15432

Development of Immunity to Reinfection During Chemoprophylaxis of Trypanosomiasis with a New Antimony Derivative.

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The sodium salt derived from *p*-(2,4-diamino-1,3,5-triazinyl-6) aminophenylstibonic acid (Compound 122), exercises a long-lasting prophylactic activity in mouse-trypanosomiasis, according to Friedheim and Berman.[1] The duration of this protection varied with different animals, and in our own experiments we were able to protect mice treated with single doses of .05 g/kg for a period of up to 330 days. The mouse which was protected for the longest period finally succumbed from trypanosomiasis after it was previously inoculated 45 times without incident. Friedheim and Berman suggested that the prophylaxis against a first infection could be explained by a slow elimination of the drug. Indeed, at the site of injection a deposit of the free acid is formed which is resorbed very slowly. However, we also obtained a very long-lasting prophylactic effect when the product was given by mouth, in 25 and 50 mg/kg doses administered on 5 consecutive days; the mice were protected for a period of 2 months or more against 5 or less consecutive reinoculations. Further experiments are necessary before the mechanism of this prophylaxis can be determined.

During and at the end of the period of protection, certain phenomena occurred which indicate that immunologic processes had developed in the protected animals. First: In all control mice, the trypanosomes appeared in the blood stream 24 hours after the inoculation, whereas in all treated and protected mice, there was an interval of 4 to 8 days between the inoculation of trypanosomes and their appearance in the blood stream. Secondly: In several of the protected mice, trypanosomes appeared in the blood stream for a short time, as already pointed out by Friedheim and Berman. In other mice, they appeared as above, 4 to 8 days after a reinoculation, but did not multiply and disappeared spontaneously after a short interval.

The following experiments were made in order to determine whether these phenomena were due to a specific action of Compound 122, or to processes independent of the nature of the therapeutic agent.

1. Ten normal, uninfected mice received a subcutaneous injection of 50 mg/kg of Compound 122. Three weeks later their blood was collected and pooled and the serum obtained injected into normal mice. Twenty-four hours later these mice were inoculated with trypanosomes and succumbed, as usual, after 3 to 4 days. In a second series, 10 mice were inoculated with trypanosomes and 48 hours later, at the height of infection, treated with 50 mg/kg of Compound 122. Three weeks later the animals were bled, the blood pooled and the serum so obtained injected into untreated mice. These mice were protected against a subsequent infecting dose with trypanosomes.

Serum of normal mice served as a control and did not, as it has long been known, interfere with the infection. Repetition of these experiments under different conditions, especially varying the time between the curative treatment with Compound 122 and the collection of the serum, always gave the same results and showed that anti-trypanocidal effect was obtained 5 days after the treatment of the infected mice with Compound 122. The intensity of the protective and trypanocidal effect of the immune mouse serum was compared *in vitro* and *in vivo* with that of human serum and it was found that the immunologic power was almost the same with both sera.

[1] Friedheim, E. A. H., and Berman, Rose L., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 131.

TABLE I.
Passive Immunity After Prophylaxis with Compound No. 122.

Mice treated with	Inoculation	Appearance of Trypanosomes. Days after injection of serum										
		0	1	2	3	4	5	6	7	8	9	10	11
1. Normal serum			+	++	+++	d							
			(+)	+	++	++++	d						
			++	++	+++	d							
2. Protected mice:			0	0	0	0	+	+++	d				
No. 122 + Trypanos.			0	0	0	0	++	++++	++++	d			
			0	0	0	0	0	0	0	0	+++	++++	d
			0	0	0	0	0	0	0	0	0	0	0
3. No. 122 alone			+	++	++++	d							
			(+)	+++	++++	++++	d						
4. Neoarsphenamine + Trypanosomes			0	0	0	0	+	+++	++++	d			
			0	0	0	0	++	++++	d				
			0	0	0	0	0	0	++++	++++	d		
5. Controls			(+)	+	++	+++	d						
			(+)	++	+++	d							

0 = No trypanosomes; (+) - ++++ = Different quantities of trypanosomes in the blood; d = Dead.

TABLE II.

Passive Immunity After Immunization with Dead Trypanosomes.

Serum obtained days after active immunization, days	Delay of reinfection, days
0	0
3	0, 0, 3
5	3, 4, 5
10	2, 3, 3
14	5, 5, 5

These results indicate that the trypanocidal power of the serum of infected mice treated with Compound 122 is, apparently, not due to circulating antimony, but to other processes, probably immunologic in nature, and induced by the chemotherapeutic agent. But this action is not characteristic of the antimony compound used, since an experiment in which Neoarsphenamine was used instead of Compound 122 produced the same result. (Table I).

2. The development of anti-trypanocidal activity in the mouse-blood is probably an effect of any successful treatment of dourine with subsequent death of the protozoa. Immunization with killed trypanosomes, with results similar to those obtained in our experiments with Compound 122, has often been obtained, for instance, in mice infected with *Trypanosoma brucei* and treated with arsacetin.[2] We had the same results after immunization with *Trypanosoma equiperdum.* Fifteen mice were exsanguinated at the height of the infection, the blood citrated, pooled, and the contained trypanosomes killed by repeated freezing and thawing. This sterile blood, incapable of producing infection, was injected, 0.5 ml doses, into normal mice and groups of 3 inoculated with living trypanosomes immediately and after 3, 5, 10 and 15 days. The results are seen in Table II.

Conclusion. It is confirmed that a single intraperitoneal or subcutaneous injection of a sodium salt derived from melaminylphenylstibonic acid (Compound 122) confers a long-lasting protection against numerous consecutive infections of the mouse with *Trypanosoma equiperdum.* This protection is also obtained by oral administration of the compound. In infected mice which have been cured with this product, an immunity to reinfection with *Trypanosoma equiperdum* is achieved. The passive transfer of this immunity has been demonstrated. A similar state is reached if the product is injected first, and subsequently, reinfections with small amounts of trypanosomes are made. It is not established whether this acquired immunity to reinfection plays a role in the prophylaxis conferred by Compound 122.

[2] Browning, C. H., and Gulbranson, R., *J. Path. and Bact.*, 1936, **43**, 479.

15433

Pectin Adjuvant for Oral Penicillin.

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Numerous adjuvants have been used individually and in various combinations in attempts to increase the absorption of orally administered penicillin.[1-3] For the most part the relative efficacy of these substances has been determined by comparing the average blood concentrations obtained in small groups of subjects at various intervals following a dose or on the average amount of penicillin recovered from the urine. Individual variations under these conditions, how-

[1] Cutting, W. C., *et al., J. A. M. A.*, 1945, **129**, 425.

[2] Free, A. H., Parker, R. F., and Biro, B. E., *Science*, 1945, **102**, 666.

[3] McDermott, W., *et al., Science*, 1946, **103**, 359.