hages and H. A. Scheraga, *J. Biol. Chem.*, **205**, 255 (1953).

been expected from previous results. The first peak accounted for 75% of the ovalbumin, and the second peak for 80% of the BSA, while the third peak contained also the denatured ovalbumin and BSA in addition to methylated BSA. The total yield of the three peaks matches very closely the total amount of the material initially introduced onto the column.

3. Growth Hormone and BSA.—After preliminary investigations where a separation of growth hormone and BSA was effected with a buffer of *p*H 5.2, which adsorbs the former but not the latter, the following experiment was undertaken wherein both proteins are adsorbed and subsequently eluted separately.

A 6-g. Hyflo Super-Cel column was equilibrated with buffer no. 1 at *p*H 4.0, containing 5% SAS; 4.6 mg. of BSA and 3.8 mg. of somatotropin was then introduced onto the column. A *p*H gradient was instituted with buffer no. 9. Results shown in Fig. 6 indicate two distinct peaks whose maxima coincide with that of both proteins individually. There is total recovery of material in both peaks, and the first peak, corresponding to BSA, was found to be biologically inactive while the second peak possessed the growth-promoting activity, when they were assayed in hypophysectomized rats.

Discussion

Among various proteins investigated, bovine serum albumin, methylated BSA and growth hormone were found to be adsorbed on the Hyflo Super-Cel column at a *p*H below the isoelectric point of these proteins.[33] Others, like ovalbumin, ribonuclease and ACTH preparations, were not adsorbed, whatever the *p*H of the initial buffer. Those proteins which were adsorbed onto the column could be eluted in yields varying from 80–90%, with no indications of extensive denaturation when the *p*H was shifted to values above their isoelectric point.

The observations from these experiments and previous work, to the effect that proteins which are adsorbed below the isoelectric point can be eluted by increasing the *p*H, suggests that an ion-exchange mechanism may be postulated for the observed elution analyses. This view is further supported by the fact that washing with a strong acid is necessary to get the maximal adsorption, as emphasized above. Another type of adsorption, like salting-out adsorption,[9,13,14] may also be operative on this type of column.[34]

However, there are certain observations which might seem to contradict the concept of ion exchange as the mechanism whereby the column operates; for example, if ion exchange were operating, one would expect a lessening of adsorption when the *p*H was lowered to the acid range. However, in our experiments, adsorption of growth hormone was found to be as good at *p*H 4 as at *p*H 5.2, and the adsorbability of BSA was the same at *p*H 3 as at *p*H 4. This seems to indicate that it is not the actual charge of the adsorbent but the net charge of the protein which is the important factor in this behavior. This same anomaly was recently observed by Boardman and Partridge[35] on ion-exchange resins, and tentatively explained by these investigators on the hypothesis that at low *p*H's short range adsorptive forces, which keep the protein from eluting, are operative between the adsorbent and the protein, instead of the electrostatic bonds which ordinarily obtain just below the isoelectric point. This hypothesis may also explain the observation that Hyflo Super-Cel is incapable of adsorbing ovalbumin, ribonuclease and ACTH, even at a *p*H far below their isoelectric points, owing to the lack of short-range forces on the part of these proteins when they are adsorbed on Hyflo Super-Cel.

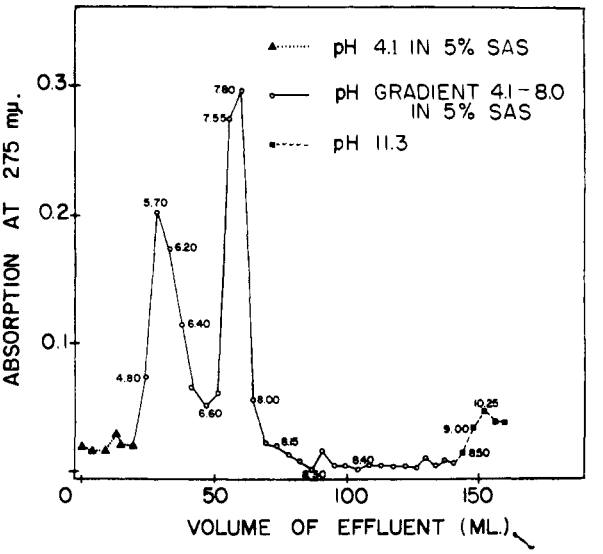

Fig. 6.—Separation of growth hormone (3.8 mg.) and BSA (4.7 mg.) with a continuous *p*H gradient on a 6-g. column; figures along the curve represent the *p*H values of the effluent; the first peak, BSA; the second peak, growth hormone.

Whatever the mechanism of adsorption may be, it is clear that a satisfactory separation of certain proteins can be achieved on a column employing Hyflo Super-Cel as the adsorbent. Furthermore, no extensive denaturation occurs when the protein is adsorbed and eluted from the column. It is particularly interesting to note that the recovered somatotropin had the same growth-promoting potency as the unadsorbed hormone, since it is well-known that the hormone is very labile with respect to denaturation.[30] As it may be seen in Figs. 1 and 2, and in Table I, no fractionation of the hormone protein or separation of biological activity from the protein occurs in these adsorption experiments with somatotropin. This might be taken as additional evidence for the purity of the hormone protein.

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(33) A recent study by A. D. McLaren (private communication) on the absorption of proteins on Kaolinite, has yielded similar conclusions. However, not all the proteins which were found to be adsorbed on Kaolinite could be adsorbed on Hyflo Super-Cel.

(34) This mechanism appears to be unlikely, since with somatotropin and BSA, particularly the latter, varying the salt concentration has no effect on the adsorption and elution phenomena other than a slight sharpening of the elution peak at higher salt concentrations. As a matter of fact, this sharpening of the elution peak gives support of itself to the theory of an ion exchange as the operating mechanism of the column.

(35) N. K. Boardman and S. M. Partridge, *Nature*, **171**, 208 (1953).