Attempts to Induce Double Resistance to Drugs in the Flagellate, Chilomonas paramecium

By

R. P. HALL

With 2 Figures in the Text

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For microorganisms in general (Abraham 1953; Bishop 1959; Dean and Hinselwood 1957), acquired resistance to drugs tends to be specific to some degree, but with at least occasional cases of cross-resistance, and to be fairly stable for some time in the absence of the inducing drug. Chilomonas paramecium conforms to this general pattern. Induced sulfonamide-resistance in this flagellate (Hall 1957a) has persisted with no significant change through more than 250 transfers (over a period of 5.5 years) after return to drug-free medium (Hall 1961).

Attempts to induce resistance to a second drug in sulfonamide-resistant strains of C. paramecium have yielded varied results. In one case, secondary adaptation to p-aminobenzoic acid caused a loss of the original resistance to sulfanilamide; this resistance could be partially restored by a second gradual adaptation to sulfanilamide (Hall 1961). Analogous losses of resistance induced by adaptation to a second drug have been reported previously, especially in cultures of bacteria (Abraham 1953). As described below, exposure to certain drugs other than p-aminobenzoic acid has not decreased sulfonamide-resistance in C. paramecium and in a few cases a superimposed resistance to the second drug has been developed.

Material and methods

An axenic Pringsheim strain of Chilomonas paramecium was used as the parent stock.

The basal medium contained: NH₄Cl, 0.19; KH₂PO₄, 0.27—0.36; and MgSO₄·7H₂O, 0.01 g/l; lactic acid, 2.0 ml/l; thiamine-HCl, 2.0 mg/l. Ca and Fe were added (as chlorides) at 1.0 mg/l. The medium was adjusted to pH 3.8—4.0 with KOH before sterilization, and different drugs were added as indicated below. The pH, as determined after inoculation, ranged from 3.7—4.3 with different types of media. Cultures were maintained in 125 × 20 Kimmie tubes with plastic screw-caps and were incubated at 22.5°C. Growth was estimated as optical density in a Coleman Jr. spectrophotometer.

* To Professor Dr. E. G. Pringsheim on his 80th birthday.

1 The writer is much indebted to Miss Marie Schmid for her assistance during this investigation.
Six compounds were tested by comparing growth in basal medium with that in presence of the drug: 8-azaguanine, benzimidazole, 5-bromouracil, 2,6-diaminopurine, isoniazid (isonicotinic acid hydrazide), and 6-mercaptopurine. In attempts to obtain adaptation, the flagellates were started in a medium containing a relatively low concentration of the drug under consideration. After several serial transfers, the strain was shifted to a higher concentration of the drug and again carried through several transfers. Continuation of this procedure resulted in the strains listed below:

- **AG 10**, derived from sulfonamide-resistant strain **S-7**, gradually adapted to 8-azaguanine at 10.0 mg/100 ml;
- **AG-12**, derived from **AG-10**, maintained with 8-azaguanine at 12.0 mg/100 ml;
- **BA-50**, derived from **S-7**, maintained with benzimidazole at 50 mg/100 ml after transfers through lower concentrations;
- **BU-15**, derived from **S-7**, maintained with 5-bromouracil at 15.0 mg/100 ml;
- **DP-2**, derived from the normal stock, maintained with 2,6-diamino-purine at 10.0 mg/100 ml;
- **GA**, derived from the normal stock, maintained with 8-azaguanine at 4.0 mg/100 ml;
- **IN-50**, derived from strain **S-7**, maintained with isoniazid at 50 mg/100 ml;
- **IN-75**, derived from **IN-50**, maintained with isoniazid at 75.0 mg/100 ml;
- **MP-3**, derived from the normal stock, maintained with 6-mercaptopurine at 6.0 mg/100 ml;
- **MP-10**, derived from **S-7**, maintained with 6-mercaptopurine at 10.0 mg/100 ml after passage through lower concentrations;
- **N-1**, derived from the normal stock, maintained with isoniazid at 100.0 mg/100 ml;
- **S-7**, derived from the normal strain, maintained with sulfanilamide at 700.0 mg/100 ml after gradual adaptation to increasing concentrations;
- **UB-2**, derived from the normal stock, maintained with 5-bromouracil at 8.0 mg/100 ml.

Each of the strains maintained in drug-containing medium was passed through a series of transfers in basal medium before being used for inoculation of experimental media. This procedure reduced the concentration of drug in the inoculum to an ineffective level.

**Results**

In concentrations up to 20 mg/100 ml, 5-bromouracil, a thymine antagonist, seemed to be slightly stimulatory—increases of 10—25 per cent at maximal density—for the strains tested (**S-7**, **BU-15**, **UB-2** and the normal strain), and there were no marked differential effects on growth. The “adaptation” of strain **BU-15** (a derivative of **S-7**) to bromouracil had no apparent effect on its ability to grow in the presence of sulfanilamide at 700 mg/100 ml.

The effects of 2,6-diaminopurine (20 and 25 mg/100 ml) on strains **S-7**, **DP-20**, **DP-2** and the normal stock were rather similar to those of 5-bromouracil on the strains mentioned above. There was no inhibitory effect on either **S-7** or **DP-20**. The normal strain showed little if any effect with the lower concentration and a slight inhibition with the higher concentration of dianinopurine, while strain **DP-2** showed a very slight,