Records for twelve complete nights' sleep of one subject were analyzed. From these records 83 movements conforming to the preceding criteria were obtained. The average heart rate for each minute of the period beginning 6 minutes before movement and terminating 5 minutes after movement is shown graphically in Fig. 1.

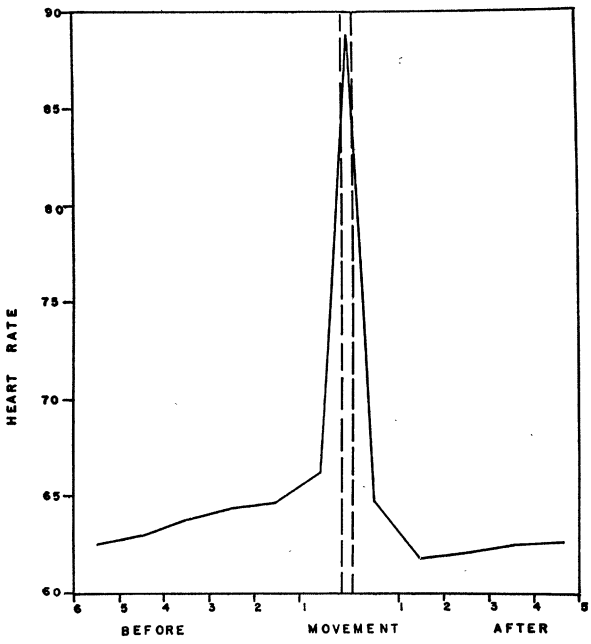

FIG. 1.

A trend of the sort suggested by the congestion hypothesis is apparent. An anticipatory increase in the heart rate is clearly demonstrated. From the curve, the rise appears to begin as much as 6 minutes before movement and the increment becomes greater until movement takes place. A minimum below that of any other period under consideration occurs soon after movement, and from this point there is a slow return to the earlier level. While of no immediate bearing on the congestion hypothesis, the much increased rate during movement is to be noted.

Analysis of the cardiotachometer records in quarter-minute intervals shows the same general trend, and the points of change are fixed more accurately in time. There is a slow rise continuing until one-half minute before movement. This is followed by a much more rapid rise that immediately precedes movement. This suggests that the anticipatory rise may be the resultant of two different functions. Statistical analysis of the data shows that both the anticipatory increase and the subsequent decrease are reliable.

These data are evidence of the correctness of the congestion hypothesis. Further experiments are planned to determine more specifically the nature of the stimulus and the mechanisms involved.

M. M. JACKSON

U. S. NAVAL TRAINING STATION,
NEWPORT, R. I.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A RAPID METHOD FOR THE DETERMINATION OF NITROGEN IN PLANT TISSUE

IN view of the imminent shortage of critical elements which are necessary in maintaining crop production, it is becoming increasingly important that guess-work be eliminated in so far as possible in determining the actual fertilizer needs of economic plants. The chemical analysis of leaves as a means of determining the nature of nutritional disorders and as a tool in determining the fertilizer requirements of crop plants has received increasing attention in recent years. The investigations of Lagatu and Maume,[1] Thomas,[2] Thomas and Mack[3] and others have placed the theory of leaf analysis or "foliar diagnosis" on a sound basis. This method has not found wide application probably because of the time and expense involved in the chemical procedures usually employed. With these factors in mind, and faced with the necessity of analyzing hundreds of apple leaf samples in connection with nutrition studies, along with specimens of nutritional disorders frequently brought to the laboratory by fruit growers, we found it desirable to devise more rapid methods for the determination of some of the common nutritional elements in plant tissue.

Nitrogen is perhaps the element that is determined most often by agricultural and biological chemists, and practically all analyses are based on the standard time-consuming Kjeldahl method. A rapid acid-digestion procedure was sought which would make it possible to determine not only nitrogen, but also phosphorus, potassium, calcium, magnesium and other elements in the same sample. The use of 30 per cent. hydrogen peroxide in the presence of concentrated sulfuric acid was found to be a remarkably fast and thorough method for digesting relatively small quantities of plant material. The entire digestion takes only about five minutes, and total nitrogen, including nitrates, can be determined in the resulting solution by the standard nesslerization procedure using a photoelectric colorimeter of the test-tube type. The

[1] H. Lagatu and L. Maume, *Ann. Ecole Nat. Agr. Montpellier*, 20: 219–281, 1930.
[2] Walter Thomas, *Plant Physiol.*, 12: 571–600, 1937.
[3] Walter Thomas and Warren B. Mack, *Penn. Agr. Exp. Sta. Bull.* No. 378, 1939.

Koch-McMeekin Nessler reagent[4] was used in this work, although others would probably be just as satisfactory. Since Beer's law was not found to be valid over a wide range, a curve had to be prepared from readings based upon standard solutions of ammonium sulfate. Either fresh or dry material was found to be satisfactory for analysis, but the use of fresh material saved considerable time in sample preparation. A leaf punch which cuts out exactly one sq. cm of leaf tissue can be used, thus saving the time required to dry, grind and weigh the sample. Ten sq. cm of leaf tissue of most fruit trees is equivalent roughly to 100 mg of the dry material, and the area basis is just as satisfactory as a dry-weight basis for comparing samples.

The procedure adopted is as follows: Transfer 100 mg of dry material or 10 cm^2 of fresh material to a 50 ml Erlenmeyer flask. Add 2 ml of concentrated sulfuric acid and heat gently over a flame until the sample is broken down and partially dissolved. If nitrates are present, continue digestion for about a minute after dense fumes have been given off to allow for complete reduction of the nitrates by the organic matter. Allow to cool and add 0.5 ml of 30 per cent. hydrogen peroxide. Heat gently—the solution should become clear and colorless. Continue the heating until dense fumes are given off—usually the solution becomes darker at this stage. Allow to cool and add 5 drops more of 30 per cent. hydrogen peroxide and heat as before. If the solution is not completely clear and colorless on further heating, add 5 drops more of hydrogen peroxide and heat again—repeat this procedure if necessary. No more than 5 drops of hydrogen peroxide should be added at one time after the first addition, because a large excess of peroxide in the absence of organic matter will oxidize some of the ammonia. When the solution is perfectly clear and colorless on continued heating, cool, dilute with water and transfer with washings to a 100 ml volumetric flask and make to volume. Transfer a 10 ml aliquot to a 50 ml volumetric flask. Add 2 ml of 2.5 N NaOH to partially neutralize the excess acid and 1 ml of 10 per cent. sodium silicate to prevent turbidity. Make to volume and mix well. Transfer a 5 ml aliquot to a colorimeter tube and add 4 drops of Nessler's reagent—mixing thoroughly after the addition of each drop. If the mixing is not thorough, additional drops of reagent will be required to obtain the maximum color. Allow to stand for several minutes before taking a reading on the colorimeter. A blue filter (Wratten No. 49) was used in this work.

Typical comparisons between the rapid and the Kjeldahl methods and recoveries of nitrate nitrogen added to apple leaf tissues are shown in Table 1.

[4] F. C. Koch and T. L. McMeekin, *Jour. Am. Chem. Soc.*, 46: 2066–2069, 1924.

TABLE 1

REPRESENTATIVE ANALYSES OF APPLE LEAF TISSUE AND RECOVERY OF ADDED NITRATE NITROGEN. EXPRESSED AS PERCENTAGE OF DRY MATTER

Sample No.	Kjeldahl method	Rapid method	1 per cent. N added as $NaNO_3$	Recovery of added NO_3
61	2.73	2.73		
	2.73	2.71		
111	.70	.75		
	.69	.74		
216	1.77	1.81		
	1.80	1.81		
291	2.22	2.23		
	2.28	2.26		
315	1.37	1.40	2.40	101 per cent.
	1.39	1.40	2.40	101 " "
		1.37	2.41	102 " "
215	1.88	1.89	2.89	100 " "
	1.89	1.91	2.86	97 " "
		1.86	2.88	99 " "

The time required to determine nitrogen by this method in routine analysis was found to be about 10 minutes per sample, making it possible for two analysts to complete at least 48 samples a day. Furthermore, the cost of reagents is only about one twelfth that of the Kjeldahl procedure. Numerous nitrogen determinations made on replicate samples of leaves of apple, pear, peach, cherry, apricot, grape and corn gave Kjeldahl accuracy. The solution obtained from the peroxide-digested material can be used not only for the determination of nitrogen, but also for phosphorus, potassium, calcium and magnesium. Rapid colorimetric methods for the determination of these elements have been worked out and are now being prepared for publication.

Since leaf analysis offers the most promising means of diagnosing nutritional deficiencies and unbalance within the tissue of the plant, and since it eliminates most of the uncertainty in determining the actual fertilizer needs of crop plants, it is hoped that by the adoption of faster methods, leaf analysis, as a tool in increasing crop production, may come to be more widely used.

R. C. LINDNER
C. P. HARLEY

BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
WENATCHEE, WASH.

BOOKS RECEIVED

FINK OLLIE E. *Conservation for Tomorrow's America.* Illustrated. Pp. 144. The Ohio Division of Conservation and Natural Resources. $.50.

MIZWA, STEPHEN P. *Great Men and Women of Poland.* Illustrated. Pp. xxviii + 397. Macmillan. $4.00.

MYERS, VICTOR C. *Laboratory Directions in Biochemistry.* Pp. 288. The C. V. Mosby Company, Inc.

PEATTIE, RODERICK. *How to Read Military Maps.* Illustrated. Pp. v + 74. George W. Stewart, Inc. $1.50.

WARD, HAROLD B. and WILLIAM E. POWERS. *Weather and Climate.* Illustrated. Pp. 112. Northwestern University Press.