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

RESEARCH LABORATORY OF THE CONNECTICUT
AGRICULTURAL EXPERIMENT STATION.

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

BY THOMAS B. OSBORNE.

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

case when tropaeolin or lacmoid is used as an indicator, more acid still remains combined.

Solutions in water of preparations of crystallized ovalbumin, in sodium chloride brine of excelsin, amandin, vignin, conglutin, glycinin, corylin, phaseolin and legumin, and in 75 to 90 per cent. alcohol of zein, gliadin and hordein,[1] which were either neutral or acid when tested with a strip of sensitive, neutral litmus paper, capable of showing distinctly the presence of 0.25 cc. of centinormal hydrochloric acid in 10 cc. of water, when made neutral to litmus, were, in every case, still acid towards phenolphthalein. With the exception of ovalbumin, these preparations had been made by the methods usually employed and had come in contact with no acid except that contained in the seeds from which they were obtained. The ovalbumin preparations were made both by Hopkins's and by Hofmeister's methods, the acidity of all of them being the same.

To render gram portions of these several protein preparations neutral to litmus required in a few cases not any, in most cases from 0.1 cc. to 1.5 cc. of, decinormal alkali ; while to make the same 1-gram portions neutral to phenolphthalein required the further addition of from 0.7 to 1 cc. of decinormal alkali, except for legumin, which required 2 cc.

This reaction with phenolphthalein is sharp and definite, like that with strong mineral acids, and is independent of dilution, the same result being obtained in a volume of 10 cc. or in one of 100 cc.

The question now arises, whether complete neutralization of the combined acid is indicated by phenolphthalein or by litmus.

Preparations of edestin, which are neutral or acid to litmus, when suspended in water and made neutral to phenolphthalein by adding potassium hydroxide are not in any perceptible degree dissolved, but yield to the solution potassium salts of simple acids, which may be obtained therefrom by evaporation. When thus freed from these acids, the edestin immediately begins to dissolve if more alkali is added.

Edestin made neutral to phenolphthalein and dissolved in sodium chloride solution reacts distinctly alkaline towards litmus. This alkaline reaction is caused by the edestin itself and not by

[1] These protein bodies are described in Reports of the Connecticut Agricultural Experiment Station, 1890 to 1899 ; also Osborne : *Am. Chem. J.*, 13, 14 and 15 ; This Journal, 16, 17, 18, 19, 20 and 21 ; also "Die Proteide." etc., Heidelberg, 1897.

organic salts of the alkali, since such preparations yield a very small amount of ash, less than 0.05 per cent., which is neutral to both litmus and phenolphthalein.

Crystalline preparations of excelsin, obtained from the Brazil nut, *Bertholetia excelsa*, are undissolved when suspended in water and made neutral to litmus, but are completely dissolved when enough alkali, either potassium or its equivalent of ammonium hydroxide, is added to render the solution neutral to phenolphthalein. With less alkali, solution is not complete, the amount of excelsin dissolved depending upon the quantity of alkali added.

Likewise, preparations of legumin from the pea, horse bean, or vetch, when suspended in water, are completely dissolved only when enough potassium, or an equivalent of ammonium hydroxide is added to neutralize their acid reaction to phenolphthalein. It is highly improbable that excelsin and legumin can form soluble compounds with potassium which are neutral to phenolphthalein, and the fact that an exactly equivalent quantity of ammonium causes solution, is also evidence that this is not so, since a much larger proportion of ammonium hydroxide than of potassium hydroxide is required to dissolve edestin. Thus, 1 gram of a preparation of edestin, which was completely dissolved by 1 cc. of decinormal potassium hydroxide solution, was not entirely dissolved by 13 cc. of decinormal ammonium hydroxide. Furthermore, the proteins being very weak acids, it is scarcely possible that these form salts with potassium which are neutral to phenolphthalein. Certainly this is not the case with edestin, as the slightest excess of potassium hydroxide above that required to neutralize the combined acid at once turns phenolphthalein red.

Solutions of all the other protein bodies that I have examined, when similarly made neutral to phenolphthalein react decidedly alkaline with litmus.

From these facts it seems certain that the proteins are true bases, as I have previously pointed out,[1] and that they are not pseudoammonium bases, as Cohnheim and Krieger assume.[2]

II. COMPOUNDS OF EDESTIN WITH ACIDS.

Since edestin, when neutral to phenolphthalein, is insoluble in

[1] Osborne : This Journal, **21**, 486 ; Report of Connecticut Agricultural Experiment Station for 1899 ; also This Journal, **22**, 402.

[2] Cohnheim and Krieger : *Ztschr. Biol.*, n. f., **22**, 95.

water, it presents an opportunity for studying the nature of the acidity of its preparations, as obtained by the methods now used, that is offered by no other protein with which I am familiar. I have, therefore, subjected edestin to an extensive study, the details of which are given in the following pages.

This study appeared to be important, as edestin is a true protein substance, presenting all the essential characteristics of these bodies, so that the reactions into which it enters with bases and acids are, without doubt, to a large extent typical of those of the proteins in general.

1.   The Degree of Acidity of Edestin Preparations.

To neutralize to phenolphthalein the acid reaction of a sodium chloride solution of 1 gram of each of twenty different prepara-tions of edestin made by all the various methods usually em-ployed in preparing this substance, it was necessary to add from 0.85 to 1.5 cc. of decinormal potassium hydroxide solution.   To neutralize eight of these solutions to litmus required from 0.2 to 0.5 cc., while the remaining twelve were neutral to litmus, the difference in the acidity of the several preparations measured by these two indicators being equal to from 0.85 to 1.10 cc. of deci-normal alkali.

The degree of acidity towards phenolphthalein is very easily determined by direct titration, since the end reaction appears promptly.   The same result is obtained by titrating the saline solution directly, by dissolving the preparation in an excess of alkali and titrating back, or by suspending the edestin in water in a stoppered bottle and adding the alkali until a red color ap-pears.   In the latter case the end-point is obtained more slowly, since the reaction takes place with one substance in the solid state.

2.   The Nature of the Acids Combined with Edestin.

Air-dried preparations of edestin, when suspended in water and treated with enough dilute potassium hydroxide solution to give a just perceptible alkaline reaction with phenolphthalein, do not dissolve, as already said, but form, on standing, dense deposits of disintegrated crystals, above which a voluminous flocculent layer of amorphous matter settles.   Some samples, when thus treated, separate completely from the solution, leaving it clear and easily filtered : others form a milky, colloidal semi-solution which can-

not be filtered.   These latter preparations, when suspended in 50 per cent. alcohol and neutralized to phenolphthalein with alkali, yield clear solutions which are easily filtered.   The residue left on evaporating the filtrate and washings of the neutralized edestin, when dried at 110°, usually equals about 1.25 per cent. of the edestin, dried at the same temperature.   The proportion of this residue obtained from several preparations was found to be as follows :

Preparation ··	4	7	9	10	11	12	13	15	18
Per cent······	1.30	1.05	1.27	1.21	1.40	1.26	1.41	1.15	1.44
					1.26	1.41		1.17	

In order to find the composition of these residues, 75 grams of preparation 19[1] were exhausted with large quantities of 75 per cent. alcohol until the final washings left, on evaporation, no notable quantity of solids.   The total residue left by evaporating the whole of these washings weighed 0.2833 gram, equal to 0.38 per cent. of the protein.   Its solution in water was made neutral to phenolphthalein by 3.4 cc. of decinormal potassium hydroxide solution, and yielded a precipitate, chiefly protein, weighing 0.1159 gram.   The filtrate from this, when evaporated, left a residue weighing 0.1674 gram, of which 0.0133 gram was potassium that had been added in neutralizing the solution.   Deducting this, we find that only 0.1541 gram of non-protein matter had been removed by washing 75 grams of edestin.   On ignition, a residue of mineral matter remained, weighing 0.0600 gram, from which again should be deducted 0.0133 gram, corresponding to the potassium contained in the 3.4 cc. of decinormal solution added, so that the total mineral matter removed by exhaustive washing from the 75 grams of edestin amounted to only 0.0467 gram, or 0.06 per cent.   The absence of more than traces of soluble mineral matter remaining in this sample of edestin having thus been demonstrated, it was suspended in pure water, and 90 cc. of decinormal potassium hydroxide solution, diluted with much water, was gradually added, which sufficed to exactly neutralize the mixture to phenolphthalein.   After being shaken in a closed flask for some hours, the undissolved edestin was filtered out and washed by decantation with alcohol of about 65 per cent. by volume, applied in six successive portions of

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[1] This was obtained by treating hemp seed with 3 per cent. brine heated to 60° and cooling the clear extract.   The protein which separated wholly in octahedral crystals, therefore constitutes a preparation of crude edestin.

1 liter each. The filtrate and washings were separately evaporated and left residues, when dried at 110°, which weighed as follows :

TABLE I.—SOLUBLE MATTER FORMED BY NEUTRALIZING EDESTIN 19
WITH POTASSIUM HYDROXIDE.

		Gram.
1st	aqueous filtrate·····················	0.2542
1st	one liter 65 per cent. alcohol ········	0.1225
2d	" " 65 " " " ········	0.0680
3d	" " 65 " " " ········	0.0750
4th	" " 65 " " " ········	0.0725
5th	" " 65 " " " ········	0.0480
6th	" " 65 " " " ········	0.0308
	Total ·······························	0.6710

These residues were united and extracted with strong alcohol, in which a part dissolved. The part soluble in alcohol was also soluble in water, forming a yellow solution which required 0.3 cc. of decinormal hydrochloric acid to neutralize it to litmus, and 0.7 cc. to phenolphthalein.

When this alcoholic solution was evaporated and the residue burned at a low temperature, alkaline vapors were evolved in small amount.

The matters soluble and insoluble in strong alcohol weighed respectively 0.1514 and 0.6173 gram, and when analyzed were found to contain the following substances :

TABLE II.—COMPOSITION OF THE POTASSIUM SALTS FORMED BY NEU-
TRALIZING A CRUDE PREPARATION OF CRYSTALLIZED EDES-
TIN 19 WITH POTASSIUM HYDROXIDE.

	Part soluble in strong alcohol.		Part insoluble in strong alcohol.	
	Gram.	Per cent.	Gram.	Per cent.
Organic matter·············	0.0825	54.42	0.1429	23.15
Potassium carbonate·······	0.0400	26.38	0.0600	9.72
Potassium sulphate·········	0.0200	13.33	0.0766	12.41
Potassium chloride·········	0.0052	3.43	0.2750	44.55
Potassium phosphate·······	······	····	0.0235	3.74
Sodium chloride···········	······	····	0.0091	1.47
Undetermined and loss·····	0.0037	2.44	0.0302	4.96
	0.1514	100.00	0.6173	100.00

The sum of the mineral matters contained in the two portions is 0.5433 gram, and had the following composition :

TABLE III.—COMPOSITION OF TOTAL INORGANIC SALTS FORMED BY NEU-
TRALIZING THE CRUDE PREPARATION OF CRYSTALLIZED
EDESTIN 19 WITH POTASSIUM HYDROXIDE.

Potassium carbonate	0.1000	18.40
Potassium sulphate	0.0966	17.81
Potassium chloride	0.2802	51.54
Potassium phosphate	0.0235	4.32
Sodium chloride	0.0091	1.67
Undetermined and loss	0.0339	6.26
	0.5433	100.00

From these figures we find that the greater part of the soluble substances formed by neutralizing this preparation consists of potassium salts of mineral acids and that 60 per cent. of the potassium in these salts is present as chloride.

We also find that alcohol dissolves a large proportion of the organic matter which consists largely of potassium salts of one or more organic acids, since the quantity of potassium carbonate formed after igniting is equal to about one-half the combustible matter. The sum of this latter and the potassium carbonate is 0.1225 gram, of which 0.0211 gram is potassium and 0.1014 gram organic. From this a maximum mean molecular weight of 188 can be calculated, which shows that no considerable part of the potassium carbonate is formed from potassium compounds of protein.

Owing to the exhaustive washing to which this preparation had been subjected before neutralization, it is improbable that this organic matter was an admixed impurity, nor was it a free organic acid insoluble in water, because the solution of its potassium salt remained clear on adding a little hydrochloric acid, showing the organic acid to be readily soluble in water.

We must, therefore, conclude that this organic matter consists of one or more of the organic acids of the seed, which had combined with the edestin.

In confirmation of these results, this experiment was repeated, with sample 20, prepared from a sodium chloride extract of hemp seed, which had been neutralized to litmus with potassium hydroxide, and dialyzed until the edestin had precipitated in crystals. This precipitate was redissolved in 10 per cent. sodium chloride brine, heated to 50°, the solution diluted with three volumes of water at the same temperature, filtered perfectly clear and cooled at 12°. The beautifully crystallized deposit which

separated was thoroughly washed, first with 1 per cent., then
with 0.5 per cent. sodium chloride solution, and finally with 50
per cent. alcohol until all the salt was removed.   This method of
washing with dilute salt solution and alcohol was necessary,
because on washing with water alone a large part of the prepara-
tion dissolved, the reason for which is later explained.

Thirty grams of the air-dry preparation were suspended in pure,
freshly-boiled water, and 24 cc. of decinormal potassium hydrox-
ide solution, likewise diluted with water, were added.   The
mixture, perfectly neutral to phenolphthalein, was allowed to
stand some time, filtered, and the insoluble edestin thoroughly
washed with dilute alcohol.

The filtrate and washings were evaporated, the residue dried
at 110°, weighed and analyzed with the following results :

TABLE IV.—COMPOSITION OF THE POTASSIUM SALTS FORMED BY NEUTRAL-
IZING A RECRYSTALLIZED PREPARATION OF EDESTIN
20 WITH POTASSIUM HYDROXIDE.

	Gram.	In per cent. of the inorganic matters.
Organic matters	0.0315	....
Potassium carbonate	0.0083	5.7
Potassium sulphate	0.0095	6.5
Potassium chloride	0.1081	73.9
Sodium chloride	0.0108	7.4
Undetermined and loss	0.0095	6.5
Total	0.1777	100.0

These figures in general confirm those previously given, but
show a larger proportion of potassium chloride and a smaller
proportion of organic salts and sulphate.   This is doubtless the
result of the recrystallization, whereby this preparation, 20, was
finally separated from a solution containing a much smaller pro-
portion of negative ions other than those of chlorine than that
existing in the solution from which 19 was crystallized.

As already stated, a part of many preparations of edestin dis-
solves in water when the associated salts are mostly washed out.
In order to determine whether or not this solubility is due to a
difference in the nature of the acids united to the soluble and to
the insoluble parts, 30 grams of preparation 20 were exhausted
with pure water and the filtered solution was neutralized with
10.6 cc. of decinormal potassium hydroxide solution, whereby the
edestin was wholly precipitated.   The clear filtrate and washings

from this precipitate were evaporated, the residue dried at 110°
and analyzed, with results as follows :

TABLE V.—COMPOSITION OF THE POTASSIUM SALTS FORMED BY NEUTRAL-
     IZING THE PART OF THE EDESTIN PREPARATION
          WHICH WAS SOLUBLE IN WATER.

	Gram.	In per cent. of the inorganic matters.
Organic matters	0.0902	....
Potassium carbonate.........	0.0021	1.4
Potassium sulphate	0.0096	6.6
Potassium chloride	0.0721	49.8
Sodium chloride.............	0.0585	40.4
Undetermined and loss	0.0025	1.8
Total	0.2350	100.0

The presence of sodium chloride, which forms so large a part
of these salts, is undoubtedly due to incompletely washing the
original preparation, which had been recrystallized several times
from dilute brine and, in order that the water-soluble edestin
compound should not be removed from it, had been washed first
with 0.5 per cent. brine and then with 50 per cent. alcohol.   The
inorganic residue obtained in a similar experiment, described on
page 49, contained only 11.41 per cent. of sodium chloride, which
shows that in the present case this is to be regarded as an acci-
dental contamination and not as a product of neutralization.

The potassium hydroxide used for neutralizing the edestin was
equivalent to 0.0791 gram of potassium chloride, of which salt
0.0721 gram appears in the analysis, even after calculating all the
other acids as potassium salts.   From this, it is evident that the
water-soluble edestin was mostly combined with hydrochloric acid.
It is to be noted that all the added alkali, in this case, was recov-
ered, whereas in the experiments first described only 70 per cent.
was found in solution.   This is doubtless due to the fact that in
these first experiments the acid neutralized was largely contained
within the body of the crystals,[1] and, as these crystals did not dis-
solve when neutralized, the potassium salts which formed within
them were washed out with great difficulty.   In this last experi-
ment the edestin was wholly dissolved at the time the alkali was
added, and consequently the potassium salts that formed were
easily separated from it.

[1] Under the microscope the greater part of the undissolved edestin is seen to consist
of fragments of crystals, most of which are of relatively considerable size, so that potas-
sium salts formed within these fragments are necessarily extracted with difficulty.

The part of preparation 20 which did not dissolve on treating with water, was next suspended in pure, boiled water and neutralized to phenolphthalein, by carefully adding 16.6 cc. of decinormal potassium hydroxide solution.   After standing some time, the solution was filtered and the undissolved edestin thoroughly washed with 75 per cent. alcohol.   The filtrate and washings, when evaporated, left a residue which, when dried at 110°, was analyzed with the following results :

TABLE VI.—COMPOSITION OF THE POTASSIUM SALTS FORMED BY NEUTRAL-
        IZING THE PART OF THE EDESTIN PREPARATION
              WHICH WAS INSOLUBLE IN WATER.

	Gram.	In per cent of the inorganic matters.
Organic matters............	0.0574	....
Insoluble mineral matter····	0.0067	5.8
Potassium carbonate........	0.0121	10.5
Potassium sulphate	0.0117	10.1
Potassium chloride	0.0710	61.5
Sodium chloride............	0.0085	7.4
Undetermined and loss	0.0055	4.7
	0.1729	100.0

In this experiment, 75 per cent. of the potassium added was recovered, of which 76 per cent. is chloride and 11 per cent. sulphate.

In the similar experiment, to be described on page 50, 85 per cent. of the potassium added was recovered, of which 47.6 per cent. was chloride and 52.4 per cent. sulphate.   Since the present experiment yielded so small a proportion of sulphate, it is evident that the insolubility of edestin in water is not simply due to combined sulphuric acid, but, as we shall later show, is chiefly due to the *greater proportion* of acid combined with the soluble part.

To make a preparation which, when neutralized, should yield only chloride, 3,000 grams of hemp-seed meal were extracted with 9 liters of brine heated to 60° and containing 3 per cent. of almost chemically pure sodium chloride.   The filtered extract was cooled to 8° and allowed to stand over night, until the large crop of crystals had been deposited.   These crystals were then collected on a filter, washed once with water, redissolved in 1 liter of sodium chloride brine, the solution filtered perfectly clear, heated to 55°, diluted with three volumes of water at the same temperature, and rapidly cooled to 0°.

The crystalline deposit which separated was suspended in water, brought on a filter, and this process repeated until all the edestin soluble in water was washed out.   The three portions of washings first obtained were almost protein-free, the fourth contained nearly one-half of the original preparation, the fifth much less, and the sixth very little.

To the fourth portion enough pure sodium chloride was added to form a 10 per cent. solution.   This caused at first an abundant precipitate, most of which dissolved when more of the salt passed into the solution, which was then filtered and dialyzed for forty hours, whereby its salt content was reduced to 2 per cent., and the edestin was precipitated in beautiful crystals.   These were filtered out, washed with 50 per cent. alcohol and finally with absolute alcohol and dried over sulphuric acid, giving 37 grams of a perfectly crystallized, dazzlingly white preparation, 22, which was mostly soluble in pure water, and wholly soluble in sodium chloride solution.

To neutralize to phenolphthalein, a quantity of this preparation corresponding to 1 gram of the water and ash-free substance, 1.22 cc. of decinormal potassium hydroxide solution was required, corresponding to an acidity which, as we shall see later, is that of a mixture containing a small proportion of the water-insoluble, with a large proportion of the water-soluble, edestin compound.

Twenty grams of this preparation, air-dry, were then treated with 600 cc. of pure water, 220 cc. of centinormal potassium hydroxide solution added, and the voluminous precipitate filtered out and washed with 250 cc. of water.   The filtrate and washings, when evaporated, left a residue, which had the following composition :

TABLE VII.—POTASSIUM SALTS FORMED BY NEUTRALIZING EDESTIN, PREPARATION 22.

	Gram.	In per cent. of the inorganic matters.
Organic matters	0.0185	....
Potassium carbonate	0.0020	1.19
Potassium sulphate	0.0065	3.89
Potassium chloride	0.1398	83.51
Sodium chloride	0.0191	11.41
	0.1859	100.00

Although the free edestin had been only superficially washed, we find over 85 per cent. of the added potassium to be contained

in these salts, 95 per cent. of which is present as chloride. The proportion of sulphate to chloride in these salts is less than 2 molecules of the former to 100 of the latter. Although this preparation was nearly free from sulphate, nevertheless a perfectly pure chloride was not obtained in this way.

Twenty grams of that part of the edestin, which, as already described, did not dissolve in water, were suspended in 600 cc. of water and neutralized by adding 12 cc. of decinormal potassium hydroxide solution. The solution filtered from the undissolved edestin, together with the washings, was evaporated and the residue analyzed with the following results :

TABLE VIII.—COMPOSITION OF POTASSIUM SALTS FORMED BY NEUTRALIZING THAT PART OF THE EDESTIN PREPARATION WHICH WAS INSOLUBLE IN WATER.

	Gram.	In per cent. of the inorganic matters.
Organic matters	0.0340	...
Potassium carbonate	none	...
Potassium sulphate	0.0479	49.6
Potassium chloride	0.0373	38.7
Sodium chloride	0.0079	8.2
Undetermined and loss	0.0034	3.5
	0.1305	100.00

In this residue we have a larger proportion of sulphate than in any previously analyzed. We shall later show edestin sulphate to be less soluble in water than the corresponding chloride, and, for this reason, it is probable that the edestin sulphate had accumulated in the fraction insoluble in water. Whether edestin sulphate is a constituent of the seed or results from sulphates contained in the sodium chloride and in the river water in which the solutions were dialyzed was not determined, but the latter seems probable, because, before its final precipitation, this substance was wholly soluble in water.

From these results it appears that edestin preparations, obtained by cooling or by dialyzing saline solutions, usually consist of mixtures of several compounds of the protein with acids, the proportion of the different compounds formed depending upon the degree of acidity of the solution and the nature of the negative ions which are present when the edestin crystallizes out.

Owing to the difficulty of obtaining edestin chloride free from sulphate, it seemed possible to obtain the sulphates free from other acid compounds.

Four kilograms of hemp-seed meal were accordingly extracted with 15 per cent. ammonium sulphate solution, the extract saturated with the same salt, and the protein thus precipitated was filtered out and dissolved in the dilute sulphate solution which resulted on adding water. The clear solution, when dialyzed, deposited relatively large octahedral crystals, which, when washed with water and alcohol and dried, weighed 440 grams, preparation 23.

Fifty grams of this preparation were suspended in water and made exactly neutral to phenolphthalein by adding 45 cc. of decinormal potassium hydroxide solution, diluted with much water. As the neutral edestin did not separate from the solution in a condition to be filtered, an equal volume of alcohol was gradually added. The clear filtrate and washings then obtained were evaporated and the residue analyzed with the following results:

TABLE IX.—COMPOSITION OF THE POTASSIUM SALTS FORMED BY NEUTRALIZING EDESTIN, PREPARATION 23.

	Gram.	In per cent. of the inorganic matters.
Organic matters	0.2269	....
Potassium carbonate	0.0480	18.98
Potassium sulphate	0.1956	77.34
Potassium chloride	0.0066	2.61
Undetermined and loss	0.0027	1.07
	0.4798	100.00

The effect of substituting sulphate for chloride in extracting edestin is plainly shown by these figures, over 75 per cent. of the recovered potassium being sulphate and less than 3 per cent. chloride. Of the potassium added in neutralizing, 66 per cent. was recovered in the above salts, which agrees well with the 70 per cent. obtained in the previously described experiments in which the edestin crystals did not dissolve but fell into relatively large fragments on neutralizing.

In order to convert, if possible, edestin sulphate into chloride, 50 grams of this preparation, 23, were suspended in 70 per cent. alcohol and 50 cc. of decinormal potassium hydroxide solution added. The edestin thus made neutral was filtered out, dissolved in 500 cc. of 10 per cent. sodium chloride solution and carefully mixed with an equal volume of the same brine containing 50 cc. of decinormal hydrochloric acid. The solution was filtered clear, dialyzed, the crystalline precipitate washed with water, until all

the soluble edestin was removed, the residue suspended in water, neutralized to phenolphthalein, and the potassium salts produced, analyzed with the following results :

TABLE X.—COMPOSITION OF THE POTASSIUM SALTS FORMED BY NEUTRAL-
IZING EDESTIN CHLORIDE OBTAINED FROM EDESTIN SULPHATE.

	Gram.	In per cent. of the inorganic matters.
Organic matters............	0.0192	....
Potassium carbonate........	0.0016	2.01
Potassium sulphate.........	0.0130	16.31
Potassium chloride.........	0.0587	73.65
Undetermined and loss......	0.0064	8.03
	0.0989	100.00

These figures show that over 82 per cent. of the potassium was recovered as chloride and less than 16 per cent. as sulphate.

The many experiments here described can leave no doubt that the acidity of edestin preparations is chiefly caused by hydrochloric and sulphuric acids and that by neutralizing to litmus only a part of the combined acids is removed.

As to the nature of the organic acid, which was present in all the salts described, no information has been obtained, too little being present to make its identification possible.

To determine whether this organic acid was in fact an acid combined with the protein substance or was a product resulting from local overaction of the alkali on the edestin molecule, I extracted hemp-seed meal with 10 per cent. sodium chloride solution containing enough baryta to cause the extract to be just neutral to litmus and then precipitated the edestin from the filtered extract by dialysis against distilled water.

The crystalline product thus obtained was thoroughly washed with water, dissolved in brine, again precipitated by dialysis and extensively washed with water. The moist product was then suspended in water, made neutral to phenolphthalein by adding very dilute potassium hydroxide solution, filtered out and redissolved in 10 per cent. sodium chloride brine. The resulting solution was mixed with an equal volume of the same brine, containing hydrochloric acid equivalent to the potassium hydroxide previously used for neutralizing and the solution dialyzed. The crystalline precipitate was filtered out, washed and neutralized as before and the entire process again repeated. The three solutions

filtered from the three successive edestin precipitates caused by neutralization were evaporated and the residues analyzed.

TABLE XI.—COMPOSITION OF SALTS RESULTING FROM THREE SUCCESSIVE
NEUTRALIZATIONS OF AN EDESTIN PREPARATION.

	I. Per cent.	II. Per cent.	III. Per cent.
Potassium carbonate......	7.4	5.1	0.8
Potassium sulphate........	4.4	2.6	23.0
Potassium chloride........	82.8	80.6	71.3
Sodium chloride......		5.2	3.6
Undetermined............	5.4	6.5	1.3
	100.0	100.0	100.0

These figures show that the organic potassium compound practically vanished after the third precipitation, and therefore it is highly probable that this is a salt of an organic acid previously combined with the edestin and not a product of the action of the alkali upon the protein molecule.

3.   The Proportion of Acid Combined with Edestin.

On page 42, I have shown that all of a large number of edestin preparations are, without exception, acid to phenolphthalein, and that many of them are also acid to litmus.   Some of these preparations are decidedly more acid than others.   Most of the more acid contain more substance soluble in water than those less acid.   Some, however, of the most acid are wholly insoluble in water.   All of these latter were prepared from solutions containing ammonium *sulphate* and, when neutralized with potassium hydroxide, yielded chiefly potassium *sulphate*, whereas the others containing substance soluble in water, yielded, on thus neutralizing, chiefly potassium *chloride*.

The difference between the degree of acidity of the part of the preparation which is soluble in water and of that which is insoluble therein is, however, marked.   In upwards of a hundred trials, I have invariably found the acidity of the part soluble in water to be equivalent to very nearly 1.4 cc. of decinormal acid per gram of the dissolved edestin, while that of the insoluble part was but half as great, provided the preparation tested was made without using ammonium sulphate. As an illustration of this, the following experiments may be given.

A quantity of an edestin preparation was made by cooling a warm sodium chloride extract of hemp seed and repeatedly

recrystallizing the precipitate from warm, dilute salt solution. The substance was then washed by decantation with water. The first, second and third solutions decanted contained no edestin, owing to the salts present in them, but the fourth and fifth contained some.

It was necessary to add 0.8 cc. of decinormal potassium hydroxide solution to 10 cc. of the fourth decantation in order to obtain a neutral reaction with phenolphthalein, while exactly twice as much was required by 20 cc. By evaporating, and drying the residue at 110°, it was found that 10 cc. contained 0.5777 gram of edestin, so that 1 gram of the soluble edestin neutralized 1.39 cc. of decinormal alkali. Similarly, 25 cc. of the fifth washings, containing 0.5320 gram, required for neutralization 0.7 cc. of decinormal alkali, equal to 1.32 cc. per gram.

Eighteen grams of that part of the original preparation which did not dissolve on exhausting with water, when suspended in water, was made exactly neutral to phenolphthalein by 12 cc. of decinormal alkali, or 0.66 cc. per gram,—that is, by just one-half the quantity of alkali required by the soluble part.

Further, another quantity of edestin from which all the compounds soluble in water had been removed and which, when subsequently dried at 110°, weighed 21.27 grams, required when freshly washed and not dried 16.6 cc. of decinormal alkali to render it neutral to phenolphthalein, or 0.78 cc. of decinormal alkali for each gram of protein.

On page 51 the method is described by which a part of preparation **23,** yielding chiefly sulphate when neutralized, was converted into a crystalline preparation, yielding chiefly chloride. The product thus obtained was exhausted with water until nothing more was removed from it, and that part which remained undissolved was uniformly suspended in water and 10 cc. drawn out and mixed, in a stoppered bottle, with 1.5 cc. of decinormal potassium hydroxide solution and phenolphthalein. A clear red solution was formed at once, which required 0.95 cc. of decinormal hydrochloric acid for neutralization, showing 0.55 cc. of the alkali to have been neutralized by the acid combined with the edestin. By evaporating and drying the residue at 110°, it was found that the 10 cc. contained 0.8152 gram of edestin, from which it is seen that 1 gram of the compound insoluble in water ad   an acidity equal to 0.68 cc. of decinormal acid. Two other

similar trials gave exactly the same result.   Ten cc. of the mixture were also added to 10 cc. of 20 per cent. sodium chloride solution, phenolphthalein added and then decinormal potassium hydroxide solution until a slight pink color formed, for which 0.55 cc. was likewise required.

Edestin preparations suspended in water and treated with an insufficient quantity of hydrochloric acid to dissolve them, yield solutions which have an acidity equal to 1.4 cc. of a decinormal solution per gram of dissolved protein.

From all these facts it is clear that the acidity of the edestin chloride, which is soluble in water, is twice that of the part which is insoluble therein.

If the edestin molecule contains two atoms of sulphur, its weight must be about 7,250, or a multiple of this.   If the acidity of the water-soluble edestin is equal to 1.4 cc. of decinormal acid per gram and if one molecule of acid unites with one of edestin to form a water-soluble compound, the molecular weight of edestin would be 7,129.   But, since the water-soluble edestin is twice as acid as the water-insoluble, the former must contain at least two molecules of acid, so that the molecular weight of edestin must be about 14,258, or a multiple of this.

We thus have, in these two acid compounds, a rational explanation for the fact, first observed by Ritthausen, that a part of most edestin preparations is soluble in pure water, while the remainder is insoluble therein.   That this was due to a chemical difference between these two parts was most probable, although preparations showing this behavior consisted wholly of crystals having apparently the same form.

That these two compounds of different composition, as well as the other salts and the free edestin, should crystallize in the same form is to be expected, since the form of the crystal is determined by the protein molecule, the weight of which is enormous compared with that of the one or two molecules of acid combined with it.   The same condition occurs with the compounds of hemoglobin, the crystals of carbon monoxide hemoglobin being isomorphous to those of oxyhemoglobin.   The same isomorphism also occurs among minerals of high molecular weight, as shown by Penfield and Foote,[1] who state: ''In tourmaline we have an isomorphous relation of a very peculiar nature, for in the acid

[1] Penfield and Foote· *Am. J. Sci.*, **8**, 122 (1899).

$H_9Al_3(BOH)_2Si_4O_{19}$ the nine hydrogens may be replaced to a large extent by the trivalent metal aluminum or by the bivalent metals magnesium and iron without any decided change in crystalline form. This leads to the consideration of a certain phase of isomorphism which, as it seems to us, has not been considered with sufficient care, namely, the mass effect of complex radicals in influencing or controlling crystallization.''

If such be the case with a mineral acid containing but $(41)_n$ atoms we certainly should expect the mass influence of a molecule containing approximately $(2,000)_n$ atoms to be all-controlling.

4. Reactions of Edestin With Larger Quantities of Acid.

It has long been known that protein substances combine with acids, and it is a common practice to determine the amount of acid combined with products of gastric digestion. In these products, however, the combined acid is chiefly held by proteoses and peptones, bodies that are known to have more basic properties than the native proteins from which they originate.[1] Panormoff[2] has recently described definite compounds of ovalbumin with different acids, but these contain a much larger proportion of acid than the salts of edestin just described, and are doubtless compounds of a different order (see preceding paper).

I have, however, obtained evidence that edestin also enters into definite reaction with similar large quantities of acid.

A study of the action of water and acids upon edestin has shown that a hydrolytic change is effected in the edestin molecule, whereby a more basic derivative is formed, which shows an entirely different behavior with alkalies and salt solutions from that exhibited by the original, unchanged edestin. It is probable, therefore, that these more acid compounds are not salts of edestin, but salts of more basic derivatives, which form intermediate steps in a series of changes leading to the so-called ''acid albumin.'' Whether or not these changes are common to the other ''native proteids'' I have not as yet ascertained with certainty, but it is probable that they are. Gram portions of the air-dry preparations of edestin, 11, 12, and 13, were suspended in water and decinormal hydrochloric acid gradually added, until a drop of the solution, evaporated on porcelain, showed with tropaeolin a distinct

[1] Cf. Chonheim and Krieger : *Ztschr. Biol.,* n. f., **22**, 95.
[2] Panormoff : *J. russ. phys.-chem. Ges.,* **31**, 556.

red reaction, which in each case appeared with 12 cc.   The tests were then repeated by adding to each solution 11 cc. of acid and afterward increasing this quantity by successive additions of 0.2 cc.   By testing with tropaeolin after each such addition, it was found that one gram of 11 had reacted with 11.2 cc., 12 with 11.5 cc., and 13 with 11.3 cc.   Calculating these figures for the preparation dried at 110° and ash-free, and adding to this the acid originally contained in them, we find that 11 had reacted with 13.9 cc., 12 with 13.7 cc., and 13 with 14.1 cc., which corresponds almost exactly to a compound of 1 molecule of edestin with 20 molecules of acid, assuming this protein to have a molecular weight of approximately 14,500, or, in other words, to exactly ten times the quantity of acid required to form a soluble compound with 1 gram of edestin.

Strong evidence of a definite reaction with about 10 molecules of acid was obtained by testing with potassium nitrite and iodide. A series of five 1-gram portions of 11 were suspended in water in small, glass-stoppered bottles, and to them were respectively added 4 cc., 5 cc., 6 cc., 6.5 cc., and 7.5 cc. of decinormal hydrochloric acid and then to each 7.5 cc. of a solution of soluble starch, containing 1 per cent. of potassium iodide and 1 per cent. of potassium nitrite.

The portion containing 7.5 cc. of acid became blue throughout within five minutes, the color first appearing at the top of the solution ; that with 6.5 cc. began to turn blue at the top within a minute and a half, and became wholly blue in twelve minutes ; that with 6 cc. showed a trace of blue on the surface after five minutes, which, even after thirty minutes, was very slight and limited to the upper surface ; that with 5 cc. showed a trace of blue on the surface after fifteen minutes, which was still slight after an hour and a half ; that with 4 cc. behaved like that with 5 cc., except that, on adding the nitrite solution, a large, permanent precipitate formed, whereas all the other solutions remained very nearly clear.   On standing over night, in the stoppered bottles, the difference between the various portions was much more pronounced, for from those to which 4, 5, and 6 cc. of acid had been added, an opaque yellowish jelly had separated, above which was a clear blue jelly, whereas the portion with 6.5 cc. formed a thin blue jelly containing but little of the

opaque substance, and presented a wholly different appearance
from those with 6 cc. and less.

From this it would appear that this edestin preparation com-
bined with the hydrogen ions contained in 6 cc. of decinormal
hydrochloric acid more firmly than with those contained in the
larger quantities. If we add the acid originally combined with
the edestin, we may conclude that the hydrogen ions equivalent
to 7 cc. of the acid were more firmly combined with the protein
than those contained in the larger quantities.

5. *Solubility of Edestin in Hydrochloric Acid.*

Having found that edestin forms a water-soluble salt with
hydrochloric acid, I undertook to determine the amount dissolved
by definite quantities of this acid. To do so, it was necessary to
make a preparation which should be as neutral as possible to
phenolphthalein, free from any of the hydrolytic derivative of
edestin, mentioned on page 56, and as free as possible from ash.

This is accomplished by extracting oil-free hemp-seed meal
with 3 per cent. sodium chloride brine, previously heated to 60°,
to which is added enough saturated baryta solution to render the
extract neutral to litmus, the requisite quantity being determined
by a preliminary experiment with 100 grams of the meal. It is im-
portant to avoid an excess of baryta, since otherwise, compounds of
edestin with basic constituents of the seed seem to be formed,
which are difficult to get rid of afterwards.

The hot extract is strained on coarse cloth and the residue
pressed. The very turbid extract is thrown on large paper filters
and allowed to stand at rest for about two hours. During this
time a part of the extract filters through and the residue settles
in the funnels so that about two-thirds of the solution can be
drawn off as a turbid liquid, which, however, contains but little
suspended matter. This is filtered by suction on thick felts of
filter pulp on perforated porcelain plates placed in large funnels,
all being previously washed with 3 per cent. salt solution heated
to 70°. By thus filtering, this part of the extract may be readily
obtained perfectly clear and the filter be washed with hot dilute
salt solution, within two hours, 2 liters being passed through
each filter. During this time, most of the residual extract passes
through the paper filters, so that what remains can be rejected
without serious loss. The clear extracts are united in a large

glass-stoppered bottle and allowed to stand over night and cool to 5° or less.   The edestin separates as a dense deposit of crystals, from which the solution is siphoned and thrown away, since very little more can be obtained from it by further dilution and cooling.

The crystallized edestin is next dissolved in 10 per cent. salt solution, best by adding a volume of 20 per cent. solution equal to that of the mother-liquor remaining with the crystals after siphoning off the greater part.   Enough 10 per cent. salt solution is then added so that the solution contains about 8 per cent. of edestin, since stronger solutions, under the subsequent treatment, do not, as a rule, yield well crystallized products.   This saline solution of edestin is heated to 50° and gradually diluted with two volumes of water, at the same temperature, whereby a perfectly clear solution results.   By again cooling to 5° the edestin is re-crystallized.   By repeating this process a very pure crystalline product is obtained, which is again dissolved in enough 10 per cent. sodium chloride brine, free from carbonic acid, to make an 8 per cent. solution of edestin.   An aliquot part of this solution is neutralized to phenolphthalein with decinormal sodium hydroxide solution, and the quantity of alkali necessary to neutralize the whole is determined.   The edestin solution is then heated to 50° in a glass-stoppered jar, and twice its volume of carbonic acid-free water at the same temperature and containing 4 or 5 cc. more than the calculated quantity of the decinormal alkali is gradually added.   The mixture, carefully protected from carbonic acid, is allowed to cool during the night to 5°, the nearly clear solution siphoned off, and the crystalline precipitate collected on a piece of Schleicher & Schüll's thick, hardened filter-paper placed on a perforated plate.   The precipitate, consisting of crystals, is very quickly sucked almost dry and washed two or three times with 1 per cent. sodium chloride solution, cooled to 0°, and free from carbonic acid, then three times with carbonic acid-free water, ten times with 70 per cent. alcohol, and many times with absolute alcohol, all the wash-water and alcohol being at 0°.   It is necessary that the washing should be complete and the dehydration with absolute alcohol thorough, so that on drying over sulphuric acid no water should be left after the alcohol has gone off, which would convert a part of the edestin into the insoluble edestan.

Owing to the physical state of the crystalline edestin, the fil-

tration, washing and dehydration of 50 to 100 grams can be accomplished within twenty minutes. Preparations made in this way were either neutral or very nearly neutral to phenolphthalein, completely soluble in salt solution, contained not more than 0.02 to 0.03 per cent. of ash, and consisted of fine powders, free from lumps, which can be uniformly suspended in water and almost instantly dissolved by the requisite quantity of acid, alkalies, or salts.

It is very difficult to keep the edestin from combining with minute quantities of carbonic acid, since during the final filtration and washing, a brief exposure to the air is unavoidable, without employing elaborate and cumbersome apparatus. As a result, from 1 to 2 cc. of centinormal alkali were required to neutralize one gram of most preparations thus made.

This process is given in detail, as I was unable to prepare edestin suitable for the experiments next to be described, until I had worked out this method of preparation in all its details.

A series of gram portions of preparation **28,** made as above described, was suspended in glass-stoppered bottles, in enough water to make a final volume of 20 cc. with the acid subsequently added. To one portion no acid was added, to the next 2 cc. of centinormal hydrochloric acid, to the next 3 cc., and then 1 cc. more to each succeeding portion, up to 14 cc.

A second, exactly similar series, was also made, commencing with 6 cc.

After frequently shaking the contents of the bottles for about two hours, they were allowed to stand at rest for two hours longer until the suspended matter had practically all deposited. From each solution 10 cc. were drawn out with a pipette, the acidity of each such 10 cc. determined with centinormal potassium hydroxide solution, and then all separately evaporated to dryness on a water-bath and the residues dried to constant weight at 110°. In this way the results given in the following table were obtained :

TABLE XII.—EDESTIN DISSOLVED BY A CENTINORMAL SOLUTION OF
HYDROCHLORIC ACID.

	0.0 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	8 cc.
I	0.0104	0.0082	0.0504	0.0526	0.0718	0.1400	0.1876	0.2576
II	....	.....	.....	.....	.....	0.1460	0.1702	0.2458

	9 cc.	10 cc.	11 cc.	12 cc.	13 cc.	14 cc.	$\frac{HCl}{100}$
I	0.3362	0.4358	0.5850	0.6816	0.7650	0.8406	gram.
II	0.3378	0.4336	0.5636	0.6590	0.7350	0.8080	gram.

The acidity of the solutions and of the residues of Series I was determined by titration with centinormal alkali and phenolphthalein, with the following results :

TABLE XIII.—DISTRIBUTION OF ACID BETWEEN THE DISSOLVED AND UN-DISSOLVED EDESTIN IN TERMS OF CENTINORMAL ACID.

0.0 cc.	2 cc.	3 cc.	4 cc.	5 cc.	.6 cc.	7 cc.	8 cc.
0.0 cc.	0.0 cc.	0.0 cc.	0.0 cc.	0.5 cc.	1.8 cc.	2.6 cc.	3.9 cc.
1.3 cc.	2.9 cc.	3.6 cc.	4.5 cc.	5.0 cc.	4.25 cc.	4.4 cc.	4.4 cc.
1.3 cc.	2.9 cc.	3.6 cc.	4.5 cc.	5.5 cc.	6.05 cc.	7.0 cc.	8.3 cc.

9 cc.	10 cc.	11 cc.	12 cc.	13 cc.	14 cc.	$\frac{HCl}{100}$
5.0 cc.	6.8 cc.	8.6 cc.	10.6 cc.	12.2 cc.	13.6 cc.	Dissolved.
4.0 cc.	3.6 cc.	2.4 cc.	1.9 cc.	1.0 cc.	0.8 cc.	Undissolved.
9.0 cc.	10.4 cc.	11.0 cc.	12.5 cc.	13.2 cc.	14.4 cc.	Total.

The degree of acidity of the dissolved and undissolved edestin, —that is, the amount of centinormal alkali neutralized by 1 gram of the edestin chloride contained in the solution and residues of each of these portions,—is given in the following table :

TABLE XIV.—THE ACIDITY CORRESPONDING TO ONE GRAM OF THE DISSOLVED AND UNDISSOLVED EDESTIN CHLORIDE OF TABLE XIII.

0.0 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	8 cc.
0.0 cc.	0.0 cc.	0.0 cc.	0.0 cc.	6.0 cc.	11.8 cc.	13.0 cc.	14.4 cc.
1.5 cc.	3.3 cc.	4.3 cc.	5.4 cc.	6.4 cc.	6.8 cc.	6.4 cc.	7.0 cc.

9 cc.	10 cc.	11 cc.	12 cc.	13 cc.	14 cc.	$\frac{HCl}{100}$
14.3 cc.	15.1 cc.	14.0 cc.	15.1 cc.	15.6 cc.	16.0 cc.	Dissolved.
7.3 cc.	8.0 cc.	8.0 cc.	9.4 cc.	8.5 cc.	......	Undissolved.

From this table it appears that 1 gram of the substance in the solutions to which from 7 to 12 cc. of acid had been added, neutralized nearly the same quantity of centinormal alkali as that calculated for a compound of one molecule of edestin with 2 molecules of hydrochloric acid, assuming edestin to have a molecular weight of about 14,500, namely 13.8 cc.

Since the amount of edestin in many of these portions was small, most of these determinations are only approximately correct. As already stated, one-half of each of the solutions in these experiments was drawn out with a pipette and the acidity and quantity of dissolved matter was determined as shown above. In order to more accurately determine the acidity of the dissolved edestin by using a larger proportion of substance, the remainder of those solutions of Series I to which from 7 to 13 cc. had been added, was decanted from the undissolved edestin, united and

neutralized by 20 cc. of centinormal potassium hydroxide solution. By evaporating, and drying the residue at 110°, this solution was found to contain 1.4459 grams of edestin, corresponding to an acidity equal to 13.8 cc. of centinormal alkali per gram, or exactly that above calculated. Similarly, the remaining solutions from Series II were neutralized by 25 cc. of centinormal alkali and contained 1.6941 gram of edestin, corresponding to an acidity equal to 14.7 cc. per gram. From these facts it is evident that the substance brought into solution by the acid has the acidity of a bichloride.

The acidity of the undissolved edestin increases with each increase in added acid, until in the portion containing 6 cc. it reaches 6.8 cc. per gram, which corresponds to the formation of a monochloride. With the larger quantities of added acid, this acidity of the undissolved edestin remains nearly constant, although a slight increase is apparent. This excess of acidity above that of the monochloride is caused by the formation of a small proportion of a hydrolytic derivative of edestin, which I have designated *edestan*. Edestan has a higher acid-combining power than edestin and forms with acid a salt which is very sparingly soluble in water. The formation and properties of edestan are described in detail in the preceding paper.

Table XII shows that a small quantity of edestin is apparently dissolved by the water to which no acid had been added as well as by that to which 2 cc. were added. This small quantity is due to a little suspended matter, drawn off with the solution, and is not to be considered as dissolved edestin. In the portions containing 3, 4, and 5 cc. of acid, some edestin appears to be dissolved, but as these, in contrast with the others, were opalescent and neutral, it is most probable that they were colloidal or false solutions caused by edestin bichloride, formed by a local overexcess of acid, which was subsequently reprecipitated on coming into contact with uncombined edestin.

By 6 cc., some edestin was dissolved, but the lower acidity of this portion indicates that the whole of this was not brought into solution by the acid. From 6 cc. upward, a uniformly increasing quantity of edestin passed into solution, until only an insignificant amount remained undissolved by 14 cc. If edestin has a molecular weight of about 14,500 and forms an insoluble chloride with 1 molecule of hydrochloric acid, about 6 cc. of centinormal hydro-
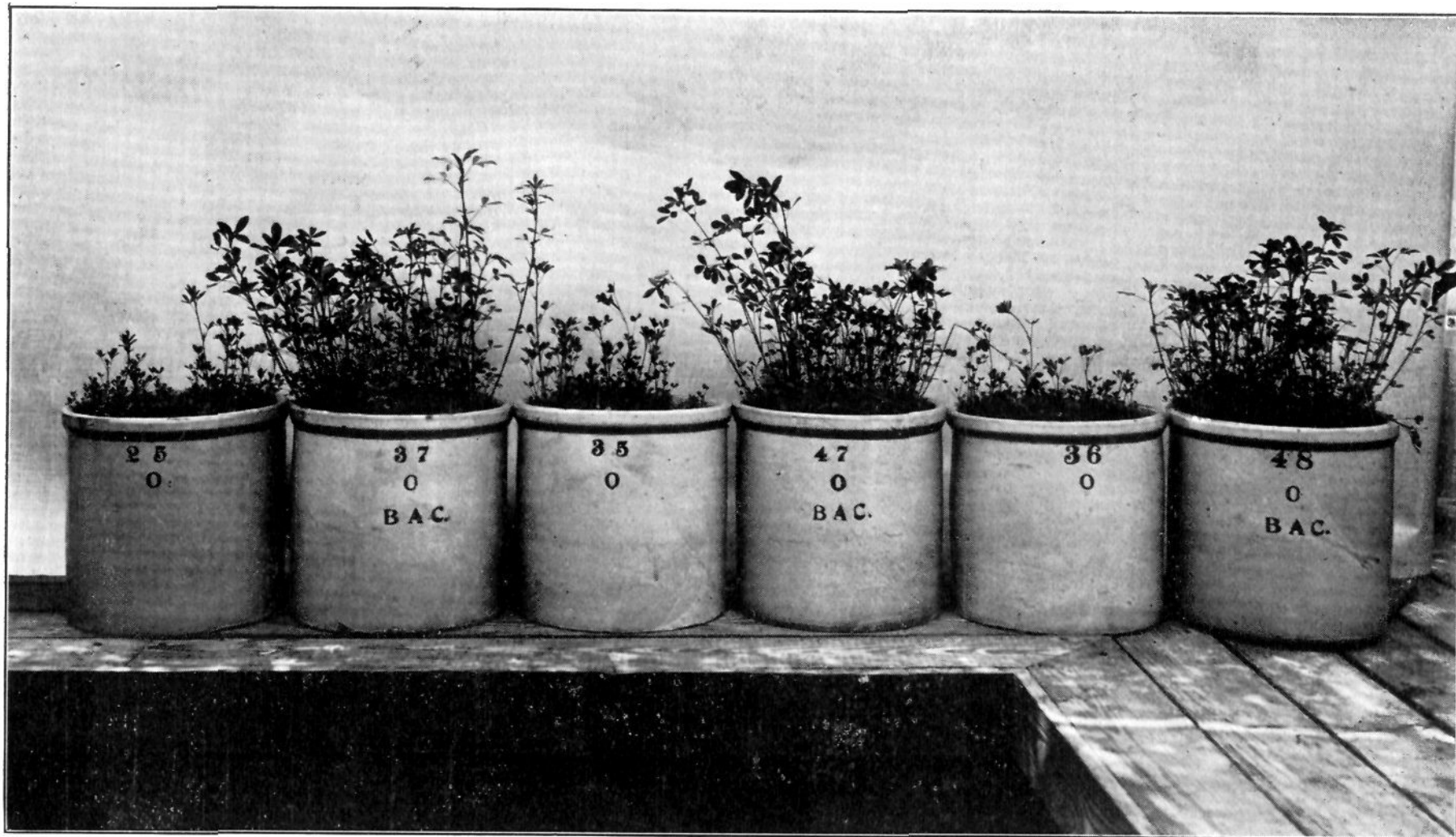
Fig. 1.—Alfalfa pot cultures, showing effect of inoculating ordinary black prairie soil with alfalfa bacteria.
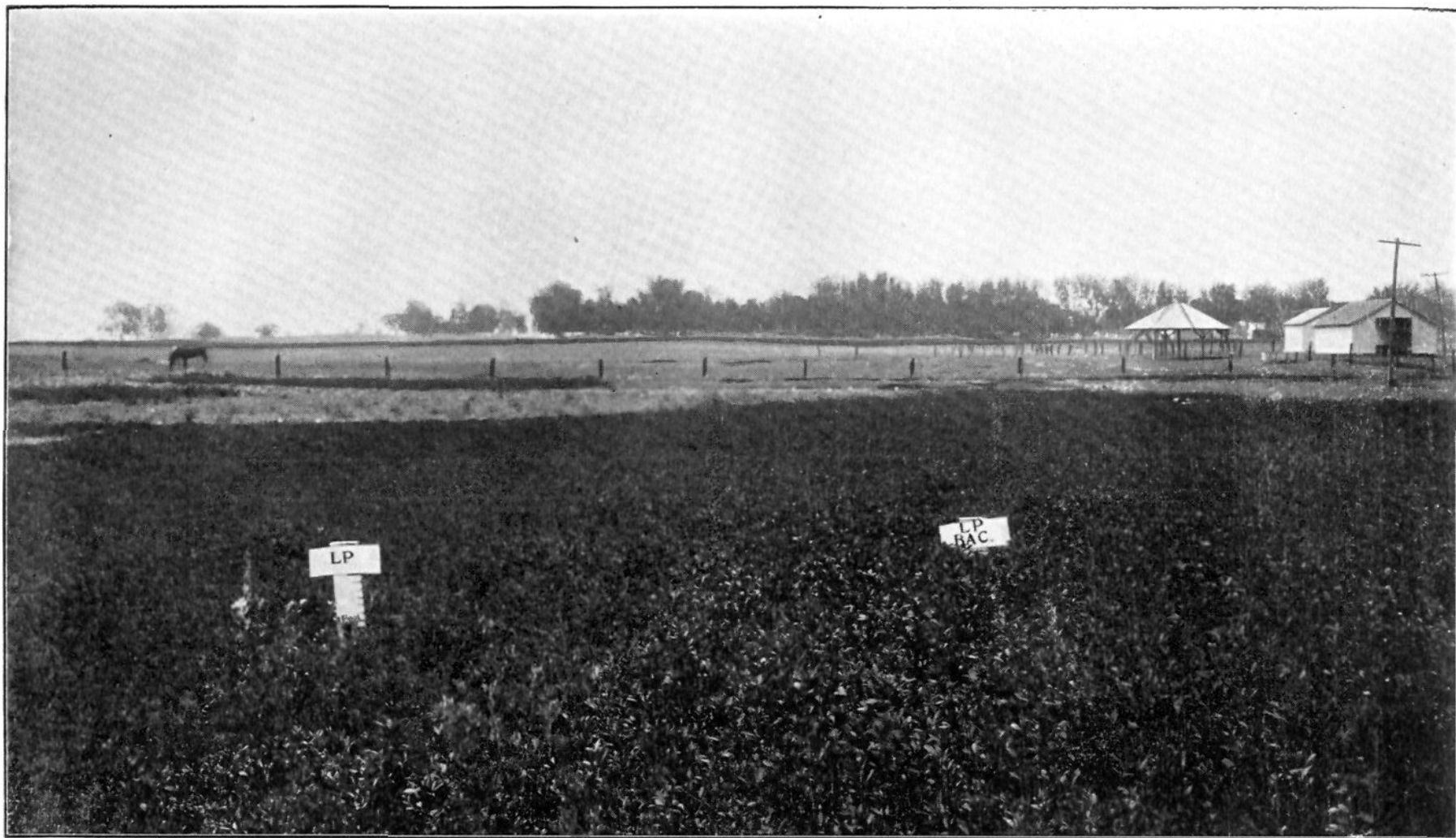
Fig. 2.—Alfalfa field experiments, showing effect of inoculating prairie soil after application of lime and phosphorus.

chloric acid should convert 1 gram of an air-dry preparation into this chloride. In Table XII it is seen that the edestin does not dissolve with less than 6 cc. of acid. Strictly, none should dissolve with less than 7 cc., but as this preparation had an acidity of about 1 cc. per gram, the hydrogen ions causing this ought, in so dilute a solution, to contribute equally with those of the hydrochloric acid in reacting with the edestin, and hence a corresponding quantity should be dissolved.

That this soluble acid compound of edestin contains twice as many molecules of acid as the insoluble acid compound, is shown by the rate at which the acidity of the solution of this substance increased in comparison with the rate at which the acid was added in these experiments. Thus, the solution produced by 10 cc. of acid neutralized 1.80 cc. more alkali than that produced by 9 cc., although but 1 cc. more acid had been added ; that with 11 cc. also neutralized 1.8 cc. more alkali than that with 10 cc.; that with 12 cc. neutralized 2 cc. more alkali than that with 11 cc.; that is, after the whole of the edestin was converted into the insoluble chloride, each molecule of acid subsequently added brought into solution another molecule of acid which had been previously combined with the edestin as an insoluble chloride.

If edestin has a molecular weight of about 14,500, each cubic centimeter of centinormal acid in excess of 7 cc. should dissolve 0.1450 gram. The quantity actually dissolved by each cubic centimeter of acid in excess of 7 cc. falls considerably short of this, chiefly owing to the formation of the more basic derivative, edestan, mentioned on page 56.

Edestan forms sparingly soluble salts with larger quantities of acid than does edestin. In these experiments about 8 per cent. of edestan was formed and consequently the amount of edestin dissolved per cubic centimeter of acid was less than that calculated.

From these results it would appear that edestin, like a true base, enters into ionic reactions with hydrochloric acid. That a weak base should enter into such complete reaction with hydrochloric acid is due to the dilution of the solution, for if its molecular weight is 14,500, a solution containing 5 per cent. of this substance is nearly equivalent to a $1/_{300}$ normal solution.

This method of determining the quantity of edestin brought into solution by hydrochloric acid was next applied to prepara-

tions of the chlorides obtained by the methods formerly employed for the preparation of edestin. The only difference in the manipulation of these experiments consisted in filtering the acid solutions on small felts of washed paper pulp, with help of a pump, and washing each filter and residue with 20 cc. of water.

As a part of each of these preparations was soluble in water,— that is, consisted of water-soluble acid compounds of edestin,— the amount dissolved by water alone was deducted from that obtained from each portion to which acid had been added, the difference being the quantity of edestin dissolved by the given quantity of acid. The results thus obtained are given in Table XV.

TABLE XV.—EDESTIN CHLORIDE DISSOLVED BY CENTINORMAL HYDRO-CHLORIC ACID.

Preparation.	1 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.
No. 2. I........	0.1041	0.2306	0.3291	0.4726	0.5426	0.6301
II.......	.....	0.2451	0.3279	.....	0.5301	0.6025
No. 4.	0.0920	0.2300	0.3260	0.4425	0.5681	0.6380
No. 11. I.......	0.1194	0.2540	0.3679	0.4445	0.4950	0.5085
II.......	0.0738	0.1968	0.3398	0.4498	0.5369	0.6068
No. 15. I.......	0.0916	0.2235	0.3250	0.4332	0.5528	0.5762
II.......	0.1015	0.2291	0.3455	0.4445	0.5365	.....
No. 20. I.......	0.1295	0.2655	0.3761	0.4790	0.5115	.....
II.......	0.1140	.....	0.3484	.....	.....	.....
III.......	0.0870	0.2095	0.3335	0.5125	0.6035	.....
IV.......	0.0745	0.2055	0.2935	0.4050	0.4625	.....

AVERAGE OF THE ABOVE FIGURES.

Preparation.	1 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.
No. 2	0.1041	0.2409	0.3285	0.4726	0.5364	0.6163
No. 4	0.0920	0.2300	0.3260	0.4425	0.5681	0.6380
No. 11	0.0966	0.2254	0.3539	0.4472	0.5160	0.5577
No. 15	0.0966	0.2263	0.3352	0.4388	0.5446	0.5762
No. 20	0.1012	0.2268	0.3379	0.4648	0.5258	.....

Although notable irregularities occur among these figures, the results, on the whole, are as uniform as perhaps we could expect them to be under the conditions of the experiments. It is to be noted that the greater differences are found for the smaller and for the larger quantities of acid employed, that is, in those cases where slight differences in manipulation would naturally cause the greatest variation in the results.

In the table only 60 per cent. of the edestin is shown to be dissolved by the acid employed. This is due to the fact that the

remaining 40 per cent. consisted of moisture, ash, acid compounds of edestin which were soluble in water, and a little of the hydrolytic derivative, edestan, which is insoluble in salt solutions. It was necessary, therefore, to add only about 6 cc. of acid in order to bring into solution all the edestin monochloride con·tained in each preparation.

The following table gives the average weight, in grams, dissolved per cubic centimeter of centinormal hydrochloric acid in each portion to which the designated quantity of acid was added.

TABLE XVI.—EDESTIN CHLORIDE DISSOLVED PER CC. OF CENTINORMAL HYDROCHLORIC ACID SOLUTION.

Preparation.	1 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.
No. 2	0.1041	0.1204	0.1095	0.1181	0.1073	0.1027
No. 4	0.0920	0.1150	0.1087	0.1106	0.1136	0.1063
No. 11	0.0966	0.1127	0.1179	0.1118	0.1032	0.0920
No. 15	0.0920	0.1131	0.1117	0.1097	0.1089	0.0960
No. 20	0.1012	0.1164	0.1126	0.1162	0.1052	0.1022

These results, while not so accordant as those obtained with pure and neutral edestin, are nevertheless in harmony with them.

To find what effect the nature of the acid combined with the edestin would have upon the solubility of edestin in hydrochloric acid, a similar series of determinations was made with preparation **13,** which consisted mostly of edestin sulphate, having been obtained by saturating an extract with ammonium sulphate and dialyzing the solution of the precipitate so produced. The results obtained were as follows:

TABLE XVII.—EDESTIN SULPHATE DISSOLVED BY CENTINORMAL HYDROCHLORIC ACID.

Preparation.	0.0 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	8 cc.
13	0.0000	0.0150	0.0420	0.0825	0.1500	0.3070	0.4435	0.4776

9 cc.	10 cc.	11 cc.	12 cc.
0.5616	0.6860	0.7521	0.8378

From these figures we see that very little edestin dissolves with less than 6 cc. of hydrochloric acid, that is, it does not dis·solve until the .sulphate has been converted into the chloride. Above 7 cc. solution proceeds at about the same rate as with edes·tin chlorides. It is evident that the combined sulphuric acid contributes but little to the solution of the edestin, since the sulphate corresponding to the bichloride is insoluble in water.

Plate I shows at a glance the solubility of these several preparations of edestin and its salts.
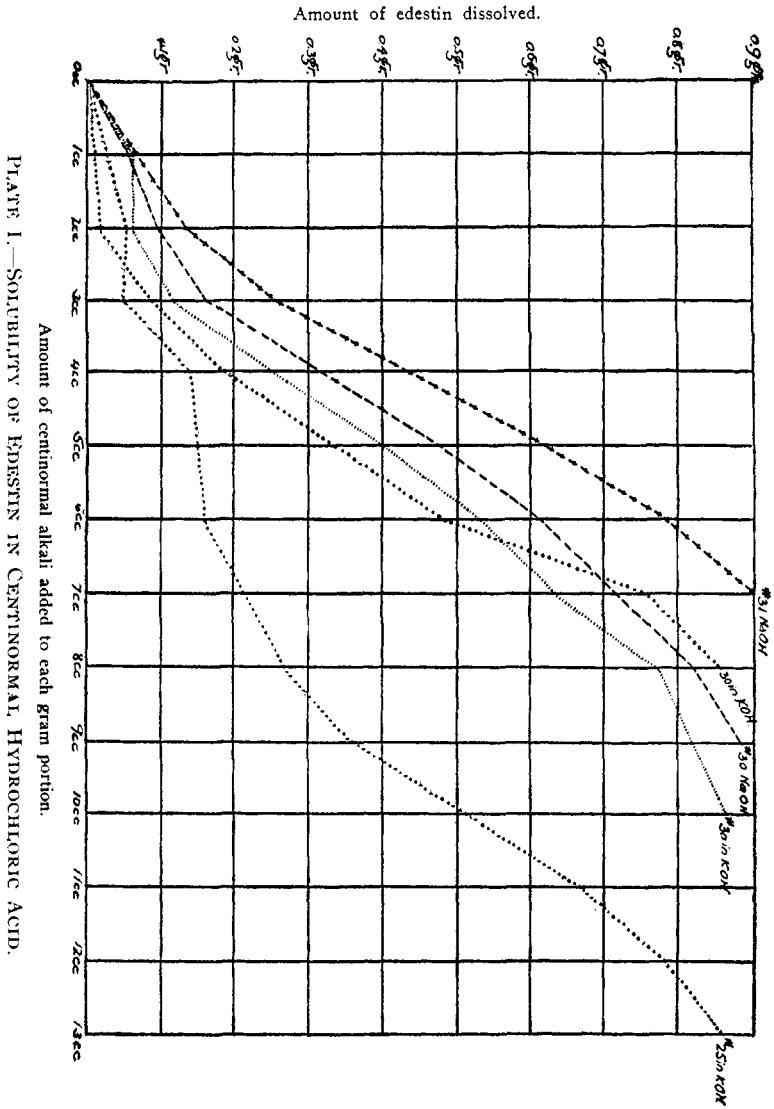
Amount of edestin dissolved.

Amount of centinormal alkali added to each gram portion.

PLATE I.—SOLUBILITY OF EDESTIN IN CENTINORMAL HYDROCHLORIC ACID.

6. *Solubility of Edestin in Sulphuric Acid.*

Preparation 23, which I have shown on page 51 to be nearly pure edestin *sulphate*, required nearly ten times as much decinormal *sulphuric* acid for solution as edestin *chloride* required of decinormal *hydrochloric* acid. It was, therefore, necessary to use a decinormal acid in carrying out experiments similar to those just described, but in the following table the amount of acid is stated in terms of a centinormal solution, in order to avoid possible confusion on comparing the figures with those of the preceding tables. This preparation had an original acidity equal to 10 cc. of a centinormal solution per gram and, as none of it dissolved in water, this amount of acid is added to that applied to the preparation, so that all the acid present with which the edestin could react is here taken into account.

TABLE XVIII.—EDESTIN SULPHATE DISSOLVED BY CENTINORMAL SULPHURIC ACID.

Amount in Grams of Dissolved Edestin Sulphate.

	10 cc.	20 cc.	30 cc.	40 cc.	50 cc.	60 cc.	70 cc.
No. 23.	0.0000	0.0705	0.1750	0.4355	0.6245	......	0.9075
	0.0000	0.0510	0.2280	0.4740	0.6950	0.8680	0.9088

Amount of Edestin Sulphate Dissolved Per cc. of Centinormal H_2SO_4 Solution.

No. 23.	0.0000	0.0035	0.0058	0.0109	0.0125	......	0.0130
	0.0000	0.0026	0.0076	0.0118	0.0139	0.0145	0.0130

Acidity of the Solution of Edestin Sulphate in cc.

No. 23.	0.0	3.0	6.0	18.0	33.0	...	67.0
	0.0	2.0	8.5	21.5	37.5	57.0	64.0

Acidity of the Solution Per Gram of Dissolved Edestin in cc.

No. 23.	0.0	43.0	35.3	41.3	52.9	...	73.7
	0.0	39.2	37.3	45.4	54.0	65.7	71.0

From these figures it is plain that a much larger quantity of sulphuric acid is required to dissolve a given amount of edestin than of hydrochloric acid. The edestin sulphate corresponding to the bichloride is insoluble in water. Whether the soluble compound formed with a sufficient quantity of sulphuric acid is a salt of edestin or whether a hydrolytic derivative of edestin is first produced which forms soluble compounds with the larger quantity of sulphuric acid, was not ascertained, but the behavior of edestin with hydrochloric acid would indicate this to be the case.

7. *The Solubility of Edestin in Phosphoric and Acetic Acids.*

Phosphoric acid reacts with edestin as a monobasic acid, in accordance with its dissociation into the ions H and H_2PO_4.

One gram of the air-dry preparation, suspended in 6 cc. of water, was completely dissolved when treated with 14 cc. of a centinormal solution of phosphoric acid. With 13 cc., 0.8920 gram was dissolved, which is very nearly the calculated quantity, namely, 0.9230 gram.

Acetic acid likewise reacts with almost the calculated amount of edestin, since I found that 13 cc. of a centinormal solution dissolved 0.8804 gram.

Both these acids dissolve somewhat more edestin than does an equivalent quantity of hydrochloric acid, 13 cc. of a centinormal solution of which dissolved about 0.7770 gram. This difference appears to be due to the formation of different proportions of the more basic edestan, as the following experiments indicate. To each of 3 gram portions of edestin suspended in 6 cc. of water, were respectively added 14 cc. of a centinormal solution of each of these acids. After standing for about two hours at 25°. the acid in each was exactly neutralized, and an equal volume of 20 per cent. sodium chloride solution was added. The amount of the insoluble edestan present in the portion with hydrochloric acid was 0.1786 gram, in that with phosphoric acid 0.1484 gram, and in that with acetic acid 0.0565 gram. These results are approximately in accord with the degree of ionization of these acids and appear to explain the relative incompleteness of the reactions with the respective acids.

8. *The Solubility of Edestin in Nitric Acid.*

Nitric acid dissolves edestin chloride in nearly the same proportion as does hydrochloric acid, but a larger quantity of the former acid is required to dissolve the neutral edestin at about 20° than of the latter. At 35° one air-dry gram of neutral edestin, equal to 0.9300 gram dried at 110°, was completely dissolved by 14 cc. of centinormal nitric acid, but by 12 cc. at this temperature much remained undissolved. At 20°, 1 gram was completely dissolved by 20 cc., all but a very few milligrams by 19 cc., while with 18 cc. much remained undissolved.

This quantity of acid is in such close agreement with that required for the formation of a trinitrate that it strongly suggests.

that such is formed, but it is not safe to assume this without other evidence to confirm it.

That a compound with 2 molecules of nitric acid should exist which is more soluble in warm than in cold water, is in harmony with the known behavior of this acid with proteoses, some other protein substances and the histons.

A more extended study of this question is necessary, and I shall take it up as soon as possible.

III. COMPOUNDS OF EDESTIN WITH ALKALIES.

It is well known that protein substances react with alkalies as well as with acids, in which respect they closely resemble the purin bases, which, as pronounced bases, form salts with acids and are also able, like weak acids, to form with bases definite compounds, of which the silver salt is one of the best known, since it is used for the separation of these bodies from their ammoniacal solution.

Many unsuccessful attempts have been made to obtain definite compounds of the proteins with bases, especially with the heavy metals. The chief reason that these failed probably lies in the fact that the small quantity of acid which these substances still contain when their solutions are made neutral to litmus has been overlooked, and also to the fact that salts of the heavy metals are hydrolytically dissociated to such an extent as to make it difficult or impossible to maintain suitable conditions for the formation of definite metallic compounds with the proteins.

The experiments next to be described show that edestin enters into definite reaction with potassium and sodium.

1. Solubility of Edestin in Sodium Hydroxide Solution.

Preparation 31, which was strictly neutral to phenolphthalein, was used to determine the solubility of edestin with definite quantities of sodium hydroxide, in a manner similar to that employed in determining the solubility of edestin with definite quantities of hydrochloric acid.

Gram portions of the air-dry preparation were suspended, in glass-stoppered bottles, in enough water, free from carbonic acid, to make 20 cc. with the alkali solution to be afterwards added. To the first, 2 cc., to the second, 3 cc., and so on up to 7 cc., of centinormal sodium hydroxide solution were added. After agitating frequently for an hour, the solutions were allowed to stand

at rest for another hour, during which time the undissolved
matter settled, leaving the solution nearly clear. From each
portion 10 cc. were drawn out with a pipette, evaporated to dry-
ness, and the residues dried to constant weight at 110°. The
amount dissolved by each quantity of alkali added is shown in the
following table:

TABLE XIX.—AMOUNT OF EDESTIN 31 DISSOLVED BY A CENTINORMAL
SOLUTION OF SODIUM HYDROXIDE.

2 cc.	3 cc.	4 cc.	5 cc.	6 cc	7 cc.	$\frac{NaOH}{100}$
0.1244	0.2564	0.4358	0.6086	0.7834	0.8990	gram dissolved.

The amount, in grams, of edestin dissolved per cubic centi-
meter with each quantity of alkali added, was the following:

TABLE XX.—AMOUNT OF EDESTIN 31 DISSOLVED PER CC. OF CENTI-
NORMAL SODIUM HYDROXIDE.

2 cc.	3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	$\frac{NaOH}{100}$
0.0622	0.0855	0.1090	0.1217	0.1306	0.1283	gram dissolved.

These figures show that the amount dissolved per cubic centi-
meter steadily rises, until with 6 and 7 cc. it reaches a maxi-
mum. This is due to the difficulty with which all of the soluble
sodium edestin is separated from the relatively large quantity of
edestin remaining undissolved in those portions to which the
smaller amounts of alkali had been added, since the great extent
of surface presented by the fine crystalline powder strongly
adsorbs the soluble sodium edestin and also, to the indiffusibility
of the substance, since any sodium edestin formed within the
solid particles is removed with difficulty. With increasing quan-
tities of alkali the proportion of undissolved edestin diminishes
and the proportion dissolved per cubic centimeter correspondingly
increases until it reaches a quantity but little less than that cal-
culated for a complete reaction between one molecule of edestin
and one of sodium hydroxide.

Another series of gram portions of preparation 30, 1 gram of
which had an acidity requiring 2 cc. of centinormal sodium hy-
droxide solution for neutralization to phenolphthalein, was treated
in the same way as the preceding and the following amounts were
found to be dissolved:

TABLE XXI.—AMOUNT OF EDESTIN 30 DISSOLVED BY CENTINORMAL
SODIUM HYDROXIDE SOLUTION.

1 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	8 cc.	9 cc.	$\frac{NaOH}{100}$
0.0490	0.0940	0.1636	0.3208	0.4800	0.6060	0.7120	0.8188	0.8782	{ gram dissolved.

In this case the effect of the carbonic acid contained in this preparation is plainly manifested, since 9 cc. of the alkali were required to dissolve the same amount that 7 cc. dissolved of the perfectly neutral preparation **31**. The small quantity appearing to be dissolved by 1 and **2** cc. consisted mostly of suspended matter unavoidably drawn off with the solution. The agreement between these results and those obtained in the first experiment is best shown by the solubility curves given in Plate II.

2. Solubility of Edestin in Potassium Hydroxide Solution.

Preparation **28**, 1 gram of which required 2 cc. of centinormal alkali for neutralization to phenolphthalein, was treated with a centinormal solution of potassium hydroxide in the way described for experiments with sodium hydroxide. Table XXII shows the weight in grams dissolved by the several quantities of alkali.

TABLE XXII.—AMOUNT OF EDESTIN **28** DISSOLVED BY CENTINORMAL POTASSIUM HYDROXIDE SOLUTION.

1 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	8 cc.	$\frac{KOH}{100}$
0.0000	0.0150	0.0938	0.1944	0.3294	0.4772	0.7592	0.8500	gram dissolved.

The amount dissolved per cubic centimeter above 2 cc. was as follows :

TABLE XXIII.—AMOUNT DISSOLVED PER CC. OF CENTINORMAL POTASSIUM HYDROXIDE.

3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	8 cc.	$\frac{KOH}{100}$
0.0938	0.0972	0.1098	0.1193	0.1518	0.1417	gram dissolved.

In this, as in the experiments with sodium hydroxide, the proportion of dissolved edestin increased as the proportion of undissolved edestin diminished, the amount dissolved by 8 cc. being nearly equal to that calculated for a complete reaction between equal numbers of molecules of each substance. The somewhat higher figure found for 7 cc. is doubtless due to a slight error of manipulation, as indicated by the rise in the curve given in Plate II, showing the results of this experiment.

Another similar series of gram portions of edestin **30**, treated with centinormal potassium hydroxide in the same way as **28** had been treated, gave the following results :

TABLE XXIV.—AMOUNT OF EDESTIN 30 DISSOLVED BY CENTINORMAL POTASSIUM HYDROXIDE SOLUTION.

1 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	8 cc.
0.0586	0.0574	0.1146	0.2496	0.3984	0.5220	0.6292	0.7726
		9 cc.	10 cc.	$\frac{KOH}{100}$			
		0.8120	0.8558	gram dissolved.			

These results are nearly the same as those obtained with preparation **28**, the amount dissolved by less than 7 cc. being slightly more than in the first series, while that dissolved by more than 7 cc. is slightly less.

Assuming that true solution begins only with 3 cc., we have the following quantities, in grams, dissolved per cubic centimeter with each quantity of alkali added :

TABLE XXV.—EDESTIN 30, DISSOLVED PER CC. OF CENTINORMAL POTAS-
SIUM HYDROXIDE SOLUTION.

3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	8 cc.	9 cc.	10 cc.	$\frac{KOH}{100}$
0.1146	0.1248	0.1328	0.1305	0.1258	0.1288	0.1160	0.1060	gram dissolved.

As this table shows, with from 5 to 8 cc., the amount dissolved per cubic centimeter is about 15 milligrams less than that calculated for a complete reaction between equal numbers of molecules of the protein and alkali. The rate at which this preparation dissolved is more uniform than that at which **28** dissolved, the effect of adsorption being much less in these experiments with **30** than in those with **28**. Probably this is due to the fact that the crystals of **30** were much larger than those of **28**, and therefore a much smaller surface was exposed by the former on which the soluble edestin could be adsorbed than by the latter preparation.

A similar experiment with another preparation, **25**, was also made with the following results.

TABLE XXVI.—AMOUNT OF EDESTIN 25, DISSOLVED BY A CENTINORMAL
SOLUTION OF POTASSIUM HYDROXIDE.

2 cc.	3 cc.	4 cc.	6 cc.	8 cc.	9 cc.	10 cc.	12 cc.	13 cc.	$\frac{KOH}{100}$
0.0058	0.0048	0.1380	0.1588	0.2694	0.3594	0.5044	0.7790	0.8678	$\left\{ \begin{array}{l} \text{gram} \\ \text{dissolved.} \end{array} \right.$

This preparation, which, when first precipitated, was perfectly neutral to phenolphthalein, became so acid, after washing and drying without precautions to exclude carbonic acid, the necessity of which was not recognized at the time the preparation was made, that 4 cc. of centinormal alkali were required to neutralize 1 gram of it.

From the solutions, to which 2 and 3 cc. of alkali had been added, the insoluble edestin settled out, leaving the solution very nearly clear and showing no opalescence whatever. Unlike **28**, this preparation at first dissolved slowly, only 0.02 gram more being dissolved by 6 cc. than by 4 cc. but from 6 cc. upwards,

solution proceeded at nearly the same rate as in the other experiments.

That more edestin was not dissolved by the potassium carbonate first formed with the carbonic acid previously combined with the edestin, is due to the slight degree of hydrolytic dissociation occurring with this salt, an insufficient amount of hydroxyl ions being formed to cause a noticeable solution of the edestin.

Plate II, page 74, shows the relations of these experiments with alkalies to one another and makes very evident the effect of the small amount of carbonic acid which some of the preparations contained.

Preparations consisting of mixtures of edestin mono- and bichlorides are completely dissolved when suspended in water and an excess of centinormal potassium or sodium hydroxide solution is added, equivalent to about 7 cc. per gram of edestin. The proportion of such edestin preparations dissolved by a smaller excess of alkali cannot be determined, as the solution from which the undissolved edestin chloride deposits on standing is milky and cannot be filtered because supersaturated with free edestin arising from the water-soluble bichloride.

Solutions of sodium or potassium edestin become turbid after several hours and gradually deposit a part of the protein from the supersaturated solution thus formed.   Solutions of calcium or barium edestin, which are formed only in the presence of a much larger proportion of these inorganic bases, are far less stable than those of sodium or potassium edestin and soon deposit a large proportion of the dissolved protein, together with a considerable part of the mineral base.   Probably the substance which separates from solutions of these compounds is a derivative of edestin, but this has not been determined.

Solutions of sodium or potassium edestin are precipitated by extremely small amounts of neutral salts, but are unaffected by non-electrolytes, such as sugar or alcohol, unless the latter is added in large quantity.   The precipitate thus produced is readily and completely dissolved by adding a sufficient quantity of sodium chloride.   In this saline solution these compounds of edestin show the properties of globulin.

Towards salts, potassium and sodium edestin behave like edestin bichloride.   In the absence of salts, the aqueous solutions
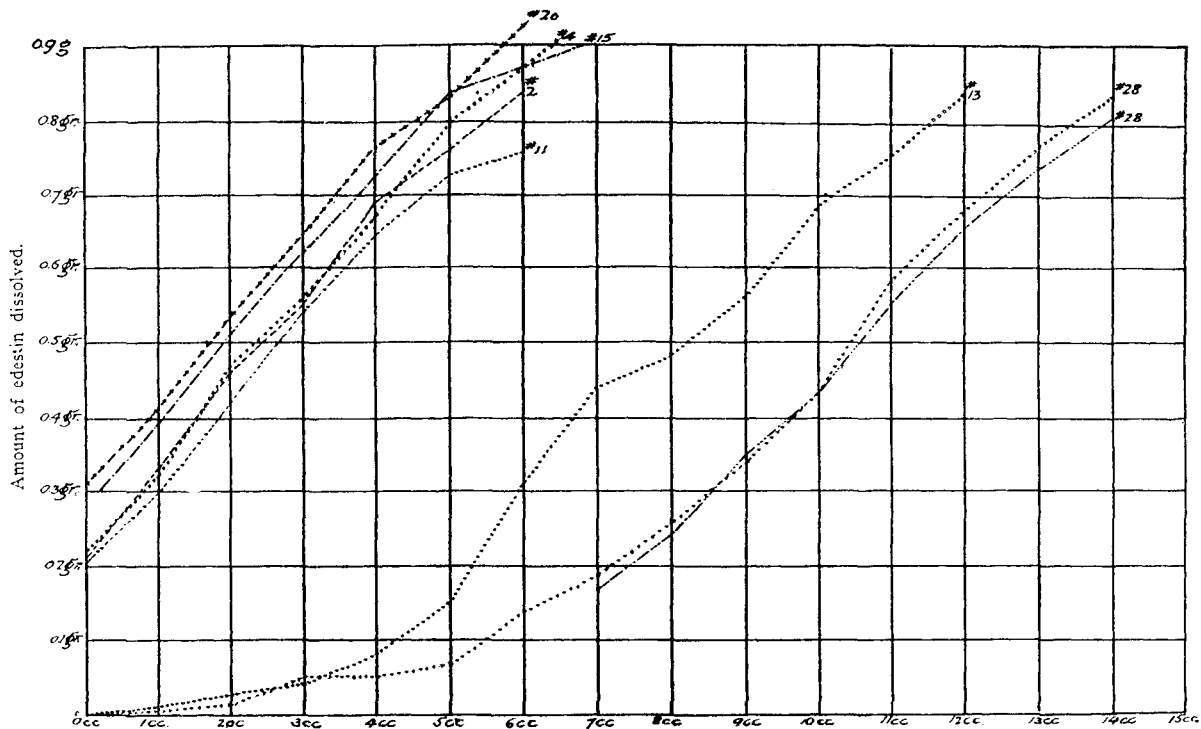
Amount of centinormal hydrochloric acid added to each gram portion.

PLATE II.—SOLUBILITY OF EDESTIN IN CENTINORMAL ALKALI.

of all these compounds are clear and in no way resemble opalescent colloidal solutions.   The solutions produced by strong sodium chloride brine are likewise clear.

3.   Solubility of Edestin in Sodium Carbonate Solution.

Each one of a series of gram portions of edestin 30 was suspended, in a stoppered bottle, in water enough to make a final volume of 20 cc. with the decinormal sodium carbonate solution to be afterwards added.   After adding the amounts of decinormal sodium carbonate solution stated in the table and frequently shaking, the solutions were allowed to stand until the suspended matter had settled.   As the portions containing 0.2 cc., 0.4 cc., and 0.6 cc. settled slowly, these were filtered through pure paper, and 10 cc. of each solution of the entire series were evaporated, the residue dried at 110°, and the following quantities were found to have been dissolved :

TABLE XXVII.—AMOUNT OF EDESTIN DISSOLVED BY A DECINORMAL SODIUM CARBONATE SOLUTION.

0.2 cc.	0.4 cc.	0.6 cc.	1.0 cc.	1.4 cc.	1.8 cc.	2.2 cc.	2.4 cc.	2.5 cc.	$\frac{Na_2CO_3}{10}$
0.0090	0.0376	0.0580	0.2202	0.4722	0.6036	0.7510	0.8160	0.8618	gram.

Very little edestin is dissolved by less than 0.6 cc. of the sodium carbonate solution, doubtless because of the small amount of carbonic acid contained in this preparation.   Above 0.6 cc. the edestin dissolved at a nearly uniform rate, as shown by the following table, which gives the amount in grams dissolved in each portion per 0.1 cc. in excess of 0.6 cc.

Thus the amount dissolved by 1 cc. was 0.2202 gram or 0.1622 gram more than was dissolved by 0.6 cc., therefore one-fourth of this quantity, 0.0405 gram, was dissolved by each 0.1 cc. in excess of 0.6 cc.

TABLE XXVIII.—EDESTIN DISSOLVED PER 0.1 CC. OF DECINORMAL SODIUM CARBONATE SOLUTION IN EXCESS OF 0.6 CC.

1 cc.	1.4 cc.	1.8 cc.	2.2 cc.	2.4 cc.	2.5 cc.	$\frac{Na_2CO_3}{10}$
0.0405	0.0518	0.0455	0.0433	0.0421	0.0423	gram dissolved.

From these figures it is seen that the amount of edestin dissolved is proportional to the amount of sodium carbonate added in excess of 0.6 cc.

A larger proportion of sodium carbonate is required to neutralize a preparation of edestin chloride than of potassium hydroxide.   Five grams of preparation 3 were suspended in water, 5 cc.

of decinormal sodium carbonate solution added, the mixture shaken for some time, the edestin filtered out, and to the filtrate and washings 5 cc. of decinormal hydrochloric acid added. The solution was then boiled, cooled, and neutralized to phenolphthalein by 3.8 cc. of potassium hydroxide solution, from which it is evident that only 3.8 cc. of the sodium carbonate solution had been neutralized by the combined acid of the edestin chloride, which contained sufficient acid to neutralize 5 cc. of *caustic alkali*.

Whether the edestin is dissolved by sodium carbonate solely in consequence of the hydrolytic dissociation of this salt, or whether this salt acts also as a solvent, after the manner of neutral salts such as sodium chloride, requires further investigation into the relations of globulins to neutral salts, which is now in progress in this laboratory.

IV.   CONCLUSIONS.

1. The proteins are basic bodies and enter into ionic reactions with acids with which they form true salts.

2. Preparations of native proteins, as usually obtained from solutions slightly acid or neutral to litmus, are salts of the basic protein substance.

3. The acid of these salts can be separated from those proteins that are insoluble in water, by making them neutral to phenolphthalein with potassium or sodium hydroxide. The acid combined with the protein can then be identified in the aqueous solution, by filtering out the insoluble protein, evaporating the solution and analyzing the alkaline salts thus obtained.

4. Preparations of edestin, made by the methods commonly employed, contain chiefly chlorides, when crystallized from sodium chloride solutions,—chiefly sulphates, when crystallized from a solution containing ammonium sulphate. The salts of edestin which crystallize out are those of the negative ions present in the solution, the predominating salt being that of the negative ion most abundant at the time of crystallization.

5. Edestin preparations, as usually made from sodium chloride solutions, generally dissolve to a considerable extent when washed with pure water. The part that dissolves is twice as acid toward phenolphthalein as the part that remains undissolved.

6. The degree of acidity of the part insoluble in water is equal to that of a compound of 1 molecule of edestin with 1 of hydrochloric acid, assuming that the former has a molecular weight

of about 14,500, which is twice as great as the simplest one that can be calculated from its analysis, if there are 2 atoms of sulphur in its molecule.

Edestin, therefore, forms salts corresponding to a mono- and bichloride.

7. The crystals of edestin, as well as those of its different salts, are, so far as has been determined, isomorphous, the mass influence of the protein molecule being so great as to prevent the small amount of combined acid from effecting a change in crystal·line form.   In this respect, edestin behaves like hemoglobin, the compounds of which with oxygen and with carbonic acid are also isomorphous.

8. The free base edestin, when suspended in pure water, is dis·solved by nearly the calculated quantity of hydrochloric acid required for a complete reaction between 1 molecule of edestin and 2 of hydrochloric acid.   On adding the acid in successive small quantities, no solution takes place, until one-half the required amount has been added.   On adding the second half of the acid, solution takes place at a rate proportional to the amount of acid added.   The acidity of the solution obtained with the second half of the acid increases at twice the rate at which the acid is added, in accordance with the conversion of an insoluble monochloride produced by the first half of the acid into a soluble bichloride formed by the second half.

9. Somewhat more than the calculated quantity of hydrochloric acid is required to dissolve a given quantity of edestin, because a more basic hydrolytic derivative of edestin, sparingly soluble in water, is formed by the hydrogen ions set free by hydrolytic dissociation of the chloride.

10. Since solutions of edestin bichloride do not appear to be precipitated by hydrolytic dissociation, it is probable that edestin hydroxide may be formed and remain in solution under the conditions of the experiments tried.

11. Edestin sulphates are less soluble than the corresponding chlorides and, consequently, preparations obtained from solutions containing ammonium sulphate are not soluble in water.   Ten times more sulphuric acid is required to dissolve a given quantity of edestin than of hydrochloric acid.   Definite compounds with sulphuric acid have not yet been obtained.

12. Hydrochloric acid dissolves more nearly the calculated

quantity of edestin than does acetic acid, since the latter, being less ionized than the former, produces, in a given time, only one-third as much of the more basic hydrolytic derivative as does the former.

13. Phosphoric acid reacts with edestin as a monobasic acid, in accordance with its dissociation into the ions H and H_2PO_4. But little more than the calculated quantity of this acid is required to dissolve a given amount of edestin.

14. Edestin forms a salt with nitric acid, which corresponds to the bichloride. At 30° edestin binitrate is much more soluble in pure water than at 20°, so that a clear solution containing 5 per cent. of this salt yields a considerable precipitate on cooling.

15. Edestin reacts with potassium or sodium hydroxide in a proportion equivalent to that with which it forms the monochloride with hydrochloric acid.

A given quantity of edestin is dissolved by an amount of centinormal potassium or sodium hydroxide solution, which corresponds closely to a proportion of 1 molecule of the base to 1 of protein.

Solutions of potassium and sodium edestin, probably in consequence of hydrolysis, become turbid after standing some time and gradually deposit some of the dissolved protein.

16. About thirteen times as much ammonium hydroxide is required to dissolve a given quantity of edestin as of sodium or potassium hydroxide.

17. About three times as much sodium in the form of carbonate is required to dissolve a given quantity of edestin as of sodium in the form of hydroxide.

18. Edestin conforms strictly with the definition of a globulin, being insoluble in pure water, but readily soluble in neutral solutions of sodium chloride of sufficient strength.

19. Edestin monochloride is, likewise, insoluble in water, but readily soluble in saline solutions. Edestin bichloride and potassium or sodium edestin are soluble in pure water, but insoluble in the presence of a small proportion of a neutral salt. In the presence of a larger proportion of the neutral salt they are soluble, and in such solutions they show the properties of globulin.

20. The fact that edestin, as well as its salts with strong acids, is soluble in perfectly neutral solutions of sodium chloride shows that the solubility of a globulin does not depend on the presence of alkali, as Starke has recently asserted.