XIX. A CHEMICAL STUDY OF LEAF CELL CYTOPLASM.

I. THE SOLUBLE PROTEINS.

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Our knowledge of the chemistry of vegetable cytoplasm rests primarily on the classic research of Reinke [1881] on the cytoplasm of the slime-mould *Aethaleum septicum*. This simple organism, which has no cell wall and contains a very large number of nuclei, is highly specialised, and it is doubtful if plant physiologists have any right to assume, as they have done, that the cytoplasm of the normal vacuolated plant cell has a similar composition.

The present research has been undertaken with the object of gaining some insight into the composition and physical properties of leaf cytoplasm. Such knowledge should not only throw light on the metabolism of the cell but should also help to explain the mechanism of cell permeability. The cytoplasm from vacuolated cells which do not contain those highly specialised inclusions—the chloroplasts—would have been preferable in some respects, but leaves have been chosen, not only because they can be obtained in large quantities, but also because the elasticity of the cell wall enables the cytoplasm to be extracted in a state free from cell wall and vacuole material.

In the investigations reported in the present paper it has been necessary to treat the leaves as a whole unit. The objections to this are obvious: not only may the individual cells vary in composition, but the chloroplasts and other inclusions are not differentiated from the protoplasm. At the present stage of the investigation this cannot be avoided, for, from the practical standpoint, it is impossible to obtain a good preparation of cytoplasm *in bulk* from unicellular organisms. If we are successful in obtaining a fairly comprehensive analysis by the methods now employed, it may be possible at a later stage of the investigation to apply micromethods to individual cells.

Before making as complete an analysis as possible of the cytoplasm from one particular species of leaf, we have thought it advisable to make a general survey of the relations between the cytoplasm and sap of a large number of different species. The ease with which the cytoplasm can be extracted from the leaf cells, the amount of "combined" and "soluble" protein that the cytoplasm contains and the relation of the isoelectric point of the proteins

to the hydrogen ion concentration of the leaf sap form the basis of the present communication. More comprehensive analyses of the proteins, substances soluble in alcohol (fats, lipins, etc.) and the inorganic material of leaf cytoplasm are in progress.

General Experimental Methods.

Materials employed. The leaves were gathered on the morning of the experiment and used within one or two hours.

Methods of manipulation (separation of cytoplasmic proteins and vacuole material). *Method A.* Details of this method have already been published [Chibnall, 1924, 1] and need only brief reference here. The leaves were first cytolysed with ether, enveloped in filter-cloth and pressed in the Buchner press. The leaf residues were then washed free from the remainder of the vacuole material by alternate soaking in water and pressing. The cytoplasm, from which some water-soluble substances may have been removed, was then dispersed into colloidal solution by grinding with water in a meat chopper, the cellular matter being afterwards strained off by squeezing through fine gauze. This colloidal solution of cytoplasm contains protein, which it is possible to separate into two fractions by filtration through paper pulp. That fraction retained on the filter pad is in some loose combination with substances soluble in alcohol, whilst that in the filtrate appears to be uncombined and present in solution as an anion. This would seem to be shown by the fact that it can be precipitated by the addition of acid. It is probable that both these fractions contain more than one protein and, until further research has enabled a satisfactory classification of the leaf proteins to be drawn up, it is proposed to refer to these fractions simply as the "combined" and "soluble" proteins respectively. The present paper deals almost exclusively with the "soluble" proteins extracted from different leaves.

Some leaves—from which a large amount of cytoplasm, mixed with vacuole material, could be extracted by simple grinding—gave only a small amount of colloidal cytoplasm when ground up after the ether treatment already described. What appeared to be some form of denaturing of the cytoplasmic proteins after cytolysis of the cells with ether had already been observed when investigating the leaves of *Medicago sativa* [Chibnall, 1924, 2]. To gain some insight into the amount of this supposed denaturing, a certain number of the different species of leaves were treated in the following way.

Method B. About 200 g. of fresh leaves were minced in a "Universal No. 2" mincing machine, the material being returned to the machine for a second grinding. The minced pulp was then wrapped in a small piece of stout filter-cloth and the liquid expressed in the Buchner press. The press residue was then allowed to soak up water and the operation was four times repeated. The combined extracts were made up to 500 cc. and total-N determined on an aliquot part. The remainder of the solution was filtered through a small, but thick, pad of paper pulp, which was washed with water

until the total volume of the filtrate was 1000 cc. Total-N was then determined. The difference between these two nitrogen values, after allowance for the liquid withdrawn for aliquots, gives the N of the "combined" protein retained on the filter pad. The remainder of this solution was boiled to coagulate protein and again filtered. The filtrate and washings were made up to 1000 cc. and total-N determined. Allowing for aliquots withdrawn the total-N in this last solution represents the water-soluble non-protein-N, and the difference between this value and the one immediately preceding represents the N of the coagulated ("soluble") protein. Examples of such analyses are given later in Table V.

In connection with the above procedure two points must be noted. The coagulated protein from the first filtered extract will contain not only the "soluble" protein of the cytoplasm, but also a small amount of protein that appears to be present normally in the vacuole fluid. Whilst the former seems to represent between 15 % and 25 % of the total leaf-N it has been our experience that the latter represents only about 2 %; therefore in the present paper the coagulated-N can be taken as representative of the "soluble" proteins of the cytoplasm. Secondly the success of this separation depends entirely on the efficiency of the filter pad. Very fine pulp, rammed down to a depth of one to one and a quarter inches on a six inch Buchner funnel, has been used. Filtration through such a pad is necessarily slow, but experience has shown that a more loosely packed pad allows a considerable amount of the "combined" protein to pass through. The filtered leaf extracts should be brown, without any green tinge; whilst the coagulum of protein should be grey or white. If the coagulum is ground up with 10 cc. of 90 % alcohol and filtered, the filtrate should be either colourless or only faintly green. If more than a trace of chlorophyll is present, then some of the "combined" protein has passed through the original filter pad.

Hydrogen ion concentration of the leaf sap.

Hydrogen ion concentration was determined in all cases electrometrically, using a Cambridge Instrument Company's standard portable potentiometer box and a Hildebrand type of hydrogen electrode. Readings were frequently checked with a glass electrode [Kerridge, 1925], which is not subject to the errors due to the bubbling of hydrogen through the liquid, or the "killing" of the platinum electrode which occasionally occurs when working with solutions containing protein.

When possible the hydrogen ion concentration of the sap prepared by both the methods outlined above has been determined. It is more convenient to discuss the sap given by Method B first. When the leaf cells are ruptured by grinding and the resulting pulp pressed out, a thick green liquid is obtained, containing (1) the contents of the vacuole and (2) part, but not all, of the cytoplasm, which has become colloidally dispersed in the vacuole liquid. The hydrogen ion concentration of this sap then does not represent

that of either the cytoplasm or the vacuole fluid in the living cell, but a mixture of both these constituents extracted from the dead cell.

Previous experimental work has shown that the extract obtained by pressing the cytolysed leaves (Method A) consists of the vacuole fluid, mixed, possibly, with some of the more easily diffusible constituents of the cytoplasm. It might be thought that the presence of ether would affect the hydrogen ion concentration as determined by the hydrogen electrode, but experience has shown that any change produced is not within the limits of accuracy required for the present work. For example, *Heracleum sphondylium* leaf sap prepared by Method B was at p_H 6·19. After shaking this sap with 1/15th its volume of ether the hydrogen ion concentration was unchanged.

In the case of all the leaves investigated the hydrogen ion concentration of the cell contents given by Method A and the vacuole material given by Method B do not differ by more than p_H 0·2; generally the agreement is very much closer. This result shows that even if the cytoplasm and vacuole fluid in the *living* cell are at different hydrogen ion concentrations, the permeability of the cytoplasm after cytolysis rapidly brings about equilibrium between them. Therefore the hydrogen ion concentration of the sap given by both methods depends on the relative buffering power of the cytoplasm and the vacuole fluid. Our experience leads us to conclude that the buffering power of the latter is relatively much greater than that of the former so that the observed hydrogen ion concentration is a close approximation to that which would be given by the vacuole fluid in the living cell. In a later paper the relation between the hydrogen ion concentration of the cytoplasm and vacuole fluid will be discussed in much greater detail; it may be mentioned that there is already evidence that a gradient of hydrogen ion concentration exists across the cytoplasmic membrane.

Table I shows the hydrogen ion concentration of the cell sap from a number of different leaves (Method B). The variations between different samples of the same species of leaf are surprisingly small, and seldom exceed p_H 0·2. In cases where the leaves were partially or wholly etiolated greater variations were found. Thus green leaves from unheaded cabbage gave p_H 5·6 to 5·7, whereas leaves from headed cabbages varied between p_H 6·0 and 6·4. As no attempt has been made to inquire into seasonal variations, the relative ages of the plants when the leaves were picked has not been quoted.

Preparation of the "soluble" proteins.

It has not been thought necessary to give details of every preparation made; a condensed summary of one preparation from each species of leaf is given in Table II. The details for *Spinacea oleracea*, *Medicago sativa* and *Zea Mays*, which have already been published, are included for purposes of comparison.

The percentage of N shows that the purity of the protein extracted by the same method from different leaves varies. The first six proteins enumerated

Table I. *The hydrogen ion concentration of the leaf sap.*

(Figures marked * are for sap prepared by Method A; remainder by Method B.)

Species	p_H of sap
Ficus Carica (fig)	6·87 6·85* 6·73
Spinacea oleracea (spinach)	6·57 6·57* 6·57* 6·53* 6·50
Helianthus multiflorus	6·54
„ *annuus* (sunflower)	6·40
Heracleum sphondylium (cow-parsnip)	6·19 6·30*
Tetragonia expansa (New Zealand spinach)	6·06 6·08*
Phaseolus multiflorus Willd. (scarlet runner)	5·97 5·92* 6·12 6·09 6·04 6·00
Populus tremula	5·93
Atropa Belladonna (deadly nightshade)	5·80
Clerodendron trichotomum	5·83 5·78 5·62 5·72* 5·47
Wistaria chinensis	5·70
Vicia Faba (broad bean)	5·67 5·84*
Medicago sativa (lucerne, alfalfa)	5·67
Zea Mays (maize)	5·66
Brassica oleracea (cabbage) "unheaded"	5·60 5·60* 5·68
Tropaeolum majus (nasturtium)	5·60
Crambe cordifolia	5·56 5·50*
Catalpa bignonioides	5·50 5·57*
Cochlearia Amoracia (horse-radish)	5·37 5·40 5·41 4·98* 5·20* 5·45 5·05*
Acer pseudoplatanus (sycamore)	4·88 4·64*
Polygonum cuspidatum	4·52
Saxifraga cordifolia	4·22
Rheum rhaponticum (rhubarb)	4·00 4·62* 4·36*
Pterocarya caucasia	4·15
Parthenocissus quinquefolia	3·70
„ *tricuspidata*	3·56
Rumex acetosella	3·19
Vitis vinifera (vine)	3·21 3·04 3·02* 3·03* 3·12

in the table do not give the Molisch test for carbohydrate; the remainder give a small amount of furfural on boiling with hydrochloric acid, and a carbohydrate impurity of the nature of pectin is possibly present. This impurity does not seem to denature in the same way as the cytoplasmic proteins, because the smaller the yield the less pure is the "soluble" protein from the same species of leaf. In some cases, where the yield of protein by Method A is small, as in *Cochlearia Amoracia*, the protein prepared by Method B has a higher N content (N 15·70 %, ash-free). As a general rule it has been found that the amount of impurity increases as the leaf ages.

The leaves of *Helianthus multiflorus*, *Tropaeolum majus* and *Atropa Belladonna* have been examined qualitatively. The cytoplasm was easily extracted by Method A and soluble proteins were prepared from them.

Analysis of the "soluble" proteins.

Table III gives the analyses of the proteins by Van Slyke's method, as recently modified by Plimmer and Rosedale [1925]. The analyses show that a close relationship exists between all these proteins, the only significant variations being in the amide-N and histidine-N.

Theoretically it should be possible for a protein to undergo amidisation or deamidisation without any change in the internal structure of the protein molecule. Such little evidence as has yet been collected shows that these proteins undergo deamidisation as the leaf becomes more mature. Thus the

amide-N of a preparation from young leaves of *Phaseolus multiflorus* was 7·36 %, from mature leaves 5·69 %, from young leaves of *Heracleum sphondylium* 7·95 % and from aged leaves 6·46 %. In the latter case no significant change was observed in the distribution of the basic-N.

Table II. *Showing details of "soluble" proteins prepared by Method A.*

Species	Weight of leaves taken kg.	Yield of "soluble" protein g.	"Soluble" protein Ash %	"Soluble" protein Ash-free N %
Spinacea oleracea	22·7	70·0	1·72	16·25
Vicia Faba	3·0	6·1	4·27	16·77
Phaseolus multiflorus	2·2	16·0	3·12	15·85
Heracleum sphondylium	4·3	11·8	6·20	15·90
Medicago sativa	5·5	32·3	0·20	15·76
Crambe cordifolia	3·0	7·0	2·20	15·70
Brassica oleracea	1·5	2·0	5·90	14·65
Zea Mays	6·7	29·6	3·28	14·40
Cochlearia Amoracia	2·5	1·0	1·28	14·37
Tetragonia expansa	5·5	5·0	5·07	14·46
**Helianthus annuus* (Method B)	2·4	14·2	10·4	13·70
Ficus Carica	3·0	4·0	9·7	13·40

* Old leaves showing signs of chlorophyll degeneration.

Table III. *Van Slyke analysis of the "soluble" proteins.*

(In percentages of total nitrogen; nitrogen figures corrected for solubility of bases.)

	Spinacea oleracea	*Medicago sativa*	*Zea Mays*	*Vicia Faba*	*Cochlearia Amoracia*	*Crambe cordifolia*	*Brassica oleracea*	*Tetragonia expansa*	*Ficus Carica*	*Heracleum sphondylium*	*Phaseolus multiflorus*	*Helianthus annuus* (aged leaves)
Amide-N	6·93	5·51	7·44	7·49	6·37	6·51	6·37	7·36	6·96	7·95	5·69	6·82
Acid humin-N	0·76	1·22	1·91	1·87	2·36	2·16	1·74	1·81	3·99	2·40	3·12	3·61
Basic humin-N	1·71	1·48	2·66	1·51	1·99	1·34	1·29	1·93	2·99	1·90	1·06	4·92
Arginine-N	13·80	15·32	14·69	16·08	14·44	16·75	14·29	15·75	14·54	15·15	16·08	13·08
Histidine-N	3·89	3·09	4·70	9·02	5·23	7·30	7·05	8·15	7·36	10·20	6·24	6·45
Lysine-N	10·47	10·28	9·07	6·99	8·82	8·84	10·07	8·15	8·84	7·60	10·07	6·77
Mono-amino-N	58·42	59·11	56·29	50·00	55·24	53·07	56·87	53·93	50·68	50·36	53·85	52·46
Non-amino-N	2·58	3·19	2·04	6·90	4·23	3·20	1·51	2·39	1·50	4·00	3·40	3·23
	98·66	99·20	98·80	99·86	98·68	99·17	99·19	99·47	96·86	99·56	99·51	97·34
Total basic-N	28·16	28·69	28·46	32·09	28·49	32·89	31·41	32·05	30·74	32·95	32·39	26·30

This variation of amide-N, which has not been observed in any of the seed proteins, may be due to the fact that the "soluble" proteins are mixtures. In spite of this, however, the general agreement between the analyses given in Table III shows that the composition of this fraction of the cytoplasm is very similar in all the leaves investigated.

Properties of the "soluble" proteins.

The proteins extracted from the different leaves outlined above are similar in most respects to that from the leaves of *Spinacea oleracea*. They all belong to the ill-defined class known as glutelins.

Between the limits p_H 4·0 and 5·0 the solubility of the proteins is at a minimum and in the case of spinach is of the order 0·0014 %. Below p_H 4·0

the proteins are slowly soluble, the solubility increasing with decrease of p_H to 2·5, beyond which they are again precipitated. In such an acid solution the proteins are sensitive to the presence of salts, though not all to the same degree. The protein from spinach will dissolve with difficulty at about p_H 3·0 in the vacuole sap from the same leaves, whereas those from cabbage and horse-radish will not dissolve in an acid solution containing as little as $M/50$ sodium chloride.

Above p_H 5·0 the proteins are readily and increasingly soluble with increase of p_H, and are precipitated from solution only by salts which are used for this purpose in general protein chemistry (ammonium sulphate, etc.).

The isoelectric point of the "soluble" proteins.

The isoelectric point has been determined by the method of maximum precipitation. The original protein solution as prepared by Method A has a p_H between 6 and 7. On account of the rapid denaturing that some of these proteins undergo in acid or alkaline solution the isoelectric point was determined with as little delay as possible. 50 cc. portions were run into a series of graduated tubes and increasing amounts of $N/10$ hydrochloric acid added to them successively. In all the preparations made the protein was completely precipitated between the limits p_H 4·0 and 5·0 within one quarter of an hour; on standing a few hours some of them were completely precipitated over a range as wide as p_H 3·5 to 6·5. Attempts to obtain a narrower range for the isoelectric point by using buffered solutions and measuring the volume of precipitated protein led to inconclusive results. It is possible that the wide precipitation zone of these proteins, like that of the globulin edestin, is due to the relative insolubility of the protein acid compounds in the neighbourhood of the isoelectric point [cf. Cohn, 1925]. Alternatively, as there is no evidence that the proteins being investigated are not mixtures, the range p_H 4·0 to 5·0 may represent the limits within which the constituent proteins of the mixture are isoelectric.

The p_H of the sap from which these proteins were extracted varied from 6·87 in the case of *Ficus Carica* to 5·37 in the case of *Cochlearia Amoracia*. Therefore the proteins must have been present in the cytolysed cells as anions, but at a p_H not far removed from that of their isoelectric point. The ease with which the cytoplasm dispersed into colloidal solution immediately after the cells were broken open indicates that the proteins in the living cell were present also as anions.

The denaturing of the "soluble" proteins.

The (presumed) denaturing which these proteins undergo in certain circumstances has been the cause of considerable trouble during the present investigations. By Method B, which separates the "soluble" protein from the cellular material and the remainder of the cytoplasm within a few minutes,

the "soluble" protein represents between 15 % and 25 % of the total leaf-N. But by Method A, which requires an hour or more for the ether cytolysis, pressing and washing, the yield is often very low, due to some change in the character of the cytoplasm, rendering it less easy to disperse into colloidal solution. A comparison of the yields of "soluble" protein by the two methods is given in Table IV.

No satisfactory explanation of this denaturing has yet been found. It is not caused by the ether used in the cytolysis, for the expressed cytoplasm obtained by Method B, if shaken with ether to the point of saturation, has no greater tendency to flocculate than a similar extract not so treated. Although the hydrogen ion concentration of the vacuole fluid may have some effect, it is not the determining factor, for leaves of the runner bean, the proteins of which do not appear to undergo any denaturing, have a vacuole fluid at p_H 6·0; whilst the leaves of New Zealand spinach, the proteins of which denature very rapidly, have a vacuole fluid at p_H 6·08. Further, it has been found by experience that the pressure applied in the Buchner press has no appreciable effect on the yield of "soluble" protein. Thus no factor which will account for this denaturing has been found, and it is possible that the denaturing is due to differences between the individual leaf cells, the vacuole fluids of which diffuse from one to another after cytolysis.

Table IV. *Comparison of the yields of "soluble" protein.*

(Protein-N as a percentage of total leaf-N.)

Species	Method A %	Method B %
Spinacea oleracea	14·1	16·7
Vicia Faba	4·0	—
Phaseolus multiflorus	16·0	16·4
Heracleum sphondylium	5·1	—
Medicago sativa	8·6	15·1
Crambe cordifolia	6·0	27·0
Brassica oleracea	4·0	15·4
Zea Mays	12·0	—
Cochlearia Amoracia	1·0	19·4
Tetragonia expansa	4·5	17·5
Helianthus annuus	—	13·7
Ficus Carica	1·5	—

The rôle of the "soluble" proteins in the metabolism of the leaf cell.

As the amount of "soluble" protein extracted from the leaf must necessarily depend on the number of ruptured cells, the yields given in Table IV are minimum values. When the methods now being employed for the extraction of the cytoplasm were being investigated, the various operations were frequently followed with the aid of the microscope. There is no doubt that the N remaining in the leaf residues is due to unextracted cytoplasm, which has not been scoured out of the ruptured cells by the grinding. Within the limits of accuracy possible at this stage of the investigations there seems no reason to assume that this unextracted cytoplasm differs in composition

from that which has been extracted. Table V shows how the yield of "soluble" protein compares with that of the "combined" protein. It might at first be thought that, as the "soluble" protein is not in any apparent combination with the fats, etc. in the cytoplasm, it is not necessary for the vital functions of the living cell and that its sole function is to act as a reserve of N which can be utilised by the cell as required. The following experiment does not support such a contention, and shows that the functions of the "soluble" and "combined" proteins in the leaf are not dissimilar.

Some leaves of *Phaseolus multiflorus* with long petioles were cut from the plant and left exposed to diffuse daylight for four days with the petioles dipping in water. Previous experiment [Chibnall, 1924, 3] had shown that under these conditions considerable degradation of the cytoplasmic proteins occurs, giving rise to soluble products, chiefly asparagine. Table VI shows the distribution of the protein-N and non-protein-N obtained by Method B. The non-protein-N has increased from 15·8 % to 28·5 %, yet the ratio of "soluble" to "combined" protein extracted shows no great variation. As mentioned above, there is no reason to assume that the cytoplasm unextracted by the methods employed differs materially from that which has been extracted, and it would appear therefore that the increase of non-protein-N has taken place at the expense of both the protein fractions of the cytoplasm, and not from the "soluble" protein alone.

Table V. *Showing the amounts of "combined" and "soluble" protein extracted from various leaves.*

(Percentage of total leaf-N.)

Species	*Cochlearia Amoracia*	*Phaseolus multiflorus*	*Brassica oleracea*	*Spinacea oleracea*
"Combined" protein-N	18·4	35·4	14·0	19·1
"Soluble" protein-N	19·4	16·4	15·4	16·7
Water-soluble non-protein-N	13·5	15·8	31·7	26·7
Unextracted-N	48·7	32·4	38·9	37·5

Table VI. *Showing the change in the distribution of N in leaves placed with their petioles in water.*

Phaseolus multiflorus.

(Percentages of total leaf-N.)

	Leaves kept with their petioles in water for 4 days	Freshly picked leaves
"Combined" protein-N	33·5	35·4
"Soluble" protein-N	14·9	16·4
Water-soluble non-protein-N	28·5	15·8
Unextracted-N	23·3	32·4

Leaves from which no "soluble" protein could be prepared.

No "soluble" proteins were extracted from the following two groups of leaves by the methods outlined above.

Group 1. Leaves of *Populus tremula, Wistaria chinensis, Rheum rhaponticum, Clerodendron trichotomum, Polygonum cuspidatum, Saxifraga cordifolia, Catalpa bignonioides* and *Pterocarya caucasia.* By Method B an extract charged with cytoplasm was obtained, which flocculated fairly readily on standing. The filtered extract gave but very little coagulum on boiling. Method A gave only a very small amount of cytoplasm. In the case of leaves with a fairly acid sap, such as rhubarb, p_H 4·0, a considerable amount of "soluble" protein could be obtained by using $N/100$ sodium hydroxide in place of distilled water during the grinding which extracts the cytoplasm. In the case of rhubarb the yield corresponded to 6 % of the total leaf-N, the preparation containing 5·7 % of ash and, ash-free, 14·3 % of N. This protein flocculates fairly sharply on adding acid at about p_H 3·5, and is insoluble in excess of acid. It is probable that part, at least, of this preparation is derived from the "combined" protein by the mild hydrolytic action of the alkali, for the alcoholic washing contained solids equivalent to 11 % of the final preparation.

Group 2. Leaves of *Parthenocissus quinquefolia, Parthenocissus tricuspidata, Rumex acetosella* and *Vitis vinifera.* These leaves are distinguished from all the leaves mentioned above, not only by the extreme acidity of the sap, but also by the fact that they contain a considerable amount of what appears to be a mucilage. The presence of this material interferes with the extraction of the cytoplasm both by methods A and B: some modification of the technique employed will have to be devised before the cytoplasmic proteins can be extracted in a state of purity.

Reviewing all the above results it will be seen that we have been able to extract proteins in a state of purity only from the leaves of herbaceous plants having a leaf cell sap which is alkaline with respect to the isoelectric point of the proteins.

Summary.

The proteins of leaf cytoplasm can be separated into two fractions: (A) "combined" proteins, which are in some loose combination with substances soluble in alcohol, and (B) "soluble" proteins, which are uncombined, and which pass fairly readily into solution when the cytoplasmic gel is ground with water.

The "soluble" proteins of a large number of different leaves have been investigated. Preparations sufficiently pure for chemical analysis have been made from the leaves of eleven herbaceous plants and one ligneous plant.

These proteins are glutelins, and although they may be mixtures, their chemical properties are very similar. All of them have an isoelectric range from p_H 4·0 to 5·0 in which their solubility is at a minimum. The hydrogen ion concentration of the leaf cell sap was in all cases alkaline with respect to this isoelectric range, so that the proteins were present in the cytolysed cells, and probably in the living cells, as anions.

Brief mention is made of leaves from which it has not yet been possible to prepare pure "soluble" proteins.

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