time of his publication, he estimated (on the basis of weight differences) that about 1 g of fat would be the maximum available for migration. This amount would provide energy for a flight of only about 385 miles, not enough to get the bird across the Gulf of Mexico. However, the four premigrant hummingbirds in Table 2 actually had an average fat content of 2.1 g, as compared with about 0.4 g for summer individuals. Using Pearson's metabolic rate figures, 2.1 g of fat would allow a flight of 800 miles. Even if 0.4 g (the summer level) were unavailable for flight, the remaining 1.7 g would last for 655 miles, more than enough to span the Gulf.

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4. One of the premigrant hummingbirds was obtained on the Gulf coast of Florida 4 Nov. by Herbert Stoddard, and three were captured near Augusta, Ga., on 26 Sept. by Robert Norris.
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9 November 1955

## Effect of Foliar Sprays of Maleic Hydrazide on Photosynthesis

Maleic hydrazide (1,2-dihydropyridazine-3,6-dione) was found to decrease the number and increase the size of chloroplasts in lettuce (*1*). We are now able to report that this compound produces similar effects in other plants, and that there is an accompanying effect on photosynthesis.

In the experiments reported here (*2*) two concentrations of maleic hydrazide (MH) were used, 0.375 g/lit and 3.0 g/lit (*3*). Swiss chard was raised from seed in the greenhouse. In the cotyledon stage, or after the formation of two to three leaves, the seedlings were sprayed with the MH solution. Leaves that developed subsequent to the treatment were used for photosynthesis studies (*4*). Tobacco plants were sprayed in a similar manner, except that the plants had five to six leaves at the time of treatment.

Table 1. Number and size of chloroplasts in normal swiss chard leaves and in leaves treated with maleic hydrazide.

| Chloroplasts | Normal | Treated | $LSD_{.01}$* |
|---|---|---|---|
| No. per cell | | | |
| Palisade | 73.0 | 42.5 | 19.6 |
| Spongy parenchyma | 81.3 | 57.1 | 21.2 |
| Diameter (μ) | 5.74 | 7.04 | 0.464 |

* Least significant differences at the 0.01 level of probability.

Table 2. Rates of photosynthesis and respiration and chlorophyll concentrations of normal tobacco leaves and leaves treated with maleic hydrazide.

| Item | Normal | MH-treated (0.375 g/lit) | MH-treated (3.0 g/lit) |
|---|---|---|---|
| Photosynthesis (μlit $O_2$/$cm^2$ hr) | 9.17 | 12.08 ($LSD_{.01}$ = 2.14) | 14.42 ($LSD_{.01}$ = 1.72) |
| (μlit $O_2$/mg of chlorophyll per hour) | 1128 | 1928 ($LSD_{.01}$ = 438) | 1545 ($LSD_{.01}$ = 441) ($LSD_{.05}$ = 307) |
| Respiration (μlit $O_2$/$cm^2$ hr) | 1.93 | 1.74 | 1.04 |
| Chlorophyll (mg/$cm^2$) | 0.00813 | 0.00636 | 0.00933 |

Chloroplast structure in normal and treated leaves was studied microscopically. Disks of leaf tissue were macerated in a mixture of 1*N* hydrochloric acid and 50 percent ethyl alcohol. Chloroplast size was determined by means of an ocular micrometer, and the number of chloroplasts per cell was determined by counting the chloroplasts in a large number of cells.

Oxygen evolution by leaf disks was measured manometrically at 25°C. Disks were punched with a cork borer, and ordinarily 10 $cm^2$ was suspended in 0.1*M* $KHCO_3$ in standard Warburg flasks. Two Lumiline tubes, supported in the bath 1 cm below the vessels, provided a light intensity of 200 ft-ca at the level of the leaf tissue. Respiration measurements made in the dark were used to correct the oxygen evolution. Dry weights of the tissue used in the vessels, or of separate aliquots, were determined by oven-drying to constant weight. Chlorophyll was determined with the Beckman DU spectrophotometer, using the method of Arnon (*5*).

Leaves that developed subsequent to treatment were noticeably darker green than normal leaves. The alterations in chloroplast morphology found by Callaghan (*1*) in lettuce were found also in swiss chard and tobacco. Representative data from one treatment are presented in Table 1. Tobacco chloroplasts are somewhat smaller than those of swiss chard, but the effect of MH is about the same as that shown in Table 1.

Rates of photosynthesis are significantly increased by pretreatment with MH, as is shown in Table 2. At the lower concentration of MH there is relatively little effect on dry weight, respiration rate, and chlorophyll concentration. The increase in the rate of photosynthesis, however, is quite spectacular. It is to this increase that we attach the most significance.

At the higher concentration, the depression of respiration and the somewhat higher chlorophyll concentration account for a part of the apparent increase in photosynthetic rate. In leaf disks from plants treated with the higher concentration of MH, the dry weights per unit area were quite variable and depended on the recent history of the plants. Thus, chlorophyll or area is a better basis upon which to compare photosynthetic rates.

Swiss chard yielded values comparable to those shown for tobacco.

Maleic hydrazide has many effects on higher plants, most of which result from changes in the developmental sequence in the terminal meristem (*6*). At the lower concentration used here, however, there was little obvious morphological change in size or shape of leaves. The measured increase in photosynthetic rate seemed to be a modification of the physiology of the individual leaf cells. At present it is impossible to tell how the observed changes are brought about. The great change in rate per unit of chlorophyll suggests an alteration in the photochemical mechanism of photosynthesis. The low light intensity used here contributes to this suggestion. It is possible that this technique will provide an approach to the problem of energy transfer in photosynthesis.

The increase in photosynthetic rate has practical implications. Indeed, Mikkelsen *et al.* (*7*) found an increase in yield of sucrose from MH-treated sugar beets. However, they suggested only casually that there might be a direct effect on photosynthesis. In other experiments (*8*), they found increased sucrose concentration but no increase in yield.

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**References and Notes**

1. John J. Callaghan, M.S. thesis, Cork University, Cork, Ireland (1953).
2. Contribution No. 202 from the department of botany and plant pathology, Pennsylvania State University, University Park.
3. Concentrations of the active ingredient in a solution of the diethanolamine salt of MH. Alconox detergent was added as a spreading agent.

Controls were sprayed with water containing the detergent.

4. The leaves were studied after they reached mature size. The growth usually required 4 to 5 weeks.

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## International Comparisons of Radioactivity Standards

During the period May 1952 to November 1954, international comparisons have been made of standard samples of the radioactive nuclides $Na^{24}$, $P^{32}$, $Co^{60}$, $Sr^{90}+Y^{90}$, $I^{131}$, and $Au^{198}$. The organizations taking part in these measurements and comparisons have been the U.S. National Bureau of Standards, Atomic Energy of Canada Limited, and four British laboratories (the National Physical Laboratory; the Atomic Energy Research Establishment, Harwell; the Royal Cancer Hospital, London; and the Medical Research Council, London, coordinated through the NPL Advisory Committee on Radioactive Standards). Periodic meetings between representatives of the three countries have been arranged by the U.S. National Research Council Subcommittee on Beta- and Gamma-ray Measurements and Standards. Several papers on the work have already appeared (*1–12*).

The samples used for the comparisons were standard solutions prepared by the NBS, except the $Sr^{90}+Y^{90}$ and $Au^{198}$ samples, which were prepared by the AERE. The solutions generally contained a few microcuries per gram. In the case of $Co^{60}$ and $I^{131}$, stronger solutions containing of the order of 1 millicurie per gram were provided. The disintegration rates of the samples were determined at each of the laboratories by one or more of the following methods: total $\beta$ counting in a $4\pi$ solid angle, $4\pi\beta\gamma$ and $\beta\gamma$ coincidence counting, and $\gamma\gamma$ coincidence counting. The results of measurements under what are considered to be the best conditions attainable at present are in agreement to within about $\pm 2$ percent except for $4\pi$ counting of low-energy $\beta$ rays.

In measurements of $Na^{24}$ and $Au^{198}$ by $4\pi\beta$ counting, the fractional losses of $\beta$ particles by absorption are small and the results agree within $\pm 2$ percent. They also agree within these limits with $4\pi\beta\gamma$ and $\beta\gamma$ coincidence results. The basic counting measurements for $I^{131}$ are in agreement within 1 percent, but there is some uncertainty concerning the allowance to be made for self-absorption. No such allowance is applied by the British and Canadian laboratories, but it has been calculated by the NBS to be about 2 percent. Owing to the complex disintegration scheme of $I^{131}$, there is unfortunately no ready means of providing a cross check. The apparent systematic difference of about 3 percent between the NBS results for $I^{131}$ and the standard of this nuclide at present adopted in Great Britain and at Chalk River is thus attributable to the correction assumed for self-absorption.

Agreement to within $\pm 1$ percent was obtained in the measurements of $Co^{60}$ by $\beta\gamma$ and $\gamma\gamma$ coincidence counting. A wide range of values by $4\pi\beta$ counting was reported, however, by the British laboratories. Subsequent investigations at the NBS showed that owing to the relatively low energies of the $\beta$ particles, the apparent disintegration rates obtained by $4\pi\beta$ counting were critically dependent on the amount of solid material in the source and on its distribution. Consideration of the $Co^{60}$ results leads to the conclusion that owing to the precautions necessary in source preparation, and the uncertainties in the absorption corrections, the $4\pi\beta$ counting method of standardizing this nuclide is at present less reliable than the coincidence method. However, it is considered that the distintegration rate of the solution distributed was determined by the coincidence method to an accuracy approaching $\pm 1$ percent.

Ionization chamber equipment for preserving standards of the nuclides used for these comparisons has been set up at the NBS, and similar equipment is being calibrated at the NPL. The cooperation between the NBS, AECL, and the British laboratories on all matters relating to the establishment and the maintenance of radioactivity standards is to be continued in order to check the agreement already attained and to extend the comparisons to other nuclides. It is hoped that standardizing laboratories in other countries will participate in future comparisons; those interested should write to the most convenient of the authors of this letter.

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## Explanation for the So-Called "Ascending Impulses" in the Pyramidal Tract

In 1953 Brodal and Kaada (*1*) reported responses to peripheral nerve stimulation in the medullary pyramid of the cat. They related this unorthodox finding to previous studies in which ascending fibers had been demonstrated histologically (*2*). The possibility that their responses were related to activity in the medial lemniscus was considered and rejected. A further evaluation of their experiments was suggested by the observation that the antidromic cortical responses to medullary pyramidal tract shocks are seriously complicated by spread of the stimulus current to the sensory pathway in the adjoining lemniscus.

In adult cats that had been lightly anesthetized with Surital, the sigmoid motor cortices and superficial radial nerves were exposed for stimulation, and the ventral medullary surface was exposed for recording. The animal was placed in a supine position, the space ventral to the medulla being filled with Tyrode's solution. Responses of medullary points were mapped following both a constant ipsilateral cortical stimulus and a maximal contralateral nerve shock.

The electrodes were 75-$\mu$ steel wires cemented together with the tips 1 mm apart. Bipolar recording proved more effective, but monopolar recording of the "active" lead against an indifferent lead in the cervical soft tissue was also used. The area adjacent to the midline was explored from the origin of the