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Chemotherapy and Akinetoplastic Form Induction Test with Furazolidon in *Trypanosoma gambiense* infected Mice

TADASUKE ONO and SHOZO INOKI

Department of Protozoology, Research Institute for Microbial Diseases, Osaka University, Yamada-kami, Suita, Osaka

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Introduction

Members of the Protozoa belonging to the family Trypanosomatidae contain a self-duplicating body known as the kinetoplast which seems to be a region of the large mitochondrion (Steinert, 1960., Clarke and Wallace, 1960). The kinetoplast is a permanent organelle, but the organisms devoid of the kinetoplast (Akinetoplastic form, hereafter referred as AK form) may appear on treatment with certain dyes, such as acriflavine and ethidium bromide (Werbitzki, 1910., Inoki, 1956).

Inoki (1956) has analysed the origin of the AK forms of *Trypanosoma gambiense* appearing in mice after p-rosaniline treatment.

The AKfrom inducing compounds have been found to bind with DNA and inhibit DNA synthesis.

Endo *et al.* (1963) observed that Nitrofurazone derivative (NFT) inhibits DNA synthesis in such concentration as not to inhibit RNA and protein synthesis in *Escherichia coli*. A number of workers have already demonstrated the curative effect of Nitrofurazone (Furan) in mice infected with *T. equiperdum*, *T. rhodesiense* and *T. gambiense* (Dodd, 1946. Packchanian, 1955. Evens *et al.*, 1957). However, the effect of this drug on the kinetoplast in trypanosoma has not been reported yet.

We have an interest in the action of Furazolidon as one of the Nitrofurazone derivatives and attempted to examine the effect of Furazolidon on the course of trypanosoma infection and the induction of AK forms in infected mice.

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Materials and Methods

Two clones (p-rosaniline sensitive and resistant clones) of *Trypanosoma gambiense* (Wellcome strain) were employed in the present study. These two were isolated by the single cell inoculation technique (Inoki, 1960.) and maintained in this laboratory by passage through ddo mice. The original clone was sensitive to p-rosaniline (hereafter referred as the WS). A clone of *T. gambiense* resistant to 85 mg p-rosaniline per kg mouse body weight (WR) has been obtained by the short passage method from the Wellcome strain (Inoki and Matsushiro, 1959).

The following methods to examine the effect of Furazolidon on trypansosmes in mice were used in this experiment. (1) The first method was performed to examine the effect of Furazolidon or Nitrofurazone on the inhibition infection or the prolongation of the prepatent period in mice. The blood from one of the infected mice was diluted with glucose-citrate-saline (Inoki and Matsushiro, 1959) to contain about one million motile trypanosomes per ml. Mice inoculated intraperitoneally with 0.1 ml of this suspension were immediately given various doses of Furazolidon orally. Five to 12 mice were used for each experiment. As a control, Nitrofurazone was employed. (2) The second

method was to observe if the survival days of infected mice could be prolonged by treatment with 200 mg Furazolidon after the appearance of trypanosomes in the circulating blood. Twenty-two mice were inoculated with WS or WR to examine the effect of Furazolidon. Forty-eight hours after inoculation, mice were given orally with Furazolidon. Ten mice were used as control.

The blood of each of the treated and untreated mice was examined microscopically for the presence of trypanosomes. After treatment with 200 mg/kg Furazolidon, blood smears were taken at time intervals to see if AK forms have appeared.

In order to examine the effect of Furazolidon at 200 mg/kg and at 300 mg/kg on the ultrastructures of the kinetoplast, electron microscopic observations were made 24 hours and 40 hours after treatment of the infected mice. Electron microscopy was done following the technique of Inoki *et al.* (1969).

Furazolidon (N-(5-nitro-2-furyliden)-3-amino-2-oxazolidon) was obtained from Ueno-Seiyaku Co. Ltd. Research Section (Fig. 1) and Nitrofurazone (Furacin) was given, courtesy of Dr. T. Karasaki, Toyama Chemical Inductry Co. Ltd.

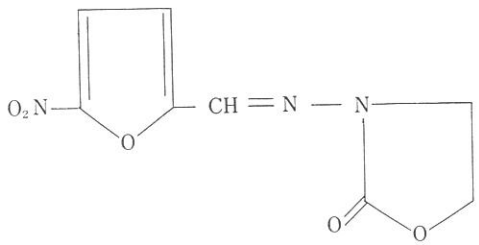


Fig. 1 Chemical structure of Furazolidon.

Results

The effect of Furazolidon on the inhibition of WS and WR infection in mice are summarized in Table 1. The untreated control showed trypanosomes in the peripheral blood in about 48 hours after inoculation with WS. The number of trypanosomes increased progressively and all 10 inoculated mice died on the fourth days and fifth days after inoculation.

The course of infection in 10 untreated control mice following the inoculation with WR is the same as in 10 untreated control mice inoculated with WS. In WS infected mice treated with 100 mg/kg Furazolidon, the trpyanosomes appeared between 3 and 9 days after inoculation and all mice died within 11 days. However, the survival rate of mice treated with 200 mg/kg Furazolidon was 100 % and microscopic examination of the tail blood of surviving mice was negative for trypanosomes.

Similarly, the effect of Furazolidon on the course of WR infection in mice was examined. Trypanosomes first appeared between 3 and 10 days following inoculation of mice treated with 200 mg/kg Furazolidon. All mice treated with 200 mg/kg Furazolidon died within 12 days. At 300 mg/kg Furazolidon the parasites did not appear at all.

Doses of 50 mg to 200 mg/kg of Nitrofurazone as used by other workers (Dodd, 1946. Packchanian, 1955. Evens *et al.*, 1957) were employed to compare its therapeutic effect with that of Furazolidon.

Table 2 shows the effect of Nitrofurazone on trypanosome infection in mice. A dose of 100 mg/kg and 200 mg/kg Nitrofurazone was necessary to completely inhibit WS and WR infection in mice, respectively.

In the experiment mentioned above, although the untreated controls showed the same course of infection for both WS and WR clones, trypanocidal actions of both Furazolidon and Nitrofurazone were found stronger against WS clone than against WR clone.

The next experiment was performed to examine in detail the difference in the effect of the drug on WS and WR. Table 3 shows the effect of Furazolidon on mice showing parasitemia. As shown in the Table, the untreated controls for both WS and WR clones died within 4 to 5 days after inoculation. Although 200 mg/kg Furazolidon failed to produce a permanent recovery from experimental trypanosomiasis, the dosage inhibited the multiplication of the parasites in varying degrees and resulting in a delay of the death

Table 1 Effects of Furazolidon on WS and WR injected mice, administered immediately after inoculation of the parasite.

strain	Doses /kg mice	Days after parasite inoculation and Furazolidon treatment												
		1	2	3	4	5	6	7	8	9	10	11	12	13
		−	−	+	++	D								
		−	−	−	−	++	++++	D						
		−	−	−	−	+	++++	D						
		−	−	−	−	++	D							
	100 mg	−	−	−	−	+	++++	D						
		−	−	−	++	++++	D							
		−	−	−	++	D								
		−	−	−	−	+	+++	++++	D					
		−	−	−	−	−	−	−	−	+	+++	D		
		−	−	−	−	−	−	−	−	++	++++	D		
		−	−	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−	−	−
WS	200 mg	−	−	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−	−	−
		−	+	++++	D									
		−	+	+++	++++	D								
		−	++	+++	D									
		−	++	+++	D									
	None	−	++	++++	D									
		−	+	++	++++	D								
		−	+	+++	++++	D								
		−	+	++	+++	D								
		−	+	+++	D									
		−	+	++	++++	D								
		−	−	+	+++	D								
		−	−	−	++	++++	D							
		−	−	−	++	+++	++++	D						
		−	−	+	++	D								
		−	−	−	−	++	+++	D						
	200 mg	−	−	−	−	+	+++	D						
		−	−	+	+++	D								
WR		−	−	−	−	+	+++	D						

Table 1-2

strain	Doses /kg mice	Days after parasite inoculation and Furazolidon treatment												
		1	2	3	4	5	6	7	8	9	10	11	12	13
		−	−	−	+	##	D							
		−	−	+	###	D								
		−	−	−	−	−	−	−	−	−	##	###	D	
		−	−	−	−	−	−	−	−	−	+	##	D	
		−	−	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−	−	−
	300 mg	−	−	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−	−	−
		−	##	###	D									
		−	+	##	D									
		−	+	##	D									
		−	##	###	D									
	None	−	+	##	###	D								
		−	+	##	D									
		−	+	##	###	D								
		−	+	##	###	D								
		−	+	##	D									
		−	+	##	###	D								

− ; trypanosomes are not found in 10 fields under the microscope (×400)
+ ; less than one parasites per one field. ## ; 2~10 parasites. ### ; 11~50 parasites.
; more than 51 parasites.
D ; Death
WS ; *Trypanosoma gambiense* p-rosaniline sensitive clone
WR ; *Trypanosoma gambiense* p-rosaniline resistant clone

of the infected mice.

Blood smears were taken at various time following 200 mg/kg Furazolidon treatment to examine its effect in the induction of AK forms in WS or WR infected mice. Table 4 shows the percentage of appearance of AK forms at 4 and 24 hours after the treatment with Furazolidon. The percentage of AK forms obtained 4 hours after treatment were not significantly different from that obtained before treatment. However, 24 hours after treatment, the increase was significant. The percentage of AK form in mice infected with WS is higher than that with WR infections.

Photo. 1-a shows the kinetoplast of *T. gambiense* before treatment with Furazolidon. The kinetonuclear fibers are seen within a two layered envelop. Photo. 1-b shows the kinetoplast 24 hours after treatment with 200 mg/kg Furazolidon. Photo. 1-c shows the parasite 40 hours after treatment with 300 mg/kg Furazolidon. Fragmentation and disappearance of the kinetonuclear fibers occurred, but the envelop was not affected by the drug at all.

Table 2 Effects of Nitrofurazone on WS and WR injected mice, administered immediately after inoculation of parasite.

strain	Doses /kg mice	Days after Nitrofurazone treatment										
		1	2	3	4	5	6	7	8	9	10	11
WS	50 mg	−	−	+	++	D						
		−	−	−	−	−	−	−	+	+++	D	
		−	−	−	−	+++	D					
		−	−	−	−	−	−	+	+++	D		
		−	−	+	+++	D						
		−	−	−	+	+++	D					
	100 mg	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−
	None	−	++	+++	D							
		−	−	++	+++	D						
		−	+	+++	D							
		−	+	++	+++	D						
		−	+	+++	D							
		−	++	+++	D							
WR	100 mg	−	−	−	+	+++	D					
		−	−	−	−	+	+++	D				
		−	−	−	+	+++	D					
		−	−	−	+	++	D					
		−	+	+++	D							
		−	−	−	−	+	+++	D				
	200 mg	−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−
	None	−	+	+++	D							
		−	++	+++	D							
		−	+	+++	+++	D						
		−	+	+++	D							
		−	++	+++	D							
		−	−	++	D							

− ; trypanosomes are not found in 10 fields under the microscope (× 400)
+ ; less than one parasite per one field. ++ ; 2～10 parasites. +++ ; 11～50 parasites.
+++ ; more than 51 parasites. D ; Death
WS ; *Trypanosoma gambiense* p-rosaniline sensitive clone
WR ; *Trypanosoma gambiense* p-rosaniline resistant clone

Table 3 Effects of Furazolidon on WS and WR injected mice, administered after the parasite appeared in the blood.

strain	Dose /kg mice	Degree of infection	Days after Furazolidon treatment											
			1	2	3	4	5	6	7	8	9	10	11	12
WS	200 mg	+	+	+	++	−	+	+++	D					
		+	++	+++	+++	D								
		++	++	+	−	−	−	+	++	++	D			
		++	++	++	−	−	−	+	+++	D				
		+	++	+	−	−	−	−	−	+	+++	D		
		+	++	++	−	−	−	−	−	+	+++	D		
		+	++	+	−	−	−	++	+++	D				
		+	++	+	+	+++	D							
		+	++	++	+++	D								
		+	++	+++	+++	D								
		+	++	+	−	−	−	−	+	+++	D			
		+	++	++	+	+++	D							
		+	++	++	−	+	++	+++	D					
		++	++	−	−	−	−	++	+++	+++	D			
		+	+++	++	−	−	−	+	+++	D				
		+	++	−	−	−	−	+	++	D				
		+	+	−	−	−	−	−	−	−	+	+++	D	
		+	++	+	−	−	−	−	+	++	D			
		+	++	+	−	−	−	−	−	−	++	D		
		+	+	+	−	−	−	−	−	+	+++	D		
		+	++	+	−	−	+	+++	+++	D				
		++	+++	++	−	−	−	++	D					
	None	+	+++	D										
		+	++	+++	D									
		++	+++	D										
		+	+++	D										
		+	+++	+++	D									
		+	++	+++	D									
		+	+++	+++	D									
		++	+++	D										
		+	+++	D										
		+	+++	D										
WR	200 mg	+	++	++	D									
		+	++	+++	D									
		+	+	++	+++	D								
		+	+	+++	+++	D								
		+	++	−	−	−	−	+	++	+++	D			
		+	+	−	+	+++	D							
		+	+	−	+	+++	D							
		+	+	+	+++	D								
		+	++	−	−	−	+	+++	D					

Table 3-2

strain	Dose /Kg mice	Degree of infection	Days after Furazolidon treatment											
			1	2	3	4	5	6	7	8	9	10	11	12
		++	+++	++	+++	+++	+++	+++	+++	D				
		+	+++	−	−	++	+++	+++	D					
		+	++	+++	+++	D								
		++	+++	+++	+++	D								
		+	+++	+++	+++	D								
		+	++	+++	D									
		+	++	+++	−	−	++	+++	D					
		+	++	++	++	++	+++	D						
		++	+++	+++	+++	+++	D							
		++	+++	+++	+++	D								
		+	+++	+++	+++	D								
		+	++	+++	D									
		+	+++	++	+++	+++	D							
	None	++	+++	D										
		+	++	+++	D									
		+	+++	D										
		+	++	+++	D									
		+	++	+++	D									
		+	++	D										
		++	+++	D										
		+	++	+++	D									
		+	+++	D										
		+	++	+++	D									

The degree of infection means the number of parasites in the blood before Furazolidon treatment.

− ; trypanosomes are not found in 10 fields under the microscope (× 400)

+ ; less than one parasite per one field. ++ ; 2~10 parasites. +++ ; 11~50 parasites.

+++ ; more than 51 parasites

D ; Death

WS ; *Trypanosoma gambiense* p-rosaniline sensitive clone

WR ; *Trypanosoma gambiense* p-rosaniline resistant clone

Table 4 The percentage of appearance of AK forms in WS and WR infected mice before and after Furazolidon treatment.

	WS		WR	
before Furazolidon treatment	0.50[a]	0.30≤m≤0.70[b]	0.63	0.33≤m≤0.93
4 hr after Furazolidon treatment	0.75	0.41≤m≤1.09	0.81	0.51≤m≤1.11
24 hr after Furazolidon treatment	15.45	15.02≤m≤15.88	11.41	10.67≤m≤12.15

WS ; *T. gambiense* p-rosaniline sensitive clone

WR ; *T. gambiense* p-rosaniline resistant clone

a ; the mean percentage of appearance of AK forms (%)

b ; 99% reliability limit

Discussion

The present work shows that Furazolidon is very effective in inducing AK forms and in inhibiting *T. gambiense* infection in mice when the drug is given immediately after inoculation of the parasites. Although the dose required is higher than that of Nitrofurazone (Furan), Furazolidon is considered a better drug, since its LD_{50} in mice is 4100–4500 mg/kg as compared with that of Nitrofurazone which is 545 mg/kg (Dodd, 1946).

When given after the appearance of the parasite in the blood, Furazolidon, at a dose of 200 mg/kg, was found to suppress the multiplication of the parasite resulting in a delay of the death of infected mice.

In previous studies on the mode of AK form induction by means of dyes and antibiotics (Inoki, 1956. Sakamoto, 1963), the AK forms in *T. gambiense* and *T. evansi* were seen to appear 2 hours after administration of the inducing substances. The number of AK forms were found to increase gradually with time. In this study, however, AK forms appeared 24 hours after treatment of 200 mg/kg Furazolidon. It is possible that Furazolidon differs from other compounds such as p-rosaniline, acriflavine and ethidium bromide, in the mechanism of AK induction.

Electron microscopy shows the fragmentation and disappearance of the fibrous structure of the kinetonucleus in the AK forms obtained by Furazolidon treatment. Similar features have been demonstrated in the AK forms resulting from the use of p-rosaniline (Inoki *et al.*, 1969), acriflavine (Mühlpfordt, 1963. Trager and Rudzinska, 1964), ethidium bromide (Delain and Riou, 1969) and hydroxystilbamidine (Delain *et al.*, 1971).

Inoki *et al.* (1969) showed that the administration of 10 mg/kg p-rosaniline to mice infected with p-rosaniline sensitive *T. gambiense* resulted in the disappearance of both the kinetonuclear fibers and the kinetoplast envelope. The effect of Furazolidon on the ultrastructure of kinetoplast resulted in the fragmentation and disappearance of kinetonucleus and did not result in the disappearance of the envelope. The mechanism of difference in the effects of Furazolidon and p-rosaniline on the kinetoplast envelope is still undetermined.

Summary

The therapeutic effect of Furazolidon on *Trypanosoma gambiense* infected mice was examined employing both the p-rosaniline sensitive and the resistant clones.

Furazolidon was found to show therapeutic effect for both clones, but apparently more effective for the sensitive clones than for the resistant ones.

The AK induction test (Inoki and Matsushiro, 1959.) revealed that Furazolidon was able to increase AK forms (dyskinetoplastic forms) in both clones.

Ultrastructural studies showed that the fragmentation and disappearance occurred in the kinetonuclear fibers after treatment with Furazolidon.

References

1) Clarke, T. B. and Wallace, F. G. (1960) : A comparative study of kinetoplast ultrastructure in the *Trypanosomatidae*. J. Protozool., 7, 115–124.

2) Delain, E., et Riou, G. (1960) : Ultrastructure der altérations du DNA du kinétoplaste de *Trypanosoma cruzi* traité le bromnre d'éthidium. C. R. Acad. Sc, Paris., 268, 1327–1330.

3) Delain, E., Brack, Ch., Riou, G. and Festy, B. (1971) : Ultrastructural alterations of *Trypanosoma cruzi* kinetoplast induced by the interaction of a trypanocidal drug (Hydroxystilbamidine) with the kinetoplast DNA. J. Ultrastruc. Res., 37, 200–218.

4) Dodd, M. C. (1946) : The chemotherapeutic properties of 5-nitro-2-furaldehyde semicarbazone (Furacin). J. Pharmacol. Exptl. Therap., 86, 311–323.

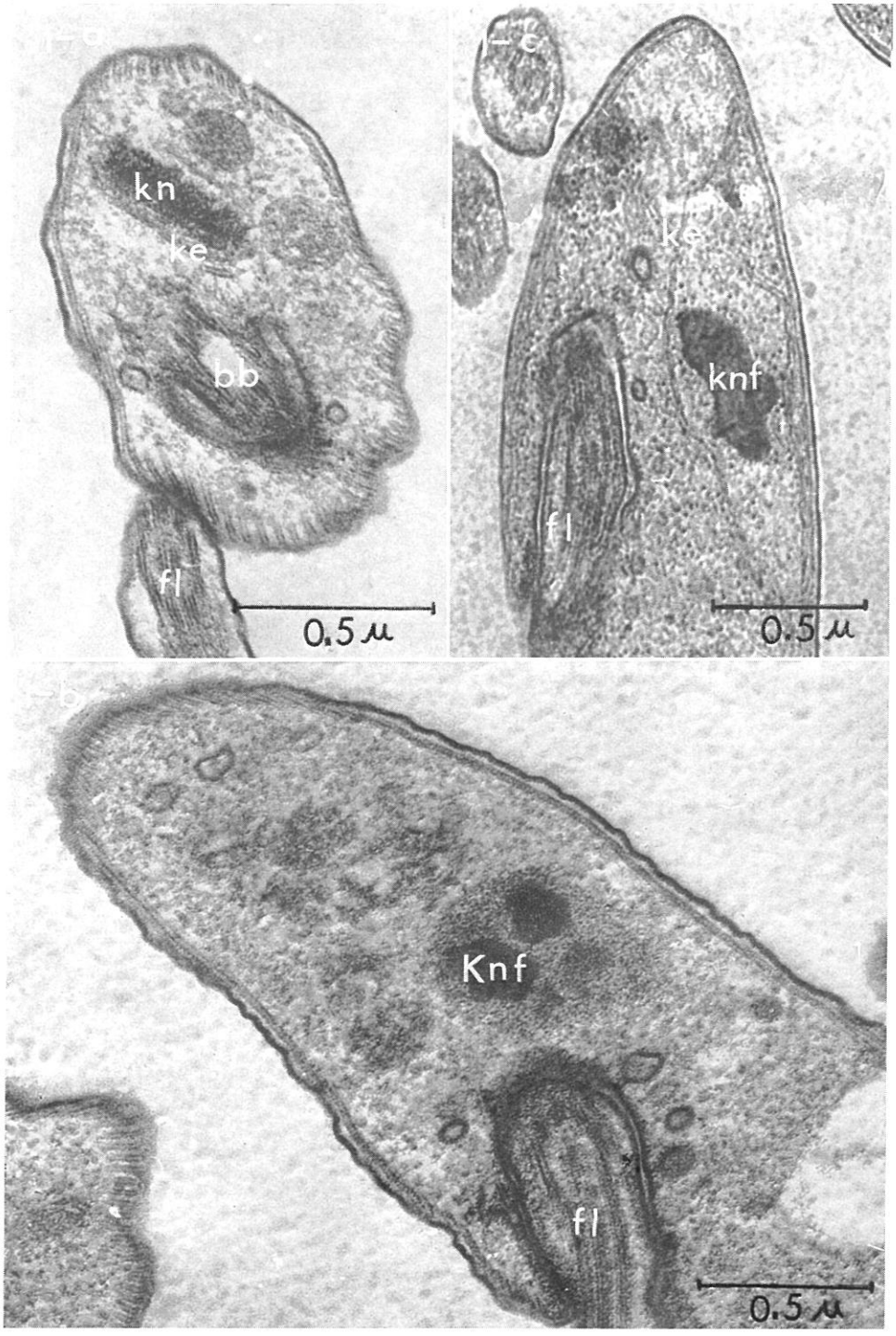
5) Endo, H., Ishizawa, M., Kamiya, T. and Kuwano, M. (1963) : A nitrofuran derivative, a new inducing agents for the phage development in lysogenic *Escherichia coli*. B.B.A., 68, 502–505.

6) Evens, F., Hiemegeers, K. and Packchanian, A. (1957) : Nitrofurazone therapy of *Trypanosoma gambiense* sleeping sickness in man.

Amer. J. Trop. Med. Hyg., 6, 665–678.
7) Inoki, S. (1956) : Origin of the akinetoplastic strain of *Trypanosoma gambiense*. Cytologia, Supplt. Vol. (Proceedings of international symposia, Tokyo, Japan) 550–554.
8) Inoki, S. and Matsushiro, A. (1959) : Relationship between kinetoplast elimination and pararosaniline resistance in *Trypanosoma gambiense*. Biken J., 2, 371–374.
9) Inoki, S. (1960) : Studies on antigenic variation welcome strain of *Trypanosoma gambiense*. I. Improvements in technique. Biken J., 3, 215–222.
10) Inoki, S., Ono, T. (1969) : Effects of p-rosaniline on the ultra-structure of the kinetoplast in *Trypanosoma gambiense* and *Trypanosoma evansi*. Biken J., 12, 187–199.
11) Mühlpfordt, H. (1963) : Über die Bedeutung und Feinstrukture des Blepharoplasten bei parasitischen Flagellaten. Z. Tropenmed. Parasitol., 14, 357–398.
12) Packchanian, A. (1955) : Chemotherapy of African sleeping sickness. I. Chemotherapy of experimental *Trypanosoma gambiense* infection in mice (Mus musculus) with nitrofurazone. Amer. J. Tropenmed. Hyg., 4, 705–711.
13) Sakamoto, H. (1963) : Genetic studies on the mode of akinetoplastic-form induction in *Trypanosoma evansi* by mean of injecting mouse with some chemical agents. Jap. J. Parasitol., 12, 1–12. (in Japanese).
14) Steinert, M. (1960) : Mitochondria associated with the kinetonucleus of *Trypanosoma mega*. J. Biophys. Biochem. Cytol., 8, 542–546.
15) Trager, W. and Rudzinska, M. A. (1964) : The riboflavin requirement and the effects of acriflavin on the fine structure of the kinetoplast of *Leishmania tarentolae*. J. Protozool., 11, 133–145.
16) Von Brand, T., Tobie, E. J., Mehlman, B. and Weinbach, E. C. (1953) : Observations on the metabolism of normal and arsenic-resistant *Trypanosoma gambiense*. J. Cell Compt. Physiol., 41, 1–22.
17) Werbitzki, F. W. (1910) : Über blepharoblastlose Trypanosomen. Zentr. Bakteriol. I. Abt. Orig., 53, 303–315.

Explanation of Photo.

Photo. 1-a. The kinetoplast of p-rosaniline sensitive *T. gambiense*. The typical fibrous structure is seen within the envelope.
Photo. 1-b. The kinetoplast of p-rosaniline sensitive *T. gambiense*. 24 hours after treatment with 200 mg/kg Furazolidon.
Photo. 1-c. The kinetoplast 40 hours after the treatment with 300 mg/kg Furazolidon.
Fragmentation and disappearance of the fibrous structure occur, but the envelope persists.
Kn, Kinetonucleus (fibrous inclusion). Ke, Kinetoplast envolope.
Knf, Kinetonuclear fragment. fl, flagellum. bb, basal body.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Trypanosoma gambiense 感染マウスに対する Furazolidon の治療効果および AK 型原虫誘発効果について

小野忠相・猪木正三
（大阪大学微生物病研究所　原虫学部門）

アフリカ睡眠病は *Trypanosoma gambiense* の感染によっておこるが，その治療薬の1つとして Nitrofurazone (Furacin) があり，従来より用いられている．この実験では Nitrofurazone の誘導体である Furazolidon を使用し，この薬剤がマウスにおける *T. gambiense* 感染を阻止するかどうか，又，すでに感染しているマウスに対して治療効果をもつかどうかを調べた．その結果，Furazolidon は原虫感染の阻止をおこすが，それに要する量は Nitrofurazone よりも多いことがわかつた．しかし，マウスに対する LD_{50} を考慮した場合，Furazolidon の方が治療薬として実際に使用し易いのではないかと思われる．この実験では *T. gambiense* の p-rosaniline 感性と耐性の両 Clone が用いられたが，後者は Furazolidon による治療に対して前者よりも強い抵抗性を示した．

これまで，Trypanosoma の kinetoplast に作用してこれを欠いた原虫（AK 型原虫）を誘発する効果をもつ薬剤が数種類知られている．これらの薬剤は DNA と結合しあるいは DNA 合成を阻害する．DNA 合成の阻害効果をもつた Nitrofurazon 誘導体の存在が報告されている所から，この実験では Furazolidon の AK 型原虫誘発効果を期待し，詳細に調べた結果，*T. gambiense* 感染マウスに 200 mg/kg の Furazolidon を投与すると24時間後に AK 型原虫が誘発されることがわかつた．AK 型原虫誘発効果をもつ薬剤としてすでに知られているものはいずれも薬剤投与後4時間目頃までに AK 型原虫の誘発を示すのに対して Furazolidon では4時間目には未だ AK 型原虫の誘発を示さず，24時間後に AK 型原虫を誘発させた．それ故，他の薬剤とは誘発機序が異なるかも知れない．

なお，Furazolidon による AK 型原虫出現率は p-rosaniline 感性の Clone が耐性の Clone よりも高い率を示した．これは治療試験で後者が前者よりも Furazolidon に対して抵抗性を示した成績を一そう支持するものと思われる．

Furazolidon によつて誘発した AK 型原虫の電顕像は kinetonucleus の線維状構造の fragmentation あるいは消失を示し，これは他の薬剤によつて誘発した AK 型原虫でみられる所見と同じある．