Biochemical Study on the Autumn Fall of the Leaf

By

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Numerous investigations have been reported by physiologists and chemists on the autumn fall of the leaf, a remarkable feature of the plants growing in countries with alternating wet and dry, or warm and cold seasons[1]. Among these, the research work by S. Kaeriyama[2], dealing with the Ginko tree (*Ginko biloba, L.*) and the cherry tree (*Prunus donarium, Sieb.*), is of great interest; estimating the reducing sugars and starch and dextrin in the fallen leaves and branches at the same time, and comparing the leaves and stems gathered in May and in December, with respect to the content of crude protein, ether extract, crude fibre, total ash, and especially of the composition of the ash, he came to the conclusion that the object of the autumnal shedding of the leaves is not thrown away substances useless for the life of the plant, though the yellow coloured leaves, as other investigators have reported[3], contain in their ash constituents more Na, Ca and S but less K, N and P than the green ones. However, he said nothing with regard to the hypothesis put forward by Sachs in his study of plant physiology, but opposed by Wehmer[4], that substances useful for the life of the plant migrate from the leaves to the stems before winter comes.

Other plant biologists have supposed that when the leaves are exhausted of the mineral and organic substances required for leaf-

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1. Michel-Durand: Variation des Substances hydrocarbonees dans les feuilles. 1917.
2. J. Chem. Soc., Tokyo, **32**, 964 (1902).
3. E. Stahl: Zur Biologie des Chlorophylls, s. 139 (1909).
4. Ber. D. Bot. Gess., **10**, 152 (1892).

activity, or when winter comes to reduce the risk of desiccation of the plant, the evaporation of moisture from the leaves is prevented by the forming of a layer of cork near the base of the leaf stalk, and the leaves are then broken off by the atmospheric agencies or drop by their own weight to the ground.

The object of this experiment was to find out what chemical changes in the leaf constituents take place in the life history of the leaves, and if possible to find the physiological meaning of the autumn leaf-fall. The leaves of the ginko tree were therefore examined, since this tree is very common in Japan and moreover, the leaves change their colour quite uniformly and then almost all the leaves fall simultaneously.

The samples of the leaf used for the experiment, except the last dated one, shown in the following table, were plucked once a month at 10 o'clock on fine mornings, from the southern branches—about 3–4 meters above the ground—of the two ginko trees growing side by side in the garden of the Buddhist temple, Hyakumanben Tion-di, located about 100 meters from the laboratory. The last sample was gathered at 8 o'clock on Nov. 19th from the leaves fallen on the ground under the trees which had been cleared of fallen leaves at 5 o'clock the previous evening; the fallen-leaves, among which there were no green-tinged ones, were quite uniform in maturity.

In these samples, the hydrogen-ion concentration of the cell-sap and the content of moisture, ash, ether-soluble matter, nitrogenous matter, chlorophyll, carbohydrates and crude fibre were determined by the following methods :—

1. pH-value: the cell-sap of the leaves was prepared by crushing them in a mortar and its pH-value was determined by the electrometric method, since the concentration of leaf-sap, in the case of leaves poor in juice-content, varies, as observed by Y. Yamaguchi[1], according to the method of preparation, and crushing the leaves in a mortar gives good results for the present purpose. In the determination of the pH-value, the experiment was carried out on the day on which the sample for analysis was collected, and again on the day after, and the mean value of the two determinations was taken.

2. Moisture: about 5 gms. of the fresh leaves were dried to a

1. J. Chem. Soc., Tokyo, **41**, 729; and also refer to Atkins, "Some Recent Researches in Plant Physiology", p. 86 (1916), and H. H. Dixon, "Transpiration and the Ascent of Sap in Plants". p. 175 (1914).

constant weight in an air bath heated at 103–105°, and the loss of weight was designated moisture-content.

3. Ash-content: the sample, dried as above, was incinerated in the usual way.

4. Ether-soluble matter: the sample was finely pulverised after being dried in a vacuum sulphuric acid desiccator and treated with ether to extract fatty compounds.

5. Nitrogen-content: the total nitrogen in the fresh leaves was determined by Kjeldahl's method and also the nitrogen-content in both water and alcohol extracts was estimated.

6. Chlorophyll-content: the leaves, dried in a vacuum desiccator, were treated with acetone to extract chlorophyll and its content was calculated by multiplying the nitrogen-content in the extract by 150.

7. Carbohydrates: 50 gms. of the fresh leaves were dipped in alcohol, containing calcium carbonate to neutralize organic acids in the tissues, to dry the leaves, filtered and then minced finely, and treated successively with 90 % alcohol at a temperature of about 60°, with hot water, with a hot 1 % hydrochloric acid and finally with a hot 1.25 % caustic soda solution to extract simple sugars, disaccharides, starch-like substances and pentosan respectively.

The two alcohol solutions were put together and the solvent was distilled off under reduced pressure. The residue was then dissolved in water and the solution clarified with neutral lead acetate solution and sulphuretted hydrogen, and the sugar content in the clarified solution was estimated by determining the reducing power before and after hydrolysis with 2.5 % hydrochloric acid.

The hot water extract was clarified as before with lead acetate solution and sulphuretted hydrogen, and the content of maltose in the solution was estimated by its reducing power after being hydrolysed with 2.5 % HCl.

The starch-content in the leaves was estimated with the 1 % HCl extract by determining the reducing power of the solution after it had been clarified in the usual manner and neutralized with caustic soda.

8. The cellulose-content: the residue of the alkaline extract was washed with water and dried, and the difference between this and the ash-content was designated crude fibre.

In the determination of carbohydrates, the reducing power of the solution was determined by the Munson-Walker method[1] and the results were expressed as d-glucose.

1. J. Am. Chem. Soc., **28**, 663 (1906).

The experimental results are shown in the following table. In the table the content of ether-sol. matter, crude fibre, and other constituents are expressed on an ash-free and dry basis.

Date	Appearance of leaf	pH	Moisture %	Ash %	Ether extract %	Chlorophyll %
June 18	green	3.7	72.74	1.37	—	1.51
Aug. 8	green	3.1	73.32	1.65	12.55	1.56
Sept. 8	green	3.1	74.57	1.84	14.79	1.23
Oct. 8	gr., slightly yellow	3.6	75.21	1.89	14.28	1.14
Nov. 7	greenish yellow	3.5	76.99	1.76	16.68	0.99
Nov. 19	almost pure yellow	3.2	73.51	2.48	15.12	0.46
Nov. 21	yellow slightly brown	3.6	71.88	2.14	17.55	0.50

Date	Total N	Soluble N		Insoluble N	Soluble N (% in Total N)		
		alc. extract	water extract		alc. extract	water extract	Total
June 18	2.70	0.31	0.15	2.24	11.4	5.7	17.1
Aug. 8	2.45	0.39	0.20	1.86	16.0	8.2	24.2
Sept. 8	2.68	0.19	0.08	2.41	7.1	2.8	9.8
Oct. 8	2.22	0.17	0.09	1.96	7.7	4.1	11.8
Nov. 7	1.42	0.26	0.12	1.04	18.6	8.5	26.1
Nov. 19	0.99	0.23	0.12	0.64	23.4	12.1	35.5
Nov. 21	0.95	0.20	0.09	0.65	21.3	9.9	31.2

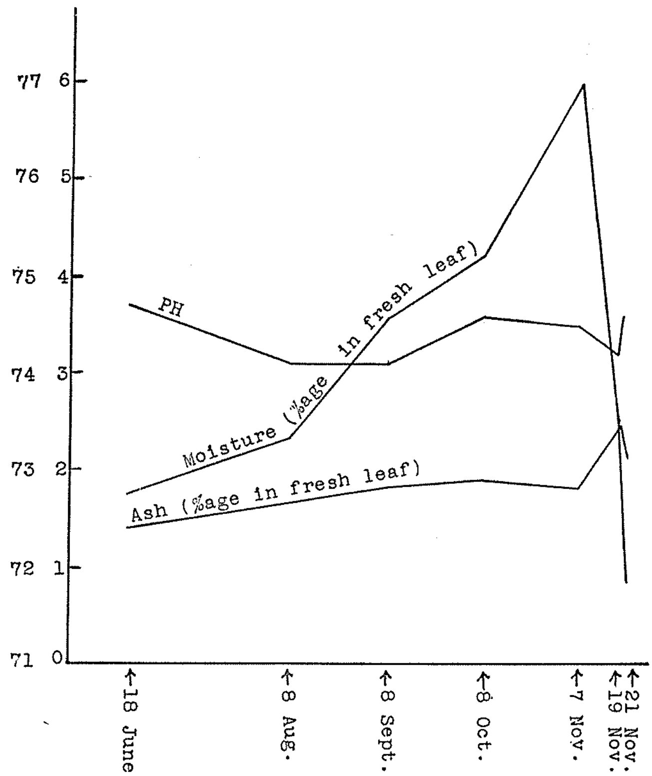
Date	Soluble carbohydrates		Starch	Total carbohydrates	Crude fibre
	reducing	non-reducing			
June 18	—	—	—	—	19.66
Aug. 8	1.44	9.87	2.76	14.07	27.37
Sept. 8	2.25	8.73	3.01	14.04	26.66
Oct. 8	2.77	9.33	2.40	14.50	28.60
Nov. 7	4.94	3.67	1.98	10.59	30.54
Nov. 19	2.25	3.70	2.75	8.70	34.44
Nov. 21	2.31	2.77	2.08	7.16	32.61

As may be seen from the experimental results, the pH-value of the press-juice was 3.7 on June 18th; this decreased gradually to 3.1 on September 8th and increased again to 3.6 in October, and the monthly variation of the pH-value is reciprocal with that of the total soluble nitrogenous substances.

The percentage of moisture increased gradually from 72.7 % on June 18th to the maximum value 77 % on Nov. 7th and then decreased suddenly to 73.5 % on Nov. 19th before the leaf-fall. The ash-content changes with age in the same way as the moisture content.

The content of reducing sugars increases gradually till the leaf begins to be coloured yellow and after this period the content decreases rapidly as yellow shade of the leaves increases.

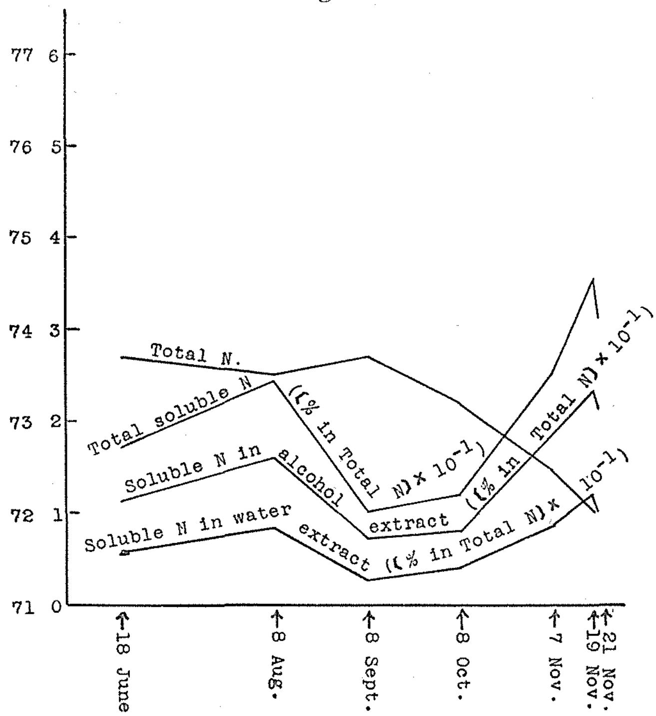
Fig. 1



The content of sugar hydrolysible with 1 % HCl, mostly a starch-like substance, changes inversely with the simple sugars. The content of non-reducing soluble sugars remains almost constant till October and then decreases rapidly with progress of time. The content of total carbohydrates consequently remains constant till October and then decreases suddenly before winter comes, while the crude fibre increases with age.

The chlorophyll-nitrogen content shows a decrease through the life-history of leaf, but on the other hand the content of ether extract increases steadily.

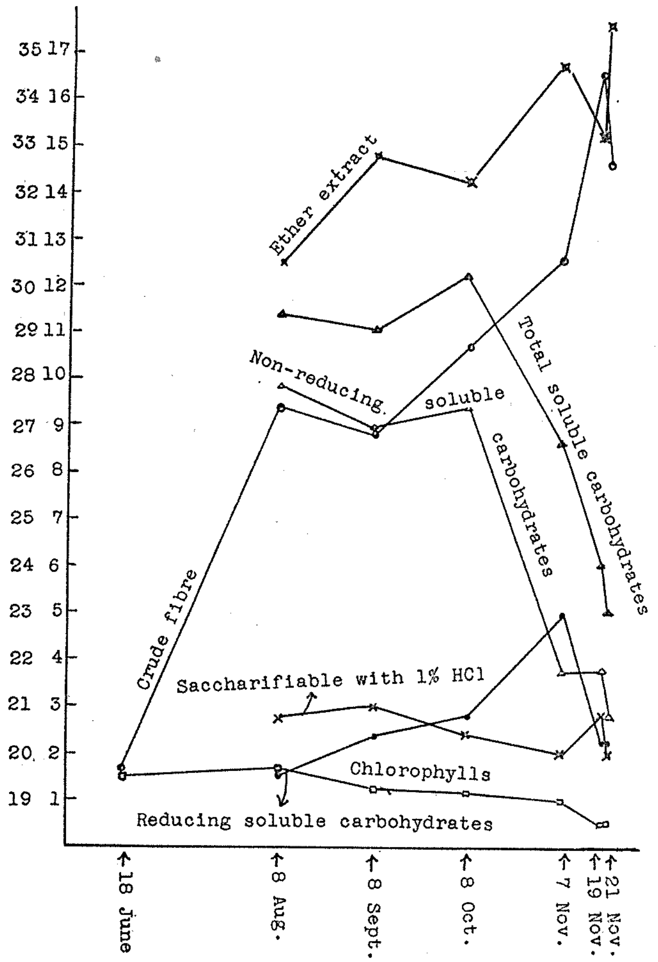
Fig. 2



Discussion of the Results

The pH-value of the cell-sap shows a close relationship, in numerical value, with the content of the water-soluble nitrogenous matter; at the end of summer the cell-sap shows strong acidity and the water soluble nitrogen in the same season shows the minimum value and the insoluble N the maximum; but when autumn comes the acidity of the cell-sap decreases suddenly owing to an increase in the soluble nitrogenous matter. The seasonal variation of the nitrogenous matters, which are usually regarded as intimately connected with the activity of plant tissues, is noteworthy. The content of the total N decreases with time though that of the soluble N shows a minimum in autumn and a maximum at the end of summer and also the beginning of winter, and we have put forward the hypothesis based on these facts that the decomposition of cytoplasmic material and the transition of the soluble nitrogenous matter formed by the decomposition of proteins from leaves to stems occurs when the leaves are mature. This hypothesis to explain the metabolism of nitrogenous substances in the leaves of higher plants is partly in agreement with the opinion of Chibnall, who investigated the diurnal variations in the protein nitrogen of Phaseolus vulgaris[1].

Fig. 3



1. Chibnall: Bioch. Jour., **18**, 387 (1924).

The moisture content, as may be seen in the table and figure, increases continuously till the end of autumn and decreases suddenly when winter comes. This finding is opposed to the observation made in plant-leaves by Andre[1], and may be explained by assuming that the respiration of the leaf-cell will take place vigorously in autumn by reason of which water, one of the products of respiration, is stored in the cell-sap without evaporation to the surrounding air owing to the high degree of humidity of the atmosphere in that season, as may be seen from the atmospheric conditions of the year, observed in the Laboratory of Agricultural Technology of Kyoto, shown in the following table.

Date	Humidity at 10 a.m.	Max. humidity preceding it	Temperature at 10 a.m.	Lowest temp. early that morning	Max. temp. of the previous day
June 18	—	—	—	—	—
Aug. 8	75	95 at 5 a.m.	28.3°	21.7°	29.4°
Sept. 8	75	99 „ 6 „	29.4	24.4	31.7
Oct. 8	90	100 „ 8 „	22.8	15.0	26.7
Nov. 7	90	100 „ 9 „	10.6	1.1	15.0
Nov. 19	—	—	18.3	11.1	17.8
Nov. 21	—	—	—	—	15.6

The metabolic changes in the nitrogenous matter in the ginko leaves seem to occur, as we understand from Figs. I & II, in an opposite way to the seasonal variation of the carbohydrate content, but the latter changes in the same way as the water-content.

The fact that the reducing sugar-content changes in parallel with the water-content suggests that the water in the tissues plays an important part in the hydrolysis of polysaccharides, and indeed the content of starch-like substance shows a maximum at the end of summer and a minimum at the end of autumn.

Conversion of the non-reducing soluble sugars into crude-fibre in the tissues is supposed to occur, from the relation of their content in various seasons, and the polymerisation of the lower saccharide into the higher molecular one—cellulose—as we learned from the heat of formation of these substances, is accompanied by the absorption of heat. As a matter of fact, the formation of cellulose in the leaves occurs mostly in summer, which is to be expected from van't Hoff's

1. C. R., **148**, 1685 (1909).

law of mobile equilibrium. The accumulation of reducing sugars and of cellulose which occurs actually in the leaf before winter comes may be explained in the following way :—the hydrolysis of starch-like substances is the chief factor in the origin of reducing sugars and the formation of cellulose is due to polymerization of non-reducing soluble sugars. Such antagonistic chemical changes in the carbohydrates—hydrolysis and polymerization—were also noticed to occur in plants by Michel-Durand, but the polymerization of carbohydrates, in her case, was expressed as characteristic feature of plants which is observed from spring to the end of summer.

Such a difference between our results and those of European investigation in the water content of the leaves in autumn and also in the carbohydrate content between ours and Europian investigators' must be attributed to the difference in the environmental conditions of Japan and the West.

It seems that *one of the most important functions of the autumn leaf-fall is to enable the plant to get rid of inorganic materials—ash—so that the accumulation of the ash constituents is prevented from controlling the metabolic changes of the tissue materials and the plant is able to live a normal life in winter.*

But the fate of the plant ash in vegetation is a matter of speculation when we observe ever-green trees, the leaves of which remain attached to the tree till, after some years, one by one they fall from old age. This problem will be discussed again in the near future on an article from this laboratory.

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