Relationship between Kinetoplast Elimination and Para-Rosaniline Resistance in *Trypanosoma gambiense*

The kinetoplast is a self-duplicating particle at the base of the flagellum of the family Trypanosomidae. It has recently come to the fore in the field of the cytoplasmic inheritance (Inoki, 1956). Werbitzki (1910) first described the interesting phenomenon that aberrant forms devoid of kinetoplasts (Ak forms) increase in infected animals (with *Trypanosoma brucei*) after treaement with certain dyes, such as *p*-rosaniline. However, the important question has not yet been answered of whether such an increase in Ak forms would be produced by the direct action of the injected dyes or if the dyes merely serve to select pre-existing Ak forms.

Inoki (1956) worked on this genetic problem and studied the origin of the Ak forms. Employing a Welcome strain* of *Trypanosoma gambiense*, he proved that the increase in Ak forms after the treatment with dyes (acriflavine, *p*-rosaniline and rosaniline) is due to their direct action and not to a selective effect. It was also known that Ak forms of this species, either induced artificially by dye treatment or appearing spontaneously, cannot multiply by fission.

To study this problem, the short passage method originated by Lewy and Gurewitch (1926) was employed to make a *p*-rosaniline-resistant strain for isolation of Ak forms which could multiply. Heavily infected mice were treated with very small dose of *p*-rosaniline (1.0 mg/kg) and the parasites were transfered to normal mice one hour after this intraperitoneal treatment. The same procedure was repeated increasing the dose by 5 mg/kg at each following passage. In this way, the dose was increased up to 85 mg/kg. A larger dose than this was so toxic that the mice died within one hour. Thus, a pure line of the parasites was isolated from mice treated with this maximum dose, employing the single cell isolation technique (Inoki, unpublished). During this experiment, the percentage of induced Ak forms was calculated in the course of passage in mice by the method already reported (Inoki, 1956). The result showed that the percentage was variable and usually higher than the normal level (less than one per cent). However, it returned to the normal level in several mouse passages after the single cell isolation. Therefore, the attempt to isolate Ak forms which could multiply was unsuccessful even by this method. The character of this induced resistance has lasted for three years in the course of passage in mice. The resistant strain obtained will be called " 85 p " in the following experiments.

The Ak forms did not increase in 85 p infected mice, even by the treatment with sufficient dose (10 mg/kg) of dye to induce Ak forms in the sensitive (original strain). Fig. 1 shows the rate of the appearance of Ak forms in sensitive and resistant cells. 240 minutes after dye injection, the rate of appearance is about 20 per cent in the sensitive, and only about 5 per cent in the resistant cells (See Fig.

* This strain was given by courtesy of Dr. Max C. McCowen, The Lilly Research Laboratories, Indianapolis, Indiana, U.S.A.

Fig. 1. The Ak Induction Test

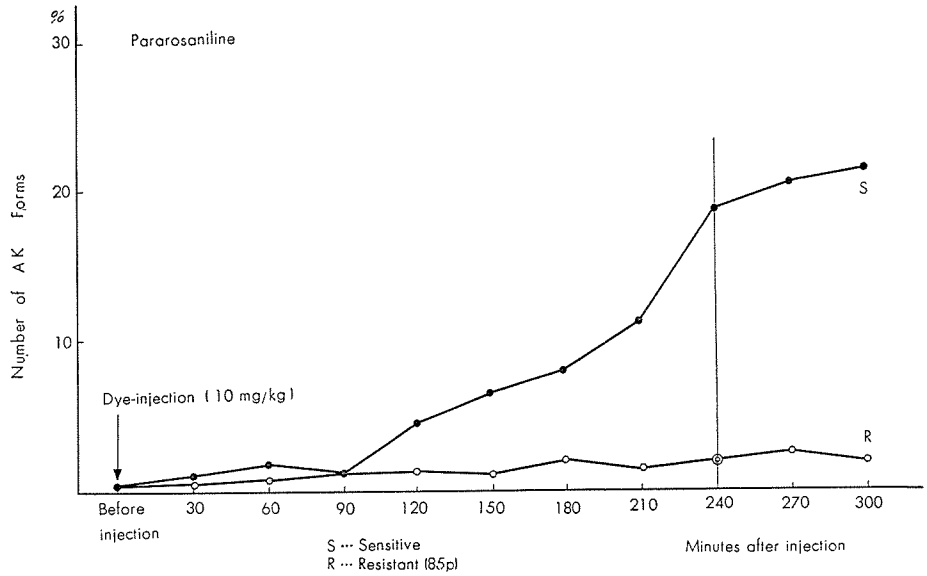
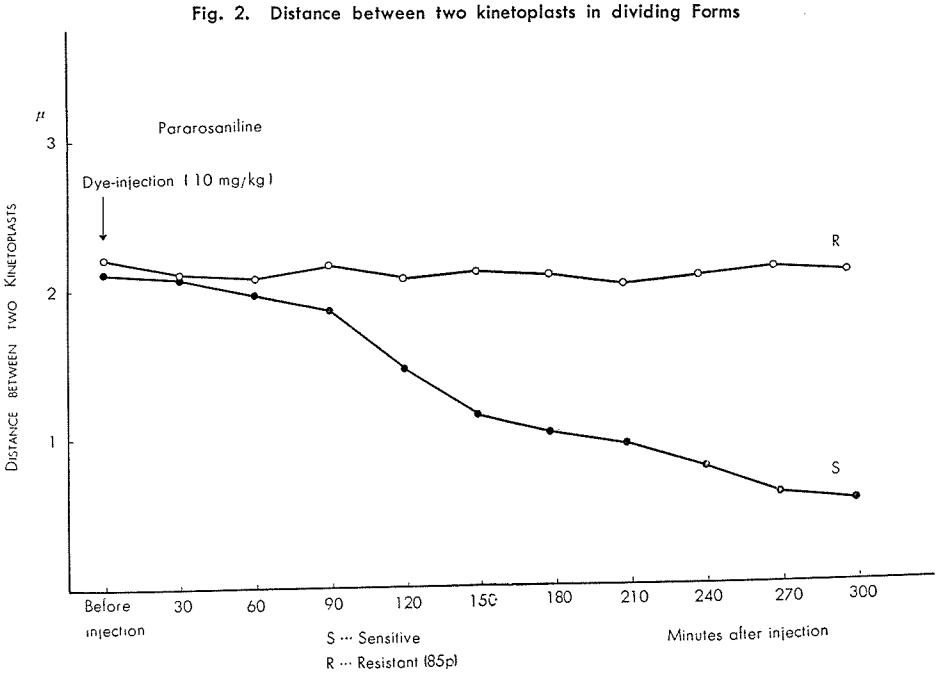


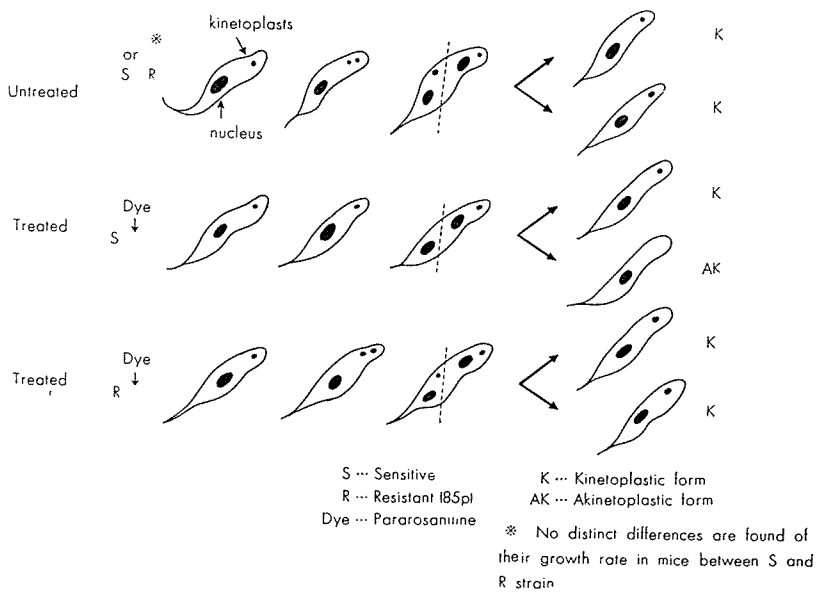
Fig. 2. Distance between two kinetoplasts in dividing Forms



1). Thus, the Ak induction test above-mentioned could be used to determine the grade of resistance of the parasites in mice. In this test, the resistance of the parasites can be demonstrated by the percentage of Ak forms 240 minutes after dye injection. If the percentage of Ak forms at that time is lower than that of the sensitive cells, parasites are resistant. This test was more convenient and more reliable than routine tests in which resistance is indicated by the days of survival of infected mice after dye treatment.

To analyse this phenomenon, the distance between two kinetoplasts in each dividing form was measured by micrometer. The measurement was made on the stained blood smears taken from treated mice every 30 minutes after the dye injection, and has already been reported by Inoki (1956). This experiment showed that the distance was not changed in the resistant forms (85p) even 240 minutes

Fig. 3. Mechanism of the Appearance of Ak Form



after injection of 10 mg/kg *p*-rosaniline, but it decreased gradually in the sensitive forms (See Fig. 2). Therefore, it is clear that the division of the kinetoplast in the 85p resistant as in the sensitive strain is not inhibited by this induction test, and the division of the cell, the nucleus and the kinetoplast, seems to take place normally. Fig. 3 explains the mechanism of the appearance of Ak forms schematically (See Fig. 3).

In short, this test is very convenient to determine the grade of *p*-rosaniline-resistance in *Trypanosoma gambiense*, and it has been employed successfully for further advancement of this study.

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