recovery of the E.E.E. virus from the mosquito *Mansonia perturbans* Walker taken in nature.

During the summer of 1948, a few horses in different parts of Georgia were diagnosed as having encephalomyelitis. The brains from two animals were sent to the U. S. Public Health Service Virus Laboratory in Montgomery, Alabama, where the virus of eastern equine encephalomyelitis was recovered from them. During the same period, mosquitoes were collected from different areas in the state by members of the Epidemiology Division of the U. S. Public Health Service in collaboration with the Georgia State Department of Health. Forty-three *Mansonia perturbans* were taken from farms in Burke and Jenkins Counties, where sick horses previously had been reported. These mosquitoes were put into glass ampules, which were sealed in a flame and then quickly frozen with dry ice and alcohol. They were sent in this frozen state to the laboratory in Montgomery. There they were pooled, washed with buffered saline, suspended in 3 ml of buffered saline containing 30% normal rabbit serum, and spun in the angle centrifuge for 20 min at 13,000 rpm. The supernatant fluid was removed and 0.03 ml was inoculated intracerebrally and 0.1 ml intra-abdominally into nine 12-day-old white Swiss mice. After 2–4 days, three animals died and four became sick and were killed. The brains were removed but, because of a bacterial contamination, the suspension of brain tissue was passed through a Seitz filter before inoculation of a second group of mice. The latter animals either died or showed typical convulsions in 2–3 days. Cultures of the brains were negative for bacteria, so that a third passage was made to mice. All of these animals either died or showed symptoms of an acute encephalitis. In a further passage the titer was 10^{-8} in mice.

This virus was then identified as that of the eastern equine encephalomyelitis strain, both by means of the neutralization test in mice and by cross immunity inoculations into immune guinea pigs. The virus was neutralized by the E.E.E. antiserum but not by that of the W.E.E. or St. Louis strains. Guinea pigs, previously proven immune to the stock E.E.E. strain by intracerebral inoculation of 70,000 lethal mouse doses of virus, withstood a dose of the new strain that was fatal for the control animals and for those immunized to the western equine virus. Moreover, an antigen prepared from a mouse brain suspension of this virus gave a positive complement-fixation against E.E.E. antiserum but not against those of the W.E.E. or St. Louis strains. The antigen titer was 3+ in a dilution of 1: 32 against the E.E.E. serum.

It should be stated here that the Venezuelan equine virus has been recovered by Gilyard (*2, 3*) from another species of the genus *Mansonia* (*Mansonia titillans*) taken in nature in Trinidad. Both *M. titillans* and *M. perturbans* are persistent feeders on warm-blooded animals, including horses and chickens. This makes these mosquitoes potentially dangerous vectors of equine encephalitis viruses if they are true vectors, rather than merely transient carriers. Further studies to establish the role of *M. perturbans* in the epidemiology of eastern equine encephalitis are planned for the coming season.

In summary, a filterable virus, proven to be antigenically and immunologically identical with that of the eastern equine encephalomyelitis virus, has been recovered from wild-caught specimens of *Mansonia perturbans* Walker. The infected pool of these mosquitoes was collected in Burke and Jenkins Counties, Georgia during the summer of 1948.

References

1. Davis, W. A. *Amer. J. Hyg.*, 1940, **32**, 45.
2. Gilyard, R. T. *U. S. Army Med. Dept. Bull.*, 1944, **75**, 96.
3. ———. *J. Amer. Vet. med. Ass.*, 1945, **106**, 267.
4. Howitt, B. F., *et al. Proc. Soc. exp. Biol. Med.*, 1948, **68**, 622.
5. Merrill, M. H., Lacaillade, D. W., Jr., and TenBroeck, C. *Science*, 1934, **80**, 251.

A Convenient Microsyringe

A. W. Woodrow

Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture, Davis, California

An accurate microsyringe is useful for dispensing minute quantities of fluids in laboratory experiments. This type of instrument was used for inoculating individual honeybee larvae in the honeycomb by adding spores of *Bacillus larvae* to the food in which the larvae float during the first two days of life (*1, 2*). Recently it has been improved to increase its accuracy and convenience, and has been used for feeding minute quantities of DDT suspended in 50% sugar syrup to individual adult worker bees. By this method as little as 1 μg or less of DDT can be fed to each bee. The unit of discharge is 1 μl.

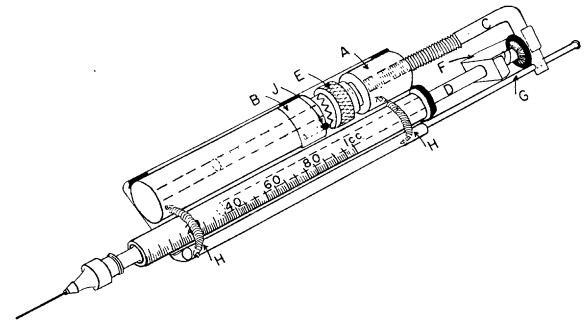

Fig. 1.

The complete instrument is diagramed in Fig. 1. The syringe is a 1-cc glass tuberculin type hypodermic syringe, the 1-cc calibration occupying a linear distance of 56.5 mm. The syringe holder is made of sheet brass formed to accommodate and hold the syringe in position. Two bushings, *A* and *B*, serve as bearings to guide the 3/16-in. feed screw *C*, which regulates the movement of the plunger *D* of the syringe. The screw is threaded 3/16-32. The feed device *E* is a knurled nut tapped to fit this screw, and on one end are cut notches or gear teeth. A hole is drilled

in the bushing *B* under the teeth of the nut to accommodate a 5/64-in. steel ball *J* with a coil spring placed under it. The ball enters the space between the teeth as the nut is turned, resulting in a definite click.

The instrument is held in the right hand and operated by turning the nut with the thumb or forefinger. The syringe holder is cut away so that the nut may be reached from underneath. The clip *F* and a coil spring hold the plunger in position against the screw rod. The rod *G* is firmly attached to the syringe holder and serves as a guide for the screw rod *C* through which it passes.

Two light coil springs *H* attached to the central portion and hooked over two prongs soldered to the outside of the holder serve to hold the syringe firmly in place. The springs are unhooked for removal of the syringe.

With a feed nut of 14 teeth and a 32-thread screw there are 448 clicks per in. of screw, or 997 clicks to the 1 cc occupying a distance of 56.5 mm. This is equivalent to 0.0009965 cc per click or, for all practical purposes, 1 μl. By proper selection of the number of threads per in. in the screw, the number of teeth in the gear of the nut, and the diameter of the syringe, various sizes of drops may be obtained. The quantity of dissolved or suspended materials for a given dose may be calculated on this basis.

A fine long needle of 25 or 27 gauge, cut off bluntly, has been used for the small drops fed to bees. For accuracy it is essential that all air be removed from the syringe and that the temperature remain constant.

References

1. Woodrow, A. W. *J. econ. Ent.*, 1942, **35**, 892.
2. Woodrow, A. W., and Holst, E. C. *J. econ. Ent.*, 1942, **35**, 327.

The Caries-producing Capacity of Starch, Glucose, and Sucrose Diets in the Syrian Hamster

William G. Shafer[1]

Division of Dental Research and Department of Pathology, School of Medicine and Dentistry, University of Rochester

The reports of experiments with the cotton and albino rat, in which various carbohydrates were incorporated in the diets to determine their relative cariogenic effectiveness, are either inconclusive or inconsistent (*2*, *6*, *7*, *8*).

It has been shown that the Syrian hamster on a high carbohydrate diet will readily develop gross lesions of the teeth which are comparable to human dental caries (*1*, *4*), and the use of this animal seems to have several advantages over that of the rat (*5*). An experiment was designed, therefore, in which the hamster was used for testing the caries-producing capacity of a monosaccharide, a disaccharide, and a polysaccharide.

Sixty-two hamsters (36 males and 26 females) were divided into three main groups with as nearly equal sex and littermate distribution as possible. At the initiation of the experiment, the ages of the animals ranged between 31 and 34 days. Beginning at this age, each of the three groups received diets which varied in composition only in the type of carbohydrate present. Group I received the following diet: raw cornstarch, 61%; whole powdered milk, 35%; powdered alfalfa, 3%; sodium chloride, 1%. In the diet of group II, the 61% cornstarch was replaced by an equal quantity of powdered α-glucose, and in the diet of group III, the starch was replaced by sucrose in the form of confectioner's sugar.

[1] National Institutes of Health senior research fellow.

TABLE 1

Caries Scores on Carbohydrate Diets

	No. of animals	Mean caries score	Standard deviation	Critical ratio Starch	Critical ratio Sucrose
Starch diet					
Males	12	2	7	..	9
Females ...	9	0	0	..	11
Glucose diet					
Males	12	72	35	7	5
Females ...	8	44	27	4	7
Sucrose diet					
Males	12	163	57	9	..
Females ...	9	170	42	11	..

After an experimental period of 111 days, the animals were sacrificed, the teeth examined under a dissecting microscope, and the carious lesions charted and scored according to the method of Keyes (*3*). The mean caries scores are shown in Table 1. The difference in mean caries scores of the males on the various diets, and also the difference in the mean caries scores of the females on the various diets, are of a high order of statistical significance.

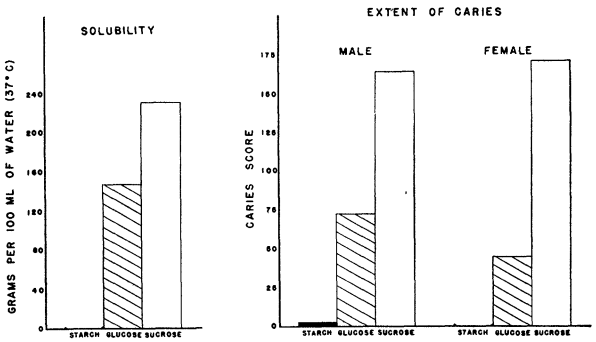

Fig. 1.

Thus it is seen that raw starch plays little if any significant role in the initiation of gross dental caries in the Syrian hamster. The feeding of a diet high in sucrose results in the highest caries scores, whereas a diet high in glucose results in caries scores intermediate between the starch and sucrose groups. Although the severity of dental caries in these three groups is roughly proportional to the solubility in water of the three carbohydrates (Fig. 1), it must not be inferred that solubility is the controlling factor in this experiment. No evidence is available except that cited above and no conclusions