Properties of Adsorbed Chlorophyll

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Most of the investigations on chlorophyll have been done in solution in such fat solvents as ether, ethanol, methanol, acetone, etc. As such, the chlorophyll is deep green in color, shows much red fluorescence, and is rapidly decomposed by light.

In the experiment described in this preliminary report, chlorophyll in solutions of acetone and ethanol was adsorbed on charcoal (norit A), talc, fuller's earth, infusorial earth, casein, agar, gelatin, Ottawa sand, absorbent cotton, filter paper, calcium carbonate, egg albumin, cornstarch, and supercel. No attempt was made to purify the chlorophyll solution, to separate the extraction mixture into *a* and *b* components, or to remove the carotenoids.

The best method of adsorbing chlorophyll from a solution was that used by Willstätter and Stoll (*2*), who adsorbed chlorophyll onto the surface of talc from an acetone solution. The chlorophyll in dry, powdered nettle leaves was extracted in 80 per cent acetone. Talc (about 1 gram to each 50 ml. of acetone solution) and then distilled water were added to dilute the acetone to 55 per cent. Upon standing (2–15 hours) and filtering, the talc residue on the filter paper was thoroughly washed with distilled water and was observed to have a deep green color, comparable to the color of many leaves. As the talc dried, the color became much lighter, as it does in leaves as they are dried. The dried talc with a green coating of chlorophyll is quite comparable in physical appearance to the powder of dried nettle leaves.

Charcoal is very effective in adsorbing chlorophyll from a diluted solution. Although it was the most effective adsorbent of those tried, it has the disadvantage of remaining black in color. Also, chlorophyll cannot be eluted from charcoal surfaces with the ordinary solvents, such as acetone, ethanol, methanol, ether, etc. According to the author's present knowledge, pyridine is the only substance which will do so.

Talc, fuller's earth, infusorial earth, cotton, supercel, and egg albumin have proved to be quite effective adsorbents of chlorophyll in 55 per cent acetone. The egg albumin was dissolved in water and then added to acetone to make a 55 per cent aqueous solution of acetone, from which the egg albumin precipitated. Sand may be just as effective if the amount of chlorophyll is correlated with surface. Casein and gelatin are somewhat less effective and have the disadvantage of becoming hardened into a tough, brittle, plastic-like material from which there can be no easy extraction of the chlorophyll. Agar, which is about the poorest adsorbent, loses what little chlorophyll it adsorbs when the agar residue is washed to remove the acetone. Calcium carbonate, cornstarch, and filter paper are also rather poor. The frayed edges of the filter paper are much more effective.

The best adsorbents are talc, fuller's earth, infusorial earth, and adsorbent cotton. These substances, when moist with water, take on the green leaf-like color and, when dry, resemble the light green color of dried leaf powder. Furthermore, except for cotton, which was used in its usual fibrous form, they retain their powdered form. The chlorophyll in all four cases can be re-extracted as easily as it is extracted from whole leaves or from such material as finely powdered, dry nettle leaves. Of these four, talc seems to be the best.

The properties of chlorophyll adsorbed on these adsorbents are very much like those of chlorophyll in the leaf, as found by Fishman and Moyer (*1*). The adsorbed chlorophyll has a natural green color, a reddish fluorescence, an electronegative charge, and great photostability. The reddish fluorescence was observed in the dark by irradiating a suspension of chlorophyll-coated talc in distilled water with ultraviolet light from a Cenco ultraviolet light source. The fact that chlorophyll adsorbed on talc, as well as chlorophyll in living cells, displays a red fluorescence is a crucial indication that chlorophyll in its natural state is adsorbed on the surface of chloroplast material, most likely the grana.

TABLE 1

ABSORPTION SPECTRA OF CHLOROPHYLL IN VARIOUS AQUEOUS DILUTIONS OF ACETONE

Acetone (%)	Absorption bands			
	First*	Second*	Third*	Fourth*
80, 75, 70	6825–6360 (6593)	6230–6030 (6130)	5875–5675 (5775)	5435–5295 (5365)
65	6895–6365 (6630)	6260–6050 (6155)	5895–5680 (5788)	5460–5310 (5385)
60	6915–6400 (6658)	6270–6070 (6170)	5915–5705 (5810)	5480–5350 (5415)
55	6950–6425 (6688)	6290–6070 (6180)	5900–5705 (5803)	5485–5340 (5413)
50	6940–6440 (6690)	6285–6070 (6178)	5895–5690 (5793)	5500–5350 (5425)
40	6870–6445 (6658)	6280–6090 (6185)	5900–5675 (5788)	5485–5360 (5423)
20	6845–6480 (6663)	Shadow		5485–5370 (5428)
10	6810–6490 (6650)			Shadow

* Averages are in parentheses.

Another important property of adsorbed chlorophyll is the similarity of its absorption spectrum with that of chlorophyll in living leaves. A study of the absorption spectra of chlorophyll in 80, 75, 70, 65, 60, 55, 50, 40, 20, and 10 per cent acetone shows that there is a shift in the position of the absorption bands between approximately 70 and 55 per cent. The shift begins slightly above 70 per cent acetone, and continues gradually and in direct proportion to any dilution between 70 and 55 per cent, becoming complete at 55 per cent (Table 1). This characteristic seems to indicate why 55 per

cent acetone is the most effective dilution from which to adsorb chlorophyll on talc or any other adsorbent.

The total shifts in the bands are: 95 A. for the first, 50 A. for the second, 27 A. for the third, and 47 A. for the fourth.

Upon successive dilutions, ethyl alcohol (95 per cent) extract of chlorophyll from fresh green corn leaves shows the same shift of the absorption bands toward the red end of the spectrum: 98 A. for the first, 52 A. for the second, 27 A. for the third, and 57 A. for the fourth. A 95 per cent ethanol extract of chlorophyll from dry nettle leaves does not show a shift in the spectrum upon dilution with water, probably because of allerimization.

The reason for the shift in the spectrum as a result of dilution with water is not known definitely, but it appears that it may be due to the adsorption of (or the chemical union with) water on the surface of the chlorophyll molecule.

The absorption spectrum of chlorophyll adsorbed on the different adsorbents moistened in water is quite similar to that of chlorophyll in 55 per cent acetone or in solutions with less percentage of acetone. There is a shift toward the red end of the spectrum, quite similar to that in living leaves. The absorption spectra of chlorophyll on dry talc and on dry cotton show no change in position of the bands as compared with that of adsorbed chlorophyll on water-moistened talc and cotton. Thus, the water which possibly originally caused the shift may be retained and may be the principal factor causing the chlorophyll to adhere to talc, cotton, or any adsorbent.

References

1. FISHMAN, M., and MOYER, L. S. *J. gen. Phyisol.*, 1942, **25**, 755.
2. WILLSTÄTTER, R., and STOLL, A. *Investigations on chlorophyll*, 1913. (Trans. by Schertz and Merz.) Lancaster, Pa.: Science Press, 1928. Pp. 290–291.

Daily Nitrogen Urinary Excretion in People of the Working Class of Caracas

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Studies on the elimination of nitrogen and on nitrogen balances are as old as the science of nutrition. The first investigators who involved themselves in these problems had the opportunity of working with very exact methods, and many of their results fit perfectly into the science of today. The work of Boussingault (*3*), Liebig (*10*), Bidder and Schmidt (*1*), and especially Voit and his collaborators (*11*) showed the relationship between nitrogen ingestion and eliminated nitrogen. The notion of nitrogen ingestion equilibrium was established.

It is well known not only that the diet of the Venezuelan people is poor in high-protein-content foods and especially low in proteins of rich biological value (*5*), but also that total serum proteins in the popular class are low, resulting in a high incidence of hypoproteinemia (*7*), especially during pregnancy (*4*) and lactation (*6*). For this reason it seemed interesting to establish the daily urinary excretion of nitrogen, this being the most exact procedure available for determining the amount of metabolized proteins in the body. These determinations were recommended by the International Conference of Berlin in 1932 (*12*) as a very accurate measure of protein intake, although, as Bigwood (*2*) stated, it is sometimes difficult to obtain a complete sample.

For the whole group it was found that 53 per cent of the determinations were under the average value, and 85 per

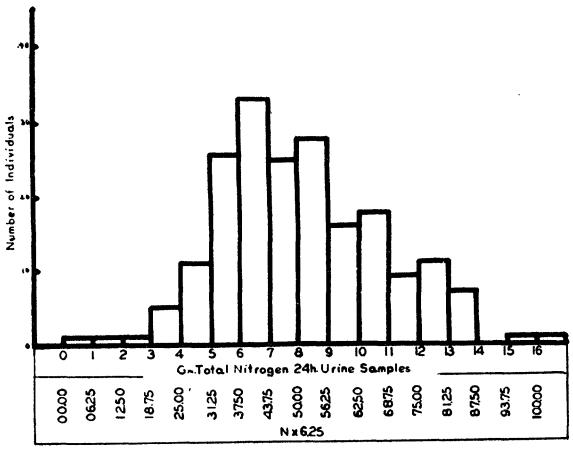

FIG. 1

cent eliminated less than 11 grams of nitrogen in the urine over a period of 24 hours.

METHODS

Determinations were made of total nitrogen in urine eliminated over a period of 24 hours by 194 apparently healthy people attending the Health Certificate Service. Of these, 118 were females and 76 were males. The ages ranged from 11 to 57 years, but the majority were young adults. Special care was taken in order to obtain complete

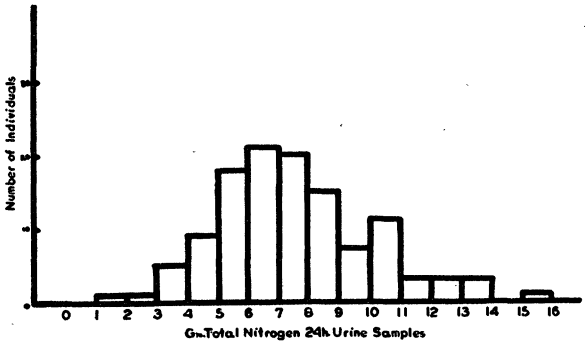

FIG. 2

24-hour samples. The subjects were notified that daily elimination is greater than one liter, and bottles for the purpose were provided. Samples suspected of being incomplete were rejected. Determinations of creatinine were made in all the samples. In a group of 37 women eliminating less than one liter of urine the average creatinine value was the same as that in a group of 92 women eliminating more than one liter. The variation coefficient was smaller in the first group. Almost all the men eliminated more than 1,000 cc. of urine.