THE THERAPEUTIC IMMUNITY REACTION IN THE DIFFERENTIATION OF TRYPANOSOME SPECIES.[1]

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When mice infected with the parasites of caderas, dourine, nagana, surra of India, or surra of Mauritius are properly treated with various therapeutic agents, a temporary immunity is produced. As the immunity is specific (1), the hope was at one time entertained that the reaction might be of service in differentiating trypanosome species.

This expectation, however, almost vanished when the delicacy of the reaction began to be appreciated. By means of it, Ehrlich[2] (2) and Browning (3), and subsequently the writer (4), were able to distinguish strains of trypanosomes known to have had a common origin, but rendered resistant to various medicaments. With equal clearness the reaction enabled the writer to differentiate normal trypanosomes supposed to have had a common origin, *i. e.*, the organisms of surra of India and surra of Mauritius.

Although this extreme delicacy apparently rendered the reaction useless in determining species, objections could be raised to drawing this conclusion from the evidence cited. First, the resistant strains differentiated were no longer normal. As they had acquired new characteristics it seemed scarcely permissible to draw from them inferences as to the behavior of organisms that had never come in contact with therapeutic agents. Second, the common origin of surra of Mauritius and surra of India is still questioned, although the majority of the authorities seem to be of the opinion that the Isle of Mauritius became infected through the importation of

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[2] In this article Ehrlich expresses the opinion that the immunity reaction, although specific, would not suffice to show that different trypanosome strains belong to different species.

cattle from India (see Laveran and Mesnil (5)). As long as even a slight doubt remains concerning the origin of the Mauritian virus, conclusions from experiments with this and the Indian strain will fail to convince.

The experiments to be reported here are not open to the objections raised against the preceding. The trypanosomes employed had never been in contact with medicaments of any sort and the common origin of the strains was beyond question.

Two distinct species were employed. Caderas was selected because its trypanosomes are morphologically distinguishable from the others with which I have worked. The purity of the virus could, therefore, be controlled by the microscope.

For quite a different reason surra of India was chosen. Against the parasites of this infection an efficient immunity is easily obtained. If a spontaneous change in the virus occurred, it seemed probable that the strength of the immunity would enable one to detect this with ease and bring it into prominence.

Each of the two species was inoculated into two guinea-pigs, and each guinea-pig was placed in a separate cage. Cages 1 and 2 contained the caderas virus, cages 7 and 8 the parasites of surra of India. The virus in these four cages was kept separate and preserved for twelve months by successive passages through guinea-pigs.

The immunity reaction was now resorted to in order to determine whether the trypanosomes of common origin had become differentiated during the year they had been kept separate. The first experiments were with the organisms of caderas.

Nine mice (see Table I) were infected with the virus from cage 1, and seven were then twice treated with mixture 1.[3] After the seven had received a second inoculation with virus No. 1, to increase the immunity, five of them were tested on the eleventh day with the parasites of caderas (cages 1 and 2) and surra of India (cage 8). The inoculation with surra of India was intended as a control on

[3] Mixture 1 consisted of equal volumes of acetyl atoxyl 2 per cent. and amidonaphtol disulphonic acid 1.8.3.6. plus dichlorbenzidine (alkaline.alkaline) 1 per cent. The mixture was injected subcutaneously, the dose being 1 c.c. for a mouse weighing 20 grams.

the immunity. Although all of the mice became infected, a comparison of the incubation periods showed that the two caderas strains were as sharply distinguished as if they were different species. Against caderas No. 1 alone was an immunity present. The mice inoculated with this virus (148.1 and 148.2) became infected five and eight days later than their control (147.6). On the other hand, in the animals tested with caderas No. 2 (148.3 and 148.4), the parasites appeared as quickly as in their control (147.7). Nevertheless, in these mice for six and seven days the trypanosomes remained less than five per field. They then increased rapidly and killed the animals one and two days later. The mouse inoculated with the parasites of surra of India (148.5) became infected at once and died on the same day as its control (147.8).

In order to offer a possible explanation for the long infection in the case of 148.3 and 148.4 it is necessary to refer to unpublished experiments. Mice immunized to one species of trypanosomes were tested with the parasites of another. As a rule, these animals became infected at once. Under certain conditions, however, they remained negative. On testing them again, it was found that an efficient non-specific immunity could develop in three days. For this reason in examining for specific immunity, the results of the first forty-eight hours following the test were usually regarded as crucial. The fact that two mice (148.3 and 148.4) inoculated with the virus from cage 2 became infected as soon as their control indicates that there was little or no immunity against this virus on the eleventh day. That the trypanosomes in these mice remained few in number on the fourteenth, fifteenth, sixteenth, seventeenth, eighteenth and nineteenth days (148.4) may indeed have been due to immune bodies. In my opinion, however, these were elicited, not by the cure of the virus from cage 1, but as a result of the test with caderas from cage 2.

In the next experiment, the virus from cage 2 was inoculated into six mice (see Table II). Five of these were then twice treated (first and third day), reinoculated with caderas from cage 2 (fourth day) and tested for immunity on the eleventh day with virus from cages 2, 1 and 8. Again the two caderas strains were clearly distinguished, although the differences were not as marked as in the

first table. The test with No. 2 had an incubation period of four days (control 2); that with No. 1, two days (control 1); and that with surra of India, one day (controls 1 and 2).

It should be stated that the treatment used to elicit the immunity in Tables I and II, has been excellent against certain species of trypanosomes (*e. g.*, surra of India and nagana), but less effective against caderas. This had been noted in previous work and is clearly shown by the course of mouse 153.1 in Table II. This animal was a control on the treatment. Instead of remaining negative, as all other mice similarly treated have done, it had relapse on the fifteenth day and died two days before the mouse tested with No. 2 (152.5). If a stronger immunity could have been elicited in the tests given in Table II, it is probable that the distinction between caderas from cage 1 and that from cage 2 would have been much more pronounced.

When it was seen that the organisms in cages 1 and 2 could be distinguished by the immunity reaction, the microscope was resorted to in order to forestall the possible objection that my strains of caderas had become contaminated. Stained specimens of the trypanosomes in these cages were made and studied. They were indistinguishable and the parasites had the morphologically characteristic minute centrosome. The conclusion was that no contamination had occurred and that the organisms in cages 1 and 2 were those of caderas.

In Table I and the following tables:

0 = No parasites in at least 20 fields (Zeiss, lens "D," ocular No. 4).
+ = Parasites present but less than 5 per field.
++ = 5 to 20 parasites per field.
+++ = More than 20 parasites per field.
Cad 1 = An injection of the trypanosomes of Caderas from cage 1.
Cad 2 = An injection of the trypanosomes of Caderas from cage 2.
SI 7 = An injection of the trypanosomes of surra of India from cage 7.
SI 8 = An injection of the trypanosomes of surra of India from cage 8.
Dour = An injection of the trypanosomes of dourine.
Mx 1 = An injection of mixture in the therapeutic dose.

In these three tables the volume of the diluted virus introduced was always one-fourth of a c.c., and with the exception of the inoculations on the twelfth day in Table III, all of the injections were intraperitoneal. On the twelfth day, however, the inoculations were subcutaneous, the parasites being about one in twenty fields in the virus from cage 8, none in twenty fields in that from cage 7, and one in three fields in the tests with dourine. On the eleventh day in Tables I and II, the parasites introduced were about one per field.

TABLE I.

Day.	Tested for immunity.						Treated controls.				Untreated controls.					
	148.1	148.2	148.3	148.4	148.5	148.6	148.7	149.1	149.2	143.14	143.15	143.16	147.6	147.7	147.8	
	Cad. 1	Cad. 1	Cad. 1	Cad 1	Cad. 1	Cad. 1	Cad. 1			Cad. 1	Cad. 1					
1	+	+	+	+	+	+	+			+	+					
2	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1							
3	0	0	0	0	0	0	0			+	+					
4	0	0	0	0	0	0	0			++	++					
5	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1							
6	0	0	0	0	0	0	0									
7	Cad. 1	Cad. 1	Cad. 1	Cad. 1	Cad. 1	Cad. 1	Cad. 1	Cad. 1	Cad. 1	+++	+++	Cad. 1				
8	0	0	0	0	0	0	0	0	0	Dead	Dead	++				
9	0	0	0	0	0	0	0	0	0			+++				
10	0	0	0	0	0	0	0	0	0			Dead				
11	0	0	0	0	0	0	0	0	0				Cad. 1	Cad. 2	Sl. 8	
12	Cad. 1	Cad. 1	Cad. 2	Cad. 2	Sl. 8	0	0	0	0				0	0	0	
13	0	0	0	0	++	0	0	0	0				++	++	++	
14	0	0	++	++	+++	0	0	0	0				+++	+++	+++	
15	0	0	+	+	Dead	0	0	0	0				Dead	Dead	Dead	
16	0	0	+	+++		0	0	0	0							
17	0	0	++	++		0	0	0	0							
18	0	+	++	++		0	0	0	0							
19	0	++	++	++		0	0	0	0							
20	0	+++	Dead	Dead		0	0	0	0							
21	++	Dead				0	0	0	0							
22	++					0	0	0	0							
23	+++					0	0	0	0							
24	+++					0	0	0	0							
25	+++					0	0	0	0							
26	Dead					0*	0*	0*	0*							

* = No reappearance of parasites up to 70th day.

TABLE II.

Day.	Tested for immunity.				Treated controls.			Untreated controls.						
	152.4	152.5	152 6	152.7	153.1	153.3	153.2	147.12	154.5	154.6	156.9	156.10	156.11	156.12
	Cad. 2	Cad. 2	Cad. 2	Cad. 2	Cad. 2			Cad. 2						
1	+	+	+	+	+									
	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	+						
2	o	o	o	o	o			+						
3	o	o	o	o	o									
	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	+++						
4	o	o	o	o	o									
	Cad. 2	Cad. 2	Cad. 2	Cad. 2	Cad. 2			+++	Cad. 2	Cad. 2				
5	o	o	o	o	o			Dead	+	+				
6	o	o	o	o	o				+++	+++				
7	o	o	o	o	o				+	+++				
8	o	o	o	o	o				+++	Dead				
9	o	o	o	o	o				Dead					
10	o	o	o	o	o									
11	o	o	o	o										
	Cad. 2	Cad. 1	Cad. 1	SI. 8	o	Cad. 2	Cad. 1				Cad. 2	Cad. 1	SI. 8	SI. 8
12	o	o	o	+	o	+	+				o	+	o	+
13	o	+	+	++	o	+	+				+	++	+	++
14	o	++	+++	+++	o	++	++				++	+++	++	+++
15	+	+++	+++	Dead	+	+++	+++				+++	+++	+++	Dead
16	+	+++	+++		++	+++	Dead				Dead	Dead	Dead	
17	+	Dead	+++		+++	+++								
18	++		Dead		+++	Dead								
19	+++				Dead									
20	+++													
21	+++													
22	Dead													

TABLE III.

Day.	Tested for immunity.					Treated controls.		Untreated controls.					
	150.3	150.4	150.5	150.6	150.7	151.1	151.2	147.3	147.9	154 9	154.10	154.11	154.12
	SI. 8	SI. 8	SI. 8	SI. 8	SI. 8			SI. 8					
1	o	o	o	o	o			+					
2	+	+	+	+	+			++					
	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	+++					
3	o	o	o	o	o								
4	o	o	o	o	o								
	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Mx. 1	Dead					
5	o	o	o	o	o								
	SI. 8	SI. 8	SI. 8	SI. 8	SI. 8				SI. 8				
6	o	o	o	o	o								
7	o	o	o	o	o				+				
8	o	o	o	o	o				++				
9	o	o	o	o	o				Dead				
10	o	o	o	o	o								
11	o	o	o	o	o								
12	o	o	o	o	o								
	SI. 8	SI. 7	SI. 7	SI. 7	Dour.	SI. 8	SI. 7			SI. 8	SI. 7	SI. 7	Dour.
13	o	o	o	o	o	o	o			o	o	o	o
14	o	o	o	o	o	o	o			o	o	o	o
15	o	o	o	o	o	o	o			o	o	o	o
16	o	o	o	o	o	o	o			o	o	o	o
17	o	o	o	o	o	o	o			++	+	+	o
18	o	o	o	o	o	+	+			+++	+++	++	+
19	o	+	+	o	o	+++	+++			+++	Dead	+++	+++
20	o	+	o	o	o	Dead	Dead			Dead		+++	+++
21	o	+++	o	+	o							Dead	+++
22	o	+++	+	++	o								Dead
23	o	Dead	+++	+++	+								
24	o[1]		Dead	Dead	o[1]								

[1] No reappearance of parasites up to sixty-second day.

A single experiment was also carried out with the surra of India in cages 7 and 8 (see Table III). The mice were inoculated with the organisms from cage 8, treated as in the previous experiments and tested on the twelfth day. Here the clearest possible distinction was made between the organisms in cage 8 and those in cage 7. The test with the former (150.3) failed completely, while three inoculations with the latter (150.4, 150.5 and 150.6) infected and killed. The control inoculated with dourine (150.7) was positive on the twenty-third day. It then became negative and in its blood the parasites have not yet reappeared (sixty-second day).

The changes here noted in the caderas and surra of India virus are quite possibly due to the parasites having become serum fest (see Ehrlich (6), and Mesnil and Brimont (7)).

Whatever the explanation may be, it is evident that the guinea-pig is an unfavorable animal in which to preserve the virus, *if the therapeutic immunity reaction is to be employed in the differentiation of trypanosome species;* for the above experiments clearly show that trypanosomes of common origin, never in contact with medicaments of any sort, may behave like different species after having been preserved in these animals for one year.

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