able hydrogens will then all be exchanged for deuterium.

Different conditions with respect to temperature, time, presence of suitable surface catalysts, etc., will be needed in different cases, but the principle is evidently of very general application. An example would be the bubbling of NH_3 gas through a train of bubble-tubes containing deuterium water and with suitable provision for drying the gas between tubes. From the end of such a train, deuterammonia, NH^2_3, would be evolved.

Similarly, if benzoic acid be shaken with successive portions of pure deuterium water it will probably be quantitatively converted into the corresponding deuterium acid, probably benzodeuteric acid, $C_6H^1_5COOH^2$. To prepare deuterobenzoic acid, $C_6H^2_5COOH^2$, would probably require a higher temperature and a suitable catalyst. This acid, if shaken with successive portions of pure protium water, (H^1_2O) would probably yield deuterobenzoprotic acid, $C_6H^2_5COOH^1$.

The comparative ease with which many new compounds can be prepared in this way gives further emphasis to the great need of provision for the large-scale production of heavy water. To equip a small plant having a capacity of 6 to 10 gallons of 95 per cent. deuterium water per year would cost something like $25,000. Labor costs would be about $5,000 per year, and power costs (40 KW) would depend upon location. Such a plant would be much more economical than the small-scale laboratory outfits now in use at a number of universities and would produce sufficient heavy water to allow many chemical and biological investigations to be carried out.

In contrast with the hundreds of millions which are being spent in new projects by the Federal Government and by private industry, the amount of money involved is almost infinitesimal. Yet probably in no other way could the expenditure of an equal amount of money be productive of greater advances in chemistry and possibly biology and medicine, not to mention physics, which requires only relatively small amounts of the heavy water.

EDWARD W. WASHBURN
BUREAU OF STANDARDS

ALFALFA YELLOWS

FOLLOWING the publication of the abbreviated discussion on "alfalfa yellows" (SCIENCE, October 27, 1933) we have been informed by Professor E. M. Searls, of the Department of Economic Entomology of the University of Wisconsin, that he had secured data from the entomological view-point, which lead essentially to the same conclusions with reference to leafhopper populations and time of cutting alfalfa, as expressed by us. This lends much emphasis to the validity of the findings, and because Professor Searls has not yet published his results we take this opportunity to provide for a simultaneity of recognition for his contribution.

L. F. GRABER
V. G. SPRAGUE
UNIVERSITY OF WISCONSIN

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A PORTABLE VACUUM TUBE VOLTMETER FOR MEASUREMENT OF GLASS ELECTRODE POTENTIALS WITH EXAMPLES OF pH ESTIMATIONS[1]

SIMPLICITY of design, small cost, accuracy and stability of the zero point are factors which justify a brief description of an equipment which has been found extremely useful in measuring the pH of biological fluids, food products and the like. Any laboratory possessing a suitable potentiometer and galvanometer may be provided with equipment for measurements with a glass electrode for a sum not exceeding ten dollars. The necessary parts are: One R. C. A. tube No. 232, thirteen flashlight cells, four No. 6 dry cells, two single-pole single-throw switches, twenty-five feet of rubber insulated wire No. 16 B. and S. gauge, and a wide mouth bottle, with tight-fitting rubber stopper.

The vacuum-tube is kept in a dry atmosphere by mounting in the wide mouth bottle in the following manner: An eighteen-inch length of insulated wire is soldered to each of the four base prongs, and a twelve-inch length to the cap of the tube. The wires attached to the prongs are bent so that when the tube is in a vertical position the five wires may be passed through small holes in the rubber stopper, in which they should fit snugly. A dry atmosphere is maintained either by a thin layer of phosphorus pentoxide in the bottom of the bottle, or by means of phosphorus pentoxide contained in a side arm connecting through the rubber stopper.

The wiring diagram is shown in Fig. 1. The tube filament is supplied with 1.5 volts, four No. 6 dry cells connected in parallel to give sufficient capacity for the maintenance of a constant filament temperature. Seven flashlight cells, connected in series to the filament battery, furnish twelve volts to the screen or space charge grid. Four flashlight cells in series furnish six volts to the plate, acting through a galvanometer which has a sensitivity of 0.025 microamperes

[1] Food Research Division Contribution No. 176.

per millimeter of scale deflection, a coil resistance of 1,000 ohms and a period of 3 seconds.

The negative terminal of the plate battery connects with the positive galvanometer terminal of the potentiometer and the negative side of the tube filament, and with a ground if necessary. The cap, or control grid, is given a -1.5 volt charge by a flashlight cell connected in series with the negative galvanometer terminal of the potentiometer. Single-pole single-throw switches are placed in the filament circuit and the potentiometer storage cell circuit for convenience of operation. The zero point of the galvanometer is adjusted by utilizing the torque of the galvanometer suspension to counterbalance the rotation of the coil produced by the plate current.

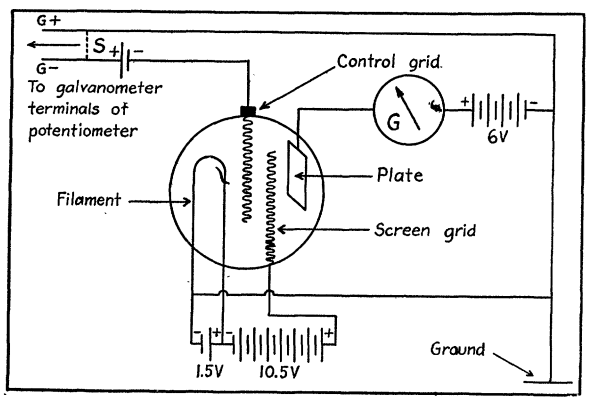

FIG. 1. Wiring diagram of vacuum-tube voltmeter, using R. C. A. 232 tube.

Differences in tube characteristics may occur among tubes of the same type, and therefore it may be necessary to alter slightly the voltages specified for the plate and space charge grid. This may be easily accomplished by keeping the charge applied to the cap or control grid constant at 1.5 volts $\pm$ 10 millivolts and varying the voltages in question until throwing the potentiometer out of balance 10 millivolts causes a deflection of the beam of light of at least four millimeters on the galvanometer scale.

By introducing a counter E.M.F. to control the zero point of the galvanometer, it becomes possible to alter the voltages applied to the plate and space charge grid so as to obtain a much greater sensitivity, but this complicates the circuit and offers no great practical advantage.

If a Leeds and Northrup type K potentiometer is used, the terminals marked G− and G+ in Fig. 1 are joined to the galvanometer terminals of the potentiometer. Use of the student type potentiometer, or in fact any potentiometer not provided with a short circuiting key, necessitates the use of a short circuiting switch at S to connect G− and G+ when the zero setting of the galvanometer is being adjusted.

Keeping the tube in a dry atmosphere eliminates erratic behavior due to electrical leakage over the tube and base. By using the galvanometer specified above with enclosed lamp and scale, a deflection of five millimeters is obtained when the potentiometer is thrown out of balance ten millivolts.

Several months' experience with this simple equipment has shown that the zero point is stable, even in the absence of shielding and ground connections. The elimination of elaborate shielding, the use of No. 6 dry cells and flashlight cells in place of storage cells, and the method of balancing the galvanometer in the plate circuit makes possible a simple piece of equipment which may be made portable by assembly in a small box.

The thickness of membranes in routine use ranges from 0.25 to 1.0 millimeter. However, as discussed in a previous publication,[2] precautions are taken to eliminate the "deviation film." The presence of this film not only requires the use of thin membranes but is a cause of erratic behavior. Several years of experience with the glass electrode has shown that a proper design of the electrode, making possible the elimination of the "deviation film," is more important than the use of extremely thin membranes and elaborate shielding of the measuring equipment.

The close agreement of a thick glass electrode and a hydrogen electrode was demonstrated by titrating a fifth molar disodium phosphate solution with 5 per cent. hydrochloric acid. The pH measured with the hydrogen electrode changed from 8.25 to 6.84, eleven measurements being made during the titration. The E.M.F. of the glass electrode measured against the hydrogen electrode remained constant at 303 ± 1 millivolts throughout the titration.

Recent studies[3] in this laboratory concerned with the toxic action of dinitrophenol, an agent which stimulates tissue metabolism, heat production and respiration, afforded an opportunity to observe the hydrogen-ion activity of the blood with the glass electrode. Two control measurements thirty-five minutes apart showed a pH of 7.30 for the arterial blood before injection of the dinitrophenol. Fifteen minutes after administration of the dinitrophenol the respiratory rate increased and the pH rose to 7.34, remaining constant for thirty minutes. Then a change toward acidity developed, as shown by pH values of 7.16, 7.04 and finally 6.94 for a sample of blood taken one minute after death.

These observations show the usefulness of the glass electrode and vacuum-tube voltmeter for demonstrating the constancy of the pH of arterial blood during

[2] H. Kahler and Floyd DeEds, *Jour. Am. Chem. Soc.*, 53: 2998, 1931.

[3] M. L. Tainter, J. H. Boyes and F. DeEds, *Arch. Internat. Pharm. et Therap.*, xlv: 234, 1933.

the control period, the shift toward the alkaline side during the period of over-ventilation, and the subsequent shift to acidity when the alkali reserve and the ventilation fail to compensate for the trend toward acidity caused by increased katabolism. The value of 6.94 for the pH of arterial dog blood removed as soon as possible after death is in close agreement with previously reported observations[4] in which thinner membranes and a much more elaborate measuring equipment were used.

This equipment, used in conjunction with sturdy glass electrodes, should extend the usefulness of the glass electrode, as for example in the determination of soil acidity, and the measurement of hydrogen-ion activity in such materials as canned meats, vegetables and fruits by direct insertion of the electrode without separation of the liquid and solid material.

FLOYD DEEDS

BUREAU OF CHEMISTRY AND SOILS
U. S. DEPARTMENT OF AGRICULTURE
AND
STANFORD UNIVERSITY SCHOOL OF MEDICINE

SPECIAL ARTICLES

ISOLATION OF A CRYSTALLINE PROTEIN FROM PANCREAS AND ITS CONVERSION INTO A NEW CRYSTALLINE PROTEOLYTIC ENZYME BY TRYPSIN

KÜHNE and Heidenhain showed that the proteolytic enzymes of the pancreas are completely inactive in fresh pancreas or in freshly secreted pancreatic juice.[1] The enzymes become active when mixed with the enterokinase of the small intestine, as found by Schepowalnikow, or when the pancreas is allowed to stand in slightly acid solution. According to Vernon, activation may also be brought about by small amounts of active trypsin. The mechanism of this activation has been the subject of controversy for many years.

This note describes the isolation from fresh pancreas of an active crystalline protein which is converted by minute amounts of trypsin into a powerful proteolytic enzyme. This enzyme has also been obtained in crystalline form. The inactive protein has been called chymo-trypsinogen and the active protein chymo-trypsin.

Pancreas was removed from cattle immediately after slaughter and immersed in M/8 cold sulfuric acid. The pancreas was then minced and extracted for 24 hours at 5° C. with two volumes M/8 sulfuric acid. This extract has no measurable proteolytic activity but becomes highly active upon the addition of enterokinase or upon the addition of relatively large amounts of active trypsin. The addition of relatively small amounts of active trypsin does not cause activation. The extract contains a protein which is soluble in 0.4 saturated ammonium sulfate but insoluble in 0.7 saturated ammonium sulfate. This protein may be crystallized from 0.25 saturated ammonium sulfate by the addition of saturated ammonium sulfate and adjustment of the pH to about 5.0. It crystallizes in the form of elongated prisms. About 1 gm of crystalline material may be prepared from one beef pancreas. The protein prepared in this way can not be activated by enterokinase but becomes powerfully active upon the addition of a very small amount of crystalline trypsin[2] or of any crude trypsin solution. The crude extract and the mother liquor from the crystals, on the other hand, are completely activated by kinase but not by small amounts of trypsin. This apparent contradiction is due to the fact that crude extracts contain some material which inhibits trypsin so that small amounts of trypsin are completely inactivated. When kinase is added to such crude extracts sufficient active trypsin is formed to overcome the inhibiting effect and this active trypsin changes the chymo-trypsinogen to chymo-trypsin.

CONVERSION OF CHYMO-TRYPSINOGEN TO CHYMO-TRYPSIN

Three grams of crystalline chymo-trypsinogen were dissolved in 400 ml. M/30 pH 7.6 phosphate buffer, 1 mg of crystalline trypsin added and the solution kept at 5° C. The activity increased rapidly and after 24 hours had reached a constant value of about 1,000 times that of the trypsin added. The time rate of increase in activity is logarithmic and not auto-catalytic. This indicates that the chymo-trypsinogen can not be activated by chymo-trypsin and control experiments confirm this conclusion. No measurable hydrolysis of the chymo-trypsinogen occurred during activation. The active protein was precipitated from this solution by bringing to 0.7 saturated ammonium sulfate. The filter cake was dissolved in twice its weight of M/100 sulfuric acid, ammonium sulfate added to slight turbidity, and the pH adjusted to about 4.0 with sodium hydroxide. The solution was allowed to stand at 22°

[4] Carl Voegtlin, Floyd DeEds and H. Kahler, Public Health Reports, 45: 2223, 1930.

[1] For review of the literature see Carl Oppenheimer, "Die Fermente und Ihre Wirkungen," G. Thieme, Leipzig, fifth edition, Vol. II, p. 917.

[2] John H. Northrop and M. Kunitz, SCIENCE, 73: 262, 1931; *Jour. Gen. Physiol.*, 16: 267, 1932.