data of mineral deficiency studies with green plants (*1*, *2*).

The increases in free amino acids are considered to be evidence that these chemical elements participate in protein metabolism, and that formation of symptoms is primarily due to the localized action of excessively accumulated normal metabolites. Growth responses and chloroses with amino acids are of sufficient variety to include many of the individual symptoms comprising the syndromes displayed with mineral deficiencies. Except possibly for magnesium, breakdown of chlorophyll in mineral deficiency is, therefore, not necessarily indicative of mineral participation in chlorophyll formation, as has often been assumed. The probable participation of the chemical elements in the enzymes regulating protein metabolism warrants the exercise of caution in associating specific mineral deficiencies with physiological processes in the plant. Drastic interference in the basic function of the plant should cause a breakdown in all physiological processes at varying rates.

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Annual Variation in Nicotine Content of Tobacco

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In connection with the change in nicotine content during fermentation of cigar leaf tobacco of the Pennsylvania Seedleaf variety, Frankenburg (*1*) has given data for crops of four different years. This percentage is given before and after "sweat" by the manufacturer, which is usually referred to as "final forced fermentation." In addition to reduction in nicotine content during this stage, there is a reduction in the curing by the farmer before the manufacturer purchases the tobacco. Then there is reduction during storage fermentation by the manufacturer before the tobacco is put into final forced fermentation. In our laboratory, tobacco, as received by the manfacturer from the farmer after shed-curing, has been analyzed for alkaloids routinely for a period of more than 20 years. Table 1 shows the variation from year to year over that period.

The data in the table for the years 1936, 1938, 1939, and 1941 are somewhat higher than those reported by Frankenburg for the same years. This is to be expected, inasmuch as the data were obtained with tobaccos as received from the farmers, so there was no loss from any processing by the manufacturer.

The results include nor-nicotine and other alkaloids which are present in trace amounts and for that reason the percentage is reported as total alkaloids. Each year's crop represents an average of tobaccos grown by about 100 different farmers. Five leaves were taken from each of three hands distributed throughout one bundle from each farmer's tobacco. The leaves from all these locations were ground and mixed to produce a uniform sample. By quartering, a final representative sample was obtained for analysis.

TABLE 1

ALKALOIDS IN TOBACCO AFTER CURING

Crop	Total alkaloids (oven-dried basis)	pH
	%	
1927	3.1	..
1928	2.8	..
1929	4.6	..
1930	6.0	5.8
1931	3.3	6.9
1932	5.0	5.2
1933	2.5	5.8
1934	3.0	7.3
1935	4.5	6.5
1936	4.3	6.4
1938	3.1	..
1939	3.0	..
1940	3.2	6.2
1941	4.1	5.4
1942	3.1	6.7
1943	5.0	6.2
1944	3.6	6.5
1945	2.8	6.8
1946	2.9	6.5
1947	4.2	6.3
1948	3.15	6.5

In addition to the representative sample for each year's crop, individual farmers' tobaccos have been analyzed. It has been found that from farmer to farmer there is variation in any given year, with some tobaccos having as little as half of the average for the year and others as much as 50% more. However, normally over 90% of the individual farmers' tobaccos do not vary more than about ±10% from the average for the year. This uniformity is to be expected, inasmuch as the tobacco represents only that grown in the general vicinity of Lancaster, Pennsylvania. Where variations do occur, they represent differences from field to field, depending upon the soil, fertilizer, seed, and variations in rainfall occurring between the time of early and late harvesting of crops by different farmers. There is no relation between pH and alkaloid content.

Plotting the average alkaloid content for the various years against the amount of rainfall during the growing season shows a definite trend of higher alkaloid content in tobaccos grown in dry seasons. By breaking this down into months, it is found that the influence of rainfall on the alkaloid content is somewhat greater towards the end of the growing season, as the time of harvest approaches.

As a further point of interest, individual crops have been followed through complete manufacturing operations, including all fermentation. The average amount of alkaloid reduction is about 40%, all based on oven-dried samples. In certain extreme conditions, this may be as little as 20%, or as much as 80% reduction. This

amount of reduction may be controlled and generally it is kept close to the average percentage mentioned.

Reference

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Simplified Preparative Electrophoresis at Room Temperature[1]

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Many attempts have been made to adapt the separation of electrically charged ions like proteins to simple equipment operable at room temperature. Membranes, sand barriers, gels, and jellies have been used to immobilize the material and thus prevent convection from mixing the protein in a solution, P, with the adjacent buffer solution, B. Tiselius (*1*) points out that aside from optical observations his method increases the potential gradient in the **U**-tube without undesirable heat convection, simplifies sampling, and avoids disturbing electrolytic processes.

The equipment for the preparative method described here costs less than $50. It suitably embodies the advantages of the Tiselius method just mentioned, and is especially adaptable for use at room temperature without any temperature control. The technique employs a modification of the classical **U**-tube with large side vessels for electrodes (carbons or reversible electrodes). Tail-hole stopcocks are used for withdrawing samples at the side, or a two-way stopcock at the bottom of the **U**-tube is employed for forming the boundary and draining the electrophoretically purified fractions from either side of the **U**-tube. The method depends upon controlling solutions B and P so that they will have different densities, viscosities, pH's, and conductances as follows: solution B (supernatant) will have a high coefficient of viscosity at the pH of the isoelectric point of the protein contained in solution P, to be immobilized in the lower part of the **U**-tube; solution B will have a density considerably less than solution P, so that as the boundary P-B is formed, a well-defined boundary, stable enough for preparative electrophoresis results. Typical solutions used by the writer and his co-workers (*2*) for the past year for the separation of trifidin and artefolin (*1*), the unpigmented fractions of giant and dwarf ragweed extracts, have been: B = 40% glycerol at pH 6.8; P = 50% glycerol extract of ragweed pollen. The electrical conductance of solutions B and P in the separation should be regulated so that as little electrolyte as convenient is in P, with a suitable amount in B, to control the pH and drop in potential at the beginning of the separation. For example, in the case of ragweed extracts, no saline was added to the ragweed to be fractionated in solution P. The 40% glycerol in B contained M/15 phosphate buffer at pH 6.8. Thus initially the main drop in potential was across the material to be separated and fractionation was facilitated. A striking experiment demonstrating this technique may be made by having solution B at about pH 6.8 employing M/15 phosphate buffer in 40% glycerine, with solution P, a mixture of hemoglobin and T-1824 (a negatively charged dye), in 50% glycerine. At this pH, the isoelectric point of hemoglobin is electrophoretically fixed at the boundaries, whereas the dye T-1824 migrates out of the mixture to the anode, leaving the hemoglobin with a fairly sharp boundary at the negative side after 24 hr.

It may be emphasized that our method utilizes Newtonian or truly viscous liquids. The convection ordinarily resulting from the application of 450 volts to a **U**-tube, 60 cm long with an internal diameter of 1.0 cm, at room temperature is avoided by the use of only high viscosities, not plasticities. No membranes, jellies, or similar mechanical devices are required. The temperature in the laboratories at Cold Spring Harbor during the summer of 1948 varied considerably over 24 hr but did not disturb the boundaries for preparative purposes during 4-day electrophoretic separations. The apparatus can be readily used in an ice chest. If room temperature does not destroy the material to be studied, it is preferable not only because of simplicity but also because the mobilities are greater at this temperature.

TABLE 1

COMPARISON OF FORMATION OF CLASSICAL TISSUES BOUNDARIES AND THOSE OF THE MODIFIED TECHNIQUE

Tiselius method	Method reported herein
Conductance B* = conductance P†	Conductance B >> conductance P
Viscosity B = viscosity P	Viscosity B < viscosity P
Density B = or slightly less than P	Density B < density P
pH of B = pH of P	The pH of B at or close to isoelectric point of protein to be separated in solution P
Temperature controlled at or near 4° C	Room temperature fluctuations—no temperature control

* Supernatant solution.
† Lower protein solution. Note that both P and B have Newtonian flow.

Table 1 summarizes the differences in preparative procedures with the classical method of Tiselius and the method herein described.

The fractionation experiments based upon this technique will soon be reported in detail (*2*). The early experiments were done with C. Reiter and M. Loebl.

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