heat and acids, it seems likely that the decompositions noted above are all closely related to enzyme hydrolysis.

Summary

The phytin contents of 57 samples of foodstuffs have been estimated by the method of Heubner and Stadler. It was found preferable to use 2% hydrochloric acid for extraction. Heating, soaking and steaming were found to bring about a very perceptible decrease in the amount of phytin as estimated by titration. The course of purification of phytin when separated by Anderson's procedure has been studied to show the effect of the various steps upon the loss and purity of the different fractions.

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[CONTRIBUTION FROM THE BIOCHEMICAL DEPARTMENT, UNIVERSITY COLLEGE, LONDON]

LEAF CYTOPLASMIC PROTEINS[1]

BY ALBERT CHARLES CHIBNALL

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The nutrition of any organism, whether animal or vegetable, is essentially a problem of cell biochemistry. Each organism utilizes its own foodstuffs, which may be presented to it in various ways, yet the nutrition of the single cells making up such an organism depends on the selective absorption of certain fairly simple substances, inorganic salts, sugars, amino acids, etc., from some medium external to the cell into the cell itself.

We are, as yet, far from having a clear idea of the mechanism of this selective absorption or, in other words, of cell permeability; it is this want of knowledge which has prevented the plant physiologist from gleaning very much beyond an empirical knowledge of plant nutrition.

As Stiles[2] has recently pointed out in his useful summary of the literature on permeability,

> "Research on the problems involved has proceeded along two rather distinct lines. In one the whole living organism has been the unit of experimentation, while in the other isolated cells and tissues have been employed..... The methods as employed today have provided a quantity of empirical information on the relation between the amount of growth of plants and the constitution of the medium external to their roots; as far as permeability problems are concerned they have not led us much further than the experiments of Sachs, Knop and other workers of their time....."

While it is probable that the cell membrane is impermeable to certain substances, there is no doubt that the protoplasmic membrane is the seat

[1] Read at the Chemistry and Plant Life Symposium, Los Angeles, August 3, 1925.

[2] Stiles, "Permeability," New Phytologist Reprint No. 13, Wheldon and Wesley, London, 1924.

of the selective absorption observed in the intake of substances into the organisms from the surroundings, and their passage from the cell into the external medium. Therefore it seems to the writer that the time is now ripe for the problem to be attacked by a third method, namely, by a thorough investigation of the physicochemical properties of cytoplasm.

In 1881 Reinke[3] showed that the cytoplasm of the plasmodium of the slime mould *Aethalium septicum* contained 55% of protein, 12% of fat and 13% of carbohydrate. Until quite recently physiologists seem to have been content with this analysis, for those who have propounded theories of permeability based on either lipoids or proteins do not seem to have investigated these substances as they actually occur in the cytoplasm. It remained for Loeb to point out the significance of the physicochemical properties of the proteins; since then there has been an awakening among physiologists to a need for more information concerning the physiologically active proteins in the living cell.

The writer's researches on leaf proteins during the past few years have been confined to methods of preparation and purification, but one of the ultimate objects has been an investigation of their physicochemical properties under conditions approximating those in the living cell. It must be admitted that the method of treating whole leaves as a single unit takes no account of possible variations among the individual leaf cells, but it is not easy to obtain cytoplasm *in mass* from unicellular organisms, so that one is more or less forced—at any rate, in the early stages of an investigation of this nature—to ignore these possible variations.

Methods have already been described whereby the cytoplasm of leaf cells can be obtained as a flocculent precipitate free from cell wall material and the water-soluble products of the vacuole.[4] Table I gives a preliminary analysis of the cytoplasm from spinach leaves. So far, it has been possible to investigate only the proteins of this complex. Part is "free," that is, it can be separated from all fatty material and carbohydrates by filtration through paper pulp. The remainder appears to be in some kind of loose combination with fatty substances, which can be removed by washing with alcohol. Analyses, given in Table II, would indicate that the "combined" protein does not differ materially from the "free" protein in amino acid composition.

Further investigations on the chemistry of this cytoplasm will be published later: in this paper is discussed only the relation between the hydrogen-ion concentration of the contents of the leaf cells and the isoelectric point of the cytoplasmic proteins that can be obtained "free"

[3] Reinke, "Studien über das Protoplasma." Unters. bot. Lab. Univ. Göttingen, vol. 2, pp. 77–184, **1881.**

[4] Chibnall, *J. Biol. Chem.*, **55**, 333 (1923); **61**, 303 (1924).

from fatty substances. Practical details will be published later, but it may be mentioned that (1) all determinations of hydrogen-ion concentration have been made electrometrically, and (2) the iso-electric point was obtained by the method of maximum precipitation.

TABLE I

ANALYSIS OF SPINACH LEAF CYTOPLASM

	Alc. sol. subs.	Ash	Protein	Undetermined	Total
%	12.7	15.1	64.6	7.6	100

TABLE II

VAN SLYKE ANALYSIS OF SPINACH CYTOPLASM. PERCENTAGES OF NITROGEN

	Subs. washed free from alc. sol. subs., %	"Free" protein. %		Subs. washed free from alc. sol. subs., %	"Free" protein %
Amide N	6.57	6.93	Arginine	14.11	13.80
Humin N in acid	2.87	0.76	Histidine	6.90	3.89
Humin N in lime	0.94	1.46	Lysine	7.47	9.63
Humin N in amyl alcohol	0.06	0.25	Amino N in filtrate	56.00	58.09
Cystine	0.94	1.27	Non-amino N in filtrate	3.07	2.58
				98.93	98.66

TABLE III

THE ISO-ELECTRIC POINTS OF SOME LEAF CYTOPLASMIC PROTEINS AND THE HYDROGEN-ION CONCENTRATIONS OF THE CONTENTS OF THE LEAF CELLS

	Spinach	Hogweed	Broad bean	Cabbage	Rhubarb	*Vitis vinifera*
P_H, protein iso-elec. pt.	5.0–4.0	5.0–4.3	5.1–4.3	4.7–4.0	3.5[a]	4.8–4.4[b]
P_H, cell contents	6.57	6.19	5.69	5.60	4.00	3.02

[a] Point of complete precipitation; this protein was insoluble in acid solution.

[b] Point of apparent maximum precipitation of the cytoplasm. The proteins of this leaf are still under investigation, and the value quoted is given with reserve.

Some results are given in Table III. The cell contents of the leaves were highly buffered and variations in the hydrogen-ion concentration were found to be small. Spinach leaves from several different farms gave a Sörensen value (P_H) of 6.5 ± 0.1. No variation greater than P_H 0.3 has been observed in any variety of leaf. Hoagland and Davis[5] found the reaction of *Nitella* sap to be extraordinarily constant at P_H 5.2, with extreme limits of 4.8 and 5.8.

In no variety of leaf so far examined has the hydrogen-ion concentration of the cell contents coincided with that of the iso-electric point of the proteins. In *Vitis vinifera* the proteins may possibly be present as cations; in all the other leaves so far examined they are definitely present as anions. It is to be noted, however, that with each sample mentioned in Table III the Sörensen value of the cell contents is not far removed from the protein iso-electric point. While small variations in this re-

[5] Hoagland and Davis, *J. Gen. Physiol.*, **5**, 629 (1923).

action are to be expected, it is probable that a change sufficient to bring the cytoplasmic proteins to their iso-electric point can result only in the death of the cell. In the first place, all of the cytoplasmic proteins that have been examined are completely precipitated at the iso-electric point, so that it is almost certain that under these conditions disintegration of the cytoplasmic complex would occur. In the second place, experiments with living cells have shown that when the external medium is made sufficiently acid, injury to the cell occurs. Hoagland and Davis[5] showed that the reaction of *Nitella* sap remained constant, and the cells suffered no injury, when the hydrogen-ion concentration of the external medium was varied between P_H 9.4 and 5.0 but when the acidity was increased beyond this value, to P_H 4.4 or lower, injury to the cell resulted. Pearsall and Ewing[6] give the iso-electric point of *Nitella* cytoplasm as P_H 4.7, so it is possible that in this case the injury was due to the precipitation of the cytoplasmic proteins. These latter authors also showed[7] that rapid outward diffusion of chlorine ions ensues when living plant tissue is brought to a hydrogen-ion concentration equal to or greater than that at which the chief proteins are iso-electric. More recently Mrs. M. R. Lewis[8] has shown that the same phenomena occur in embryonic cells. These can live even if the reaction of the external medium is as alkaline as P_H 9.0; but at P_H 4.6 coagulation of the cytoplasm and nucleus slowly takes place, probably because the penetration of acid has increased the hydrogen-ion concentration to the iso-electric point of the constituent proteins.

Until we know more of the chemistry of cytoplasm—not only of its proteins, but its fatty substances, carbohydrates and inorganic matter as well, it is perhaps unwise to draw conclusions. All that can be said is that the cytoplasm in the living cell appears to be in contact at its inner surface with a solution sufficiently buffered to keep its reaction fairly constant, while its outer surface is in contact with a medium whose reaction can vary according to the environment of the particular cell. Further, it is probable that any change of reaction must be such that the cytoplasmic proteins do not become iso-electric.

Loeb has shown that in the region of the iso-electric point the physical properties of proteins, such as viscosity and swelling, show a comparatively large change with a small variation of hydrogen-ion concentration—it is possible that an investigation of the physico-chemical properties of cytoplasm proteins in solutions of hydrogen-ion concentration not far removed from the iso-electric point may furnish data which may help to throw light on the mechanism of permeability.

[6] Pearsall and Ewing, *Biochem. J.*, **18**, 329 (1924).

[7] Pearsall and Ewing, *New Phytol.*, **23**, 193 (1924).

[8] Quoted by Streeter, *Carnegie Inst. Year Book*, **23**, p. 11 (1924).

Summary

It is pointed out that an examination of the physico-chemical properties of cytoplasm may help to explain the mechanism of cell permeability.

The relationship between the iso-electric point of some leaf cytoplasmic proteins and the hydrogen-ion concentration of the contents of the leaf cells has been determined and is briefly discussed.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

SOME AMIDINES OF THE HOLOCAINE TYPE. I[1]

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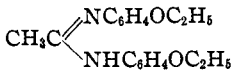
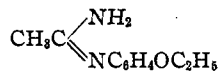
Although "Holocaine" (I) is regarded as an efficient local anesthetic



I



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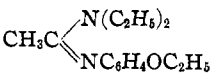

III

for ophthalmic purposes, comparatively little work has been carried out with a view to modifying its structure in order to remove the undesirable toxicity and irritability which have militated against its use.

Taube[3] has prepared a series of compounds in which methoxy and ethoxy groups occupy the *ortho* (or *para*) position in one ring, and the *para* (or *ortho*) of the other in all possible combinations, while Goldschmidt[4] has synthesized the prototype of the series in which methyl is replaced by hydrogen.

The fact that none of these compounds possesses any marked advantages over Holocaine suggests the desirability of preparing a series of amidines in which portions of the molecule are systematically replaced by groups known to exert a favorable influence on the physiological properties of local anesthetics.

An investigation has therefore been undertaken with the above-stated object in view; this paper deals with the synthesis of the following new types.

A. Compounds in which the methyl group of Holocaine is replaced by ethyl, propyl, butyl, *iso*butyl and benzyl. B. Those in which one phenetidine group is replaced by amino (II). C. Those in which one

[1] This investigation has been conducted in coöperation with the National Research Council Sub-Committee on Local Anesthetics (A. J. Hill, Chairman).

[2] This paper is constructed from the dissertation presented by Isadore Rabinowitz to the faculty of the Graduate School of Yale University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[3] "Chemische Technologie" (Wagner), **41**, 620, 621 (1895).

[4] Goldschmidt, Ger. pat. 97,103 (1898); 103, 982 (1899). *Chem.-Ztg.*, **26**, 743 (1902). *J. Chem. Soc.*, **82**, 785 (1902).