A NOTE ON THE USE OF FREE-LIVING PROTOZOA AS TEST ORGANISMS FOR DRUGS

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This method was developed in order that the toxicities of known anti-protozoal drugs could be determined on certain free-living organisms and compared with the activities of these drugs against pathogenic organisms. The results of this investigation are published elsewhere (James[1]).

The principle of the method employed was to determine the weakest concentration of drug that killed all the organisms in a standard amount of culture in a given time. Strains of *Paramoecium caudatum* and of *Colpidium colpoda* were used; the former was derived from a single individual isolated from soaked hay, and the latter from a pure bacteria-free culture kindly supplied by Prof. R. A. Peters. Cultures of each organism were grown on a medium of diluted hay infusion to which 0·04 per cent. of acetone-dried yeast had been added. The hay infusion had been boiled on two consecutive days before dilution to ensure freedom from other infusoria. Subcultures were prepared by adding to the fresh medium one-twentieth of its volume of a culture of the organism, and leaving the freshly prepared subcultures exposed to the atmosphere for a period of 48 hours to ensure adequate bacterial contamination. Several attempts to propagate *P. caudatum* on a medium devoid of bacteria failed, and the production of rich cultures of both organisms on the medium described was dependent on the bacterial contamination of the culture medium. Thus arose the necessity of a further procedure, namely, testing at the end of the experiment for surviving bacteria and so making sure that the activity of the drug was not an indirect one through the destruction of the bacterial flora of the culture.

Earlier work in this field had shown that tests carried out with the same organisms on different occasions would often give rise to variable results. It was desirable, therefore, to establish what factors were responsible for these variations, and so to standardise a procedure that would involve the minimum of errors. The considerable variation in the susceptibility of *C. colpoda* to atropine was shown by Prowazek[2] to be due to the age of the culture. Groupe[3] showed that the ciliate *Tetrahymena geleii* became less resistant to mepacrine as the culture aged and this he attributed mainly to an increase in *p*H, but partly to the presence of metabolic products.

At the time of inoculation the cultures showed *p*H values of 6·0 to 6·2, which became steadily less acid as growth proceeded, reaching a value of 6·8 to 6·9 on the seventh or eighth day. At this stage the

cultures showed the greatest number of organisms (*P. caudatum*, 450 to 600 per ml.: *C. colpoda*, 5,000 to 7,000 per ml.) and their motility was maximal. A period of stability ensued for 6 days, after which the culture became alkaline (reaching a final *p*H value of 7·5), the organisms became sluggish and collected at the bottom of the culture. Eight to 14 day-old cultures were selected and used throughout this work for three reasons:— (a) they gave a constant *p*H, (b) the organisms showed a maximum degree of activity, and (c) the number of organisms in successive subcultures did not vary by more than 40 per cent.

Preliminary experiments indicated that an increase from 20° to 25°C. in the temperature at which the experiment was conducted decreased the resistance of the organisms to most of the drugs tested to such an extent that their activity appeared doubled. Experiments were therefore conducted at a constant temperature.

Drugs strongly acid or alkaline in solution should be neutralised beforehand, particularly when high concentrations have to be used. This factor was not involved in the work on antiprotozoal drugs, partly on account of the fact that the solutions used were sufficiently dilute and partly on account of the slight buffering effect of the culture medium.

Procedure

The method of preparing drug dilutions was that described by Gunn and Simonart[4], whereby twelve 0·5 ml. serial dilutions of a 1 per cent. stock solution of 5 drugs were prepared and transferred to small tubes of 1 to 2 ml. capacity set up in suitable racks. 0·5 ml. of a 8 to 14 day-old culture of *Colpidium* or of *Paramœcium* was added to each tube, the contents well mixed and the tubes placed in an incubator at 20°C. The time was taken when the mixing was complete and the tubes were examined for survivors after 20 minutes, 1, 3, 6 and 24 hours' exposure to the drugs, and again each day until there was no further change. The tube containing the weakest dilution in which there were no survivors was noted at the end of each period of time and the final concentration of drug (half the initial concentration) in the tube recorded. This is referred to as the minimum lethal concentration.

This procedure using 12 tubes is convenient for preliminary use as it will almost certainly cover the range of activity of any drug. Afterwards it can be shortened as a rule by using only 6 dilutions of the drug.

Each experiment was repeated on 4 further occasions at different times and using different cultures. The agreement was found to be good, for in practically all cases at least 4 of the 5 results tallied.

The results were represented on a graph where the logarithm of the reciprocal of the minimum lethal concentration was plotted against time.

Colpidium was found to be more resistant to the antiprotozoal drugs than was *Paramœcium*, particularly in the case of the more active drugs. Their effects against the two organisms, however, showed a parallelism so that these drugs fell into the same order of activity when either organism was used in the test.

The test for aerobic bacteria in the drug-culture mixture consisted in

inoculating tubes of broth infusion with a few drops of the contents of the tubes from the above test after its completion and incubating for 48 hours. A control was carried out using a 1 in 2 dilution of untreated culture. The rate of appearance of turbidity in the tests was compared with that in the control. This test, when applied to the antiprotozoal drugs, showed that the bacteria were unaffected by concentrations which destroyed the protozoa.

SUMMARY

1. A simple method of testing the activity of drugs on certain infusoria is described, whereby cultures of the organisms are exposed to varying dilutions of the drugs and the lethal concentrations recorded after different periods of exposure.

2. The smaller organism, *Colpidium*, was found to be more resistant to the action of the drugs than was *Paramœcium*, but the results obtained with both species gave the same relative order of activity.

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