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Studies on the Ak Forms of *Trypanosoma evansi*

II. Effect of *p*-Rosaniline on the Appearance of Ak Forms*

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SUMMARY

1. When mice infected with *Trypanosoma evansi* are treated intraperitoneally with appropriate quantities of *p*-rosaniline, there was an increased appearance of the Ak form (akinetoplastic form) in the peripheral blood.
2. The dose of *p*-rosaniline resulting in an increase in the appearance of Ak forms in *Trypanosoma evansi* is similar to that causing the appearance of Ak forms in *Trypanosoma gambiense*. The dose differs from that of acriflavine required to cause a similar phenomenon.
3. The cause of the increase in Ak forms after *p*-rosaniline treatment is thought to be due to the direct action of the dye on the kinetoplast. Thus the effect is different from that due to acriflavine, since the latter is thought to act by selection of spontaneously mutating Ak forms.

INTRODUCTION

The preceding paper (Inoki *et al.*, 1961b) reported on the effect of acriflavine on the appearance of Ak forms of *Trypanosoma evansi* (*T.e.*). The dose range of acriflavine necessary for the increase of Ak forms in infected mice was established. The increase in the number of Ak forms was explained as the result of selection due to different effects of acriflavine on the Ak and K (kinetoplastic) forms of the cells.

This paper reports comparative studies on the effect of *p*-rosaniline on *T.e.*-infected mice.

MATERIALS AND METHODS

The same pure clone of *T. e.* (Taiwan strain) was used in this study as was employed for previous work (Inoki *et al.*, 1960; 1961a; 1961b). Normally there were about 6 per cent of Ak forms of this clone in host mice. Ak forms were counted on stained blood smears from treated mice as described previously (Inoki, 1956). To study the increase in the proportion of Ak forms after treatment with *p*-rosaniline, the distance between the two kinetoplasts in dividing forms carrying two nuclei was measured by micrometer every 30 minutes after dye treatment. The details of

* These results were reported at the 15th Meeting of Japanese Parasitologists in the western area of Japan, at Hiroshima, in November, 1959.

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this technique are given in preceding papers (Inoki, 1956; Inoki and Matsushiro, 1959; Inoki *et al.*, 1961b).

RESULTS

1. *Appearance of Ak forms*

Various doses of *p*-rosaniline were injected into mice showing moderate infections with *T.e.* to determine the dose range causing an increase in the percentage of Ak forms. Fig. 1 shows that there was an increase with doses of 5 mg/Kg, 10 mg/Kg and 20 mg/Kg body weight. The percentage of Ak forms increased to about 20 per cent, 240 minutes after dye-injection. A dose of 1 mg/Kg was too low and a dose of 50 mg/Kg too large to cause any increase. The increase in the percentage of Ak forms after *p*-rosaniline treatment usually occurs a few days later in *T.e.* than in *T. gambiense* (*T. g.*). This may be due to differences in rates of growth, because it is known that *T. g.* grows faster than *T. e.* in mice (Inoki *et al.*, 1960). The dose of *p*-rosaniline necessary to induce an increase in the percentage of Ak forms was the same in the two species of trypanosomes.

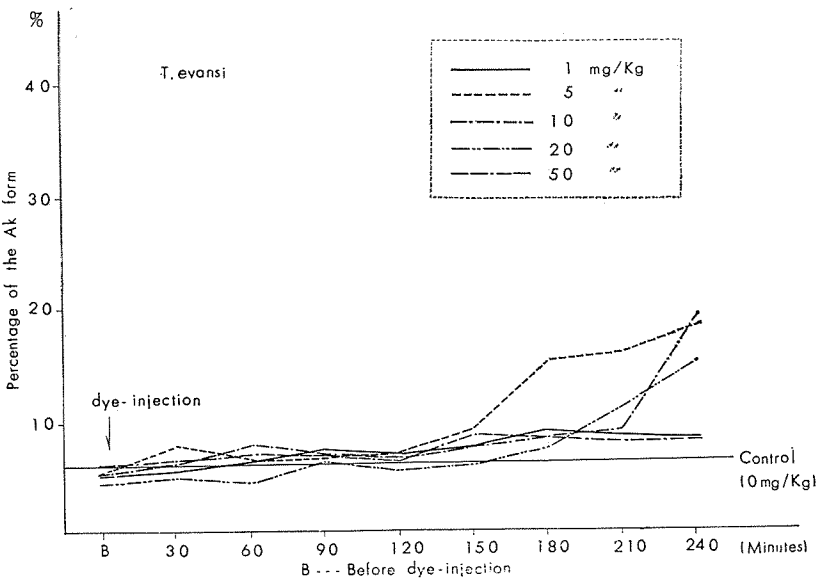


Fig. 1. Effect of *p*-Rosaniline

2. *Possible direct effect of p-rosaniline on kinetoplast*

To study the increase in the percentage of Ak forms, tests were made on whether effective doses of *p*-rosaniline inhibited duplication of kinetoplasts. The distance between the two kinetoplasts in dividing *T. e.* was measured by micrometer

in blood smears taken every 30 minutes after treatments, with doses of 5 mg/Kg, 10 mg/Kg, 20 mg/Kg and 50 mg/Kg of *p*-rosaniline. The distance was found to decrease gradually. However, a dose of 1 mg/Kg caused no decrease in the distance between the kinetoplasts (see Fig. 2).

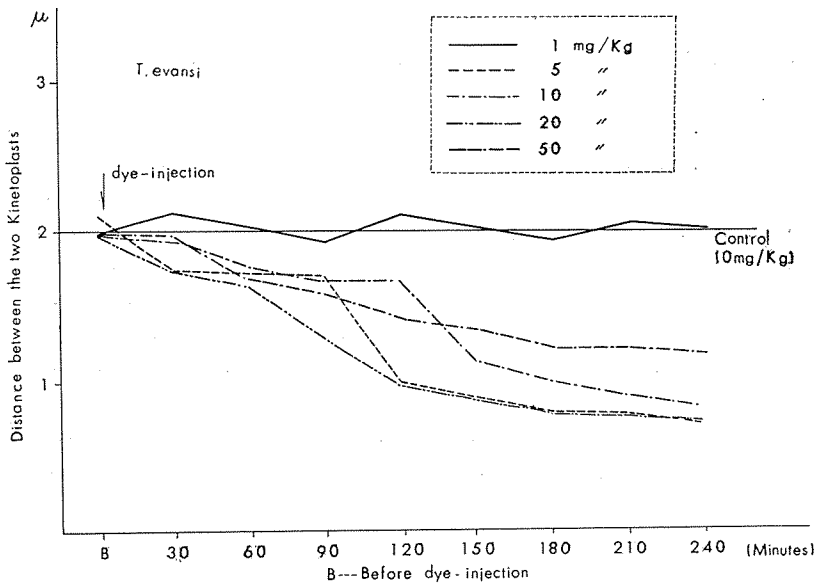


Fig. 2. Distance between the two Kinetoplasts in Dividing Cells

As mentioned in the preceding paper (Inoki *et al.*, 1961), this result indicates that the increase in the percentage of Ak forms after *p*-rosaniline treatment can be explained as due to the direct action of the dye on the kinetoplast. It is assumed that *p*-rosaniline treatment inhibits the division of kinetoplast in the same way in the two species of trypanosomes. A dose of 50 mg/Kg did not induce an increase in the percentage of Ak forms, although it inhibited the duplication of the kinetoplast. However, this may be explained by the fact that a large dose of dye also inhibits cell division (see Table 1).

Table 1. Effect of *p*-Rosaniline on Cell Division

Dye dosage	Percentage of dividing K type cells		Percentage of dividing Ak type cells	
	Before dye-injection	240 minutes after dye-injection	Before dye-injection	240 minutes after dye-injection
10 mg/Kg	5.2 %	4.3 %	5.3 %	2.3 %
50 mg/Kg	5.7	1.7	6.0	1.0

DISCUSSION

An increase in the percentage of Ak forms after treatment with certain dyes is already known to occur in *T. evansi* (Laveran, 1919; Jirovec, 1929; Mühlpfordt, 1959). However, no analytic studies have ever been made on the cause of this increase.

Inoki (1956) studied the origin of these forms from the genetical angle. He showed that both *p*-rosaniline and acriflavine inhibit kinetoplast duplication in *T. gambiense*. Employing the same techniques, Inoki *et al.* (1961) tested the effect of acriflavine on the appearance of Ak forms in *T. evansi*, and found unexpectedly that acriflavine does not inhibit kinetoplast duplication in this species.

Therefore the present study was on the effect of *p*-rosaniline on the division of the kinetoplast in *T. evansi*. It was found that this dye inhibits kinetoplast duplication in *T. evansi* just as in *T. gambiense*. Therefore, as in the preceding paper (Inoki *et al.*, 1961), it was concluded that the increase in the percentage of Ak forms after *p*-rosaniline-treatment could be explained as due to the direct action of the dye on the kinetoplast.

Acriflavine and *p*-rosaniline have different actions on *T. evansi*, but same actions on *T. gambiense*, although they cause an increase in the percentage of the Ak form.

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