A HYDROLYTIC DERIVATIVE OF THE GLOBULIN EDESTIN AND ITS RELATION TO WEYL'S ALBUMINATE AND THE HISTON GROUP.

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IT is well known that globulins, after precipitation from salt solutions, either by dilution or dialysis, usually do not redissolve completely in solutions of a neutral salt.

Weyl[1] states that, on long contact with water, globulins gradually become insoluble in neutral sodium chloride solutions of every concentration, and designates the substance thus formed as "albuminate." Very recently Starke[2] calls attention to the action of water on globulin and states that the precipitated globulin, when washed but a few times with water, always becomes nearly, or quite, insoluble in saline solutions, whereas globulin, which is precipitated by saturating its solution with neutral salts, can be kept for months in the saturated brine without losing its solubility.

In the presence of a very little acid this change from a soluble to an insoluble state appears to take place more rapidly. Thus a globulin thrown down by carbonic acid from a salt solution very soon becomes, to a large extent, insoluble in neutral solutions of sodium chloride. Myosin is rapidly changed into an insoluble form by the acid which develops in the muscle substance after death, and legumin extracted from leguminous seeds is soon converted into a form insoluble in salt solutions, unless the acid is neutralized as soon as the extract is made.

The following investigation makes it probable that the insoluble product, in the case of edestin at least, results from the hydrolytic action of hydrogen ions, and that this change in the protein molecule is the first of a series which leads to the formation of "acid albumin."

In the case of edestin and other proteins of the endosperm, this change from a soluble to an insoluble form takes place much less readily than in proteins from physiologically active animal tissues. It is also true that the proteins of the wheat *embryo*, which is likewise capable of great physiological activity, are also much

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[1] *Ztschr. physiol. Chem.*, **1**, 72 (1877.)

[2] Starke: *Ztschr. Biol.* n. f., **22**, 425.

more prone to become insoluble, in the way described, than those of the *endosperm* of wheat and other seeds.[1]

Since this derivative of edestin is a definite substance, well characterized by its properties and reactions, I propose to call it *edestan*, and if, as seems probable, the other protein bodies yield similar derivatives, these may be named in a like manner by changing the termination *in*, usually applied to the protein substance, to *an*. The group of substances belonging to this class may be called proteans, thus following the practice whereby the more altered and basic hydrolytic protein derivatives are designated proteoses, and the individual members. albumose, caseose, etc.

It is important that a distinction should be made between these proteans and those products which result from a more profound change in the protein molecule, caused by the action of stronger acids and alkalies, and which are now known as acid and alkali albumin.

A. ACTION OF WATER ON EDESTIN.

Pure water, because it is but slightly ionized, has little effect on pure edestin[2] at the room temperature. If, however, carbonic acid is present, edestan is formed in decidedly larger amount.

Gram portions of pure and perfectly neutral edestin were suspended in water, agitated frequently and exposed to different temperatures for definite periods of time. An equal volume of 20 per cent. sodium chloride solution was then added to each, and the solution made neutral to phenolphthalein, by which the unaltered edestin was at once dissolved and the formation of edestan was stopped. The edestan was allowed to settle over night and was then easily collected on a filter and thoroughly washed with 10 per cent. sodium chloride brine, until the washings showed no trace of the xanthoproteic reaction.

Nitrogen was determined in the residue and the amount of edestin calculated by multiplying the nitrogen by 5.4, since, as will be shown later, edestan contains 18.5 per cent. of nitrogen. The results obtained were the following:

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[1] *Cf.* Martin : *Jour. Physiology*, **8**, viii, (1887).

[2] For the properties of edestin, and its relations to acids and alkalies, the paper following this should be consulted, in which will be found in detail the evidence on which are based many of the statements made in this paper, concerning edestin and its compounds. Also, Osborne, "On Some Definite Compounds of Protein Bodies," This Journal, **21**, 486.

TABLE I.—PERCENTAGE OF EDESTAN FORMED BY CONTACT WITH WATER.

	Treated with sodium chloride solution after 6 hours.			
10 per cent. NaCl at 20°.	Water + CO_2 at 20°.	Pure water at 20°.	Pure water at 30°.	Pure water at 50°.
2.16	6.75	4.32	7.11	29.00

The portion which was treated with sodium chloride solution stood exactly as long as the others before filtration. The insoluble matter which this contained consisted of a little edestan which was originally present in the preparation and of edestan which was formed by treatment of the edestin with the salt solution. The above figures show that even at 20° a notable quantity of edestan is formed by water alone and that the quantity is decidedly greater if the water contains carbonic acid. At 50° about four times as much edestan was formed as at 30°, and nearly eight times as much as at 20°, which agrees with the fact that the velocity of such a reaction is about doubled by each increase of 10° in the temperature.

B. ACTION OF ACIDS ON EDESTIN.

Edestin combines with small, but definite, quantities of acid to form salts in which the edestin molecule is unchanged. In the presence of an excess of acid, above that required to form these salts, edestin is converted by the free hydrogen ions into edestan, as the following experiments show.

Gram portions of pure, neutral edestin were suspended in water enough to make a final volume of 20 cc., and to the different portions the quantity of centinormal acid stated in the following table was added. After being frequently agitated during the times indicated, the acid was neutralized by an equivalent quantity of decinormal potassium hydroxide solution and the amount of edestan that had formed was determined, as in the preceding experiments with water.

TABLE II.—PERCENTAGE OF EDESTAN FORMED BY ACIDS AT 20°.

9 cc. $\frac{HCl}{100}$		14 cc. $\frac{HCl}{100}$		18 cc. $\frac{HNO_3}{100}$	19 cc. $\frac{HNO_3}{100}$	20 cc. $\frac{HNO_3}{100}$
3 hours.	20 hours.	3 hours.	20 hours.	24 hours.	24 hours.	24 hours.
9.01	12.15	29.80	33.55	68.38	75.20	79.02

These figures, compared with those of Table I, show that edestin yielded much more edestan in contact with acids than in contact with water, and also that the percentage of edestan produced increased with the amount of acid.

One gram of this air-dry edestin preparation (equivalent to

0.9300 gram of water-free edestin) can combine with 13 cc. of centinormal hydrochloric acid, so that in the portion containing 9 cc. only that amount of acid was free which was produced by the hydrolytic dissociation of the compound formed, whereas the portion with 14 cc. of acid contained, in addition, a small amount of free acid, the effect of which is shown by the greater amount of edestan formed in it. That, in both cases, only a little more edestan was formed during twenty hours than during three hours is explained by the fact that edestan unites with a larger proportion of acid than does edestin, and consequently, as the proportion of edestan increases the proportion of free acid diminishes. With 20 cc. of nitric acid 79 per cent. of the edestin was converted into edestan.

From the salts of edestin, such as usually constitute the crystalline preparations as heretofore made by the usual methods, edestan may be prepared just as from the pure and neutral edestin which was used in the preceding experiments. One gram of a preparation consisting of edestin mono- and bichloride, chiefly the latter, and which already contained 6.32 per cent. of edestan, that had been formed during its preparation, was suspended in water and brought into solution by adding 3 cc. of centinormal hydrochloric acid. The added acid, as well as that originally combined with the edestin,[1] was at once neutralized, an equal volume of 20 per cent. sodium chloride solution added, which dissolved the unaltered edestin, and the edestan was determined as described above. Deducting the edestan originally present in the preparation, it appeared that during the very brief action of the acid, 3.49 per cent. of edestan had been formed. In a similar experiment with 3 cc. of acid, which was left in contact with the preparation for twenty hours before neutralization, 29.5 per cent. of edestan was formed. Using 10 cc. of acid, instead of 3 cc., 13.32 per cent. of edestan was formed at once, and 70.46 per cent. after twenty hours' contact.

That the amount of edestan formed in a given time depends on the degree of ionization of the acid was shown by suspending gram portions of neutral edestin in 6 cc. of water, adding 14 cc. of centinormal hydrochloric, phosphoric and acetic acids, respectively, and, after frequently agitating for about two hours at 20°, determining the amount of edestan formed in each.

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[1] One gram of this preparation contained acid equivalent to 11 cc. of $\frac{HCl}{100}$.

TABLE III.—PERCENTAGE OF EDESTAN FORMED BY EQUIVALENT QUANTITIES OF DIFFERENT ACIDS UNDER THE SAME CONDITIONS.

HCl.	H_3PO_4.	$H_4C_2O_2$.
19.29	16.02	5.65

The solution of phosphoric acid used in this experiment contained 0.98 gram of H_3PO_4 per liter, being made on the assumption that this acid behaves towards edestin as a monobasic acid. The much smaller quantity of edestan formed by acetic acid, compared with that formed by hydrochloric acid, is in accord with the lesser ionization of this acid.

C. COMPOSITION OF EDESTAN.

Ten grams of a preparation of crystallized edestin chloride were suspended in water, in a glass-stoppered bottle and 30 cc. of decinormal hydrochloric acid were gradually added. After the resulting clear solution had stood at the room temperature for about two hours, it was made neutral to phenolphthalein by adding 38 cc. of decinormal potassium hydroxide solution, the 8 cc. in the excess of the 30 cc. of added acid being required to neutralize the acid originally combined with the edestin preparation.

The curdy precipitate that formed on neutralizing, was washed thoroughly with 10 per cent. sodium chloride solution and then with water, until chlorides were removed, and finally with absolute alcohol. Dried over sulphuric acid, this formed preparation 1, which weighed 6.82 grams.

This experiment was repeated with another preparation of edestin chloride, the acid solution allowed to stand over night at a temperature below 10°, and then 50 cc. of decinormal potassium hydroxide solution were added. Although this excess of alkali was more than sufficient to dissolve the entire quantity of substance which was precipitated by neutralization, had this been unchanged edestin, nevertheless very little protein matter was dissolved by it. The precipitate was filtered out, washed with water, dehydrated with absolute alcohol and found to weigh 8 grams after drying over sulphuric acid. This formed preparation 2.

Preparations of edestin chlorides, which contain the water-soluble bichloride, yield aqueous solutions, from which the protein matter is precipitated by a little sodium chloride. The precipitate thus formed is never wholly soluble again in stronger solutions of sodium chloride, a part being converted into the so-called "albuminate" of Weyl. In order to establish the relations of this substance with that produced by the action of acids on edestin, under known conditions, a quantity of edestin, that had been obtained as a crystalline precipitate by cooling a warm dilute sodium chloride extract of hemp-seed meal, was washed by decantation with water until the sodium chloride was largely removed, whereupon the edestin began to dissolve.

When most had passed into solution, enough sodium chloride, in substance, was added to the clear aqueous solution containing the edestin to form an 8 per cent. brine. The edestin, at first precipitated by partial solution of the salt, mostly redissolved in the stronger brine that formed when all the salt had gone into solution.

The part that did not dissolve was filtered out and washed thoroughly with salt solution until all the globulin had been removed and then extensively with water. As the salt was washed away, the residue became gelatinous and dissolved slightly, so that it could no longer be washed on a filter. It was therefore suspended in water and made exactly neutral to phenolphthalein with very dilute potassium hydroxide solution. This converted it into a curdy precipitate which was easily filtered out and washed. In this condition it resembled, in all respects, the edestan obtained by neutralizing the hydrochloric acid solutions just described. After washing thoroughly with water and dehydrating with absolute alcohol, this preparation, **3**, was dried at 110° and analyzed with the results given below. Preparations **1** and **2** were likewise dried at 110° and analyzed.

TABLE IV.—COMPOSITION OF EDESTAN.

	1	2	3	Edestin.
Carbon	51.48	51.91	51.69	51.50
Hydrogen	6.91	6.96	6.98	7.04
Nitrogen	18.51	18.49	18.49	18.69
Sulphur	1.00	0.99	0.92	0.88
Oxygen	22.10	21.65	21.92	21.89
	100.00	100.00	100.00	100.00
Ash	0.55	0.06	0.14	

Between these analyses and that of edestin no sufficient difference exists to enable us to detect any change in ultimate composition caused by its conversion into edestan. A strict comparison of the reactions of **1** and **2** with those of **3** showed that these were one and the same substance.

D. REACTIONS OF EDESTAN.

Edestan prepared as just described is a voluminous, dusty, white powder which swells somewhat in water and forms a colorless, transparent jelly with very dilute hydrochloric acid. Whether a true solution was formed by the dry powder in this extremely dilute acid could not be ascertained since the opalescent fluid that resulted could not be filtered clear.

Dry, neutral edestan is scarcely soluble, even in strong ammonia, but the gelatinous mass, formed by treating the substance with very dilute hydrochloric acid, is slightly more soluble, though in either case the amount dissolved by ammonia is very small. The solution in ammonia yields a precipitate with ammonium chloride ; consequently when hydrochloric acid is added to the ammoniacal solution a precipitate forms, even when much of the ammonia is unneutralized. The ammoniacal solution is not precipitated by sodium chloride.

A strong solution of edestan, which, however, probably contains a little unaltered edestin, may be prepared by dissolving edestin in centinormal hydrochloric acid in the proportion of 30 cc. to each gram and allowing the solution to stand for at least twenty-four hours. One-third of the acid may then be neutralized by adding the requisite quantity of very dilute potassium hydroxide solution. An opalescent, unstable, supersaturated solution results, which can be filtered nearly clear and, if abundantly diluted, yields no precipitate within several hours. Such a solution of edestan chloride yields a precipitate with 10 per cent. ammonia, which is soluble in a considerable excess, the resulting solution being readily precipitated by ammonium chloride. By decinormal ammonia solution the substance is precipitated but not redissolved, even by a very large excess of this ammonia solution.

The aqueous solution of edestan chloride yields a precipitate with a very little ammonium or sodium chloride, the latter being readily, the former with difficulty, dissolved by an excess of ammonia.

With nitric acid, edestan gives a precipitate completely soluble on warming but reprecipitated on cooling.

With potassium phosphotungstate, sodium phosphomolybdate or sodium picrate, edestan chloride forms slimy precipitates. With a solution of ovalbumin, edestan chloride yields an abundant precipitate.

Mercuric chloride forms no precipitate in a dilute solution of edestan chloride, nor in a relatively strong one, unless a considerable amount of this reagent is added.

Very dilute hydrochloric acid does not precipitate edestan, but the strong acid gives a precipitate, which dissolves in a sufficient excess of concentrated acid.

E. THE AMOUNT OF ACID WITH WHICH EDESTAN COMBINES.

Edestan exists in preparations of edestin chlorides in combination with acid. The amount of acid with which this substance forms a compound, sparingly soluble in water, appears to be definite, as the following experiments show.

A quantity of a preparation of edestin chloride was dissolved, as far as possible, in 10 per cent. salt solution and the insoluble edestan was allowed to settle. This was first washed thoroughly with salt solution, until the edestin was removed, and then with water, in which a very little dissolved, while the remainder formed a gelatinous mass. The latter was suspended in water and dialyzed until the sodium chloride was removed. The dialyzer then contained an opalescent fluid and a voluminous precipitate. Of this fluid, 25 cc. were made neutral to phenolphthalein by adding 2.5 cc. of a centinormal solution of potassium hydroxide and by evaporating and drying the residue at 110° was found to contain 0.1165 gram of substance. The same volume drawn from the bottom of the dialyzer, which contained much of the undissolved matter, was made neutral by 11 cc. of the alkali and contained 0.4710 gram of substance. These quantities correspond to an acidity of 21.5 and 23.4 cc., respectively, of a centinormal solution per gram of edestan. A repetition of this experiment gave essentially the same result.

A quantity of the insoluble matter which remained on treating another preparation of edestin chloride with 10 per cent. sodium chloride brine was thoroughly washed with salt solution, once with water and finally with dilute alcohol, until all the salt was removed. A quantity of the still moist substance was then suspended in water, finely divided by straining through fine bolting-cloth, and 4 cc. of decinormal potassium hydroxide solution added, which was decidedly in excess of the quantity necessary to make the mixture neutral to phenolphthalein. After agitating continuously for some time, the excess of alkali was neutralized by 2.1 cc. of decinormal hydrochloric acid, which showed that 1.9 cc. of the alkali had been neutralized by the acid combined with the substance. By evaporating and drying the residue at 110° it was found that 0.9430 gram of edestan was present in the mixture, from which it appears that its original acidity was equal to 20.1 cc. of a centinormal solution per gram.

Another portion of this substance was suspended in water in a finely divided state and brought into solution by adding 2 cc. of decinormal hydrochloric acid and shaking for some time. The solution was then made neutral to phenolphthalein, by adding 3 cc. of decinormal potassium hydroxide solution, and the amount of dissolved edestan found to be 0.5039 gram, from which it is seen that its original acidity was equal to 20 cc. of a centinormal solution per gram.

This quantity is just three times the acidity of edestin monochloride and one and a half times that of the bichloride ; consequently if edestan is formed from edestin without any notable change in molecular weight, this substance occurs in preparations of edestin chlorides as a trichloride, assuming edestin to have a molecular weight of about 14,500.

Bang[1] has recently reviewed the reactions of the histons and concludes that for the present these may be defined as follows : In neutral solution the histons are precipitated by the cautious addition of ammonia, the precipitate in the presence of an ammonium salt very soon becoming insoluble in an excess of ammonia. They give a precipitate with nitric acid, which dissolves on heating and reappears on cooling. They are precipitated from neutral solution by heating, if their solutions contain some sodium chloride, not, however, if they are poor in salts. Neutral solutions of histons are precipitated by the alkaloid reagents and by solutions of ovalbumin and other proteins. In this group of bodies Bang includes goose-blood histon, thymus histon, scombron, and globin.

I have shown that edestan has all these reactions with the exception of the precipitation of the solution by heat in the presence of moderate quantities of neutral salts. This reaction could not be obtained with edestan as it is insoluble in water, and the

[1] Bang : *Ztschr. physiol. Chem.*, **27**, 463.

aqueous solution of its chloride gives a precipitate with a very little salt which is insoluble in stronger saline solutions.

The most important difference between these histons and edestan is that the former appear to be soluble in water when neutral, whereas neutral edestan is very insoluble in water, the reactions, which I have described, being given by aqueous solutions of its chloride. This combined acid, however, is present in such small proportion that it can be detected only in concentrated solutions by using very delicate litmus paper, so that it might easily be overlooked were its presence unknown. Bang gives no evidence that his solutions did not contain a similar proportion of acid and it seems probable that some of them at least did contain it, since the substances were extracted by dilute hydrochloric acid and the solutions made "neutral" presumably to litmus, this indicator being the one commonly employed by physiologists for such purposes. That any near relation exists between edestan and the bodies enumerated by Bang as histon is not probable, except in the case of globin, which seems to be more nearly related to edestan than to the histons since globin is a true protein substance, obtained from hemoglobin by the action of acids under conditions similar to those leading to the formation of edestan. With thymus histon and with scombron, it would seem that edestan and globin have little in common, since the two former yield little or no proteoses on pepsin digestion, whereas edestin yields such abundantly, and globin doubtless does the same.

From the facts now at our command it is evident that we have two different classes of bodies which conform pretty closely to the reactions characteristic of the histons. It is important to recognize this fact, since otherwise confusion will result if these two classes are not distinguished from one another, and protein derivatives produced by the acid used in preparing these substances may be regarded as actual constituents of the tissues.

SUMMARY.

By the action of water or very dilute solutions of acids, the globulin edestin is converted into a substance insoluble in saline solutions of moderate concentration.

This derivative of edestin is formed by hydrolysis, the amount formed being proportional to time and the concentration of the solution in hydrogen ions. In pure water less is formed in a given time than in water containing carbonic acid. More is formed by a given quantity of hydrochloric acid than by an equivalent quantity of phosphoric acid, and by either of these acids much more is formed than by an equivalent of acetic acid.

This substance is the same as that designated as "albuminate" by Weyl, which is formed in greater or less amount in preparations of crystallized edestin made in the usual manner, and is without doubt the first product of the hydrolytic changes leading to the formation of the so-called acid albumin.

It is probable that the products insoluble in saline solutions which are formed from other globulins, originate from the same cause, and that these form a distinct class of hydrolytic derivatives of the native protein molecules.

For this derivative of edestin the name edestan is proposed.

The ultimate composition of edestan is the same, so far as can be determined by analysis, as that of edestin from which it originates.

Edestan forms salts with hydrochloric acid which react acid toward phenolphthalein to the full extent of the combined acid. One salt, having an acidity equivalent to 20 cc. of a centinormal solution per gram, is very sparingly soluble in water. It is this salt which forms the so-called "albuminate" found in edestin preparations. If edestan has a molecular weight near that of edestin, assumed to be about 14,500[1] this acidity would correspond to that of a trichloride, being just three times that of edestin monochloride and one and one-half times that of the bichloride.

Edestan is insoluble in water, far less soluble in solutions of potassium hydroxide than is edestin and insoluble in ammonia water, unless the solution of the latter is relatively very strong.

The aqueous solution of edestan chloride, when concentrated, reacts acid with litmus. It is precipitated by neutralization, the precipitate being soluble in strong ammonia, yielding a solution which is precipitated by ammonium chloride but not by sodium chloride.

The aqueous solution of edestan chloride gives a precipitate with nitric acid which dissolves on warming and reappears on cooling; a precipitate with ovalbumin solutions with the alkaloidal reagents and with sufficient mercuric chloride if its solution is concentrated. These reactions agree closely with those given

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[1] See Osborne: This Journal, **21**, 486 (1899); also the paper following.

by Kossel as characteristic of histons, but with the true histons edestan has little in common.

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THE BASIC CHARACTER OF THE PROTEIN MOLECULE AND THE REACTIONS OF EDESTIN WITH DEFINITE QUANTITIES OF ACIDS AND ALKALIES.

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I. INTRODUCTION.

The Basic Character of the Protein Molecule.

THAT the proteins are ionized and highly reactive bodies is indicated by the rapidity with which they unite with both bases and acids, by the readiness with which, in many cases, they respond to changes in the ionization of their solutions, and also by the fact that they are, chemically, the most active constituents of protoplasm.

That they are neutral bodies, like the carbohydrates, is not in harmony with what is known of them.

Nevertheless, it appears to be generally assumed that a solution containing protein matter, which shows neither acid nor alkaline reaction with litmus, is chemically neutral.

Observations are on record which show that some protein solutions, when neutral to litmus, are acid to phenolphthalein and alkaline to lacmoid. It is also well known that a notable quantity of acid can be added to a protein solution before an acid reaction with tropaeolin, alizarine, or phloroglucin and vanillin appears.

The fact that acids combine with protein bodies is, therefore, well known, and, in making preparations of these substances, the necessity of removing such acids has long been recognized. This has been supposedly accomplished by adding potassium or sodium hydroxide or carbonate until the reaction with litmus becomes neutral. I am not aware that any one has offered any evidence, however, that by this procedure this object is fully accomplished. It is of importance, therefore, to know whether litmus can be used to determine the point when all combined acid has been converted into neutral salts of potassium or sodium and all the protein substance has been set free, or whether, as we know is the