THE TIME OF APPEARANCE OF A POSITIVE HINTON REACTION IN SYPHILITIC RABBITS

As a preliminary to experimental work on syphilis in rabbits, a study was made to determine the value of the Hinton test in detecting syphilis in these animals. We shall describe our findings with the Hinton test and, in addition, shall make a comparison of our results with those obtained by some of the workers who have used the Wassermann, Meinicke and Kahn tests on normal and syphilitic rabbits.

In general, data based on the Wassermann test are hard to compare because so many different modifications of the test have been used. Wakerlin and Caroll[1] have reviewed conflicting reports on the reliability of the blood Wassermann reaction in rabbits. These reports indicate that in most of the early work on rabbits, the Wassermann test was unreliable. However, Heusinkveld and Carroll[2], Wakerlin and Carroll[1] and Wakerlin and Horrall[3] concluded that the Wassermann test as performed by them, using the method of Lorenz with an acetone-insoluble antigen and active serum, was valuable not only in detecting rabbit syphilis but also in evaluating the healing powers of therapeutic agents for the disease. Kemp, Chesney and Poole[4] found that the Wassermann test, if performed according to the method used in the Johns Hopkins Hospital[5] for human serums, was satisfactory for rabbits. Of 600 uninfected rabbits, only two were positive. In infected rabbits, although the test sometimes became positive as early as two days after the first clinical symptoms appeared, the maximum serological reaction usually

occurred in from two to six weeks after the appearance of the initial syphilitic lesions. Thomsen and Christiansen[6] state that positive Wassermann reactions in rabbits developed regularly about forty days after inoculation and continued unchanged for varying lengths of time, occasionally up to six months.

Mackie and Watson[7] using Wassermann and flocculation techniques described by them found that uninfected adult rabbits gave positive reactions, but they concluded that in normal animals the reactions were always weak, as contrasted with the strong reactions obtained with the serum from rabbits with active syphilis. Similar conclusions were reached by Walker[8] who found negative Kahn reactions in only 56% of 29 presumably normal rabbits. However, the serums that showed an initial positive test gave the reaction in increased dilutions after the rabbits were inoculated with syphilitic material. In the infected rabbits, the maximum height of the serologic reaction was reached thirty to seventy days after inoculation. Observations on the use of the Kahn test in experimental rabbit syphilis were also made by Mahony, Bryant and Pickard[9]. When first tested, 114 rabbits (about 53%) out of a total of 216 showed completely negative tests, while 45% gave weakly positive Kahn tests; the remaining 2% of the animals showed strong positive reactions. By doing three tests on 179 of these rabbits, the percentage of completely negative results was lowered to about 31%. In spite of these results, Mahony et al concluded that because of marked reactions in infected rabbits, and the high specificity, simplicity and quantitative features of the reaction, the Kahn test is suitable for recording serologic changes in rabbits.

Wakerlin and Horrall[3] made a comparative study of the Kahn and Wassermann reactions in normal and syphilitic rabbits, using for the former test a method recommended by Kahn for experimental syphilis, and for the Wassermann test the method of Lorenz. In a group of 20 normal rabbits giving negative Wassermann reactions, 16 gave completely negative Kahn tests, three were virtually negative and one was positive. In spite of these false positives obtained with the Kahn test, the authors conclude that it is preferable to the Wassermann test because (1) it tends to become positive somewhat sooner and (2) because it may be used even with serums that are anticomplementary to the Wassermann. Saito[10] also concluded that the Kahn test is superior to the Wassermann reaction for rabbits. Using undiluted serums, 25 of 59 normal rabbits gave positive Kahn tests, but with serum-diluted 1:5 there were no false positives. About half of the animals gave positive Wassermann reactions.

METHOD

It was decided to try the Davies-Hinton method[11] because of the small amount of blood required for this test. Of nine rabbits on which the "capillary" Davies-Hinton test was performed by Dr. John A. V. Davies, 7 showed definitely positive reactions and two were negative. All of these same rabbits, however, gave negative reactions when the "micro-tube" (Davies-Hinton) method was used.* Consequently the "micro-tube" method was employed in the following experiments.

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*This observation is striking because the same reagents are used in both tests.

The blood was collected from the marginal ear vein of each rabbit by means of a capillary glass tube about 4 cm. in length, which had been drawn to a fine point for the purpose of penetrating the vein. About one c.c. of blood was allowed to flow through this capillary into a small test tube. Each rabbit was tested at least twice prior to inoculation, and twice a week after the inoculation they were bled and examined for testicular lesions.

The rabbits were inoculated intratesticularly with an emulsion of the testicles of a rabbit infected with *Treponema pallidum** and numbering 10 to 70 organisms per microscopic field (darkfield method). Five groups of rabbits were inoculated, each group being infected by using material from a rabbit of the preceding group, the intervals between successive transfers being from about three to twelve weeks.

Results

Out of a total of 62 uninfected rabbits, only two showed a weakly positive or doubtful reaction. These were included in the groups to be inoculated, and they later developed strong positive reactions.

(INSERT TABLE I)

Table I shows the time of appearance of a positive Davies-Hinton test and indurated testicular lesions following inoculation with *T. pallidum*. In tabulating the results in this table, the test was not recorded as positive until at least a moderate

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*A rabbit infected with this strain of T.pallidum was supplied through the kindness of Surgeon J.F. Mahoney, Director, Venereal Disease Division Laboratory, U.S.Marine Hospital, Staten Island, N. Y.

reaction had been noted. On the whole, the time of appearance of serologic and local reactions to inoculation was much the same in the five groups, although groups III, IV and V were somewhat slower than groups I and II. By referring to chart I, it may be seen that the intervals between transfers varied quite considerably and that, as a result, the animals used for transfer in each case were not in exactly the same stage of the disease. This might explain the difference in time. Another factor which may have caused some difference is the season of the year. Groups I, II and III were inoculated in the winter months, group IV in the spring and group V in the summer. It is possible that hot weather may have a depressing effect upon the speed of reaction in animals. Most of the rabbits became serologically positive during either the third, fourth or fifth weeks of the disease. Indurated lesions of the testicles appeared at approximately the same time.

Brown and Pearce as well as other workers have reported seasonal variation in the response of rabbits to infection with T.pallidum.

(INSERT TABLE II AND TABLE III)

Table II shows the data from our experiments with the Hinton test and the data from the work of Wakerlin and Carroll[1] on the Wassermann reaction and of Wakerlin and Horrall[3] on the Kahn and Wassermann reactions. Although the work was done in different laboratories and at a different time, it seems permissible to make some comparisons because the same strain of Treponema pallidum and roughly the same technique of inoculation was used. The figures show that our data on the time of development of orchitis are quite similar to theirs. However, the serologic reactions obtained in the two sets of experiments do not follow each other as closely. In Table III, the percentage of total positive reactions

occurring at the end of each week have been calculated. In no case was a positive Kahn or Wassermann test noted by the end of the third week, whereas 29% of the positive Hintons had developed by that time. By the end of the fifth week, 92% of the Hintons had become positive, while only 33% of the Kahns and 16-51% of the Wassermanns were positive.

SUMMARY AND CONCLUSIONS

Of 62 uninfected rabbits upon which Davies micro-Hinton tests were performed twice, only two showed a weakly positive or doubtful reaction. Following inoculation with Treponema pallidum, all of these 62 animals developed strongly positive tests.

A positive Hinton reaction was noted in two animals as early as the first week of the disease, and in five animals during the second week. By the end of the third week, 29% of the rabbits had become positive, 76% at the fourth, and 92% at the fifth.

Indurated lesions of the testicle appeared at approximately the same time as the positive reactions of the blood.

Data drawn from the literature concerning experimental syphilis in rabbits seem to show that positive reactions obtained by other serologic tests for the blood are somewhat slower to develop than we have found to be the case with the Hinton test, and may show more false positives in uninfected rabbits. Typical orchitis, on the other hand, occurred at very nearly the same time in our work and in the work of others, thereby showing that on the basis of speed of development of local reactions to the disease, the different sets of experiments are comparable.

The Davies micro-Hinton test is a reliable method of studying the serology of experimental syphilis in rabbits.

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TABLE I

Time of Appearance of Positive Hinton Reactions and Orchitis in Syphilitic Rabbits

Number of Rabbits Showing Positive Hinton

Weeks after Inoculation	I	II	III	IV	V	VI	VII	VIII	IX	X	Total	Interval between Transfers
Group I	1	2	4	3							10	4 weeks
" II		3	3	4							10	4 "
" III	1		3	12	3		1				20	3 "
" IV			1	5	3		1	1	1		12	12 "
" V				5	4					1	10	6 "
Total	2	5	11	29	10	0	2	1	1	1	62	

Number of Rabbits Showing Orchitis

Weeks after Inoculation	I	II	III	IV	V	VI	VII	VIII	IX	X	Total	
Group I			4	6							10	
" II		1	3	4	2						10	
" III		1	4	11	2						18	
" IV	3		1	3	3	1					11	
" V	1			6	1			1			9	
Total	4	2	12	30	8	1	0	1	0	0	58*	

*Four rabbits not included under heading of orchitis because of death in one case and failure to develop typical testicular reactions in three cases.

TABLE II

COMPARISON OF HINTON, WASSERMANN AND KAHN TESTS WITH RESPECT TO TIME OF APPEARANCE OF POSITIVE REACTION IN RABBITS FOLLOWING INOCULATION WITH TREPONEMA PALLIDUM

Weeks after Inoculation		1	2	3	4	5	6	7	8	9	10	11	12	13	Total
Data reported by us	Pos. Hinton	2	5	11	29	10		2	1	1	1				62
	Orchitis	4	2	12	30	8	1		1						58
Wakerlin and Carroll	Pos. Wassermann				15	31	23	13	5	3					90
	Orchitis			36	32	16	3	3							90
Wakerlin and Horrall	Pos. Wassermann				2		6		2	1				1	12
	Pos. Kahn				4		6		1	1					12
	Orchitis				11	1									12

TABLE III
Derived from Table II

TOTAL PERCENTAGES OF POSITIVE SEROLOGIC AND TESTICULAR REACTIONS
WHICH HAD DEVELOPED BY THE END OF EACH WEEK

	Hinton		Kahn		Wassermann		Wassermann	
	Serology	Orchitis	Serology	Orchitis	Serology	Orchitis	Serology	Orchitis
1 week	3%	7%	0	0	0	0	0	0
2 weeks	11%	10%	0	0	0	0	0	0
3 weeks	29%	31%	0	0	0	0	0	40%
4 weeks	76%	83%	33%	92%	17%	92%	17%	76%
5 weeks	92%	96%	33%	100%	17%	100%	51%	93%
6 weeks	92%	98%	83%		67%		77%	97%
7 weeks	95%	98%	83%		67%		91%	100%
8 weeks	97%	100%	92%		83%		97%	
9 weeks	98%		100%		92%		100%	
10 weeks	100%				92%			
11 weeks					92%			
12 weeks					92%			
13 weeks					100%			