pology, University of Chicago, "cultural relationships of Apache tribes"; George M. Peterson, assistant professor of psychology, University of New Mexico, "the effect of variations in the wave form of an electric stimulus on the response of conscious animals"; Otis C. Trimble, associate professor of psychology, Purdue University, "analysis of wave-form as a determining factor in auditory localization"; Wilson D. Wallis, professor of anthropology, University of Minnesota, "anatomic lag."

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

REGULATING THE FLOW OF SOLUTION FOR PLANT CULTURES

SEVERAL articles have recently appeared in SCIENCE on devices for securing a slow and accurately controlled flow of a liquid.[1] The purpose of the present note is to call attention to the simple and efficient method devised by Shive and Stahl[2] and to describe a modification of this method that permits considerable latitude in regulating the rate of flow.

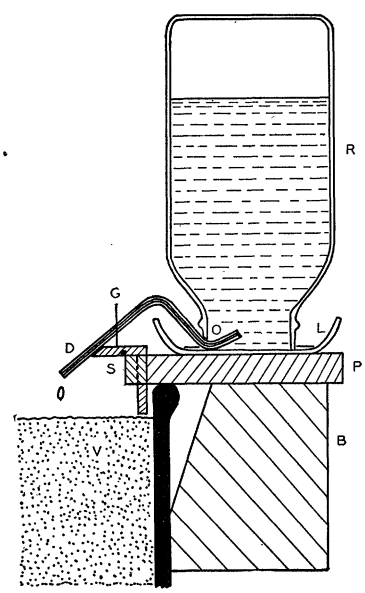

FIG. 1

Fig. 1 shows the main features of the Shive and Stahl apparatus. The solution reservoir (R) is a 2-quart Mason jar with a V-shaped orifice (O) that has been cut with a whetstone. The reservoir, acting as a Mariotte flask, maintains an approximately constant level of solution in the glass dish (L)—a Woolworth "ash tray." Solution flows at a practically constant rate through the small-bore delivery tube (D) and drips regularly into the sand in the culture vessel (V). The apparatus is supported on a platform (P) provided with a bracket (B).

The modification consists in the addition of a notched support (S) for the delivery tube. The desired rate of flow through the delivery tube (D) is then readily obtained by adjusting the position of the apparatus on the platform (P). Movement to the right raises the delivery tube (D), since this rests on the notched support (S) and is guided between two wire nails (G); this decreases the "head" and, consequently, decreases the rate of flow through the tube. To increase the rate of flow, the apparatus is moved to the left on the platform. A change in "head" of about 3.5 cm may be obtained with the apparatus illustrated. After the apparatus has been adjusted to the desired rate of flow, a mark is made with a wax pencil on the delivery tube even with the guide nails (G); this allows immediate resetting of the apparatus after the reservoir is refilled.

The addition of the tube support allows the rate of flow to be varied through a considerable range, and therefore obviates the necessity of extreme care in the selection of capillary tubing of suitable bore. In culture studies the rate of flow may easily be increased as the plants grow.

Small fluctuations in the solution level in the reservoir dish (L) occur, since air is admitted intermittently into the reservoir; changes in the temperature of the air in the reservoir also affect the solution level. These sources of variation in the rate of flow, though generally not significant in culture studies, may be avoided by employing a separate constant-level device, provided with an overflow.[3] But the simplicity, compactness and ease of manipulation of the apparatus of Shive and Stahl make it extremely useful in investigations of the mineral nutrition of plants.

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THE CHICAGO SOIL-NUTRIENT-TEMPERATURE TANK

THE Botanical Laboratory of the University of Chicago has developed the Wisconsin soil-temperature tank into a soil-nutrient-temperature tank.[1] By its use the direct pathogenic effects of deficient soil aera-

[1] J. H. Wales, SCIENCE, 79: 545–546, 1934; W. A. McCubbin, SCIENCE, 80: 144, 1934; H. F. Pierce, SCIENCE, 80: 339, 1934; R. H. Lambert, SCIENCE, 80: 361–362, 1934.

[2] J. W. Shive and A. L. Stahl, *Bot. Gaz.*, 84: 317–323, 1927.

[3] S. F. Trelease and B. E. Livingston, SCIENCE, 55: 483–486, 1922. Pierce, *loc. cit.*

[1] W. H. Tisdale, *Phytopathology*, 7: 356–360, 1917; L. R. Jones, *Plant World*, 20: 229–237, 1917; J. John-

tion, metal ions and sharply restricted root development, as well as their indirect pathogenic effects in disposing plants to other deleterious influences, are avoided. It permits growth of control plants that compare favorably in vigor, form, vegetative growth, flowering and fruiting with plants grown in the open in good soil.

The tank itself, a wooden box with a removable galvanized iron container that maintains a constant water level, is the same as that used in the Wisconsin model. A "Sylphon" metal diaphragm thermo-regulator, not shown in the diagram, is used instead of the glass-mercury regulator of the latest Wisconsin tank. Installation of relays, not shown in the diagram, and of insulated heating units is essentially that of the Wisconsin tank.

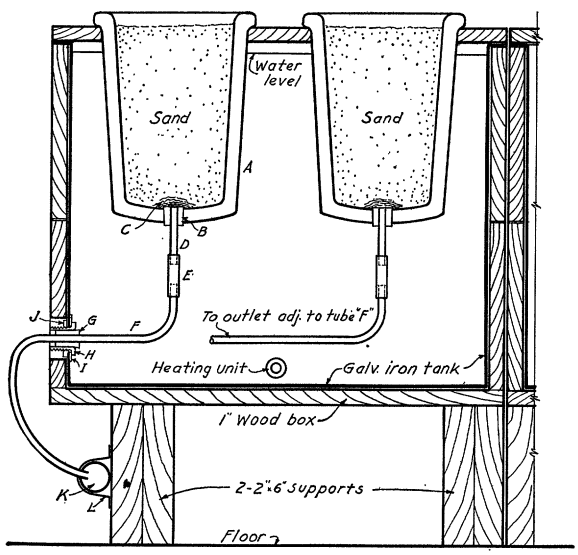

FIG. 1. Section of the Chicago soil-nutrient-temperature tank.

The critical change consists in substitution of six 8-liter glazed earthenware percolator urns for the 8 small, undrained metal containers used in the Wisconsin tank. These urns have been used by Nightingale, Robbins[2] and others of the New Jersey Experiment Station, and by Kraus, Harrison,[3] Mitchell, Shull, Eaton and others of the Chicago laboratory, for constant drip and intermittent application of mineral nutrients to plants grown in nitrogen-free quartz sand. The urns are manufactured by the American Metalware Company of Chicago for use as coffee percolators. The wide-flanged rim of the urn (A) permits its suspension from the lid of the temperature tank into the water bath, and the opening in its bottom permits connection with a drain through the side of the tank.

The drain-hole of the percolator urn is fitted with a one-hole rubber stopper (B) bearing a 3/8-inch glass tube (D) about 3½ inches long. The upper end of the glass tube is fitted flush with the bottom of the urn. Since the tanks are set up in batteries of six, two wide and three long, placed end to end, the outer side wall of each tank is perforated by six one-inch holes, one hole for each of the urns. The holes are drilled through the inner galvanized iron lining and the outer wood case, 2 to 3 inches above the floor of the tank. A ¾-inch Chase galvanized iron lock-nut bushing and an iron washer ⅛-inch thick are soldered into each hole of the metal tank. The washer (I) is placed on the inside and the lock nut (J) on the outside of the metal tank, the washer setting the bushing (H) in far enough so that the metal container is readily placed into or removed from the wooden tank. The bushing is fitted from the inside of the tank with a No. 3 rubber stopper (G) perforated with a 3/8-inch hole. A copper tube (F) 3/8-inch bore is fitted through the hole of the stopper, and the part projecting outside the tank is bent downward sufficiently to carry its end under the edge of the bottom of the tank, where it is inserted into a 1⅝-inch galvanized iron drain-pipe (K). The drain-pipes, one on each side of the battery of tanks, are fastened (L) to the wooden base which raises the tanks about 6 inches above the floor at the point of least elevation. The base is set in about 4 inches from the outer edge of the battery of tanks. The copper tubes are 18 or 24 inches long, depending upon which row of urns they drain. The inner free end of each copper tube is connected by means of a short piece of rubber tubing (E) to the lower, free end of the glass tube projecting through the stopper of the drain-hole of the percolator. The opening in the bottom of the percolator is covered with a mass of glass wool (C) or with an inverted porous flower pot whose drain-hole is covered with glass wool. Good drainage and aeration of the urn are thereby assured, and the likelihood of clogging of the copper drain tube reduced to a minimum. The glazed urn may be filled with soil, or preferably, for controlled nutrition studies, with pure white quartz sand.

The Wisconsin tank has proved an extremely useful instrument for studies in ecologic phytopathology,

son and R. E. Hartman, *Jour. Agr. Res.*, 17: 41–101, 1919; J. G. Dickson, *Jour. Agr. Res.*, 23: 837–870, 1923. In reply to an inquiry relative to the history of soil-temperature tanks, Professor Charles F. Hottes, of the University of Illinois, states that he too had developed a soil-temperature tank that was first installed "late in 1914 or early in 1915." Neither the Hottes tank nor its use has been described in publications.

[2] W. R. Robbins, *New Jersey Exp. Sta. Ann. Rpt.*, pp. 178–179, 1927.

[3] C. M. Harrison, *Plant Physiology*, 9: 83–106, 1934.

especially of diseases whose causal complex includes an infective agent. It has enabled experimental demonstration that soil temperature, moisture and reaction, singly, or in conjunction with each other and/or with other ecic factors, especially air temperature and moisture, are part of the etic complexes of infective plant diseases.[4] Culture of plants in pure quartz sand in self-draining containers has proved an extremely useful tool in physiologic and horticultural studies of the New Jersey and Chicago groups and of many others, and incidentally has contributed to analysis of the causal complexes of non-infective pathic events in plants. It permits sudden changes in nutrient treatment, such as a complete and rapid flushing with distilled water of all nutrients from the soil, with consequent rapid changes in growth status of the plant. It also permits collection of the drip for its continuous or periodic chemical analysis, for determination of the pH of the soil solution, and for reapplication, if desired. The Chicago tank combines the advantages of the Wisconsin tank with those of nutritional studies by continuous drip or discontinuous methods of applying nutrients and other ingredients to the plant substrate. Coupled with use of shades and lamps, the tank also is suited for analysis of the rôle, direct and indirect, of carbohydrate nutrition as a hygio- and pathogenic factor in development, maintenance and reproduction in plants.

The tank has been put to the following uses in the Chicago laboratory: a study of the relation of ecic factors to the processes, structures and behavior of fruit trees by Dr. G. T. Nightingale, of the New Jersey Experiment Station,[5] who put the tank to its first extensive use; production and cure of iron excess and deficiency in apple trees by Nightingale and the writer, independently; studies by the writer with the assistance of W. S. Cook and W. S. Phillips, of the relation of the carbohydrate-nitrogen nutrition of the apple tree to its positive and negative disposition to pathogenic infection by *Erwinia amylovora;* studies by Miss E. Goldberg of the relation of carbohydrate-nitrogen nutrition of susceptible and insusceptible varieties of cabbage, under controlled air and soil temperatures, to their disposition to pathogenic infection by *Fusarium conglutinans,* soil-borne fungous constituent of the etic complex of cabbage yellows; a study by W. S. Cook of the relation of nutrition of susceptible and insusceptible varieties of tomato to their disposition to pathogenic infection, under controlled air and soil temperatures, by *Fusarium lycopersici,* a soil-borne pathogenic fungus; and a study by Miss V. Eggers of the relation of nutrition of flax, under controlled soil and air temperatures, to wound healing and regeneration of an axis following experimental decapitation. In addition, to determine further the adaptability of the tank, experiments are being started to study the relation of nutrition to: (1) healing in apple and pear following non-infective and infective wounding; and (2) pathogenic infection for: (a) wheat and *Puccinia graminis tritici;* (b) cabbage and *Plasmodiophora brassicae;* (c) tomato and *Phytomonas tumefaciens;* (d) tomato and attenuated aucuba and yellow aucuba viruses; and (e) tobacco and *Thielavia basicola.* Of these experiments, 2e is being carried out by Miss F. L. Jewett, the rest by the writer and Mrs. H. W. Wilcox.

Results of the experiments indicate that the Chicago tank is well suited for studies, under controlled air and soil temperatures and controlled light and soil nutrient applications, of the relation of plant nutrition to (1) non-infective pathic events of plants, such as excess and deficiency diseases; (2) infective diseases whose etic complex includes an air-, insect-, water- or soil-borne virus, schizomycete or filamentous fungus; and (3) healing and organ regeneration following non-infective and infective wounding of plants.

The results also support the proposition that no plant should be designated immune, resistant or insusceptible, and more particularly, genotypically immune, etc., to a particular non-living factor or living agent, until its disposition to influence by that factor or agent has been tested under a wide range of factors ecically significant for the plant under consideration. The Chicago tank is suitable for such testing. The results of its use should call attention to the proposition that disposition (susceptibility, insusceptibility) is not an entity but an abstraction symbolizing the observed or probable behavior of an organism or its parts evaluated with reference to their relative capacity for reacting adaptively, non-adaptively or apparently not at all, to a given internal or external factor. Disposition is always a phenotypic expression, a resultant of an interplay of factors internal and external to the biologic system under analysis. Acceptance of this proposition is one conceptual means of bridging the chasm that exists in the thinking of many between genetics and developmental physiology. The so-called "genotypic" disposition is not a preformed material or immaterial something passed from parent or parents to progeny, but an analytic isolate from the causal complex of that phase of behavior designated as disposition. It symbolizes, in part, the relatively invariant ingredients and their organization that an individual receives from parent or parents. Possibly results obtained through use of the Chicago tank and similar tools may help to repair some of the mischief done in biologic speculation and experimen-

[4] L. R. Jones, *Trans. Wis. Acad. of Sciences, Arts and Letters,* 20: 433–459, 1922.

[5] G. T. Nightingale, *Bot. Gaz.,* 1935.

tation through unconscious hypostatization of disposition, including aggressivity, pathogenicity and virulence, and of similar concepts common to physiology, pathology and genetics.

Details of the experiments will be published elsewhere.

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SPECIAL ARTICLES

THE ROLE OF THE CARBAMINO COMPOUNDS IN THE TRANSPORT OF CO_2 BY THE BLOOD[1]

SINCE Siegfried[2] first prepared salts of carbamic acid by the reaction of CO_2 and amino-acids, and demonstrated analogous compounds of CO_2 and proteins in quite alkaline solutions, the rôle of these carbamino compounds of protein, particularly hemoglobin, as carriers of CO_2 in the blood under physiological conditions has received support by Henriques,[3] Margaria and Green[4] and others. In a recent paper, particularly, Meldrum and Roughton[5] report experiments on the reaction between CO_2 and amino-acids as well as hemoglobin. In brief, they observed that CO_2 was taken up by amino-acids or hemoglobin (to which cyanide had been added to inhibit the rapid enzyme catalysis of the hydration of CO_2 to carbonic acid) in two phases: (1) a very rapid one which they assert, correctly we believe, to be due to the formation of CO_2 in the carbamino form and (2) a slow uptake which is due to the formation of carbonic acid. From their values of carbamino-CO_2 in hemoglobin solution calculated from the *rapid* uptake, they constructed "non-bicarbonate" or carbamino-CO_2 absorption curves for hemoglobin which they "assumed to be practically the same for normal blood and for cyanide blood." They came to the conclusion, which at first sight seems strongly supported by their observations, that carbamino-hemoglobin plays a very important rôle as a CO_2-carrier in the blood. We believe, however, that the above assumption is erroneous because the equilibrium system which they studied was entirely different from the equilibrium system (*i.e.,* normal blood without cyanide) to which they applied their experimental data. Therefore, we believe that their conclusions about the physiological rôle of carbamino-CO_2 derives no support from these experiments.

This paradoxical situation arises as follows. An aqueous solution of an amino-acid, *e.g.,* glycine, to which has been added one or less equivalents of base, and which hence contains a concentration of amphanion, COO^-CNH_2, equal to the concentration of base, when suddenly allowed by equilibration to react with a CO_2 gas phase, forms carbamate, *viz.*:

(1) $COO^-CNH_2 + CO_2 = COO^-CNHCOO^- + H^+$

In addition, CO_2 reacts to form carbonic acid, *viz.*:

(2) $CO_2 + H_2O = H_2CO_3 = H^+ + HCO_3^-$

Both reactions decrease pH, since both carbonic and carbamic acid are about a thousandfold stronger than glycine. In consequence the amount of COO^-CNH_2 diminishes in favor of $COO^-CHN_3^+$ and a greater pressure of CO_2 is needed to obtain a given concentration of $COO^-CNHCOO^-$.

Now reaction 1 is very rapid even at 0° C., whereas reaction 2 is very slow. It follows then that if the equilibration is allowed to go on for a short time only (*i.e.,* about one minute at 0° C.) the carbamate reaction will be practically complete, while the carbonic acid reaction will be scarcely begun. In effect, there is an equilibrium established which is one involving CO_2, carbamate and amino-acid *but in which no carbonic acid whatever is present* (Case 1). This equilibrium affords a convenient and illuminating laboratory dissection of the reaction but has no counterpart in nature.

On the other hand, if the equilibration is allowed to go on sufficiently long, reaction 2 will be completed and the equilibrium will also include carbonic acid (as well as its ions HCO_3^- and $CO_3^=$) and will be entirely different (Case 2). This *complete* reaction is the one which occurs in the blood and therefore the only one of physiological significance.

Now Meldrum and Roughton's experiments, both on amino-acids and hemoglobin, were especially designed to bring about the first equilibrium only, but the experimental facts so elicited were applied without modification to the second equilibrium state and conclusions drawn therefrom apparently without realization that the two systems were different.

The complete dissimilarity between these two cases can be shown by our own experiments (Case 1). In Fig. 1 is shown the equilibrium curve of carbamino concentration as a function of P_{CO_2} and pH in a 0.1 M glycine solution with 0.05 M of base. The curve calculated on the supposition that *no* carbonic acid or its ions are formed agrees with our (unpublished) experiments on amino-acids and hemoglobin and Meldrum and Roughton's work on hemoglobin, under circumstances eliminating the formation of H_2CO_3. From this curve it is possible to calculate the mass

[1] From the John Herr Musser Department of Research Medicine, University of Pennsylvania, Philadelphia.

[2] M. Siegfried, *Zeits. Physiol. Chim.*, 44: 85, 1905.

[3] O. Henriques, *Biochem. Zeits.*, 200: 1 *et seq.*, 1928.

[4] R. Margaria and A. A. Green, *Jour. Biol. Chem.*, 102: 611, 1933.

[5] N. U. Meldrum and F. J. W. Roughton, *Jour. Physiol.*, 80: 143, 1933.