has been found most practical to autoclave first and then oven-dry the devices in packets of five.

Introduction of the appliance into the mouth of the animal is based on the observation that a mouse almost invariably will reach with his mouth and hold with his teeth any small article that is presented before him. In practice, the mouse is held in the conventional manner in the left hand, and the needle is held at its hub by the thumb and middle finger of the right hand with the index finger in the wire loop. With the swab drawn back into the needle (Fig. 2), the device is held before the

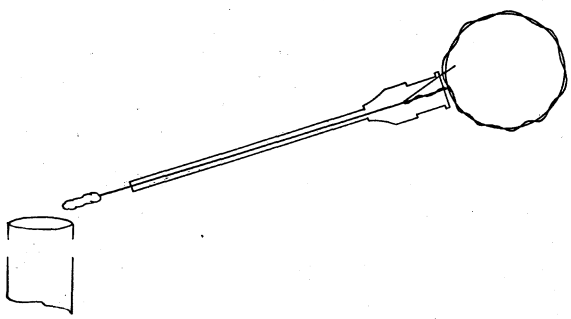

FIG. 3. Stop forced and cotton ready to be dropped into diluting fluid.

animal's face. When he reaches for it, he usually will grasp the end of the needle and begin to chew on it. At this point, the operator's index finger can push the swab gently out of the needle and into the animal's oropharynx. The stop will inform the operator when the swab is correctly positioned in the animal's mouth and will thus prevent injury caused by pushing the swab too far into the throat. It has been our experience that merely holding the swab in the mouse's mouth and allowing it to be tongued for 5 sec will insure a sample that will be as reproducible as any obtained by more elaborate manipulation. Before removal of the appliance from the animal's mouth, the swab is drawn back into the needle.

After removal, the swab is pushed about ¾ in. beyond the end of the needle by forcing the stop. Sterile forceps are used to pull the cotton off the wire and drop it into the diluting fluid (Fig. 3). After shaking the swab in the diluting fluid, an aliquot is plated on the culture medium of choice.

This appliance has been used with the Namru strain (*2*) of albino mice in studies to determine the relation between the oral flora, exposure to airborne pathogens, and the resulting degree of infection. In one exploratory test of the applicability of this technique, daily mouth swabs were cultured from 27 normal mice and from 20 mice that had been exposed for 15 min to a cloud containing *Streptococcus zooepidemicus* in a concentration of approximately 3×10^3 organisms per liter of air. The swabs were shaken in 10 ml of nutrient broth, and 0.1 ml aliquots were dallied on 5% cow blood agar and incubated at 37° C for 24 hr. Of the normal animals, 90% produced less than 3×10^3 β-hemolytic colonies per swab during the 3-week experimental period. On the other hand, counts from the infected animals showed an upward trend until, at the end of the 3 weeks, 11 of the 12 survivors produced between 2×10^4 and 2×10^5 β-hemolytic colonies per swab. The 8 nonsurvivors died within 5 days after exposure; their counts during this period were comparable to those of the survivors sampled simultaneously. In addition to colonies showing β-hemolysis, other colonial types were noted on most plates.

The fact that a trend could be discerned in the infected animals, together with the observation of various colonial types from both normal and infected animals, indicates that this mouth-swabbing technique should allow satisfactory sampling of the oral flora of mice. Further studies will be reported at a later date.

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A Method for Artificial Insemination in Viviparous Fishes[1]

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The practice of successful artificial insemination in man and in certain domesticated mammals, particularly horses and cattle, is well known, and the artificial insemination of bees is a common practice of apiculturists (*1*). The technique has also been used effectively in poultry for the study of intraspecies competition among sperm of different breeds (*2*).

Methods for the external artificial fertilization of a variety of invertebrate eggs and those of a number of oviparous poikilothermic vertebrates have been widely employed by experimental embryologists, pisciculturists, etc. However, the artificial insemination of viviparous cold-blooded vertebrates (or poikilothermic vertebrates which lay fertilized eggs) has never, to the writer's knowledge, been previously reported.[4]

In the course of studies on the sexual-isolating mechanism between the sympatric species of poeciliid fishes, *Platypoecilus maculatus* and *Xiphophorus hellerii* (*3, 4, 5*), interspecies sperm competition appeared to be a significant factor. Cross-species matings and the pro-

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[4] After this paper went to press, it was called to the writer's attention that a paper in Russian (*10*) reports hybridization between *X. hellerii* and *P. maculatus* by means of artificial insemination.

TABLE 1

RESULTS OF THE ARTIFICIAL INSEMINATION OF 19 *Xiphophorus hellerii*

♀	No. and species of donor*	Date of insemination	Dates of broods and no. of young†			Comments
			1st brood	2nd brood	3rd brood	
7	1 P.m.	2/1	3/13 (12)‡	—	—	♀ died 5/17; had no embryos
8	1 P.m.	2/1	—	—	—	No broods as of 7/24
9	1 X.h. + 1.5 P.m.	2/1	2/23 (12)	3/21 (19)	4/17 (11)	
10	1 X.h. + 1.5 P.m.	2/1	3/28 (6)	—	—	
11	1 X.h. + 2 P.m.	2/25	4/12 (23)	5/6 (15)	6/4 (22)	
12	1 X.h. + 2 P.m.	2/25	—	—	—	♀ died 4/11; had no embryos
13	1 X.h. + 2 P.m.	2/25	4/ 7 (11)	—		
14	1 X.h. + 2 P.m.	2/25	4/ 4 (14)	5/2 (3)	7/20 (1)	
18	1 X.h. + 2 P.m.	2/25	3/23 (17)§			
19	1 X.h. + 2 P.m.	2/25	3/22 (12)‖			♀ died 3/22
15, 16, 17, 20	1 X.h. + 2 P.m.	2/25				No broods as of 7/24
22	3 P.m.	3/6	4/7 (1)‡			
24	3 P.m.	3/6	4/24 (26)‡	5/22 (17)‡	6/20 (8)‡	
21, 23, 25	3 P.m.	3/6				No broods as of 7/24

* P.m. = *P. maculatus*; X.h. = *X. hellerii*.
† Except where otherwise indicated, the young are *X. hellerii*.
‡ Hybrid young.
§ One of these young is a hybrid.
‖ Embryos found in dead ♀.

duction of hybrid broods from these two species occur infrequently under limited laboratory conditions. An experimental investigation of the problem of sperm competition, therefore, could be greatly facilitated in these viviparous fishes by a method for controlled inseminations. In the past, several investigators—including the writer (*6*)—have failed to produce broods by artificially inseminating females of *P. maculatus* and *X. hellerii*, although they used large quantities of spermatophores. We have now developed a method of artificial insemination in *X. hellerii* that has proved reasonably successful.

The compact clusters of spermatozoa (called spermatophores) of the male *P. maculatus* or *X. hellerii* are squeezed out by pressing slightly on the bases of the pelvic fins and pivoting the gonopodium into the forward position. This releases hundreds of spermatophores, which usually flow into a groove formed on either the left or right side of the gonopodium. By means of a rubber tube held in the experimenter's mouth at one end, and attached to a micropipette at the other, the spermatophores can be gently sucked into the pipette and placed in a drop of 0.8% NaCl solution. The spermatophores of 1–3 males are used to inseminate each virgin female. The spermatophores are all added to the same drop of saline solution, wherein they start breaking up, and in less than 1 min highly motile spermatozoa can be observed swimming freely. The drop of saline-sperm solution is then gently sucked up and blown out of the micropipette twice in order to hasten the breaking up of the remaining clusters and to ensure adequate mixing of spermatozoa. Finally, the saline-sperm drop is injected into the genital opening of the female with the same micropipette. The females are then placed in individual aquaria and are well fed with daphnia; when the young are born they are separated from the mother to prevent cannibalism.

Table 1 shows the results of 19 attempts to artificially inseminate *X. hellerii* females. It is known that poeciliid females store sperm for long periods and may continue to drop as many as 8 broods (at approximately 28-day intervals) after being paired with a male for a day or two. It has been found, however, by the use of a smear technique to check for sperm in females after a copulation (*3*), that single inseminations resulting from normal copulations do not always produce broods (*5*). In view of these facts, the preliminary results on artificial inseminations are encouraging. The production of broods in over 50% of the trials indicates that this method may serve as a useful tool in the study of sperm competition, and further experiments are now in progress. *X. hellerii* and *P. maculatus*, as well as a number of closely related forms, are the subjects of extensive genetic studies in fishes (*7, 8, 9, 11*). It is hoped that this method of artificial insemination may be helpful in facilitating these genetic studies, particularly where the breeding of certain strains and hybrid combinations by natural methods has not been successful.

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