A MODIFICATION OF THE MUDD ELECTRO-ENOSMOSIS APPARATUS[1]

IN further experiments on electroenosmosis across intact dog tooth enamel membranes,[2] the writer has found the following modification of the Mudd electroenosmosis apparatus of assistance in eliminating the tendency of the rubber stoppers to loosen in the electrode chamber.

The changes in the apparatus concern the zinc electrode cell, Z (Fig. 1). The cell described by Mudd[3] makes use of a cylinder of glass, open at both ends. In the upper end is placed a perforated rubber stopper through which passes a glass tube which connects the electroenosmosis apparatus proper with the zinc electrode chamber. In the lower end of the glass cylinder is placed a second perforated rubber stopper, through which the zinc electrode passes into the glass electric chamber.

When the chamber is filled with zinc sulfate and then tightly closed top and bottom, the rubber stopper at the lower end carrying the zinc electrode tends to loosen and move out of the chamber. As a result the volume of the chamber changes, so interfering with the accuracy of the readings of the amounts of fluid transferred during the experiment.

The substitution of a sealed glass collar for holding the zinc electrode eliminates these difficulties. A pyrex glass cylinder has sealed within its lower end a piece of pyrex tubing B, slightly larger in inside

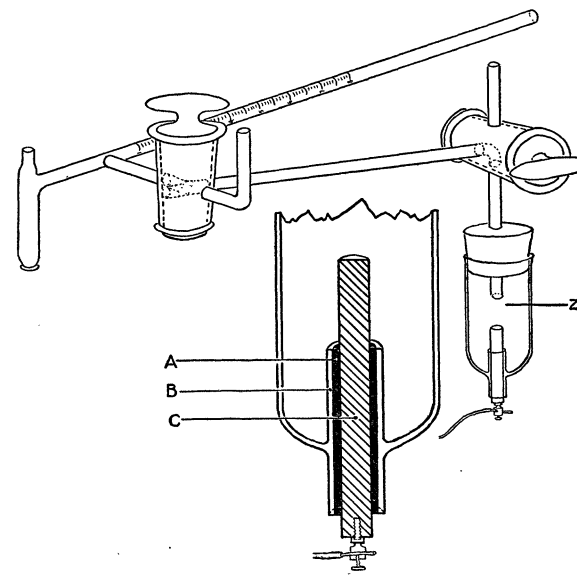

FIG. 1.

diameter than the diameter of the zinc electrode C. The lower end of the glass cylinder containing the glass collar is warmed slightly, and the zinc electrode is placed in the chamber through the glass tubing. DeKhotinsky cement A is then sealed in the space between the zinc electrode and the inside of the glass collar.

HENRY KLEIN

SPECIAL ARTICLES

PENETRATION OF GASEOUS PYRIDINE, PIPERIDINE AND NICOTINE INTO THE BODIES OF CERTAIN INSECTS[1]

THE cuticula of insects presents an effective barrier to most injurious substances which are found in the environments of these animals. Its physical structure and chemical composition afford great resistance to all but the most active chemical agents, and the view has long been held that gases and substances in solution do not penetrate the cuticula directly, but rather enter the body through the spiracles and pass from the tracheal system to the tissues.

Recently, however, it has been shown that the cuticula of some insects, at least, is not only permeable to respiratory gases but to certain toxic substances as well. For instance, Thorp[2] has found that elimination of carbon dioxide takes place largely through the cuticula of many small, thin-skinned insects, the tracheal system being in such forms of minor respiratory importance. In some adult Coleoptera, carbon dioxide seems to pass through the cuticula, although the permeable areas may be limited, and among pupal forms, the evolution from the cuticula may be slow or rapid. Hartzell and Wilcoxon[3] have shown that pyrethrins penetrate directly the cuticula of insects when they are applied to the body surface at points remote from the spiracles. Ethyl thiocyanate, methyl isothiocyanate and nicotine also were found to penetrate the cuticula in quantities large enough to provoke toxic reactions. Portier[4] immersed the tips of the antennae of butterflies in solutions of nicotine and

[1] From the Laboratory of Pathology, Yale University School of Medicine.

[2] H. Klein, *Journal of Dental Research*, 12: 87, 1932.

[3] S. Mudd, *Journal of General Physiology*, 9: 369, 1926.

[1] Rockefeller Fluid Research Fund Project No. 9, Iowa State College.

[2] W. H. Thorp, SCIENCE, 68: 1766, 433–434, 1928.

[3] A. Hartzell and F. Wilcoxon, *Contrib. Boyce Thompson Inst.*, 4: 1, 107–117, 1932.

[4] P. Portier, *Compt. rend. soc. biol.* (Paris) 105: 367–369, 1930.

observed toxic manifestations which indicated the penetration of nicotine through the cuticula. Hockenyos[5] has recently stated that sodium fluoride is absorbed directly through the cuticula of two species of cockroaches, especially through the thinner articular and intersegmental areas.

The works mentioned above treat of penetration from solids, liquids or solutions. The results of the investigation reported below are concerned with the penetration of gaseous pyridine, piperidine and nicotine into the cuticula of adult American cockroaches, *Periplaneta americana* (L.), larvae of the corn ear worm, *Heliothis obsoleta* Fab. and adult red-legged grasshoppers, *Melanoplus femur-rubrum* (DeGeer). This paper contains a preliminary statement of a part of the results obtained to date. More detailed publications will appear later.

The insects were exposed to approximately maximum vapor concentrations of the compounds in air at 30° C., the exposure times being varied as desired. After exposure, the insects were washed free of any surface-adhering compound, and were then refluxed with absolute alcohol acidified with tartaric acid. The extracted material was freed of alcohol, made alkaline with NaOH and subjected to steam distillation to recover the toxic compound. The distillate was caught in a weak tartaric acid solution and concentrated to the desired volume.

The compounds were quantitatively estimated in the distillates by successive dilution of aliquots until a limiting precipitate was obtained with phosphomolybdic acid reagent, equivalent to that given by a known solution of the compound in distilled water. The procedure was a modification of that used by Fulton[6] for the detection of alkaloids. With a knowledge of the weight of the original insect tissue extracted, the volume of the distillate, degree of dilution and concentration of the toxic compound at the limiting dilution, it was possible to express in terms of a unit weight of tissue the approximate amount of toxic compound absorbed. To confirm detection of the compound with phosphomolybdic acid reagent, the distillate was tested at the limiting dilution, or at greater dilution, with another suitable alkaloid reagent. In this manner, detection of the compound, if present, was assured, and the quantitative results were sufficiently accurate for approximate comparative purposes.

When entire insects were subjected to the vapors of the compounds, generally more pyridine, piperidine or nicotine, in mg per gram of tissue, was found in the cuticula than in the entire insects. The comparisons were made when approximately 50 per cent. of the insects had succumbed to the vapors. The results of the experiments show that relatively large quantities of the vapors of these compounds penetrate the cuticula of the insects.

To prove that these toxic gases do not necessarily first enter the body through the spiracles, pass from the tracheae to the blood and then enter the cuticula from the blood, special tests were made with corn ear worm larvae, adult cockroaches and adult red-legged grasshoppers. The posterior 3 or 4 pairs of abdominal spiracles of a corn ear worm larva were coated with beeswax, and the larva was sealed in the cork stopper of the gas bottle in such fashion that only the portion of the abdomen with covered spiracles was in contact with the toxic vapors. Control larvae, similarly fastened, lived for many hours. After 120 minutes' exposure to pyridine, an abundance of the compound was found in the bodies of the larvae. The same result was obtained with piperidine. Although it is believed the results demonstrate penetration of these compounds through the cuticula, the larvae offered certain experimental difficulties, and a more rigid test of penetration was sought. Individual grasshoppers were fastened on the cork stopper of a large-mouth gas bottle with a single posterior leg projecting through a hole in the stopper, and sealed off with beeswax. Only a part of the leg was in contact with the toxic gas. After 120 minutes, easily demonstrable quantities of pyridine were found in the bodies of the grasshoppers. Similar results were obtained with piperidine and nicotine. Cockroaches were subjected to equally rigid tests. An active circulation of blood, suggesting that in the web of the frog's foot, occurs normally in the anterior wings of the American cockroach. Cockroaches were fastened to the cork stoppers of gas bottles with only a part of one anterior wing sealed off in contact with the toxic gas. After 120 minutes' exposure to pyridine, piperidine or nicotine, the bodies of the treated insects contained sufficient of these compounds to give good quantitative values.

The results of the experiments mentioned leave little doubt that these organic bases in gaseous condition passed directly through the cuticula of the insects. The compounds have been detected, and the amounts quantitatively estimated, in muscular tissue from the wings and legs, in the entire digest tracts, in fat body tissue and in the ventral nerve cords of cockroaches, and in the blood of corn ear worm larvae which have been subjected to the gases.

CHARLES H. RICHARDSON
LOUISE H. GLOVER
LEWIE O. ELLISOR

[5] G. L. Hockenyos, *Jour. Econ. Ent.*, 26: 6, 1162–1169, 1933.

[6] C. C. Fulton, *Assn. Official Agric. Chemists*, 13: 4, 491–497, 1930.