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A NEW SIMPLE TECHNIQUE FOR REARING F_1 PROGENY FROM FEMALES OF THE SIMULIUM DAMNOSUM THEOBALD COMPLEX

Part II. Improvements and modifications to the technique

by

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1. Introduction

The first part of this paper (Raybould, 1979) described a rearing technique developed in connexion with the WHO Onchocerciasis Control Programme (OCP) in the Volta River basin area of West Africa. The apparatus comprised a simple closed system in which water was circulated by an A/C electrical centrifugal pump between a container and an inclined rearing trough. The components were simple, unbreakable, easily packed and readily transported, and could be rapidly assembled and used under makeshift laboratory conditions. The larvae were fed with living green algae (Chlorophyceae) which reduced the concentration of ammonia to which simuliid larvae are sensitive (Grunewald, 1978). The provision of a large volume of water probably helped in this respect.

During the past year this technique, with some modifications, has been in general use for rearing S. damnosum s.l. in four separate OCP laboratories located in Benin, Ghana, Upper Volta and the Ivory Coast. Good results have been obtained feeding with either algae alone (mixed with other microorganisms that "contaminate" the algal cultures) or algae supplemented with "Tetra"® tropical fish food.[3] At temperatures between about 25-32°C, rapid larval development has been achieved with pupation usually commencing from nine to 12 days after hatching. Many thousands of larvae have been reared.

Only a proportion of the eggs laid by simuliids artificially induced to oviposit in the laboratory (by immersion in water or by decapitation) normally develop and give rise to larvae. For this reason, not more than about 200 individuals have been reared from a single egg batch. When the number of developing eggs (as opposed to the total number of eggs present) has been counted, however, survival rates through to the adult have sometimes exceeded 90%. Nevertheless, for reasons not fully understood, much higher mortalities, especially of pupae, have sometimes been experienced.

The number of individuals successfully reared at one time in a single apparatus is usually limited to a few hundred, but up to about 2000 larvae, or larvae and adults, have sometimes been obtained from many egg batches.

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[3] Tetra conditioning food or Tetra Min staple food for tropical fish. Tetra Werke, Dr. rep. nat. Baensch, 452 Melle, Federal Republic of Germany.

Larvae reared by this technique are usually suitable for cytotaxonomic work, sometimes even providing "better" chromosomes than field collected material (Meredith, personal communication).

In spite of its relative success, the method as described in Part I of this document was inadequate in two respects: larvae in the rearing trough dried out rapidly during electrical power failures and the apparatus lacked an effective device for the collection of emerged adults. Improvements and modifications to the technique that have rectified these inadequacies are described below.

2. Modifications to overcome the effect of power failures

Three different modifications of the basic apparatus have been developed to overcome the effect of power failures and are used according to local conditions and requirements.

2.1 Wooden rearing troughs

The rearing troughs used in the original apparatus were cut out from corrugated plastic roofing material. Unfortunately, although strong, durable, light and easy to pack and transport, such troughs dry out quickly when water flow ceases during a power failure with the result that most larvae die in less than an hour.

Uncoated wooden rearing troughs (Figs 1 and 2) dry out slowly because of the water holding capacity of the wood and the presence of a thicker surface layer of algae and other microorganisms than normally develops on plastic. (The larvae browse on this surface layer thus supplementing their diet.) The rate of drying will, however, depend on factors such as the kind of wood used, the thickness and the composition of the covering of microorganisms and whether or not the trough is covered with an adult collecting device (see section 3.1) which reduces evaporation.

The results of tests on larval survival in wooden rearing troughs during simulated power stoppages are given in Table 1. The troughs used were made from the wood of Chlorophora excelsa, known in Ghana as "Odum". The table shows that in two tests in which troughs coated with microorganisms were covered with an adult collecting device, 73% of the larvae were still alive after about six hours. In another test (No. 3) only 36% of the larvae survived a similar period without water flow. This was partly due to fungal hyphae preventing them from rolling down the slope as the trough dried from the top downwards. Survival in "clean" troughs, not coated with microorganisms, ranged from 21 to 70% after about six hours. In general, survival was best when the larvae succeeded in rolling down the slope as the top of the trough dried. (Rolling occurs when the larvae release their hold on the substratum and wriggle as they dry out.) There is no obvious explanation for the poor result in test 4. In two other tests 2% and 3% respectively of the larvae were found to be still alive after 21-1/2 hours.

The choice of wood for troughs is important. A hardwood is required because most softwoods soon rot in water. Woods that release toxic substances should, of course, be avoided unless all toxins can be removed by prior soaking in water. A disadvantage of unpainted wooden troughs (and they need to be unpainted to prevent rapid drying) is that they render the larvae rather difficult to see. For this reason a light-coloured wood is desirable.

Rearing troughs made of wood are easily adapted for the collection of reared adults, and even in the absence of an adult collecting device, the pupae can be easily removed together with a small sliver of wood (see section 3.1).

Wooden spillways have already been used successfully for rearing simuliids in the United States of America (Brenner & Cupp, 1980) where power failures are not so frequent.

2.2 The "automatic flooding" method

In this modification of the apparatus the rearing trough (which should be a simple plane of wood or plastic without vertical sides) is positioned inside a plastic bin of about 60 litres capacity (Figs 2 and 3). Water is pumped from the bottom of the bin (where it reaches a depth of only a few centimetres) to the bottom of a second similar bin placed at a higher level. (The same Eheim centrifugal pump is used for this purpose as was employed in the original apparatus.) The upper bin fills with water to just above the level of an outlet pipe which directs the water to the top of the rearing trough in the lower bin (Figs 2 and 3). The water flows continuously down the trough providing suitable conditions for the developing larvae. For the apparatus to function properly, the correct water volume must be maintained and the level in the two bins carefully controlled. This may be done by adjusting a jubilee clip fitted around the outlet pipe from the upper bin.

When the electric current stops, the water in the upper bin flows back through the pump filling the lower bin. The larvae are submerged, but remain attached to the trough. They sway about and slowly move up the trough towards the water surface. (If a trough with vertical sides is used, the larvae climb up the sides and are stranded when the water flow resumes.)

As soon as the power returns, the water is pumped into the upper bin causing a rapid fall in level in the lower bin. The larvae are exposed and appear to respond by attaching themselves firmly to the trough. They thus withstand the sudden return of the water current, via the outlet pipe from the upper bin, without being washed away.

The results of tests on larval survival in this apparatus during simulated power stoppages, are given in Table 1. In three tests of six hours' duration, survival rates were 50, 92 and 87% respectively. In two tests, 36 and 55% respectively of the larvae survived for a 22-hour period. More information is required on the effect of temperature on survival. It may be seen from Table 1 that this method gave superior results to wooden rearing troughs.

This technique requires no additional specialized apparatus and is relatively easy to adapt for the collection of adults.

2.3 Automatic switch-over to an alternative battery power supply

This technique uses a standby circulation system powered by a 12-volt battery and D/C centrifugal pump (Fig. 1). While the main A/C power supply is on, contacts of a solenoid switch are held open. As soon as the A/C power supply fails, the contacts close to complete the D/C 12-volt circuit bringing the standby circulation system into operation. When the A/C power is restored the D/C circuit is disconnected again. A Stuart Turner No. 10 pump[1] has been found to be suitable for the standby circulation system. Although it only delivers up to about 9 l/min, compared to 22 l/min for the Eheim A/C pump, this is adequate for limited periods. A larger capacity pump would run down the battery too rapidly. One fully charged battery will operate the Stuart Turner No. 10 pump for between 12 and 14 hours.

This technique enables the larvae to feed normally throughout.

3. Adaptations for the collection of reared adults

Well-developed (i.e. black) pupae can be removed from the wooden rearing trough by slicing off a sliver of wood and placing it in an emergence tube. However, many adults fail to eclose following this procedure. The rearing apparatus has therefore been adapted for the automatic collection of emerged adults.

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[1] Stuart Turner Ltd., Henley-on-Thames, England.

3.1 Adaptations to the rearing trough (Fig. 4)

The rearing trough can be rendered suitable for adult collection by:

(i) closing the upper end with a wooden partition with a hole cut for the water delivery tube (two holes if a second pump is used during power failures);

(ii) closing the lower end with gauze. This allows the escape of water but not adult simuliids. The gauze will require frequent cleaning (with a wash-bottle and brush) and should not be so fine as to block quickly;

(iii) covering the trough with an opaque lid with a hole in the lower end over which a WHO adult insecticide susceptibility test-kit tube is screwed. Formica® makes an excellent lid because of its flexibility, but painted Perspex® or plywood may also be used. The lid may be held in position with large rubber bands;

(iv) fixing a sleeve of opaque plastic sheeting, or a length of inner-tube, around the end of the trough to extend downwards to prevent light entering through the gauze.

These modifications prevent light from entering the rearing trough except via the test-kit collecting tube. The newly emerged flies are attracted by positive phototaxis into the tube which is removed and replaced when required.

Some flies drown or become stuck to wet surfaces before they succeed in reaching the collecting tube. Certain refinements to the rearing trough are therefore required to reduce this to a minimum. Most newly emerged adults are swept downstream on to the gauze closing the trough. To facilitate emergence from the water, and subsequent collection, therefore, the gauze should slope forwards with the collecting tube positioned just above. A cone-entrance, easily made from plastic netting, helps to prevent flies falling back out of the collecting tube. The use of a horizontal tube is also under investigation in this respect.

Flies tend to become stuck if the collecting tubes are splashed with water. This can be largely avoided if water enters the trough through a short metal tube, flattened and splayed out at the end to form a large flat nozzle, to spread the water as a uniform layer on the trough surface.

3.2 Adaptations to the "automatic flooding" system (Fig. 5)

This apparatus can be rendered suitable for collecting adults by simply covering the lower bin with an opaque cover with holes to fit WHO adult insecticide susceptibility test-kit tubes. Another hole in the cover is required for the tube bringing water from the upper bin. The collecting tubes operate in the manner described under 3.1. A disc of Perspex® covered with opaque plastic sheeting is particularly suitable for the lower bin cover because it is possible to look inside after lifting up part of the sheeting. Light-coloured plastic is preferable to prevent over-heating.

As many flies are unable to leave the water surface in the bin unaided, a sloping gauze is fitted across the lower end of the rearing trough just above the water level in the bin. Newly emerged adults washed downstream are retained by the gauze from which most of them are able to take off and fly towards the light and into the collecting tubes. Because some flies rest on the under side of the cone-entrance and then drop back on to the water surface, the use of a horizontal tube is under investigation.

If the water in the lower bin is allowed to become too shallow, the pump may suck air and any floating flies from the water surface. These then pass unharmed through the pump into the upper bin which should be covered with fine netting to prevent escapes. The few flies involved can easily be removed manually.

SUMMARY

The rearing technique described in Part I of this paper gave good results in West Africa but lacked a safeguard against electrical power failures and a device for collecting reared adults.

Methods recently developed to protect the larvae during power stoppages are the use of slow-drying wooden rearing troughs, a modified apparatus that automatically floods the larvae until the power returns and an automatic switch to an alternative D/C power supply and pump.

Adult collecting devices making use of the strong phototactic responses of the flies have also been developed and fitted to the rearing troughs and the modified version of the apparatus.

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REFERENCES

Brenner, R. J. & Cupp, E. W. (1980) Rearing blackflies (Diptera: Simuliidae) in a closed system of water circulation, Tropenmed. Parasit., 31, 247-258

Grunewald, J. (1978) Die Bedeutung der Stickstoff-Exkretion und Ammoniak-Empfindlichkeit von Simuliiden-Larven (Deptera) fur den Aufbau von Laboratoriumskulturen, Z. ang. Ent., 85, 52-60

Raybould, J. N. (1979) A new simple technique for rearing the F_1 progeny from single females of the Simulium damnosum Theobald complex. Unpublished document WHO/VBC/79.714 and WHO/ONCHO/79.148, 9 p.

TABLE 1. LARVAL SURVIVAL IN WOODEN REARING TROUGHS AND THE AUTOMATIC FLOODING SYSTEM DURING POWER STOPPAGES

Test	No. of larvae tested	Wooden rearing trough				Automatic flooding system	Larval survival during power stoppage			
		Clean surface	Covered with micro-organisms	With cover	Without cover		Survival after approx. 6 hours[a]		Survival after approx. 22 hours[a]	
							No.	%	No.	%
1	200		X	X			146 (6)	73	NT[b]	NT
2	100		X	X			73 (6-1/2)	73	3 (21-1/2)	3
3	100		X		X		36 (5)	36	NT	NT
4	200	X		X			42 (6)	21	NT	NT
5	100	X		X			70 (6-1/2)	70	2 (21-1/2)	2
6	374	X			X		128 (6)	34	NT	NT
7	100	X			X		59 (6-1/2)	59	NT	NT
8	100					X	50 (6-1/2)	50	NT	NT
9	98					X	90 (6)	92	NT	NT
10	98					X	85 (6)	87	NT	NT
11	110					X	NT	NT	40 (22)	36
12	100					X	NT	NT	58 (22)	58

[a] These periods are approximate. Precise periods in hours are given in the table in brackets.

[b] NT = not tested.

FIG. 1. REARING SYSTEM IN OPERATION WITH WOODEN TROUGH AND STANDBY D/C CIRCULATION WITH AUTOMATIC SWITCH-OVER

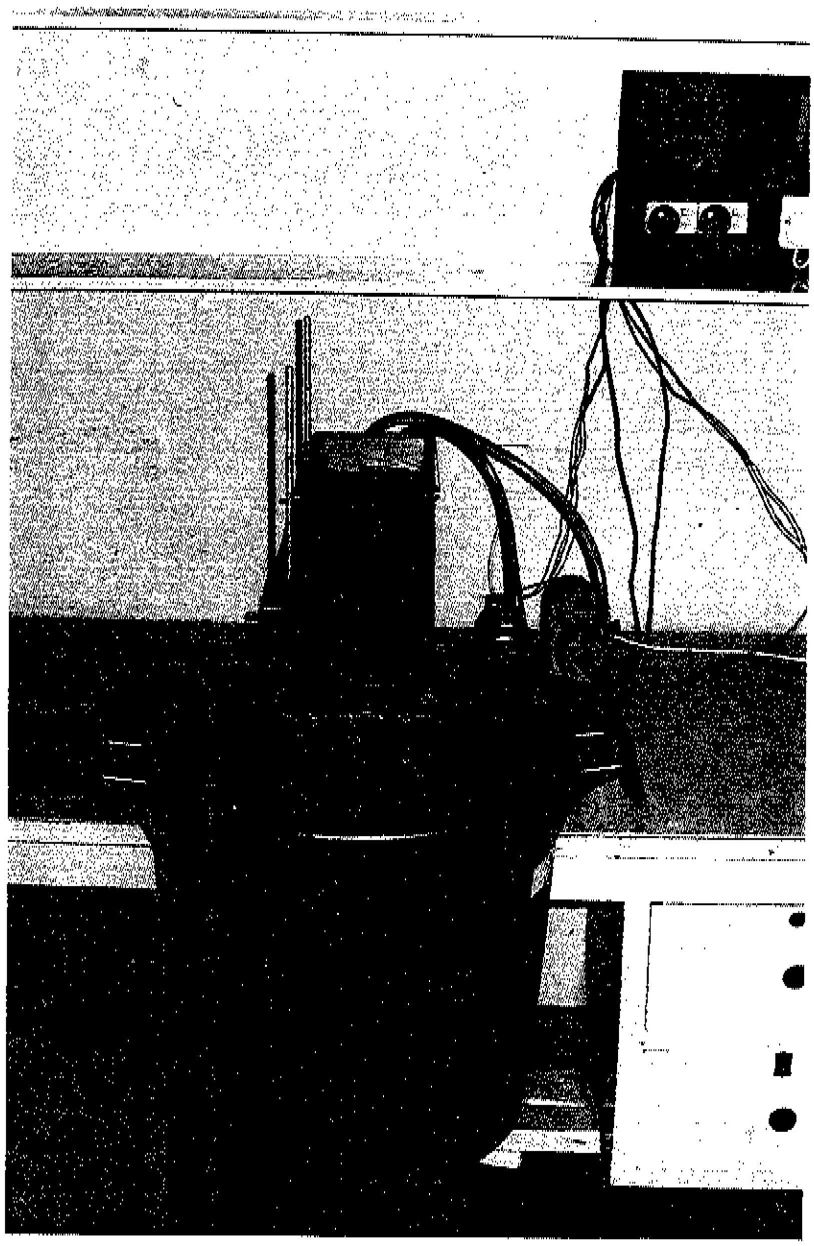

FIG. 2. AUTOMATIC FLOODING SYSTEM IN OPERATION

FIG. 3. AUTOMATIC FLOODING SYSTEM

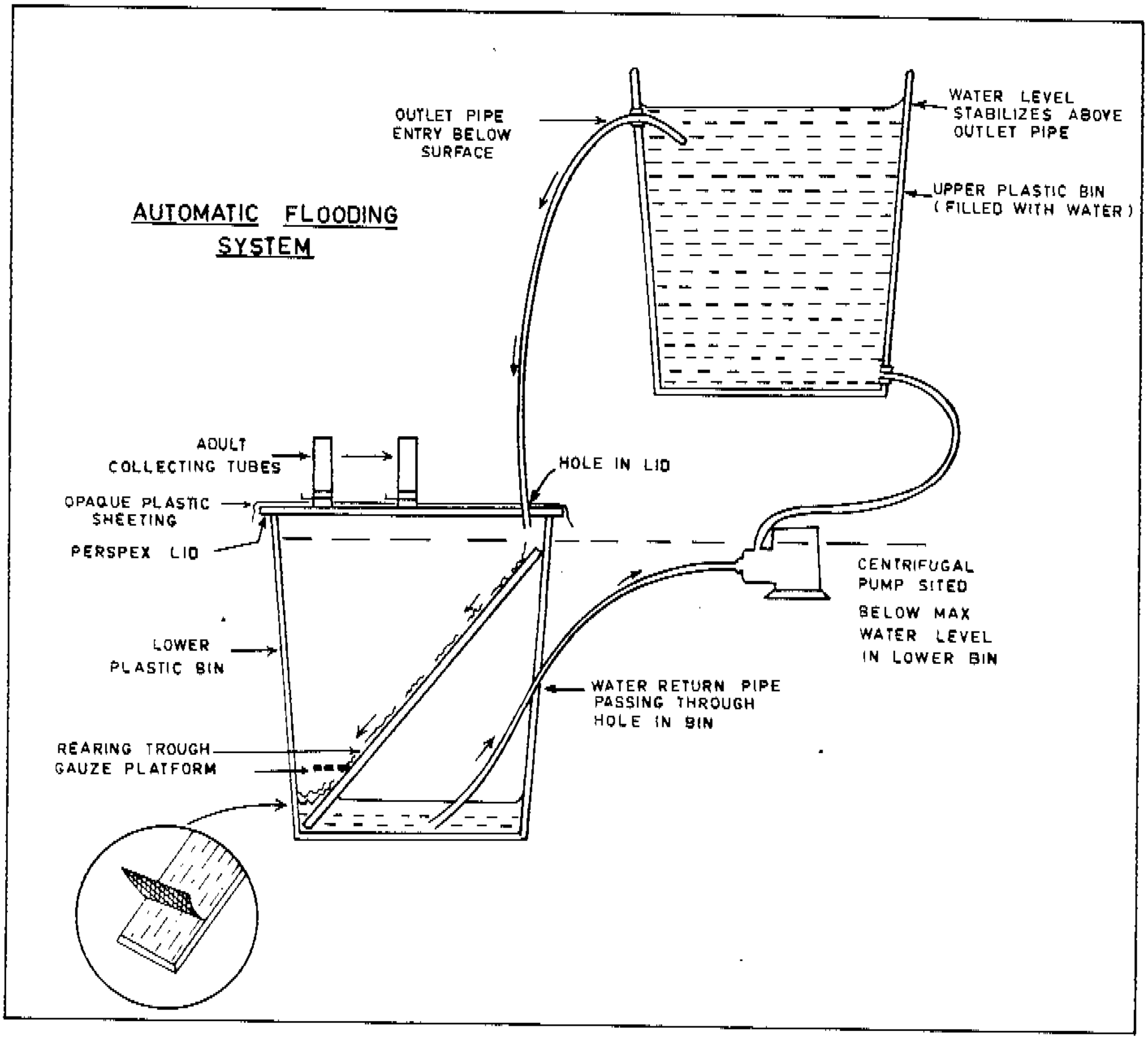

FIG. 4. TWO REARING SYSTEMS WITH ADULT COLLECTING DEVICES

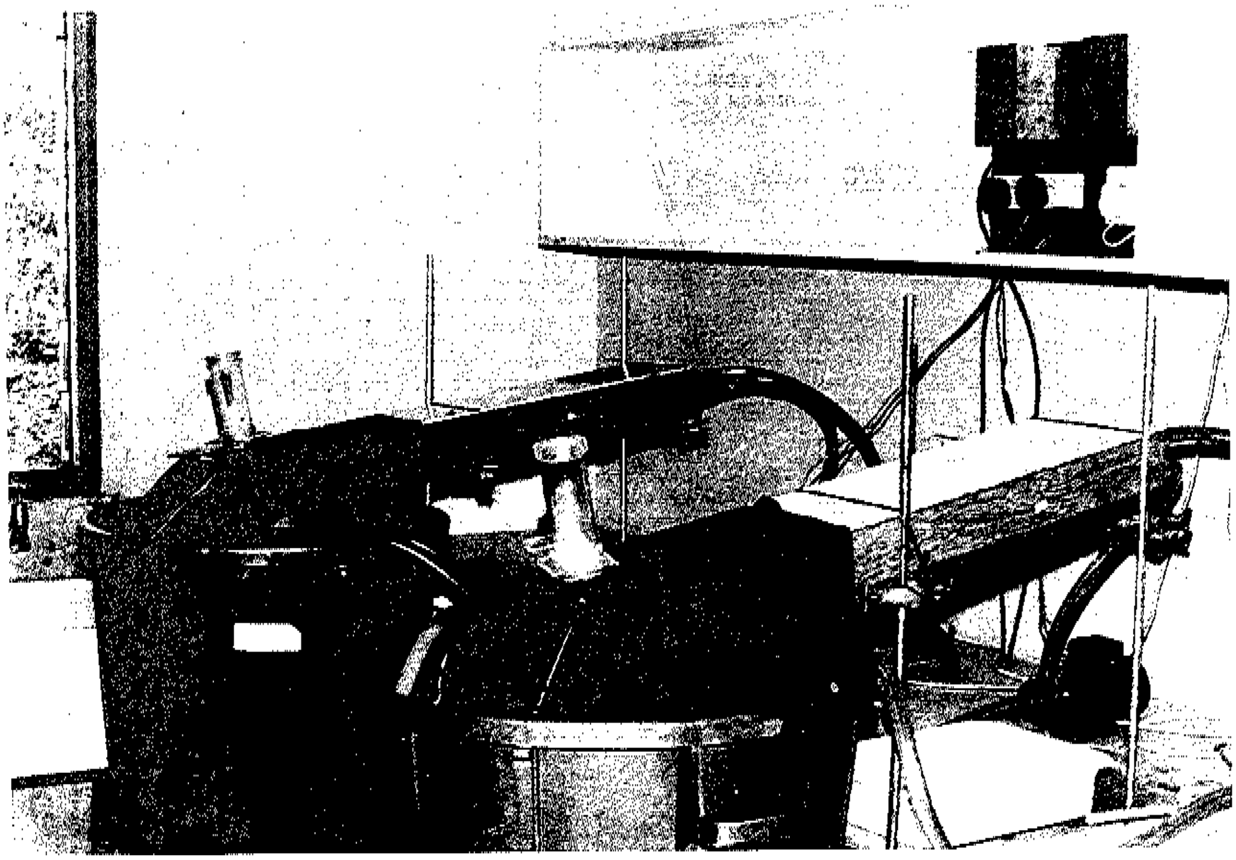

FIG. 5. AUTOMATIC FLOODING SYSTEM WITH ADULT COLLECTING DEVICE

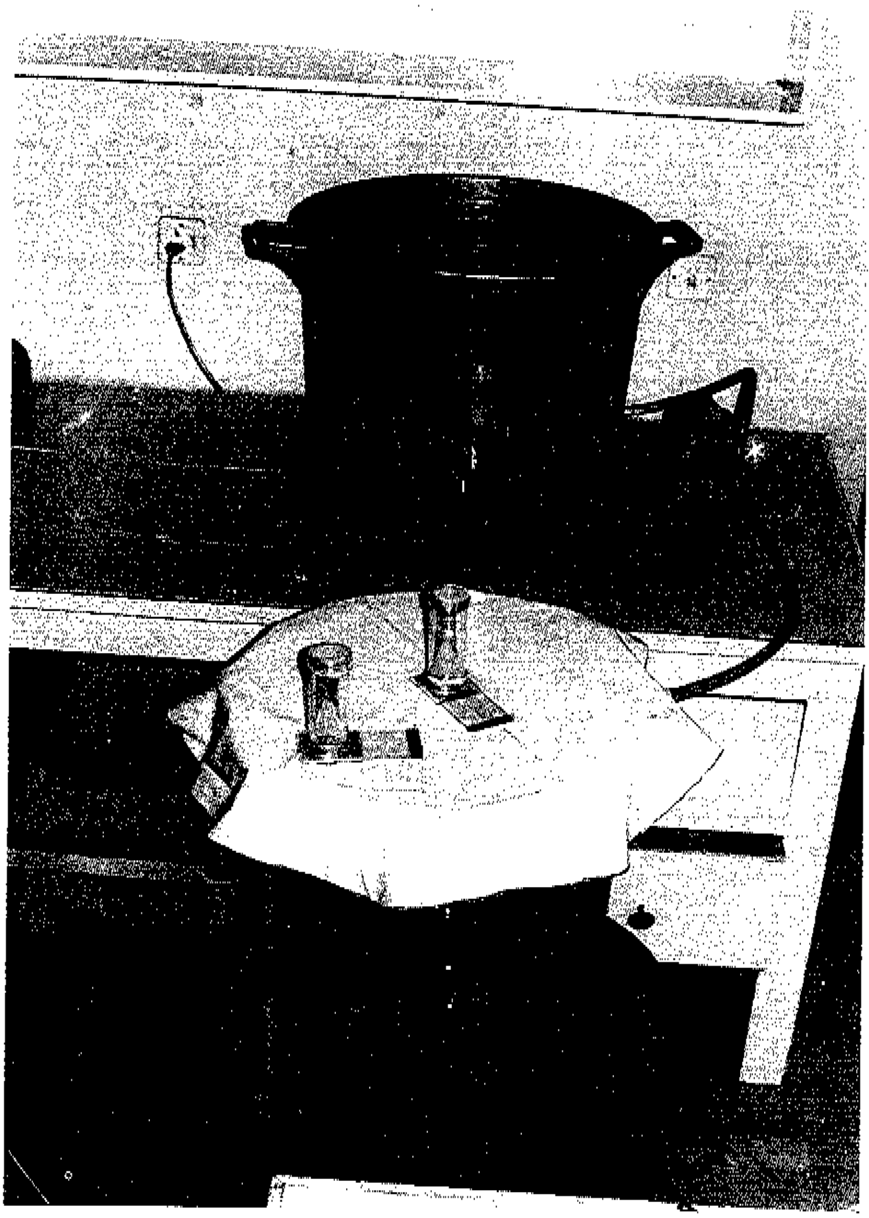

FIG. 6. FOUR REARING SYSTEMS WITH WOODEN TROUGHS AND ONE AUTOMATIC FLOODING SYSTEM IN OPERATION UNDER MAKESHIFT LABORATORY CONDITIONS

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