Technical Papers

Respiratory Studies on Mate-Killers and Sensitives of *Paramecium aurelia*, Variety 8

Myron Levine* and Jed Lee Howard
McCollum-Pratt Institute, Johns Hopkins University, Baltimore, Maryland

When stocks 130, 131, and 138 are mated to the other stocks of variety 8, *Paramecium aurelia,* the exconjugants descended from the other stocks die, but the members of the conjugating pairs descended from these three stocks survive and produce normal progeny. These three stocks are called mate-killers; the others are called sensitives. Mate-killers are distinguished from sensitives by possessing visible Feulgen-positive cytoplasmic particles. These particles, called *mu* particles, seem to depend for their maintenance and reproduction, though not for their origin, on a nuclear gene *M*. Mate-killing is thus under the control of both nuclear and cytoplasmic factors (*1–3*).

The genetics of mate-killers appears to parallel the genetics of killers in variety 4 (*4*). In that case, too, the trait is controlled by the cooperative action of a nuclear gene *K* and visible cytoplasmic Feulgen-positive particles, *kappa*. However, the conditions for killing are different. The killing action of mate-killers depends on prolonged and intimate physical contact of paramecia, and so far this killing action has not been produced by fluids in which the mate-killers have lived. With killers, such fluids are active in killing because of the liberation of a poison, paramecin, by the killers, but contact during mating does not result in death.

Simonsen and Van Wagtendonk (*5*), using the Cartesian diver technique, made a respiratory comparison of kappa-killers and sensitives in variety 4. They found that stock 51 killers respire at a rate 150 to 180 percent above that of sensitive animals of identical genic constitution. This fact, together with the lack of respiratory inhibition of killers by sodium azide in concentrations that inhibit the respiration of sensitives, as well as the low cytochrome oxidase activity of killers, led these authors to conclude that an oxidative pathway different from the normal cytochrome system operates in the respiratory metabolism of killer paramecia.

A respiratory comparison of stock 138 mate-killers and sensitives of mating type XVI was undertaken to see whether mu particles alter the metabolism of mate-killers in a manner similar to that found for kappa particles. The sensitives used in these experiments (*6*) were derived from the mate-killers and are isogenic with them. Following prolonged rapid growth at high temperature, mate-killers may lose mu particles and be transformed into sensitives without any associated genic change (*2, 3*). The only difference between the two cultures, then, is the presence of mu particles in one strain and their absence in the other.

The paramecia cultures were grown in flasks on a Ceraphyl infusion inoculated with *Aerobacter aerogenes,* according to the methods of Sonneborn (*7*). Their effective average growth rate was less than one fission per day. In preparation for examination, cultures that had last been fed 2 days earlier were harvested in a Berkefeld filter, were further concentrated by low-speed centrifugation, and were washed several times in exhausted autoclaved culture fluid or in an inorganic salt solution (*5*), depending on which was used as the respiring medium. These concentrated cultures were allowed to stand for approximately 2 hr in either of the two mediums, were washed again, and were then ready for determination of oxygen uptake. This procedure removed the bacteria as a contributing factor in the respiratory studies.

Oxygen consumption was measured with the Warburg respirometer. The number of animals examined per vessel in different experiments varied from 30,000 to 150,000 in a total volume of 3.3 ml. Total respiration varied directly with the number of animals examined. Population counts were made according to Sonneborn (*7*). All determinations were carried out in duplicate.

The data in Table 1 indicate that mate-killers and sensitives respire at about the same rate. Similar results were obtained in the two respiring mediums. Not only do mate-killers fail to differ significantly from sensitives in oxygen consumption, but preliminary experiments indicate that the cytochrome system operates similarly in both, since the oxygen consumption of mate-killers and sensitives was equally inhibited in the presence of 0.001*M* KCN.

If the Warburg technique may be compared with the Cartesian diver technique, these findings are in sharp contrast with those reported for killers. Mu and kappa particles do not seem to impose the same metabolic demands on paramecia. It is, however, possible that mu particles may alter some other metabolic system.

A possible morphological basis for the metabolic

Table 1. Rates of oxygen consumption of mate-killers and sensitives of stock 138, mating type XVI, variety 8. The Q_{O_2} is expressed in millimicroliters per animal per hour.

Respiring medium	No. of experiments	Mate-killer Q_{O_2} mean ± S.E.	Sensitive Q_{O_2} mean ± S.E.
Exhausted culture fluid	11	0.280 ± 0.027	0.260 ± 0.020
Inorganic salt solution	3	.305 ± 0.051	.266 ± 0.058

differences between killers and mate-killers is suggested by a recent demonstration by Preer *et al.* (*8*) that showed that a small proportion of the kappa particles in killers contains one or more refractile areas. These kappas are called "brights," and they are the bearers of paramecin activity. Brights are found only in animals that can produce paramecin, never in those unable to produce this toxin. Bright particles have never been found among the mu particles of mate-killers (*8*). The increased metabolic activity of killers may be a direct consequence of the production of paramecin, and the site of this altered metabolic activity may rest in the bright kappa particles or in their influence on the metabolism of the whole cell.

In this connection, it would be of interest to examine the respiratory activity of paramecia carrying a third type of particle that is neither involved in a toxic effect nor produces paramecin. This particle, called *pi*, was first discovered by Hanson (*9*) in animals that had once been killers and are in all probability mutants of kappa particles. If the higher oxygen consumption of killer animals is indeed a consequence of paramecin production and is correlated with the presence of brights, paramecia containing pi should respire at the same rate as isogenic paramecia devoid of these particles.

References and Notes

* Postdoctoral fellow of the American Cancer Society. Present address: Department of Bacteriology, University of Illinois, Urbana.

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Thermistor Electronic Thermometer

Robert W. Woods

College of Medical Evangelists, Loma Linda, California

Electronics is receiving increasing attention and finding many applications in the tools of medical research. One of our problems in instrumentation led to a solution that should find application wherever research using small animals is in progress. A research worker in physiology needed to take a record of the rectal, subcutaneous, and skin-surface temperature of experimental groups of six mice each and to observe the variation in these several temperatures over a period of time.

The temperature-measuring units need (i) to remain *in situ*; (ii) to be able to be calibrated to give comparable readings; (iii) to be fairly quick reading; (iv) to be sensitive enough to measure to 1 per-cent; (v) to be small enough physically so that an animal as small as a mouse does not experience insuperable discomfort; (vi) to be well insulated, both electrically and against moisture, and to give reproducible results.

We tried both thermocouples and thermistors and settled for the latter as being best suited to our requirements, using the W. E. No. 14B thermistor in a bridge circuit. The voltage divider can be adjusted to give the same voltage across the bridge over the life of the battery. This voltage is checked by the same vacuum-tube voltmeter that serves as the temperature indicator by substituting a voltage divider in place of the thermistor bridge (Fig. 1).

The 19 thermistor bridges (one for measuring the ambient temperature plus six sets of three for the six animals being tested) are identical (Fig. 2), each with a balancing control for setting the minimum temperature reading (in our case, 0°C) and a maximum setting control (in our case, 50°C).

This gives a 2-point adjustment of comparability between bridges. We have found this degree of comparability satisfactory, since the several thermistors that we are using track within the limits of experimental error.

These 19 bridges and the voltage-check divider are connected to the switch points of a three-bank 20-position rotary switch, making it possible to select and measure any temperature by a turn of the dial.

It is necessary to match the sensitivity of the

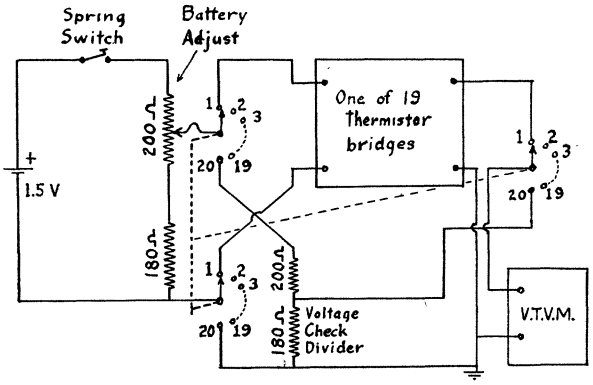

Fig. 1. Thermistor thermometer. Block diagram with supply and check-voltage source.

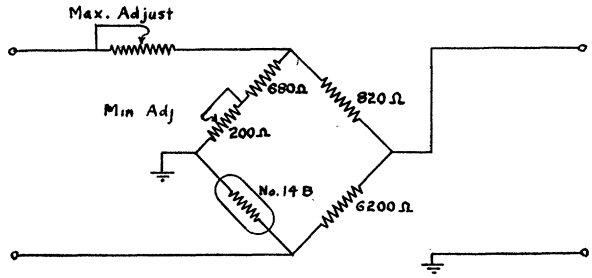

Fig. 2. Thermistor bridge. Nineteen bridges are required in the complete instrument.