

Auto-Sterilization in Trypanosomiasis.

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DURING the course of experiments on chemotherapy in Trypanosomiasis due to *Trypanosoma congolense* and *Trypanosoma vivax*, it was observed that infected animals, some considerable time after treatment or even without treatment, made spontaneous recoveries. This article is for the purpose of placing the various cases on record.

The term auto-sterilization is used to denote the attainment of a completely sterile condition, as far as the particular parasite is concerned, in the absence of any treatment or subsequent to a relapse without further treatment being instituted. It is the spontaneous cure without chemo-therapeutical interference. Obviously the determination of the sterilized state is dependent on the technique utilized for determining whether the parasite is still present or not. Microscopic tissue examinations are somewhat unreliable for in many cases careful daily blood smear examinations over long periods have failed to reveal the parasites in known positive cases. The sub-inoculation of blood into rabbits, guinea-pigs and mice is also unreliable but that into animals of the same species has given very good results although failures by this method have also been met with. Re-inoculation of the animal with the homologous parasite is of assistance and was used in a number of cases. Probably the best method is the complement fixation test but technical difficulties have interfered with its application in the trypanosomiasis other than those caused by *T. brucei* and *T. equiperdum*.

The literature of recent years gives references to auto-sterilization in the trypanosomiasis. There is a record by van den Branden (1926) of two patients who relapsed after treatment and who subsequently, without further treatment, showed no further parasites during a period of observation of two years. His examination comprised lumbar puncture and blood examination. Dyleff (1932) records his failure to detect, by triple centrifugation, trypanosomes in three Europeans who had previously been known carriers. Sen (1936) working with Surra in bovines obtained negative results when he sub-inoculated from untreated previously positive bovines into horses. Browning and Gulbransen (1935) record apparently spontaneous cure in mice infected with *T. brucei*. Manresa and Gonzalez

(1935) utilizing for the purposes of diagnosis microscopic examinations, animal sub-inoculations and complement fixation test show that a number of bovines infected with *T. evansi* made spontaneous recoveries. The period intervening from the time these animals were found to be positive to the time they gave negative results with the complement fixation test varied from 6 to 10 months. They say that revision is needed of the assumption so generally held that oxen once infected with *T. evansi* remain infected during their whole lifetime. Von Saceghem (1936) records spontaneous recoveries in calves inoculated with *T. congolense*. He tested by sub-inoculation but in his cases the recovered animals resisted subsequent re-infection.

OBSERVATIONS ON AUTO-STERILIZATION.

Auto-sterilization has been observed in *T. vivax* and *T. congolense* but not in *T. brucei* or *T. equiperdum* infections. If it were not that the complement fixation test was available for the last two there would possibly have been a decision that auto-sterilization had also occurred in these for microscopic blood examination, sub-inoculations and re-infection all were at times inefficient for diagnostic purposes.

T. equiperdum INFECTION.

The diagnosis of *T. equiperdum* infection or dourine of horses is based almost entirely on the complement fixation test, the antigen used being *T. equiperdum* obtained from small laboratory animals. The sub-cutaneous inoculation of this parasite into natural dourine cases results in an acute reaction with the appearance of the parasites in blood smears, with a severe drop in the number of red cells and in the red cell volume, with exacerbations and remissions of temperatures and with a rapid loss of condition. The infected horses in other words have no premonition against the parasite which gives a positive C.F. Test when used as an antigen. If such re-infected animals are suitably treated there is a disappearance of the parasites from the blood which is permanent when judged by smear examination, a return to previous normality of red cell count, of red cell volume, of temperature and of condition but, notwithstanding, the horse remains positive to the C.F. Test carried out 16 months later. If it were not for the availability of this test, the horse might easily be regarded as sterilized. Only in one of the numerous smears examined has *T. equiperdum* been detected in smears and that was in a smear made from the mammary gland at autopsy in May 1935 by S. W. v. Rensburg of this section. If a susceptible horse be infected as above and treated, it becomes negative subsequently to the C.F. Test.

T. brucei INFECTION.

Horses infected with *T. brucei* and then treated may survive for long periods during which time microscopic examination may give negative results. Sub-inoculation into other horses may also at times give negative results but subsequent sub-inoculations with larger quantities of blood from such an animal have given positive results. The C.F. Test, *T. equiperdum* antigen being used, however always

gives a positive reaction in such cases. In donkeys daily negative smear examination over many months has been attained without the prior utilization of drugs. Such an infected donkey, not treated, has remained in good condition for the whole period of two and three quarter years. Both sub-inoculations into horses from this donkey and the C.F. Test give positive results. The cases of auto-sterilization in equines have thus only been apparent ones.

T. vivax INFECTION.

Five bovines infected with *T. vivax*, not treated were submitted to blood and gland smear examinations with positive results. Three of these bovines were killed as negatives as judged by smears between 18th and 44th months after injection. One died of a disease other than *T. vivax* infection at 2 years and the remaining one is still alive. From the last mentioned, (bovine 2611), eighteen sub-inoculations have been carried out only the first of which gave positive results. The first negative sub-inoculation was carried out 355 days after the date of infection.

T. congolense INFECTION OF SHEEP.

Auto-sterilization has been noted in a number of sheep. All the cases which have been observed were in infected sheep which were under uniform conditions of feeding and stabling. The details are recorded in Table I.

Discussion.

The above details reveal that in all three cases sub-inoculation into sheep and bovines gave negative results, that in each case the test animals were tested for susceptibility by the inoculation of known infected blood and that in one case the susceptibility of the sheep itself was tested by the inoculation of known infected blood.

T. congolense INFECTION OF BOVINES.

Bovines infected with *T. congolense* when kept under conditions of good food and stabling pass often from the first acute stage into the chronic form of the disease. If such chronic cases be exposed to adverse conditions especially cold and rain, a change to the peracute form may occur as previously reported by the writer(1935). It was, therefore, decided to expose to similar adverse conditions, to determine whether relapses would occur, a number of bovines which were in the state of premunition, as judged by the persistence of the parasite in animals which were normal as far as their blood examination was concerned.

The arrangement of the experiment was (1) to run premune bovines in open kraals, where they would be supplied with adequate rations, day and night throughout the various seasons, and (2) to place them subsequently on natural grazing without supplementing the grazing with rations.

TABLE I.

Sheep No.	Date of Infection.	Last + Smear.	Subsequent Smears.	Sub-inoculations.		Susceptibility Test.	
				Details.	Result.	Details.	Result.
26786	7.8.31	26.6.32	—	18.11.32: Blood (10 c.c. S.C. and 40 c.c. I.V.) into sheep 34129 15.12.32: Blood (20 c.c. S.C. and 80 c.c. I.V. into sheep 34129	Negative...	7.3.33: Known infected blood (25 c.c. S.C. and 75 c.c. I.V.) into sheep 34129	Positive.
41867	26.4.35	16.6.36	243 smears up to 23.4.37	20.11.36: Blood (20 c.c. S.C.) into bovine 6026	Negative...	12.1.37: Known infected sheep blood (20 c.c. S.C.) into bovine 6026	Positive.
37096	5.12.33	15.2.35	70 smears up to 23.8.35	11.12.36: Blood (10 c.c. S.C. and 40 c.c. I.V.) into bovine 6026 2.8.35: Blood (20 c.c. S.C.) into bovine 5189	Negative...	23.8.35: Known infected sheep blood (20 c.c. S.C.) into sheep 37906 23.8.35: Known infected sheep blood (20 c.c. S.C.) into bovine 5189	Positive. Positive.

S.C. Subcutaneous inoculation.
I.V. Intravenous inoculation.

The exposure under the first heading was carried out at Onderstepoort concurrently with the exposure of chronic cases which, as a result, relapsed into peracute cases. All the premune bovines remained normal and grew satisfactorily. The winter was, that year, particularly severe and the animals were exposed to heavy winter rains and sleet. Smear examinations were regularly done and all the animals showed, on occasion, *T. congolense* in blood smears.

The exposure under the second heading was carried out on a neighbouring farm, "Kaalplaas", in a camp which was heavily infested with ticks as dipping of the cattle had not been in operation for some years. In a few cases it was necessary to treat by hand the extensive ear damage produced by the ticks. No shelter was provided. In addition to the premune animals three bovines recently infected with *T. congolense* were added to the group as controls. All these controls died within a few weeks from *T. congolense* infection. In the premune bovines, no relapses were noted and they grew satisfactorily. A number of negative controls was also introduced. No cases of transmission occurred in these.

The number of premune bovines used was nine. The period of exposure under the first heading i.e. at Onderstepoort was 17 months (May 1930 to September 1931). Under the second heading it was 14 months (October 1931 to November 1932). The animals were submitted to a weekly examination which consisted of inspection for sickness and smear examination. During the first period no losses occurred but during the second period there was some mortality due to concurrent conditions. The last positive smear cannot be regarded as an indication of the commencement of sterilization for the reason that in all cases of premunition the trypanosomes are always difficult to detect. Consequently sub-inoculations into sheep and bovines in certain of the cases were undertaken at the close of the experiment to support the negative smear examination and the obvious healthy condition of the animals.

The experiment is detailed in Table II which records the last positive smear examination of the two periods, the fate of each bovine and the various sub-inoculations undertaken.

Discussion.

From the details available in the above table, it will be noted that the sub-inoculations into bovines and sheep of the blood of six of the bovines gave negative results notwithstanding that very large amounts of blood were used in all cases except one and that only a few months previously trypanosomes were found in blood smears. A few of the animals were sold on the open market. The average live weight of these animals was 1300 pounds. At the time of infection these animals averaged 590 pounds.

A later case of auto-sterilization in *T. congolense* infection of bovines enabled a more detailed observation to be carried out. This infected bovine 5189 was injected intravenously with a trypanocidal drug (Surfen C) on 5th and 24th January, 1934. Daily blood smear

TABLE II.

Number of Bovine.	At Onderstepoort. Last Positive Smear.	AT FARM "KAALPLAAS."			Remarks.
		Weekly Smear Examination.	Sub-inoculations.		
			Details.	Result.	
2464	11/7/30	All negative.	10 c.c. blood into sheep 32342	Negative	Died of heart-water on 13/11/31 Died 3 days after calving on 14/11/31.
2468	1/8/30	All negative (additional smears made on last 3 days of life)	—	—	
2471	11/7/30	All negative	100 c.c. blood into bovine 2782 on November 14th, 16th, 18th, 21st, and 23rd, 1932 and 10 c.c. into sheep 34559 on 7th Oct. and 16th Nov., 1932	Negative	
2714	24/2/30	All negative with exception of a positive smear on 9/4/32	Into bovine 3507 and sheep 34272 same quantities on same dates as in case of bovine 2471	Negative	
2994	16/6/30	All negative	Into bovine 2805 and sheep 34099 same quantities on same dates as in case of bovine 2471	Negative	
3525	23/3/31	All negative (smears on last 3 days of life)	—	—	Piroplasmosis on 14/11/31.
3637	16/1/31	All negative	—	—	Not Trypanosomiasis on 24/9/32.
3638	23/6/31	All negative with exception of a positive smear on 25/6/32	300 c.c. blood into bovine 3020 on 11/11/32 and 10 c.c. into sheep 34123 on 7/10/32	Negative	
3684	4/5/31	All negative with exception of a positive smear on 3/6/32	Into bovine 2765 and sheep 34201 same quantities on same dates as in case of bovine 2471	Negative	

examination gave 68 negative results up to 17th April, 1934, when sub-inoculation of blood (10 c.c. subcutaneously and 40 c.c. intravenously) into two sheep gave positive results. Up to 5th March, 1935, the examination of a further 262 smears gave negative results but

the sub-inoculation of blood (25 c.c. subcutaneously and 75 c.c. intravenously) on this date into two sheep now gave negative results. From the 12th to 24th June 1,000 c.c. of blood of this bovine was injected intravenously into a susceptible bovine 6026 with negative results. It was ultimately (on 23rd August, 1935) injected with 20 c.c. known infected blood subcutaneously and reacted with an incubation period of 8 days.

This case is a striking illustration of the inefficiency of blood smear examinations. For twelve weeks smear examination carried out on six days in the week gave negative results whereas the sub-inoculation gave positive results. Further smear examination up to the 46th week gave negative results. The sub-inoculation now also gave negative results. A later sub-inoculation of a large quantity of blood again gave negative results. If the smear examination had been used as the only criterion of sterilization, this bovine would have been classed as one sterilized by the injection of the Surfen C, an obviously incorrect conclusion.

It would appear that animals may attain, with or without the assistance of drugs, a state of premunition which is an equilibrium of the animal and the parasite with the former in a state of normality and that, in time, the animal body may obtain the upper hand with the result that the parasite is destroyed.

SUMMARY.

1. No cases of auto-sterilization have been observed in *T. brucei* or *T. equiperdum* infections of equines.
2. A number of cases of auto-sterilization is recorded in *T. vivax* infections of bovines and *T. congolense* infection of bovines and ovines.

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