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Experimental Infections with African Trypanosomes: IV. Immunization of Cattle with Gamma-Irradiated *Trypanosoma rhodesiense*

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WELLDE, B. T., DUXBURY, R. E., SADUN, E. H., LANGBEHN, H. R., LÖTZSCH, R., DEINDL, G., AND WARUI, G. 1973. Experimental infections with African trypanosomes: IV. Immunization of cattle with gamma-irradiated *Trypanosoma rhodesiense*. *Experimental Parasitology* **34**, 62–68. Cattle immunized with gamma-irradiated *Trypanosoma rhodesiense* were protected against a challenge inoculation of unirradiated trypanosomes given 1 wk later. These animals were still resistant when rechallenged after 8 mo and some residual immunity persisted for 14 mo after immunization. Animals which had undergone self-cure after infection were resistant when challenged 14 mo later. No resistance was observed in animals challenged with a heterologous strain of *T. rhodesiense*. The infections observed in unprotected bovines were of a mild nature. Leukopenia and fever were common early in the course of infection, but no anemia was evident at any time.

INDEX DESCRIPTIONS: *Trypanosoma rhodesiense*; Immunization; Cattle; Gamma radiation.

Trypanosoma rhodesiense can be passaged experimentally through many different species of animals without losing its infectivity for man (Corson 1939; Fairbairn and Burtt 1946). When Heisch *et al.* (1958) experimentally transmitted *T. rhodesiense* from a naturally infected bush buck (*Trangelaphus scriptus*) to man they provided direct evidence that wild game animals are reservoirs of the parasites causing human sleeping sickness. Wilde and French (1945) showed that domestic cattle are susceptible to *T. rhodesiense* and postulated that these animals could also act as reservoir hosts for sleeping sickness. This was demonstrated during an epidemic of sleeping sickness in Western Kenya in 1963 when trypanosomes isolated from cattle produced an infection in man typical of that caused by *T. rhodesiense* (Onyango *et al.* 1966).

Duxbury and Sadun (1969) reported that irradiated *T. rhodesiense* induced a strong resistance in laboratory rats and mice to a challenging infection with the same parasite. These observations were confirmed and extended by further investigations in rodents and primates (Duxbury *et al.* 1972).

Since domestic cattle may play an important role in the transmission of human sleeping sickness, experiments were undertaken to study trypanosomiasis in cattle. Particular emphasis was placed on the induction and

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persistence of immunity produced in bovines by inoculation with *T. rhodesiense* attenuated by exposure to gamma radiation.

Materials and Methods

Experimental animals. Twenty-one cattle [2] of both sexes were used in four experiments. In a pilot experiment (Expt I) Hereford cattle were used. Animals in Expts II–IV were mainly of a Boran breed weighing between 400 and 700 lb. The cattle were provided by the Veterinary Department, Kabete, Kenya, and were maintained at the Trypanosomiasis Research Laboratory in Kabete. Laboratory rats were used as a source of immunizing and challenging inocula and mice were subinoculated with blood from the cattle to detect subpatent parasitemias.

Parasites. A monomorphic strain of *T. rhodesiense* (Wellcome) which had been maintained in laboratory rats for many years and a relatively recent isolate of *T. rhodesiense* (EATRO 1886) were used in these studies.

Parasites for immunization and challenge were obtained from infected laboratory rats. The rats were anesthetized with ether and bled by cardiac puncture with a syringe containing heparin. In the first experiment whole infected rat blood was irradiated and inoculated into the cattle. In Expts II and III infected blood was centrifuged for 10 min at 1000*g* and the plasma discarded. The thick buffy coat was then transferred to a 0.01 *M* phosphate buffered saline solution (pH 7.8) containing 5% (w/v) glucose and 10% (v/v) fetal bovine serum. The buffy coat was washed twice at 1000*g* and reconstituted with the buffer. The number of trypanosomes in the resulting suspension was determined by diluting an aliquot of the sample in a red blood cell pipette with a 0.05% Nile blue sulfate solution. After 15 min the pipette's contents were mixed, expressed into a hemacytometer, and the trypanosomes counted. Immunizing inocula were irradiated in a Gammacell cobalt 60 irradiator at approximately 70 krads. Immunizing and challenging doses of *T. rhodesiense* were injected into the jugular veins of the cattle. Each immunizing inoculum of irradiated trypanosomes was also injected into rats to determine whether the parasites had been rendered noninfective by their exposure to irradiation.

Patent infections in the cattle were detected by examining approximately 200 oil immersion fields on Giemsa-stained thick blood films obtained from capillary blood of the tail. To detect subpatent infections, 0.5 ml aliquots of blood from the jugular vein were inoculated intraperitoneally into mice. Rectal temperatures were done 6 days per week while packed cell volumes and leukocyte counts were done weekly.

TABLE I

Number of Irradiated Trypanosomes ($\times 10^9$) in *Immunizing Inoculations*

Experiment no.	Animal no.	Day of immunization					
		0	4–5	7	11	13	15–16
II	6359	18.4	9.0	8.0	5.4	10.1	7.8
	6360	22.9	9.0	8.0	5.4	10.9	7.8
III	6340	4.6	11.0	6.0	5.7	6.1	4.4
	6351	4.6	11.0	6.0	5.7	4.6	4.4
	6358	4.6	11.0	6.0	5.7	6.1	4.4

Results

All cattle were tested and proven negative for subclinical trypanosomiasis by both blood films and subinoculation before their inclusion in the study.

Experiment I. Three Hereford cattle were given three injections of irradiated whole rat blood containing *T. rhodesiense.* Three $\times$ 10^9 irradiated trypanosomes were inoculated in three equal doses given 1 wk apart. One week after the third injection, the three experimental animals and three untreated con-

[2] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Scienses–National Research Council.

TABLE II

Results of Challenge with the Homologous Strain of Trypanosoma rhodesiense *(Wellcome) 1 wk after Immunization*[a]

Group	Weeks after challenge																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Exp II																						
Immunized																						
6359	−	−	−	−		−		−	−		−		−					−	−			−
6360	−	−	−	−		−		−	−		−		−					−	−			−
Control																						
6346	−	⊕	⊕	+		⊕	○	+	+		−		−					−	−			−
6364	−	⊕	⊕	⊕		⊕		+	+		+		−					−	−			−
Exp III																						
Immunized																						
6340	−		−	−		−		−		−					−		−		−			
6351	−		−	−		−		−		−					−		−		−			
6358	−		−	−		−		−		−					−		−		−			
Control																						
6350	−	○	⊕	⊕		+		+		+					−		−					
6374	−	○	⊕	+		−		+		+					−		−		−			
6390	−	○	⊕	+		+		+		+					−		−		−			

[a] Negative subinoculation (−); positive subinoculation (+); weeks (○) during which parasites were observed microscopically.

trols were each challenged with 1×10^5 viable trypanosomes. The three controls became patent on the third day after challenge and two experimental animals became patent on days 6 and 13. The third experimental animal did not develop a patent infection. No parasites could be found in this animal by blood smear examination or by subinoculating its blood into mice. When the animal was rechallenged with 1×10^5 viable *T. rhodesiense* 5 mo after the first challenge, a patent infection developed.

Experiment II and III. These experiments were conducted in order to determine whether a stronger degree of immunity could be obtained with larger numbers of irradiated trypanosomes relatively free from contaminating rat host materials (Table I). Stained smears of the immunizing suspensions contained only a few erythrocytes and leukocytes although thrombocytes were abundant. In both experiments the injections were well tolerated by the animals and no evidence of anaphylaxis was observed. Packed cell volumes, leukocyte counts and body temperatures showed no abnormal variation during the period of immunization. One week after the last immunizing injection the animals were challenged with approximately 1×10^4 viable *T. rhodesiense.* Although the control animals in both experiments developed patent infections, no parasites were seen in the blood of the cattle which had previously received irradiated parasites. Blood from the immunized cattle did not infect mice, but blood from the control animals produced infections in mice for 8–10 wk after challenge (Table II). Although the packed cell volumes did not show abnormal variation in either immunized or control animals after challenge,

TABLE III

Development of Immunity in Rats after a Single Inoculation of Irradiated Trypanosoma rhodesiense

Group	No. of trypanosomes	Time of challenge (5×10^4) (days after immunization)	Survivors	Day of death
	Immunization ($\times 10^8$) Challenge (5×10^4)		No. challenged	
1	1.6	22	2/3	14
2	3.9	17	2/3	20
3	2.1	15	3/3	—
4	2.0	11	2/3	14
5	2.5	9	1/2	13
6	2.2	7	3/3	—
7	Controls	—	0/10	5.6 (5–7)[a]

[a] Mean and (range).

a leukopenia developed in the controls during the onset of patent parasitemia but usually subsided within a week. Fevers were detected in all the controls at the time trypanosomes first appeared in the blood and in some of them at the time parasites reappeared in large numbers.

Rats were given single injections of irradiated trypanosomes from the same aliquots as those used for cattle. None of the rats became infected. When these animals were subsequently challenged with unirradiated trypanosomes, all the control rats died of the infection, but 76% of the rats that had received a single immunizing inoculation were protected against challenge (Table III).

All experimental and control cattle of Expts II and III were rechallenged approximately 8 mo after the initial challenge. Three additional animals which had never been infected with *T. rhodesiense* were also challenged at the same time for use as controls (Table IV). The immunized animals again did not develop patent infections and their blood did not infect mice after subinoculation up to 3 mo after challenge. The animals which had served as controls for the challenging inoculations in Expts II and III had also become resistant to challenge as judged both by blood smears and subinoculation. However, the three normal control animals became infected and were positive on subinoculation for periods of 4–9 wk. The animals of Expt II were challenged for the third time 14 mo after immunization along with two untreated control animals. This challenge, unlike those given 1 wk and 8 mo after immunization, produced detectable infections both in immunized and untreated control cattle. However, the prepatent periods of the immunized animals were prolonged (7 days) as compared to those of their controls (3 and 4 days). Previously infected animals which had undergone spontaneous cures again resisted the challenging infection (Table IV).

Experiment IV. To determine whether or not resistance to a different strain of *T. rhodesiense* was elicited by immunization or infection of cattle with the Wellcome strain, the animals of Expt III were rechallenged with a more recently isolated strain of *T. rhodesiense* (EATRO 1886). No obvious differences in prepatent periods or intensity of infections were observed between immunized, spontaneously cured, or untreated control animals.

Discussion

Inoculations of irradiated *T. rhodesiense* induced a strong resistance of a lasting nature in cattle. Immunized animals were considered to be parasite-free as a result of

TABLE IV

Results of Challenge with the Homologous Strain of Trypanosoma rhodesiense (*Wellcome*) *8–14 Mo after Immunization*[a]

Group and animal no.	Time of Challenge[b] (mo)	Weeks after challenge												
		0	1	2	3	4	5	6	7	8	9	10	11	12	13
Immunized															
6359	8	−	−	−	−	−			−		−		−		−
6360		−	−	−	−	−			−		−		−		−
6340		−	−	−	−	−			−		−		−		−
6351		−	−	−	−	−			−		−		−		−
6358		−	−	−	−	−			−		−		−		−
6359	14	−	⊕	⊕	⊕		+	+		+		−		+	
6360		−	⊕	⊕	⊕		+	+		+		+		+	
Self-cured															
6346	8	−	−	−	−	−			−		−		−		−
6364		−	−	−	−	−			−		−		−		−
6350		−	−	−	−	−			−		−		−		−
6374		−	−	−	−	−			−		−		−		−
6390		−	−	−	−	−			−		−		−		−
6346	14	−	−	−	−		−	−		−		−		−	
6364		−	−	−	−		−	−		−		−		−	
Susceptible controls															
204	8	−	⊕	+	⊕	⊕	⊕		+		−		−		−
6399		−	−	⊕	⊖	⊖	−	⊕	+		+		−		−
6495		−	⊕	+	⊕	⊕			−		−		−		−
6623	14	−	⊕	⊕	⊕	⊕	+	+		+		+		−	
6638		−	⊕	⊕	+		+	+		+		−		−	

[a] Negative subinoculation (−); positive subinoculation (+); weeks (○) during which parasites were observed microscopically.

[b] Time since immunization.

negative blood smears and repeated subinoculations of blood into mice. These results confirm and extend those reported by Duxbury and Sadun (1969) in rats and mice. On the basis of our findings one cannot determine with certainty whether the immunity observed in cattle to *T. rhodesiense* is indeed a sterile immunity. The possibility exists that predilection of the parasite for connective tissues as reported in other hosts might have resulted in negative subinoculations. This seems unlikely, however, in view of the fact that all control animals were positive upon subinoculation while immunized animals were consistently negative. Blood samples of some control animals were not positive on every subinoculation. Whether the number of parasites in the subinoculated blood was below the infectivity level for mice or whether the parasites were sequestered in the tissues on these occasions is not known.

When cattle were immunized by infection and chemotherapy, a resistance against challenge developed which persisted for 8 mo (Cunningham 1968). Our results are in agreement with these observations since immunity persisted at a high level for at least

8 mo in the immunized cattle. Although some degree of immunity was detected in these animals 14 mo after immunization it was not complete. The animals which had undergone infections resulting in spontaneous cure were resistant to reinfection both at 8- and 14-mo intervals after infection, indicating that the stimulus provided by the actual infection induces a more persistent immunity than that produced by irradiated parasites.

The immunity demonstrated in our experiments is surprising in view of the well-known ability of trypanosomes to vary their antigenic constituents. We considered the possibility that our monomorphic laboratory strain (Wellcome) might have lost its ability to undergo antigenic variation after repeated blood passage in laboratory animals. However, antigenic variations were found to occur with this strain (Cunningham, personal communication). Moreover, results from experiments involving mice and rhesus monkeys (Duxbury *et al.* 1972) indicate that a strong resistance could also be induced in these animals by immunization with irradiated trypanosomes of a recently isolated strain of *T. rhodesiense*.

Immunization with attenuated parasites (Sanders and Wallace, 1966; Duxbury and Sadun 1969; Duxbury *et al.* 1972) has generally been more effective than that achieved by immunizing with nonliving trypanosomal antigens (Kligler and Comaroff 1935; Weitz 1960; Johnson *et al.* 1963). Although the irradiated trypanosomes are apparently not capable of replication, they remain motile for some time and presumably retain a portion of their metabolic integrity (Halberstaedter 1938). Strain specific protection induced by exoantigens has been reported (Weitz 1960) and it is likely that release of metabolic antigens by the attenuated but living parasites is responsible for the strong immunity induced by irradiated trypanosomes.

It is possible that rat host contaminants of the immunizing inocula played a role in the resistance observed but this seems unlikely in view of the fact that the contaminants were maximal in inocula which were less protective (Expt I) and minimal in inocula which were more protective (Expts II, III). Resistance was also not present in either immunized or recovered animals when challenged by a different strain.

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