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Studies on the Immunological Variation in *Trypanosoma gambiense* V. Antigenic Constitution of the Relapse Type Strain in Respect to its Reversibility to the Original Type

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SUMMARY

To clarify the expression of antigenic type of *Trypanosoma gambiense* (*T.g.*) and its correlation to modes of antigenic variation, relapses were investigated during passages in mice by application of a combination of the cross screening method with various *in vitro* identification methods for serotypes as reported in the previous papers.

The antigenic constitution of relapse type strain (R) can be divided into single and compound antigenic systems. The latter is subdivided into the principal-accessory and the principal-latent antigenic systems, indicating the coexistence of the original type (O) with R.

It was found that the above systems are interchangeable during the course of passage in mice.

In the single antigenic system, the antigenic reversion from R to O may take place at the first relapse (one step reversion) while in the compound system the reversion does not take place by a single step but by two or more steps *via* other R types (two or more step reversion).

INTRODUCTION

Antigenic variation in trypanosomes is one of the most important problems to be solved in the field of protozoan immunology.

Since Franke (1905) reported on antigenic variation in *Trypanosoma equiperdum* in host animals, a number of investigations have been made by Ehrlich (1909), Ehrlich *et al.* (1909), Levaditi *et al.* (1910), Neuman (1911), Ritz (1916), Mutermilch and Salamon (1928), Lourie and O'Connor (1937) and others in this connection.

In a series of immunological and genetic studies by Inoki and his collaborators on the antigenic variation of *T.g.* in mice, antigenic types of the parasites were found to be maintained unchanged in the course of passage in mice. Further antigenic variations were proved to be induced as an effect of the antiserum and reversions of antigenic type from R to O were found to be dominant among relapse types in mice.

In the present paper, an analytical investigation of relapse types in connection

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with the antigenic constitution of the parasites and the mode of control over the reversibility from R to O in mice is discussed. Detailed studies on the antigenic type of the R type strain were undertaken at each generation of passage and all the relapse forms of the strain was antigenically checked. The methods employed in these experiments were the cross screening method, the agglomeration test, which was used for the determination of antigenic types of both parasites and antisera and other methods.

MATERIALS AND METHODS

1. *T. gambiense*

The *T.g.* (TG 1 strain) used in this study was propagated by serial passages in mice.

2. *Techniques routinely employed*

Inoculation of parasites into mice, treatment of infected mice with human blood plasma, collection of antisera from mice which had recovered and other procedures were carried out as reported in the previous papers (Inoki *et al.*, 1952a; Inoki *et al.*, 1959).

3. *Determination of the antigenic type of the parasites*

A drop of antiserum of known serotype (standard antiserum) was mixed with a drop of blood taken from the tail of an infected mouse with parasites of unknown serotype on a slide glass, and the occurrence of agglomeration of the parasites was detected microscopically. A positive reaction indicated that the parasites were identical in antigenic type with the standard antiserum.

4. *Serotype determination of antiserum*

The agglomeration test was performed microscopically between antiserum of unknown serotype and parasites of known type. The serotype of the antiserum could be ascertained by a positive agglomeration reaction.

5. *The cross screening method*

As fully illustrated in the foregoing report, the screening method is of the utmost significance for determining type-specificity, reinfections being negligible. This method, carried out between two test strains, is called the cross screening method and is reliable for determining whether the types of the strains being tested are identical, partially identical or different from each other, as will be described in detail later in this paper.

6. *Identification of the antigenic type of relapse parasites*

Mice infected with R type strain and treated with human blood plasma, were checked for the occurrence of relapses every day or every other day. The antigenic types of the relapse parasites were studied by the agglomeration test.

7. *Isolation of single parasites*

For the maintenance of antigenic uniformity, single cells were isolated. Serial dilutions of a drop of infected mouse blood with glucose citrate saline were made on depression slides and the diluted suspensions were then sucked into a capillary glass tube. A small drop of each was placed on a cover slip and examined microscopically on another depression slide. A drop containing one and only one living parasite was drawn into a tuberculin syringe and immediately injected into a healthy mouse. The parasites obtained from the mouse were used as an antigenically uniform strain, that is, a clone.

EXPERIMENTALS

1. *Preliminary experiments on the cross screening method*

To evaluate the cross screening method for determining antigenic type-specificity, preliminary tests were carried out between two different strains of antigenic types, O and R_2.

In Table 1, the symbols O or R_2 indicate mice which suffered from or recovered from infection with O or R_2 type strains respectively and the numbers at the top right of the symbols indicate the generation number of the passage. The same symbols are used consistently in the six other tables.

Experiment 1 in Table 1 shows that mouse O^4 received a reinoculation of O strain from infected mouse O^{21} after recovering from an initial infection with O strain but that it showed no manifestation of infection on the second inoculation. Experiment 2 demonstrates another negative reinfection in a mouse R_2^3 which recovered from infection with R_2 strain and was reinoculated with R_2 strain from a mouse R_2^{10} infected with R_2.

In cross screening experiments with two different types, O and R_2, as seen in Experiments 3 and 4, infections were observed in mice O^{21} and R_2^3 which had recovered from infections with O and R_2 were then inoculated with strains R_2 and O respectively. These infections are identified as being due to strains R_2 and O respectively by the agglomeration test. These results indicated clearly that these two strains, O and R_2 are antigenically independent.

Table 1. Preliminary experiment on the cross screening method.

Exp. No.	Infected mouse	Inoculated mouse	Infection	Agglomeration				
				Anti O serum	Anti R_1 serum	Anti R_2 serum	Anti R_3 serum	Anti R_{20} serum
1	*O^{21}	$\longrightarrow O^4$	−					
2	R_2^{10}	$\longrightarrow R_2^3$	−					
3	R_2^{15}	$\longrightarrow O^{21}$	+	−	−	+	−	−
4	O^{26}	$\longrightarrow R_2^3$	+	+	−	−	−	−

* The symbols $O^{21} \rightarrow O^4$ show that mouse O^4 which had recovered from infection with strain O was inculated with O type parasites which were present in the blood of mouse O^{21} infected with strain O.

2. *Follow-up examination of the antigenic type of R strains*

A. *Experiments on strain R_{10}*

Strain R_{10}, a relapse variant derived from strain O, was used. Single cells were isolated immediately after the advent and occasionally during the course of mouse-passages. As shown in Table 2, the results can be classified into three stages in accordance with the generation numbers, 1 to 23, 24 to 33 and 34 to 42. In the first stage,

R_{10} and O were proved to be antigenically independent by the cross screening method and only type R_{10} could be demonstrated by both the determination of the antigenic type of the parasites and the identification of the serotypes of the antisera. Furthermore, relapse parasites in the eighth and seventeenth generations were found to be type O. From these results, it can be interpreted that R_{10} is maintained unchanged throughout this stage and expressions of O type and R types other than R_{10} are definitely absent. This strain R_{10} in the first stage is to be regarded as a single antigenic system which consists of only one relapse type antigen.

In the second stage, on the other hand, apparently different expressions of type can be found. By the cross screening method, screening of $R_{10} \rightarrow O$ between R_{10} and O, was found to be positive while that of $O \rightarrow R_{10}$ was always negative. In other words, mice which have recovered from O infections are not protected from R_{10} infection but mice which have recovered from R_{10} infections are not susceptible to infection with O. This suggests the possible coexistence of O and R_{10} antibodies in the sera of mice which have recovered from infection with R_{10}. The fact that O antibodies as well as R_{10} antibodies were found in the antisera of mice which had recovered from infection with R_{10} while only R_{10} type antibodies were demonstrated by the agglomeration test in the determination of the antigenic types of the parasites supports this assumption. The relapse parasites in mice which recovered from R_{10} infection were not O type but some R type other than R_{10}. For example, as shown in Table 2, types R_1 and R_4 were induced from R_{10}^{27} and R_{10}^{29} respectively and reversions to O type were first found in subsequent relapses. Since antisera in mice which have recovered have two serotypes although the antigenic type of the parasites remained single, the parasites in this second stage are provisionally regarded as of the principal-latent compound antigenic system which consists of the principal R_{10} and the latent O.

In the third stage, cross screening was completely negative irrespective of whether it was from R_{10} to O or *vice versa*. Furthermore, in determination of the antigenic type of the parasites and serotype identifications of the antisera both type R_{10} and O were found. The relapse parasites in mice, R_{10}^{35} and R_{10}^{42}, which have recovered from R_{10} infection did not include type O but types R_{15} and R_8 were demonstrated respectively. Consequently, the parasites in this third stage may consist of two antigenic types, R_{10} and O, and the antisera of mice which have recovered from R_{10} infection may also contain the two serotypes. Hence, parasites in the third stage may be designated as the principal-accessory compound antigenic system whereby R_{10} is the principal antigen and O is the accessory one.

Strain R_{10}, as seen in experiment A (Table 2), altered its antigenic constitution from the single to the principal-latent and subsequently to the principal-accessory system in the course of mouse-passages. This constitutional alteration in the antigenic system is seemingly controlled by having O antigenic type in addition to R_{10}, the latter being consistently found to be dominant during the course of passages. The one

step reversion of antigenic type from R_{10} to O is commonly observed in the stage of the single antigenic system but not in the other two stages whereby a two step reversion *via* some R other than R_{10} takes place.

Table 2. Experiment on strain R_{10}. Examination of the antigenic constitution and antigenic reversion to O.

Generation No. of parasites in mice	$R^{1}_{10} \sim R^{23}_{10}$	$R^{24}_{10} \sim R^{33}_{10}$	$R^{34}_{10} \sim R^{42}_{10}$
Cross screening	$R^{2}_{10} \longrightarrow O^{130}+$ $O^{142} \rightarrow R^{2}_{10}+$ $R^{2}_{10} \longrightarrow O^{132}+$ $O^{142} \rightarrow R^{7}_{10}+$ $R^{11}_{10} \longrightarrow O^{148}+$ $O^{148} \rightarrow R^{12}_{10}+$ $R^{22}_{10} \longrightarrow O^{151}+$ $O^{159} \rightarrow R^{22}_{10}+$	$R^{26}_{10} \longrightarrow O^{159}+$ $O^{159} \rightarrow R^{28}_{10}-$ $R^{26}_{10} \longrightarrow O^{155}+$ $O^{162} \rightarrow R^{25}_{10}-$ $R^{28}_{10} \longrightarrow O^{156}+$ $O^{162} \rightarrow R^{27}_{10}-$	$R^{34}_{10} \longrightarrow O^{162}-$ $O^{171} \rightarrow R^{34}_{10}-$ $R^{36}_{10} \longrightarrow O^{187}-$ $O^{185} \rightarrow R^{39}_{10}-$ $R^{40}_{10} \longrightarrow O^{177}-$ $O^{176} \rightarrow R^{40}_{10}-$
*Determ. of antigenic type of parasites	R_{10} + O −	R_{10} + O −	R_{10} + O +
**Serotype determ. of serum	R_{10} + O −	R_{10} + O +	R_{10} + O +
Identification of antigenic type of relapse parasites	$R^{8}_{10} \longrightarrow O$ $R^{17}_{10} \longrightarrow O$	$R^{27}_{10} \rightarrow R^{5}_{1} \rightarrow O$ $R^{29}_{10} \rightarrow R^{3}_{4} \rightarrow O$	$R^{35}_{10} \rightarrow R^{5}_{15} \rightarrow O$ $R^{42}_{10} \rightarrow R^{6}_{8} \rightarrow O$
Antigenic constitution	Single	Principal-latent	Principal-accessory

* The antigenic type of strain R_{10} was examined by the agglomeration test with R_{10} and O standard antisera in almost every generation of passage in mice.

** The serotype was determined by the agglomeration test in sera of mice which had recovered from infection with strain R_{10}.

The following experiments were made to see whether similar constitutional changes and antigenic reversions were forthcoming with R type strains other than R_{10}.

B. *Experiments on strain* R_{20}

As shown in Table 3, strain R_{20} can be divided in four constitutionally different stages: generation 1 to 4, 5 to 14, 15 to 54 and 55 to 66. From the principal-latent system in the first stage, the antigenic constitution of the strain changed to the single system in the second stage followed by another principal-latent system in the third stage and then to the principal-accessory system in the final stage. One step reversion from R_{20} to O was observed only in the second stage and the reversions seen in the

other stages except that in the first stage, where no relapses were observed, were all two step reversions.

Table 3. Experiment on strain R_{20}. Examination of the antigenic constitution and antigenic reversion to 0.

Generation No. of parasites in mice	$R^{1}_{20} \sim R^{4}_{20}$	$R^{5}_{20} \sim R^{14}_{20}$	$R^{15}_{20} \sim R^{54}_{20}$	$R^{55}_{20} \sim R^{66}_{20}$
Cross screening	$R^{2}_{20} \longrightarrow O^{94}+$ $O^{107} \rightarrow R^{2}_{20}-$ $R^{4}_{20} \longrightarrow O^{96}+$ $O^{110} \rightarrow R^{4}_{20}-$	$R^{5}_{20} \longrightarrow O^{102}+$ $O^{110} \rightarrow R^{5}_{20}+$ $R^{8}_{20} \longrightarrow O^{105}+$ $O^{113} \rightarrow R^{8}_{20}+$ $R^{9}_{20} \longrightarrow O^{107}+$ $O^{115} \rightarrow R^{10}_{20}+$ $R^{14}_{20} \longrightarrow O^{111}+$ $O^{120} \rightarrow R^{14}_{20}+$	$R^{15}_{20} \longrightarrow O^{111}+$ $O^{120} \rightarrow R^{15}_{20}-$ $R^{16}_{20} \longrightarrow O^{113}+$ $O^{121} \rightarrow R^{17}_{20}-$ $R^{17}_{20} \longrightarrow O^{114}+$ $O^{21} \longrightarrow R^{17}_{20}-$ $R^{19}_{20} \longrightarrow O^{117}+$ $O^{123} \rightarrow R^{19}_{20}-$ $R^{35}_{20} \longrightarrow O^{132}+$ $O^{144} \rightarrow R^{33}_{20}-$ $R^{50}_{20} \longrightarrow O^{146}+$ $O^{154} \rightarrow R^{50}_{20}-$ $R^{53}_{20} \longrightarrow O^{151}+$ $O^{157} \rightarrow R^{52}_{20}-$	$R^{55}_{20} \longrightarrow O^{153}-$ $O^{157} \rightarrow R^{55}_{20}-$ $R^{58}_{20} \longrightarrow O^{155}-$ $O^{162} \rightarrow R^{57}_{20}-$ $R^{61}_{20} \longrightarrow O^{160}-$ $O^{168} \rightarrow R^{64}_{20}-$
Determination of antigenic type of parasites	R_{20} + O −	R_{20} + O −	R_{20} + O −	R_{20} + O +
Serotype determ. of serum	R_{20} + O +	R_{20} + O −	R_{20} + O +	R_{20} + O +
Identification of antigenic type of relapse parasites	no relapse	$R^{10}_{20} \longrightarrow O$ $R^{12}_{20} \longrightarrow O$	$R^{17}_{20} \rightarrow R^{8}_{1} \rightarrow O$ $R^{35}_{20} \rightarrow R^{8}_{10} \rightarrow O$	$R^{55}_{20} \rightarrow R^{10}_{1} \rightarrow O$ $R^{60}_{20} \rightarrow R^{7}_{10} \rightarrow O$
Antigenic constitution	principal-latent	single	principal-latent	principal-accessory

C. *Experiments on strain R_2*

Three stages are represented by initial and final single systems with a principal-latent system between them in this strain. One step reversion was only traceable in the stages of the single antigenic system. (Table 4).

D. *Experiments on strain R_3*

As demonstrated in Table 5, the constitutional change in this strain is from

a single to a principal-latent and then principal-accessory system. The incidence of one step reversion from R_3 to O is limited to the first stage corresponding to the single antigenic system.

Table 4. Experiment on strain R_2.

Generation No. of parasites in mice	$R_2^1 \sim R_2^{17}$	$R_2^{18} \sim R_2^{42}$	$R_2^{43} \sim R_2^{67}$
Cross screening (example)	$R_2^{15} \longrightarrow O^{21}+$ $O^{24} \longrightarrow R_2^{17}+$	$R_2^{28} \longrightarrow O^{37}+$ $O^{32} \longrightarrow R_2^{18}-$	$R_2^{63} \longrightarrow O^{69}+$ $O^{52} \longrightarrow R_2^{43}+$
Determ. of antigenic type of parasites	R_2 + O −	R_2 + O −	R_2 + O −
Serotype determ. of serum	R_2 + O −	R_2 + O +	R_2 + O −
Identification of antigenic type of relapse parasites	$R_2^6 \longrightarrow O$ $R_2^{14} \longrightarrow O$	$R_2^{31} \rightarrow R_4^{20} \rightarrow O$ $R_2^{36} \rightarrow R_{23}^{6} \rightarrow O$	$R_2^{43} \longrightarrow O$ $R_2^{64} \longrightarrow O$
Antigenic constitution	Single	Principal-latent	Single

Table 5. Experiment on strain R_3.

Generation No. of parasites in mice	$R_3^1 \sim R_3^{21}$	$R_3^{22} \sim R_3^{41}$	$R_3^{42} \sim R_3^{60}$
Cross screening (example)	$R_3^7 \longrightarrow O^{89}+$ $O^{111} \rightarrow R_3^{21}+$	$R_3^{31} \longrightarrow O^{100}+$ $O^{112} \rightarrow R_3^{22}-$	$R_3^{42} \longrightarrow O^{122}-$ $O^{122} \rightarrow R_3^{43}-$
Determ. of antigenic type of parasites	R_3 + O −	R_3 + O −	R_3 + O +
Serotype determ. of serum	R_3 + O −	R_3 + O +	R_3 + O +
Identification of antigenic type of relapse parasites	$R_3^{19} \longrightarrow O$ $R_3^5 \longrightarrow O$	$R_3^{36} \rightarrow R_{10}^{5} \rightarrow O$ $R_3^{38} \rightarrow R_5^9 \rightarrow O$	$R_3^{45} \rightarrow R_{10}^{9} \rightarrow O$ $R_3^{56} \rightarrow R_2^4 \rightarrow O$
Antigenic constitution	Single	Principal-latent	Principal-accessory

As explained in experiments A to D above, strain R indicates not only the characteristic antigenic type of the strain, but also the O type during the course of maintenance by passage through mice. The antigenic constitution of the R strain can be analyzed into the single and compound systems. The latter can be further subdivided into the principal-latent and principal-accessory systems according to the mode of coexistence with O.

In the principal-latent antigenic system, as cited above, the cross screening method between R and O is one sided, only R→O being possible. R is the only antigenic type of the parasite shown, while both R and O are demonstrated in serotype determinations of antisera. On the other hand, the cross screening method is not positive in either direction and both R and O are identified in the parasites and sera in the principal-accessory antigenic system.

It is interesting that the R type strain can show the single and compound, and principal-latent and principal-accessory antigenic systems, during passages in mice and that the reversibility from R to O is seemingly under the control of these systems.

3. *Correlation between the antigenic constitution and the reversion from R to O*

In previous papers the authors have shown that a relapse strain derived from O type strain contained only a single R antigenic type and the first relapse strain in mice which recovered from infection of the R strain reverted to O type strain. We have proposed a scheme for the direction of antigenic variations or a variation system as shown in Fig. 1.

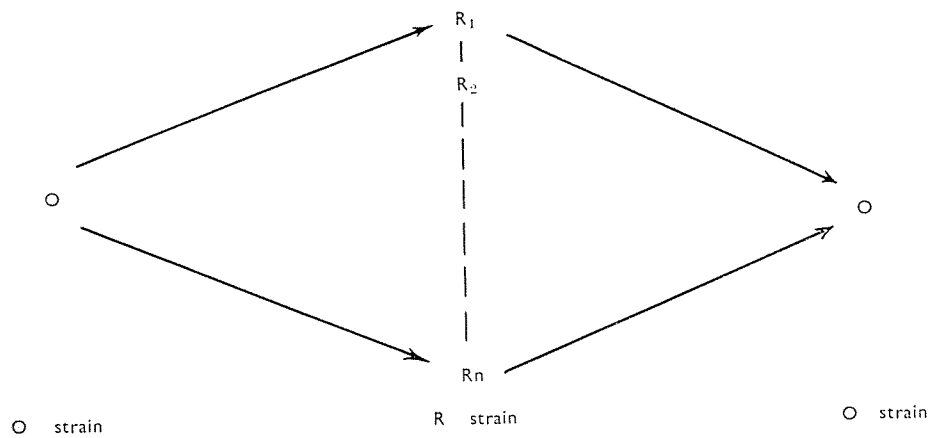


Fig. 1. General rule for the direction of antigenic variation

Since the variation from R to O was particularly convenient for the investigation of the induction of variation, it was named the one step reversion from R to O. Further detailed studies, however, have shown that one step reversion is not always the case in relapses and that variation towards some other type is sometimes noticeable.

In the present study, the antigenic constitutions of all relapse strains have been classified into three antigenic systems and the number of reversions from R to O in each antigenic system can be counted as one, and two or more steps (see Table 6). One step reversion is experienced in 95.8 per cent of the reversions in the single antigenic system while all reversions in the compound antigenic system take place by two or more steps. As to the three exceptions in the single system, it is unknown whether

they were due to some experimental mismanagement or to a more delicate constitutional control.

Table 6. Relationship between the antigenic constitution and antigenic reversion to O.

Antigenic constitution	Single	Compound	
		Principal-latent	Principal-accessory
No. of relapses in R strains	$R\frac{1}{2} \sim R\frac{17}{2}$ 9	$R\frac{18}{2} \sim R\frac{42}{2}$ 5	$R\frac{55}{20} \sim R\frac{66}{20}$ 5
	$R\frac{43}{2} \sim R\frac{67}{2}$ 7	$R\frac{67}{3} \sim R\frac{159}{3}$ 20	$R\frac{42}{3} \sim R\frac{60}{3}$ 7
	$R\frac{1}{3} \sim R\frac{66}{3}$ 30	$R\frac{15}{20} \sim R\frac{54}{20}$ 9	$R\frac{34}{10} \sim R\frac{50}{10}$ 5
	$R\frac{160}{3} \sim R\frac{166}{3}$ 5	$R\frac{22}{3} \sim R\frac{41}{3}$ 4	
	$R\frac{5}{20} \sim R\frac{14}{20}$ 4	$R\frac{24}{10} \sim R\frac{33}{10}$ 3	
	$R\frac{1}{3} \sim R\frac{21}{3}$ 4		
	$R\frac{1}{10} \sim R\frac{23}{10}$ 14		
Total No. of relapse	73	41	17
No. (%) of single step reversions in all relapses	70 (95.8)	0	0
No. (%) of two or more step reversions in all relapses	3 (4.2)	41 (100)	17 (100)

The symbols $R\frac{1}{2} \sim R\frac{17}{2}$ 9 mean that 9 relapses were found between the first and the seventeenth generation of mouse-passage in strain R_2.

From an analytical study of the antigenic constitution of *T.g.*, it was understood that R type strain was transferable by three antigenic systems and that the direction of antigenic variation in mice was sharply controlled by these antigenic systems.

DISCUSSION

Studies on immunological variation in trypanosomes first begun by Ehrlich (1909), Levaditi (1909) and others have been extensively developed by a number of investigators. Analytical studies on the mechanism of variation, however, have not been made extensively until the present.

The serial studies by Inoki and his collaborators since 1948, has proceeded not only along immunological lines but also by genetical analysis of the problem. Noteworthy among these findings is the discovery that antigenic variation of *T.g.* in mice is directly inducible by the antiserum of the serotype corresponding to the antigenic type of the parasites. Moreover, they observed that as a general rule, variation is in the direction, $O—R_{\overline{1\sim n}}O$, and a variation system (Fig. 1) leading to the advocacy of an adaptation theory for this antigenic variation was established.

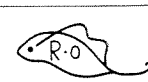
As cited above, the latter half of this general rule, the so called one step reversion from R to O, was not applicable to all relapses but variation towards some other R

type, for example from R_2 to R_4, was sometimes observed.

The lack of a very suitable method of investigation, such as the cross screening method, which is reliable for the identification of the antigenic types of two strains, prevented understanding of the mechanism of this variation system.

The present study is a supplementary analysis of the variation and the antigenic constitution of the parasites and the results obtained are summarized in Table 7.

Table 7. Antigenic constitution of *Trypanosoma gambiense*.

Antigenic constitution	Single	Compound	
		Principal-latent	Principal-accessory
Cross screening	R ⟶ O + O ⟶ R +	R(o) → O + O ⟶ R(o) −	R.o → O − O ⟶ R.o −
Determ. of antigenic type of parasites	R + O −	R + O −	R + O +
Serotype determ. of serum	R + O −	R + O +	R + O +
Antigenic reversion	One step	Two or more steps	Two or more steps
Scheme of antigenic type			

The compound antigenic system was demonstrated and the principal-latent and principal-accessory antigenic systems were distinguished mainly by the cross screening method. The finding that the antigenic constitution of the R type strain is interchangeable among these systems during the course of passage is of importance in understanding the direction of the antigenic variation. The fact that the parasites in the compound antigenic system never change the antigenic type from R to O by a single step, suggest a possible explanation for the exceptions to the R—O variation rule. The previously established R—O rule is likely to be modified preferably to such a form that the R type strain in the single antigenic system is reversible to the O type strain by a single step but that one step is not possible in the compound system. It is of much interest that the O type antigen, appearing subordinately as a latent or accessory antigen, plays a controlling roll in the reversion from R to O.

The latent antigen O is not demonstrable. However the accessory antigen O is demonstrable in R type parasites, while the O antibody is detectable either in sera of mice which have recovered from infection with parasites of the principal-accessory system or from those of the principal-latent system. These facts may indicate that there is not a sufficient quantity of the latent antigen to be detected or that, although definitely present, it is concealed inside the parasite's body. No real understanding of

the problem, however, can be gained from the facts known at present.

Among investigations involving inducible antigenic variations in microorganism by antisera, the study on *Paramecium aurelia* by Sonneborn and his co-workers is the most interesting in connection with this study on *T.g.* The antigenic type of *P. aurelia* is subject to change by the appropriate antiserum. The immobilization reaction was used in Sonneborn *et al*'s study, but the animals could react, even though very weakly, to antiserum of a different type from that of the animals. That is, there was a group reaction. This group reaction, however, had no significant correlation with the direction of variation and the change of antigenic constitution of the animals. *P. aurelia* and *T.g.* are quite different in biological characteristics, and in the investigation on *T.g.* different methods from those used for work on *P. aurelia* were used. Thus, it may be improper to compare the findings obtained in this study with those from the study of *P. aurelia.*

Antigenic variation in bacteria such as those on *Escherichia coli*, *Haemophilus pertussis*, *Diplococcus pneumoniae* and *Salmonella* group have been some of the most interesting problems in genetics. The present study on the immunological variation in *T.g.* not only from the immunological standpoint but also in relation to cytoplasmic genetics is believed to contribute to the progress of genetics in bacteria, paramecia and other protozoa.

If *T.g.*, the protozoan causing sleeping sickness in central Africa, is subject to change in antigenic type and expresses its original as well as variant types simultaneously, some complications in the immunology and epidemiology of the parasite may develop. As described by Kroó (1913), this sort of protozoa may revert in antigenic type to the same type by passing through the vector, the tse-tse fly. Even if so, it can be thought that the authors' study on *T.g.* still has significance in the field of experimental immunology and genetics.

REFERENCES

Beale, G.H. (1948). The process of transformation of antigenic type in *Paramecium aurelia* (variety 4) *Proc. Natl. Acad. Sci. U.S.* **34**, 418-423.

Beale, G.H. (1954). *Genetics of Paramecium aurelia.* Cambridge Univ. Press, London.

Ehrlich, P. (1909). Über Partialfunktion der Zelle. *Münch. Med. Wochschr.* **56**, 217-222.

Ehrlich, P., Roehl, W. und Gulblausen, R. (1909). Über serumfeste Trypanosomenstämme. Bemerkungen zu der Arbeit von Levaditi und Mutermilch. *Z. Immunitätsforsch.* **3**, 296-299.

Ephrussi, B. (1952). *Nucleo-cytoplasmic relation in micro-organisms.* Oxford Univ. Press, London.

Franke, E. (1905). Über Trypanosomentherapie. *Münch. Med. Wochschr.* **52**, 2095-2060.

Fulton, J.D. and Lourie, E.M. (1946). The immunity of mice cured of Trypanosoma infections. *Ann. Trop. Med. Parasitol.* **40**, 1-9.

Inoki, S. (1952). A new experimental methods and genetical interpretation of the antigenic variation in *Trypanosoma gambiense. Med. J. Osaka Univ.* **3**, 81-86.

Inoki, S., Kitaura, T., Nakabayashi, T. and Kurogochi, H. (1952a). Studies on the immunological variations in *Trypanosoma gambiense.* I.A. new variation system and a new expersmental method. *Med. J. Osaka Univ.* **3**, 357-371.

Inoki, S., Kitaura, T., Osaki, H. and Nakabayashi, T. (1952b). Genetical studies on the antigenic variation in *Trypanosoma gambiense. Japanese J. Genetics.* **27**, 85-92.

Inoki, S., Osaki, H. and Nakabayashi, T. (1956). Studies on the immunological variation in

Trypanosoma gambiense. II. Verification of the new variation system by Ehrlich's and *in vitro* methods. *Med. J. Osaka Univ.* **7**, 165-173.

Inoki, S., Nakabayashi, T., Osaki, H. and Fukukita, S. (1957). Studies on the immunological variation in *Trypanosoma gambiense*. III. Process of the antigenic variation in mice. *Med. J. Osaka Univ.* **7**, 731-743.

Inoki, S., Nakabayashi, T., Fukukita, S. and Osaki, H. (1959). Studies on the immunological variation in *Trypanosoma gambiense*. IV. Further considerations on the screening method. *Biken's J.* **2**, 277-283.

Inoki, S. (1960). Studies on antigenic variation in the Welcome strain of *Trypanasoma gambiense*. I. Improvements in technique. *Biken's J.* **3**, 215-222.

Inoki, S. (1960). Studies on antigenic variation in the Welcome strain of *Trypanosoma gambiense*. II. On the first relapse appearing in mice treated with human plasma, *Biken's J.* **3**, 223-228.

Kroó, H. (1925). Beitrag zur Immunbiologie der Trypanosomen. Über Stämmeinheit und Artenheit der *Trypanosoma brucei*. *Z. Hyg. Infektionskrankh.* **105**, 247-253.

Kroó, H. (1926). Weiterer Beitrag zur Immunbiologie der Trypanosomen. Zur Kritik des Kreuzinokulatiosverfahrens als immunbiologische methode der Artabgrenzung. *Z. Hyg. Infektionskrankh.* **106**, 77-82.

Levaditi, C. et Mutermilch, S. (1909). Le mécanisme de la création des variétés de trypanosomes résistants aux anticorps. *Compt. Rend. Soc. Biol.* **66**, 49-51.

Levaditi, C. et Mc Intosh, J. (1910). Mécanisme de la création de races de trypanosomes résistantes aux anticorps. *Bull. Soc. Pathol. Exot.* **3**, 368-376.

Lourie, E.M. and O'Connor, R. (1937). A study of *Trypanosoma rhodesiense* relaps strains *in vitro*. *Ann. Trop. Med. Parasitol.* **31**, 319-340.

Mutermilch, S. et Salamon, E. (1928a). Contribution à l'étude de la création des Trypanosomes du Nagana anticorps-résistantes. *Compt. Rend. Soc. Biol.* **98**, 345-347.

Mutermilch, S. et Salamon, E. (1928b). Contribution à l'étude du mécanisme de la crise chez le Cobaye Trypanosomié. *Compt. Rend. Soc. Biol.* **98**, 348-350.

Neuman, R. (1911). Zur Kentniss der Immunität bei experimenteller Trypanosomeninfektion. *Z. Hyg. Infektionskrankh.* **69**, 109-134.

Osaki, H. (1959). Studies on the immunological variation in *Trypanosoma gambiense* (serotypes and the mode of relapse). *Biken's J.* **2**, 113-127.

Ritz, H. (1916). Über Rezidive bei experimenteller Trypanosomiasis. *Arch. Schif. Trop. Hyg.* **20**, 397-420.

Sonneborn, T.M. and Lesuer, A. (1948). Antigenic characters in *Paramecium aurelia* (variety 4). Determination, inheritance and induced mutations. *Am. Naturalist.* **82**, 69-78.