

SOME CONTRIBUTIONS TO THE CHEMISTRY OF MUCOID.

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In Physiological Chemistry, one meets with a proteid that has been receiving more or less attention for several years past. This proteid is classed among the glyco-albumins and occurs especially in ligaments and tendons. Heretofore, the main problem connected with the chemistry of this substance or group of very closely related substances, as the case may be, has been the quantitative separation of the mucoid from mixtures of albumin such as blood and egg albumin, and mucoid. The usual method of separation has been to boil the neutral solution of true albumin and mucoid, thereby coagulating the blood or egg albumin and leaving the mucoid in the filtrate. By acidifying the filtrate, it yielded the mucoid as a flocculent precipitate which could be filtered and then weighed.

The purpose of this work was to ascertain first, whether mucoid was completely precipitated by the addition of a slight excess of dilute acid to the mucoid solution (the solvent being half-saturated lime water). Secondly, we wished to find out whether albumins were precipitated from a mixture of albumin and mucoid, by acidifying the mixture in the cold. Thirdly, we wished to ascertain whether mucoid coagulated by being boiled in a neutral solution in the presence of neutral salts. And lastly, we wished to see whether the various precipitations of the mucoid sample showed any differences in nitrogen content; in other words we desired to examine the homogeneity of the various acid precipitates.

The mucoid used was from several beef tendons (Achilles), and was prepared by removing all water-soluble proteids by careful washing of the tendons in tap water. The tendons were then cut into thin slices transversely and again thoroughly washed with cold water. The next treatment was to allow the slices of tendon to extract with half-saturated lime water for a day. This extract was filtered and made slightly acid with .2 per cent. hydrochloric acid, using litmus paper as indicator. The solution, with a casein-like precipitate was allowed to stand a short time when the precipitate flocked together and settled to the bottom of the container, leaving a perfectly clear supernatant liquid.

The filtered residue was dissolved in half-saturated lime water, filtered through silk and again precipitated with an excess of .2 per cent. HCl. This precipitation and solution alternation was continued until the eighth precipitation, when this precipitate was filtered by decantation and the residue was thoroughly dried by standing with absolute alcohol. The powdery white precipitate was carefully filtered and pulverized, then further dried at 100-105° C. for several hours. The bottled sample so obtained was used in this set of experiments.

In the study of the complete precipitability of tendon-mucoid by means of dilute HCl, a definite amount (2 grams) of the dried sample was weighed and dissolved in a mortar with the least quantity of half-saturated lime water necessary, about 300cc. The solution was then filtered through silk and by means of a pipette, equal portions of the filtrate were removed to respective beakers and were precipitated by varying amounts of acid. This phase of the acid precipitation was subdivided into a study of the effect of dilution of the mucoid and the effect of the use of varying amounts of acid. In each case duplicate checks were carried along on the amount of actual mucoid present and precipitable under the most favorable conditions. In every instance the mucoid precipitated by the acid was filtered on weighed papers, dried at 105° C. for several hours and weighed on the paper. The paper and mucoid were then ashed and the ash deducted from the original weight on the paper. The acid filtrates, usually about 250cc. in volume, were poured into about five liters of strong alcohol, allowed to stand 24-36 hours and filtered on weighed papers. The precipitates were washed with strong alcohol, dried and weighed; the precipitates and papers were burned respectively, and the ash, ranging from a few tenths of a milligram to a hundred milligrams, was deducted in order to get a value for ash-free mucoid material.

As results of an extended investigation of the deportment of mucoid in a half-saturated lime water solution, with .2 per cent. HCl, it was found that not all the mucoid was precipitated under the best conditions. There was always 10 to 20 per cent. of the mucoid precipitated by the strong alcohol treatment and part, perhaps 8 to 10 per cent. of the original mucoid, was not precipitated by the acid nor alcohol treatment. It was found that the more concentrated the solution of mucoid, the more complete was the precipitation. The weaker the final acidity of the solution was with .2 per cent. HCl, the less complete the precipitation. The best results were obtained with a half-saturated lime water solution saturated with the

mucoid sample (about a 2 per cent. solution), the mixture diluted slightly and made acid enough to turn fresh litmus paper red immediately. This treatment yielded a solution that filtered readily (an item of vast importance with mucoid and other gelatinous solutions), that washed rapidly and gave a filtrate yielding the minimum quantity of mucoid with strong alcohol treatment. With the use of acetic acid instead of HCl, much more acid was required to precipitate any mucoid at all, and with an excess of acetic acid the results were very unsatisfactory, much more than with even a little dilute HCl.

In testing the action of dilute acid on a mixture of albumin and mucoid, the details were as follows: A standard solution of mucoid was made and equal amounts of the alkaline solution were placed in each of several beakers. The actual amount of mucoid added to each beaker was determined by duplicate checks. To the various beakers containing equal amounts of mucoid diluted to the same volume, varying amounts of meat extract were added. The meat extract was made in the laboratory by extracting fresh meat with cold water and filtering the proteid-bearing solution through silk. Duplicates were run on the meat extract and also on each of the mixtures of meat extract and mucoid. For precipitation, the same acidity was maintained in each beaker using .2 per cent. HCl as the reagent.

By way of results, although the meat extract alone yielded no precipitate in the cold, it was found that when mixed with mucoid, practically all the mucoid and some of the albumin separated. With increased non-mucoid proteid content, the weight of material precipitated by .2 per cent. HCl likewise increased. In fact, all the precipitates from the meat extract-mucoid mixture weighed more than the amount of mucoid which the solution was known to contain. All the precipitations were made in duplicate and found to check closely with each other, and each set of duplicates in the series varied approximately the same. By knowing the position of the set of duplicates in the series, one could closely approximate the actual weight before weighing. The experiments showed that the precipitation of mucoid in the presence of albumins was inaccurate for the determination of mucoid.

With the coagulation test for mucoid, the general opinion is that mucoid does not coagulate on boiling a neutral solution in the presence of salts. To test this, a solution of mucoid was prepared by rubbing up about 10 grams mucoid in a mortar with about a liter of half-saturated

lime water. When complete solution was attained, the alkaline solution was carefully neutralized with .2 per cent. HCl. The neutral point was determined by litmus paper that was fresh and quite sensitive. No mucoid precipitated in the neutral solution, but it was strained through silk for the sake of uniformity of conditions. Duplicate samples were taken to determine the mucoid content before heating. The major portion of the solution was gently boiled under a water condenser. Every half hour, about 100 cc. of the solution was removed, allowed to cool rapidly without any loss of water vapor, filtered and duplicate aliquot portions of the filtrate were used for acid precipitation of the mucoid content.

By way of results, it was noticed that continued boiling had a fatal effect on the mucoid. At first the solution became turbid. At the end of the first half hour's heating, there was a nice coagulum in the solution. This increased gradually until about the fourth hour, when there was a heavy coagulum throughout the solution. During the process of continued heating, the solution remained neutral without the addition of any alkali or acid. The longer the heating continued, the more rapidly the solution filtered. The first few filtrates were very slightly turbid, but the turbidity gradually decreased to water-clearness in the last few filtrates.

With regard to the filtrates, on treatment with dilute acid it was found that with the initial precipitations, less mucoid was recoverable than was obtained from the unboiled mucoid solution. The amount of mucoid precipitated gradually decreased as the experiment advanced and finally filtrates were obtained from which no mucoid could be precipitated with an excess of dilute acid. This was coincident with the heavy coagulum in the major portion of the solution. This experiment seemed to show conclusively that mucoid did coagulate on heating in the presence of neutral salts. It was deemed useless to try to separate a coagulable albumin from mucoid by this method.

The work done in this research was carried out, using a mucoid sample that had been purified by solution in alkali and precipitation by acid, this alternation for eight times. To test whether the eighth precipitate might be different from the sixth or tenth precipitate, or any other precipitate in the series, about 20 grams dry mucoid that had been precipitated probably twice, were dissolved in several liters of half-saturated lime water, strained through silk and precipitated with a slight excess of .2 per cent. HCl. This was filtered, dissolved and precipitated, the whole process being repeated fifteen times. Samples of the fifth, tenth and fifteenth precipitate were re-

moved, partly dried by standing in absolute alcohol, filtered and dried in an oven at 105° C. Duplicate Kjeldahl nitrogen determinations were made on each of the respective precipitates. Each of the six duplicates were found to check quite closely, thus indicating that there was nothing gained by the continued solution and precipitation of the mucoid. Incidentally it might be mentioned that there was mucoid lost at each precipitation by incomplete precipitation. This was evident from the fact that by most careful work, starting with 20 grams mucoid, it was only possible to wind up with about 13 grams actual dry mucoid.

In conclusion, it may be stated that tendon mucoid coagulates, the amount increasing with the duration of boiling, in the presence of neutral salts; that mucoid is not completely precipitated by an excess of dilute acid; that in a mixture of albumin and mucoid, most of the mucoid and part of the albumin are precipitated by dilute hydrochloric acid in excess.

As for a remedy, nothing is offered as yet, but this work seems to show that the older methods are inaccurate.

This work was carried on largely during the last year in the laboratories of the College of Physicians and Surgeons, Columbia University, New York, where the author was associated with Dr. William J. Gies, and under whose supervision the details were worked out. Owing to the non-possession of the actual notes at present, many valuable data must be left out of this paper, but every statement made can be further demonstrated by experimental data.

