A CHEMICAL STUDY OF YELLOW ELASTIC CONNECTIVE TISSUE

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A Chemical Study of Yellow Elastic Connective Tissue.

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I. INTRODUCTION.

In order to comprehend the physiological function of any tissue, the relation which it bears to any other or to the organism of which it forms a part, and to recognize the changes which are associated with its life and growth, accurate information concerning its chemical composition is necessary. The group of connective tissues forms a class the members of which are related both as regards their mode of origin and their constituent elements. They resemble each other also in that the physical character of each is dependent on the presence in largely predominating amount of one particular constituent, usually albuminoid in nature. Until recently these tissues have been studied principally with reference to this preponderating component. Deductions regarding many of the other substances which might be associated with it have been made from inference rather than observation.

Less than any of the class has the yellow elastic tissue been studied except with reference to its main constituent, elastin. Concerning other proteid substances or those which might arise from the metabolism of proteid only the most indefinite statements are to be found. A few preliminary tests, however, have served to show us that the amount of simple (coagulable) proteid and mucoid obtainable from the ligamentum nuchae of the ox was more than would be expected from an extravascular tissue having a purely mechanical function.

With these considerations in mind we have thought it a matter of importance to subject this form of connective tissue to closer study in the hope that information gained regarding substances not already specifically investigated might furnish a rational basis for the comparison of this with other tissues, and might lead to the development of more logical methods of research into those constituents of which much is already known; and further, that a

combination of such details might throw more light on the general metabolism of the tissue in question.

In all of our investigations the source of the yellow elastic tissue has been the ligamentum nuchae of the ox.

II. COAGULABLE PROTEIDS.

At the beginning of this work we were surprised by the large amount of coagulable proteid which could be separated from the fresh ligament. If the water or saline extracts of the tissue are boiled after the addition of a trace of acetic acid, an abundant flocculent coagulum is obtained, having all the characteristics of coagulated proteid. In two determinations of the amount which could be obtained from the cleaned tissue by extraction with water we found that 0.64 per cent. of the fresh tissue or 1.93 per cent. of the dry tissue existed in this form.[50]

In order to determine, if possible, the number of these proteids we have made use of the method of fractional heat coagulation. Several extracts of the tissue were made, both aqueous and saline. 5 per cent. magnesium sulphate was most generally used for the latter type.

The method of extracting the tissue was as follows. Only such portions of the ligament as appeared free from blood were used. After a thorough cleaning of all extraneous matter—fat and connective tissue membranes—the ligaments were cut into narrow longitudinal strips and washed in cold running water for from twelve to twenty-four hours. They were then partially dried with a towel, and run through a meat chopper several times. The finely minced substance was then treated with enough fluid to cover it. At the end of twelve to twenty-four hours the fluid was strained through cloth and filtered through paper. Spectroscopic examination showed the absence of haemoglobin in every extract—an observation which implies the absence of most of the lymph proteids as well. Each fluid thus obtained became very turbid on heating, and on the addition of a drop of dilute acetic acid a flocculent coagulum separated.

In determining the temperatures of coagulation the process described by Gamgee[18] was employed—from 20-40 cc. of the extract, made faintly acid with acetic acid, being taken for each series of

determinations. The temperature was raised very gradually, never more than one degree in two minutes. When turbidity appeared the source of heat was immediately removed and the temperature held constant or raised very slowly until the turbidity had developed into a flocculent coagulum. When the separation had become distinct, usually after about half an hour had elapsed, the fluid was filtered. The filtrate in each case was as clear as water. The filtered fluid was then subjected to further heating until turbidity again ensued. This process was repeated until no more proteid could be separated from the solution. In almost every instance, an interval of several degrees occurred between the temperature at which the coagulum separated in flocculent form and the temperature at which the next succeeding turbidity appeared. Working in this way we have obtained separations at the following temperatures:

No.	Extremes of temperature.*	Average temperature.
(1)	31°-49° C.	40° C.
(2)	51°-61° C.	56° C.
(3)	60°-70° C.	65° C.
(4)	74°-76° C.	75° C.
(5)	77°-85° C.	82° C.

All of these substances were obtained from each of the types of extract used, both aqueous and saline. Nos. (4) and (5) were found in the smallest amounts.

The question naturally arose as to whether these separations represented individual proteids in the tissue. The solution of this problem we have sought in fractional separation by means of neutral salts in the methods used by Halliburton,[17] Hofmeister,[22] Kauder[25] and others.

Our results on extracts of the ligament may be summarized briefly as follows:

A. Aqueous extracts treated with $(NH_4)_2SO_4$ in substance.

(a). When the aqueous extract was half saturated with $(NH_4)_2SO_4$ a fairly heavy precipitate was obtained which consisted, theoretically, for the most part of globulins, albumin not being precipitated by this proportion of $(NH_4)_2SO_4$. The $MgSO_4$ solution of this substance when tested by fractional heat coagulation was found to contain bodies (1), (2), and (4) in the table

* These extremes represent the limits of all our observations. As a rule, the separations occurred at or about the mean temperature, with comparatively long intervals.

above. When the same precipitate was dissolved in water, the solution contained bodies (1), (3), (4), and (5).

(*b*). The filtrate from the precipitate obtained in (*a*) was saturated with $(NH_4)_2SO_4$. The substance so obtained was dissolved in water, the solution heated, and found to yield bodies (2), (3), (4), and (5).

B. *$MgSO_4$ extracts treated with $MgSO_4$ in substance.*

(*a*). When the $MgSO_4$ extract was saturated with $MgSO_4$ a heavy precipitate was obtained, which when dissolved in 5 per cent. $MgSO_4$ solution and heated yielded separations corresponding to (1) and (2).

(*b*). The filtrate from precipitate (*a*) on heating was found to contain bodies (2), (3), (4), and (5).

A comparison of these figures will show that of the total number of bodies present in the aqueous and saline extracts of the ligament, only one was separated by saturation with magnesium sulphate or by half saturation with ammonium sulphate, *viz*: No. (1), which on heating of the extracts separates at about 40° C. All of the other substances are to be found in the precipitates formed by saturation with magnesium sulphate or by half saturation with ammonium sulphate as well as in the filtrates from these precipitates.

C. *Fractional precipitation of aqueous and $MgSO_4$ extracts with $MgSO_4$ and $(NH_4)_2SO_4$ in substance and with saturated solution of $(NH_4)_2SO_4$.*

A closer differentiation of these proteids was desirable. To this end we have applied the following method. To a measured portion of the carefully neutralized fluid was added the precipitating salt, a few grams at a time. As soon as enough had been added to bring about a flocculent separation, the precipitate was filtered off and washed with a solution of the precipitating salt equivalent in strength to that of the mother liquid. To the filtrate plus enough of the washings to make it up to the volume of the fluid before filtration was again added weighed quantities of the precipitating salt. When a second precipitate appeared it was filtered off, washed, and the filtrate treated in a manner similar to that just described. This process was continued until all the proteid was removed or until the fluid was saturated. Each precipitate thus obtained was dissolved in a small amount of water with the aid of the salt mechanically adhering to it and the solution subjected to the process of heat coagulation.

(*a*). 5 per cent. $MgSO_4$ extract. Vol. 100 cc. Precipitating salt was $MgSO_4$, added in substance.

Results:

Precipitate I. 5 grams = turbidity; 25 grams = flocculent precipitate.

Precipitate II. 35 grams = turbidity; 53 grams to saturation = flocculent precipitate.

Coagulations: Solution of precipitate I. 44°-47° C. (1).
Solution of precipitate II. 64° C. (3).

(*b*). Aqueous extract was treated with equal volume of a saturated solution of $(NH_4)_2SO_4$. The resultant precipitate was washed, dissolved in water, and the solution made faintly acid with acetic acid. On standing for some time a precipitate formed which corresponded to separation No. (1) of the coagulation series. This substance was filtered off, and the filtrate accurately neutralized. The neutral filtrate was used in (*c*) and (*d*) below.

(*c*). Neutral filtrate obtained in (*b*). Volume 100 cc. Precipitating salt $MgSO_4$.

Results:

Precipitate I. 20 grams = turbidity; 42 grams = flocculent precipitate.

Precipitate II. 43 grams = turbidity; 50 grams = flocculent precipitate.

Precipitate III. 56 grams = turbidity; 63 grams = flocculent precipitate.

Precipitate IV. 73 grams = turbidity; saturation + acid = final precipitate.

Coagulations: Solution of Precipitate I. 51°-58° C. (2); 65°-67° C. (3).
Solution of Precipitate II. 68°-69° C. (3).
Solution of Precipitate III. 66°-67° C. (3).
Solution of Precipitate IV. 54°-56° C. (2); 67°-70° C. (3).

(*d*). Neutral filtrate obtained in (*b*). Volume 100 cc. Precipitating salt was $(NH_4)_2SO_4$ added in saturated solution.*

* In this series, the method of performing the experiment was the same as in those just described, except that instead of weighed quantities of the salt, measured amounts of a saturated solution were added. The figures represent the volumes of this saturated $(NH_4)_2SO_4$ solution added to 100 cc. of the fluid under observation. A determination of the exact strength of the $(NH_4)_2SO_4$ solution showed it to contain 53.67 per cent. of that salt.