paper by the usual procedure (73 percent transmission) as by the improved method (72 percent transmission).

The method, while devised initially for paper filters, is equally applicable to Millipore filters which thus can be used without the need of special holders. The 900 liters referred to above were subsequently filtered on the same funnel through Millipore filters cut to the appropriate size.

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Instrumental Conditioning of Jugular Self-Infusion in the Rhesus Monkey

Abstract. A technique is described for self-infusion of pharmacologic agents in solution through a permanently indwelling jugular catheter in the rhesus monkey. The results of an experiment utilizing this technique demonstrate that an instrumental lever response can be conditioned, extinguished, reconditioned, and brought under stimulus control and reward-schedule control with saline self-infusion as the reinforcing stimulus.

Relatively few psychopharmacological studies have focused upon a systematic analysis of the reinforcing properties of chemical compounds with known behavioral effects (*1*). Technologically, the many problems associated with drug administration and the arrangement of appropriate contingent relationships involving behavioral indicators have made experimental investigations in this area difficult to conceive and carry out. It is the purpose of this report to describe the development of a technique of potential value for investigating the reinforcing properties of drugs in laboratory animals and to present the results of an experiment designed to demonstrate its methodological feasibility. Specifically, the present report is concerned with the development of a technique for self-infusion of pharmacologic agents in solution through a permanently indwelling jugular catheter in the rhesus monkey and describes several aspects of the conditioning of an instrumental lever-pressing response with saline self-infusion as the reinforcing stimulus.

Two rhesus monkeys, restrained in chairs, served as subjects. Crackers were continually available to them. After surgical implantation of permanently indwelling catheters in the internal jugular vein of each monkey, continuous recordings of operant levels of responding on a lever switch (a modified telegraph key mounted on the chair within easy reach of the monkey's paw) were taken for both animals during an 8-day period (4 days of water available alternated with 4 days of no water available). After this operant level determination under both satiated and water-deprived conditions (Fig. 1*A*), the water bottle was removed and each lever response was programmed to activate an infusion pump and produce an injection of saline through the indwelling catheter. Under this condition of continuous reinforcement, each lever response during a 4-hour daily session delivered 1.95 ml of saline at the rate of 1 ml/min. Lever responses which occurred during a saline infusion were recorded, but otherwise had no consequence. A house light was on during each test session and off at all other times. With both animals, introduction of the saline infusion reinforcement contingency produced a significant increase in the lever-pressing response rate which remained consistently above operant levels (Fig. 1*B*) and which exhibited the typical characteristics of behavior under continuous reinforcement conditions. The subjects self-infused a daily average of 65 ml of saline during this phase of the experiment.

Extinction of the instrumental lever-pressing response was observed in both monkeys after discontinuation of the saline infusion (Fig. 1*C*). With only the tube from the pump to the catheter disconnected and all other aspects of the circuit in operation, a typical lever-pressing extinction curve was generated by both monkeys with a concomitant return to operant level response rates. After reconditioning of the self-infusion behavior by replacing the pump connection (Fig. 1*D*), both monkeys were provided with an *ad libitum* supply of water through an easily accessible bottle and mouth spout. Under these conditions of free access to water by way of the normal oral ingestion route, a marked decline in the self-infusion response rate was again observed for both monkeys (Fig. 1*E*). This finding indicates that the reinforcing properties of normal saline infused in this manner are at least partially sensitive to variations in "drive" level.

In the final phase of this experiment an attempt was made to bring the instrumental self-infusion behavior under exteroceptive stimulus control. Lever responses were reinforced with saline infusion only in the presence of a dim light stimulus presented on a panel in front of the monkey. Responses in the absence of the light were not reinforced. Five-minute periods of dim light were alternated with 5-minute periods of no light during each 4-hour daily session. After several days of exposure to these conditions, the response rates and volume of self-infused saline recorded during the dim light condition approximated levels obtained during the previous continuous reinforcement sessions. The response rates in the absence of the light approximated the operant level (Fig. 1*F*). Preliminary observations have also indicated that such self-infusion responding can be brought under reinforcement schedule control by requiring a fixed ratio of lever responses to produce each saline injection. Introduction of such a ratio requirement produced a marked increase in the response rate over both operant and continuous reinforcement levels (Fig. 1*G*).

These findings indicate quite clearly that the self-infusion of a chemical compound in solution can serve as a

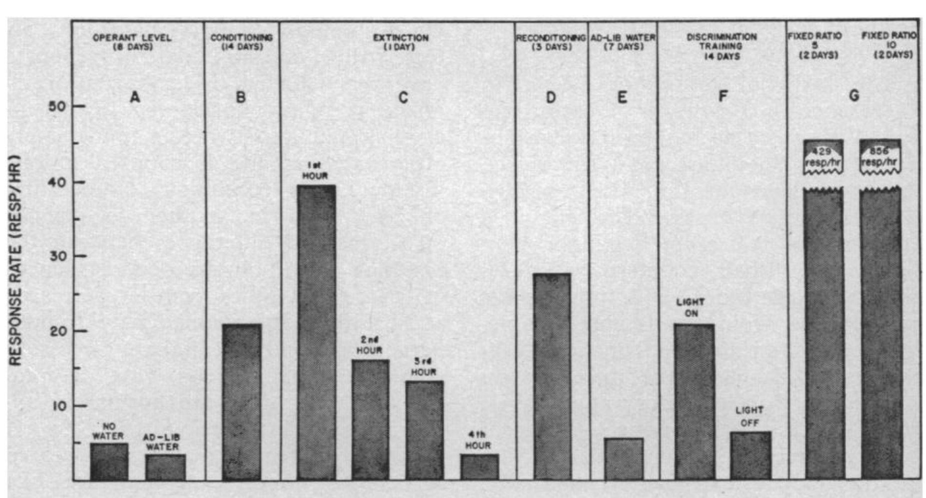

Fig. 1. Average response rate, in responses per hour, during the various phases of the experiment.

reinforcing stimulus for the establishment and maintenance of an instrumentally conditioned lever-pressing response. It is also clear that instrumental behavior maintained under these conditions is sensitive to changes in so-called "drive" states, and that it can be brought under the control of both discriminable exteroceptive stimulus conditions and reinforcement schedule contingencies. Future exploitation of this technique will permit an experimental analysis of the reinforcing properties of many pharmacologic agents under varying conditions of behavioral control.

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Reference

1. R. M. Chambers, *Am. Psychologist* **9**, 346 (19[illegible] H. W. Coppock and R. M. Chambers, *J. Comp. and Physiol. Psychol.* **47**, 355 (1954); H. W. Coppock, C. P. Headlee, W. R. Hood, *Am. Psychologist* **8**, 337 (1953); J. Olds and M. E. Olds, *Science* **127**, 1175 (1958).

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Suppression of Male Characteristics of Mosquitoes by Thermal Means

Abstract. Dimorphism in *Aedes stimulans,* a northern floodwater mosquito, may be decreased possibly to obliteration by exposing larvae for most of their lives to abnormally high temperature. Determiners for maleness fail to express themselves when larvae are exposed to a temperature of 29°C throughout their lives. Not only are male characteristics eliminated, but normal female ones such as ovaries, spermathecae, and cerci develop. The resultant adult is structurally a female. Forms showing characteristics of both sexes occur when the number of days of exposure to 29°C is lessened.

The sexes of mosquitoes differ in appearance in a number of easily recognizable ways, as has been well summarized by Snodgrass (*1*). Anteriorly, the appendages of the head are distinctive for each sex. The antennae and palpi of males are strikingly more hirsute than their counterparts in females. On the other hand, mouth parts are reduced from the female complement of seven to two functional appendages. Caudally, the male has an elaborate set of copulatory appendages, while the female has none. A pair of flaplike cerci marks the caudal portion of the female externally. Internally, the males have testes, vasa deferentia, seminal vesicles, and a bilobed accessory gland. The female has ovaries, oviducts, spermathecae, and a small saclike accessory gland. Significant changes in these structures have been brought about by treating larvae to abnormally high temperature (*2*).

Table 1. Effect of high-rearing temperature on sexual characteristics of genetically intended males of *Aedes stimulans* in the laboratory. M and F, normal male and female; a.m. and a.f., abnormal male and female; +, present; −, absent.

Imaginal parts	Sex at serial-rearing temperatures of 24° and 29°C							
	Days at 24°C:	8	5	4	3	2	1	0
	Days at 29°C:	0	3	4	5	6	7	8
Internal parts								
Gonads		M	M	M	M	F	F	F
Tubes to gonads		M	M	M	M	M,F	F	F
Sperms		+	+	+	−	−	−	−
Spermathecae		−	−	−	−	+,−	+	+
Accessory gland		M	M	M	M	M,F	F	F
Caudal parts								
Parameres		M	M	M	a.m.	a.m.	−	−
Phallosome		M	M	M	M	−	−	−
Genitalia position		M	a.m.	a.m.	a.m.	F	F	F
Cerci		−	−	−	a.f.	F	F	F
Cephalic parts								
Antennae		M	M	M	F	F	F	F
Palpi		M	M	a.m.	a.m.	a.m.	a.m.	a.f.
Mouth parts		M	M	M	F	F	F	F

Mosquitoes showing external characteristics of both sexes have been collected in different parts of the world. Such anomalies, called intersexes by Kitzmiller (*3*), number less than 40. Most of them have come from northern latitudes, and in some instances two or more individuals have been collected from the same vicinity at approximately the same time. The facts of location and repetitive occurrence suggest possible genetic or environmental causes for the anomalies. Unfortunately, specimens have been collected so infrequently that little more can be inferred.

Aedes stimulans, a snow-pool mosquito common to Canada and northern latitudes of conterminous United States, has been induced to express marked intersexual tendencies under laboratory conditions. The two sides of the responding genetic males are affected alike, and no unilateral responses have been elicited. When a uniformly mixed population of larvae (Table 1) is separated into two lots immediately after hatching and is exposed in one instance to a continuous temperature of 24°C and in another to one of 29°C, marked differences between the resulting males always occur. Genetically intended males from larvae reared at 24°C develop antennae, palpi, mouth parts, external genitalia, accessory glands, seminal vesicles, vasa deferentia, and testes that are normal in appearance and function. Larvae of genetically intended males when reared at 29°C without exception are like females in all respects except for slight differences in palpi. Internally, the anomalous males have ovaries, oviducts, and spermathecae, and they lack testes, vasa deferentia, seminal vesicles, and bilobed accessory glands. The ovaries have globular egg chambers indistinguishable from those of young genetic females (see *4*).

Abnormally high temperature exerts its modifying effect on larvae of potential males according to the duration of exposure (Table 1). High temperature applied late (last 3 days) in larval life produces no structural defects but prevents rotation of genitalia to the copulatory position. An extension of exposure to high temperature to include the last 6 days of larval life causes a series of changes that produces intersexes. Larvae exposed to 29°C for 7 days or more grow into apparent females, some of which have been inseminated by normal males. Insemination was determined by examining the spermathecae under a compound microscope for the presence of sperms.

Larvae that bear female determiners are unaffected by a temperature of 29°C. They give rise to females that are normal in appearance, copulate readily by the artificial means described by McDaniel and Horsfall (*5*), feed on blood, and develop eggs in a normal manner.

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References and Notes

1. R. E. Snodgrass, *Smithsonian Inst. Publs. Misc. Collections* **139**(8), 1 (1959).
2. Funds for support of this work were in part from a grant made by the Graduate College. Reinhart Brust has given much help in this work and our gratitude is expressed to him.
3. J. B. Kitzmiller, *Rev. brasil. malariol. e doenças trop.* **5**, 285 (1953).
4. R. F. Harwood and W. R. Horsfall, *Ann. Entomol. Soc. Am.* **50**, 555 (1957).
5. I. N. McDaniel and W. R. Horsfall, *Science* **125**, 745 (1957).

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