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Transformation of Drug-resistance in Trypanosoma*

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SUMMARY

The genetic transformation which has been described in *Diplococcus pneumoniae* and *Haemophilus influenzae* has been shown also in *Trypanosoma gambiense* and DNA was found to be the active principle. These findings are important since they are the first reports on the possible occurrence of transformation in protozoa.

INTRODUCTION

Avery *et al.* (1944) have already reported so-called type transformation in *D. pneumoniae*. DNA of the donor bacteria was characterized as an active principle. A number of further studies in the field of bacteriology were helpful in giving an understandings of type transformation in *D. pneumoniae* and *H. influenzae* and transformation of drug-resistant traits (Hotchkiss, 1957). On the other hand, no report on transformations in microorganisms other than bacteria is available.

As previously reported, Inoki *et al.* (1957) isolated a pararosaniline resistant strain. Using this isolated strain, it was also found that the Ak forms did not increase in the resistant strain, even by the treatment with sufficient dose (10 mg /kg) of pararosaniline to induce Ak forms in the sensitive (original) strain. From this, they (Inoki *et al.*, 1957; Inoki and Matsushiro, 1959) have established a new method in which the grade of resistance of the parasites is determined by counting induced Ak forms and have demonstrated a genetic continuity in the acquired resistance. By use of this technique, the authors (1958 a, b) have shown the genetic transformation of pararosaniline resistance in this protozoa.

MATERIALS AND METHODS

1. *Strains of protozoa*

A pure strain of *Trypanosoma gambiense* (Welcome strain) was used for this study, which was originated by a single parasite inoculation. The rate of spontaneous appearance of the Ak form in this strain was usually less than one per cent and in this form the parasite did not multiply (Inoki, 1956). The original strain was sensitive to pararosaniline and is designated as 'W(S)' in this report.

A pararosaniline-resistant strain was obtained by the method of Lewy and Gurewitch

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(1926) as reported previously (Inoki and Matsushiro, 1959). A clone derived from this maximally resistant strain (85 mg/kg) by single cell fishing was used and designated as 'W(R)' for convenience.

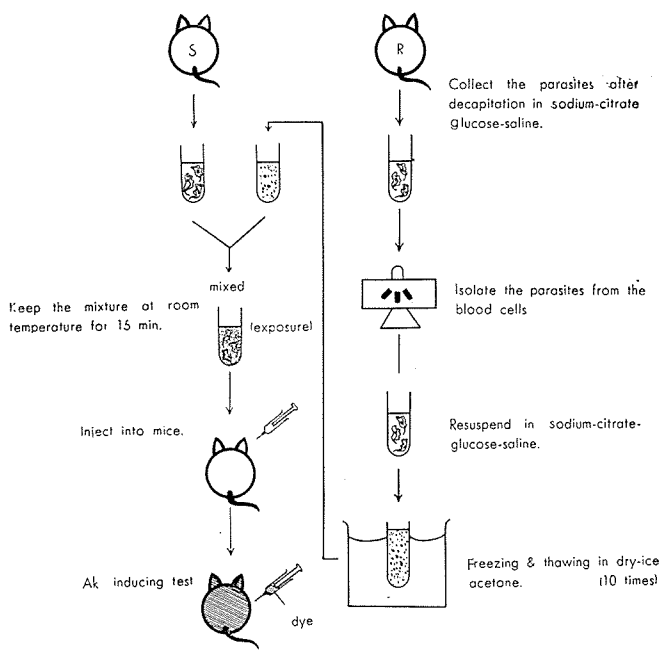
2. *Determination of drug-resistance*

As previously reported (Inoki and Matsushiro, 1959), 240 minutes after pararosaniline (10 mg/kg) injection the rate of appearance of the Ak form is about 20 per cent in the sensitive strain W(S), while it remains at about 5 per cent or lower in the resistant strain W(R). Therefore, the degree of resistance is indicated by the percentage of appearance of the Ak form after treatment with a dose of 10 mg/kg of pararosaniline.

3. *Method of transformation*

Blood collected by decapitation of mice at the maximum stage of infection with W(R) was mixed with glucose citrate saline (glucose 0.5%, sodium citrate 0.5%, sodium chloride 0.5%). The mixture was centrifuged for 10 minutes at 2,000 rpm to separate the parasites. The supernatant was discarded. New glucose citrate saline was added and the suspension was freeze-thawed 10 times using acetone dry-ice. The resulting lysate or suitable dilutions of it were used as a donor. It was injected into normal mice to test whether all parasites were killed by this treatment.

Fig. 1. Experimental procedure for inducing transformation of drug-resistance



Blood taken from a mouse severely infected with W(S) was mixed with sufficient glucose citrate saline to ensure that each microscopic field under 300 × magnification contained several parasites. This was used as the receptor.

After being mixed with the same amount of the lysate of W(R) in a small test tube, 1.0 ml of the suspension of W(S) parasites was incubated at room temperature or in a

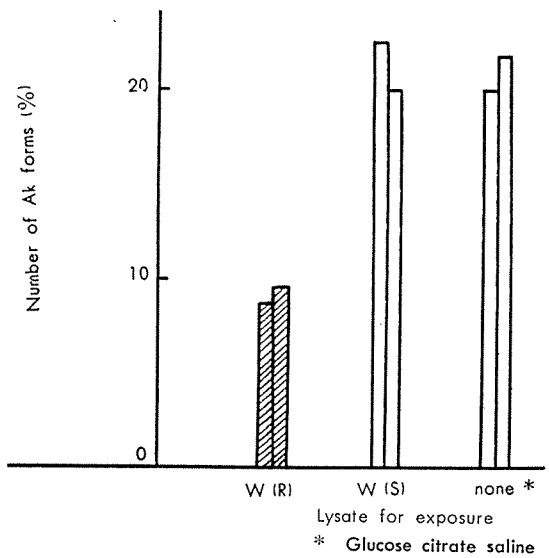
thermostat at 30°C. The untreated mixture or after treatment with DNase*, at a concentration of 500 μg per ml, was inoculated into a normal mouse and the degree of resistance was determined by the method mentioned above (See Fig. 1).

RESULTS

1. *Occurrence of drug resistant transformation*

After being exposed to the W (R) lysate, W (S) parasites were found to become resistant to pararosaniline as shown in Fig. 2. In other words, the rate of appearance of induced Ak forms in the above treated W (S) was much less

Fig. 2. Drug-resistant transformation in *Trypanosoma gambiense*



than expected. No such transforming ability was detected in the W (S) lysate. The character of transformants thus acquired was not lost by further mouse passage.

2. *Effect of the concentration of the W (R) lysate*

The influence of the lysate concentration on the transformation was investigated employing the W (R) undiluted and diluted lysate. As Fig. 3 indicates, the transforming activity was markedly diminished on dilutions of the lysate. One may postulate the existense of a transforming agent from the above results.

3. *Effect of time of exposure to the transforming agent*

In experiments on type transformation in *D. pneumoniae*, the reaction time was controled by removal of active principle by addition of a large amount of DNase 5 to 10 minutes after the begining of the reaction between viable cells and the transforming agent.

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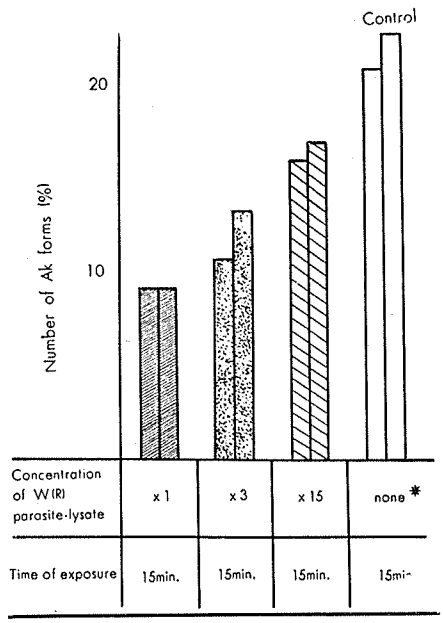
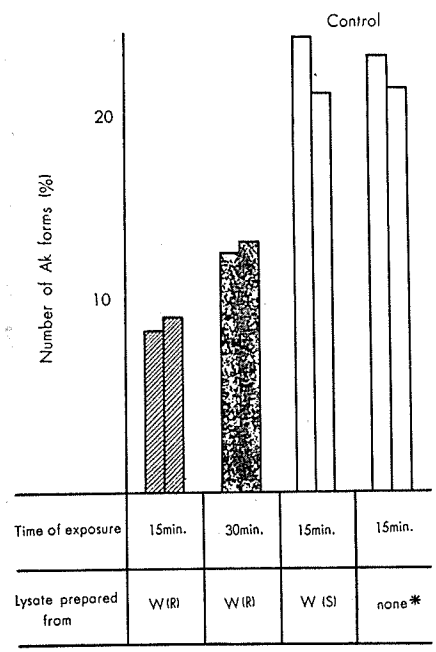
* Manufactured by the Worthington Biochemical Corp., Freehold, New Jersey, U.S.A.

Fig. 3. Effect of the dilution ot donor lysate



* Glucose citrate saline

Fig. 4. Effect of time of exposure



* Glucose citrate saline

A similar procedure was used to control the reaction time for earlier data and 15 minutes was chosen as the optimal (Fig. 4). The reason for diminution of transformation with longer exposures is not yet clear.

4. *Properties of the transforming agent*

From the above results the occurrence of transformation to pararosaniline-resistance in *T. gambiense* is certain but the nature of transforming agent is not.

Various concentrations of DNase, RNase, and various proteolytic enzymes were examined to see if they affected the transforming activity. Fig. 5 demonstrates that only DNase can inhibit the transforming activity.

Hence, it is probable that the transforming principle is DNA. Extensive fractionation studies of the transforming agent are being planned.

Fig. 5. Effect of DNase

W(S) susp.	0.2ml	0.2	0.2
W(R) lysate	—	0.5	*0.5
Rabbit serum	0.5	—	—
DN-ase 500μg/ml	—	—	0.3
Dist. water	0.3	0.3	—
Total volume	1 0	1.0	1.0

*The lysate in this column was used after treatment with 0.3 ml DNase solution (500 μg/ml) for 20 minutes at 30°C.

DISCUSSION

Since both resistant and sensitive strains employed were pure clones derived from single parasites, the selection of resistant mutants in the viable sensitive (original) recipient population by treatment can be disregarded. Recent work (Honigberg and Read, 1960) on the transformation of pathogenicity of *Trichomonas gallinae* in mice raises the problem of the genetic purity of parasites as experimental material. The results given are of much interest. They deal with delicate properties of traits based on the interrelationship between the host and the parasite, but unfortunately the conclusions are not always clear cut.

As already stated, the findings on the trait of the drug-resistance in *T. gambiense* are such that a degree of cellular resistance is detectable before cell division is completed. Then no considerations are to be paid for the possibility of selection.

In contrast to bacteria there would be a high frequency of transformation in this species of protozoa as reported here.

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