

VDRL TEST IN NORMAL ALBINO AND PIGMENTED RABBITS*†

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The rabbit has been the animal most commonly employed for syphilis research since the first report of infection of this species in 1906 (Bertarelli, 1906). Experimental ocular and neurosyphilis has been investigated in this laboratory during the past 5 years in three species—rabbits, owl monkeys, and squirrel monkeys (Smith, Singer, Reynolds, Moore, Yobs, and Clark, 1965; Taylor, Smith, and Singer, 1965; Smith and Israel, 1968). Rabbits were selected as a control to the primate experiments because of the large available literature concerning experimental syphilis in the rabbit. However, during the course of these studies, it became apparent that many rabbits purchased commercially and in apparent good health gave reactive serum reagin test results before their use in any experiment. Furthermore, this phenomenon had been previously noted by various other investigators (Kolmer and Casselman, 1913; Noguchi, 1921). This prompted a study of the incidence of reactive VDRL tests in the normal rabbit (Pannu, Rosenberg, Israel, and Smith, 1967) which revealed that, in 149 apparently healthy rabbits, the serum VDRL test was reactive in 10 per cent. and weakly reactive in an additional 30 per cent. Recent review of data

on 262 normal rabbits has shown a difference in the VDRL test results found in albino rabbits as compared with pigmented rabbits. It is the purpose of this communication to document this observation, which to our knowledge, has not been previously reported.

Materials and Methods

All rabbits were purchased from a local rabbit farm which houses over 2,000 rabbits. A medicated feed is used at the farm to cut down the level of coccidiosis. Upon arrival at this laboratory, an initial blood specimen is drawn, and each animal is examined, tattooed, and placed in a separate cage. Thereafter, ordinary rabbit chow, for obvious reasons unmedicated, is given with water *ad lib.* as is the practice at this laboratory.

Before 1967 all serological tests for syphilis done on rabbits were performed at the Venereal Disease Research Laboratory, Communicable Disease Center, United States Public Health Service, Atlanta, Georgia. Since then, VDRL tests have been performed in our own laboratory by a technician trained at the Atlanta Center. Periodical checks of sera evaluated simultaneously in both laboratories have shown good reproducibility between the two. Discrepant results were noted in only two instances in this review—one rabbit (R-LCH-2) was found to have a reactive VDRL test and another (R-LCH-3) was reported as weakly reactive in our laboratory, but both sera were nonreactive in Atlanta.

The initial VDRL test results on 262 normal rabbits

*Received for Publication January 17, 1969.
†This work supported in part by grants CC 04411-01, Communicable Disease Center, and 5 RO 1 NB 06308-03, NB 05277-09, and 1 RO 1 NB 06574-02, National Institute of Neurological Diseases and Blindness.
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TABLE
RESULTS OF VDRL TESTS IN NORMAL RABBITS, 1963-1968

Year	Albino				Pigmented			
	No. tested	Weakly Reactive	Reactive	Percentage Positive	No. tested	Weakly Reactive	Reactive	Percentage Positive
1963	1			0				
1964	4			0	3		2	66
1965	7	2		29	4			0
1966	11	1	1	18	14	6	1	50
1967	9	1		11	28	6	3	32
1968	85	14	3	20	96	30	8	40
Total	117	18	4	18.8	145	42	14	38.6

are presented in the Table, which compares the data for albino and pigmented animals. It should be noted that the criterion for differentiation of albino from pigmented rabbits was the presence of any pigmentation whatever. Thus, a white rabbit with a dark spot was called pigmented, and only pure white rabbits were called albinos.

Discussion

The importance of reactive serum VDRL tests for syphilis in the normal rabbit population is evident when one considers that the bulk of experimental work in syphilis has been carried out on this species. The phenomenon has never been adequately explained, although it has been known for a long time and has frequently been reported. Kolmer and Casselman (1913) showed that various concentrations of lipoidal extracts of syphilitic liver gave positive complement-fixation reactions in 27.5 to 56.8 per cent. of normal rabbit sera.

Mulzer (1927) discussed the Wassermann reaction in normal and syphilitic rabbit sera and the following has been extracted from his report:

Uhlenhuth and Mulzer investigated the clinical reliability and usefulness of the Weidanz modification as compared to the original method of von Wassermann in a quantity of normal and syphilitic rabbit sera. They found, as was subsequently confirmed by Schoenburg, that: "the Wassermann reaction in rabbits can hardly be used, for frequently rabbits that are quite certainly normal show positive reagin tests, whereas syphilitic rabbits do not always exhibit a positive reaction". Michaelis was the first to establish that the Wassermann reaction could give a positive result in the serum of rabbits which were not syphilitic. This finding was later confirmed by various authors (Yamanouchi, Landsteiner and Müller, Fleischmann, Truffi and Ossola, Schucht, Blumenthal, Loreda, Citron and Munk, Schilling and von Hoesslin, Manteufel and Woithe). Paul and Mulzer found the Wassermann reaction in normal rabbits to be remarkably inconstant, and noted that different results could be found in the same animal tested on two successive days. Blum discussed the opinions proposed by different authors to explain the phenomenon. Landsteiner and Müller, Schilling and von Hoesslin, and even von Wassermann thought that this represented a "physiological peculiarity or characteristic of rabbit sera". The lipoidal metabolism of rabbit was suspected. Schwarz and Flemming thought a positive Wassermann reaction might become negative through fasting the animal. Wassermann suggested that a positive reaction might spontaneously become negative when the animal's cage was changed".

The possibility that benign venereal spirochaetosis, due to *Treponema cuniculi*, was the cause of this phenomenon prompted a recent review of the literature on this organism (Smith and Pesetsky, 1967). The incidence of reagin reactivity in normal

rabbits has been rather surprisingly constant in all series. Of 42 rabbits studied in this laboratory between 1963 and 1966, the VDRL test was reactive in five, weakly reactive in six, and nonreactive in 31. Reactivity was thus found in eleven (26 per cent.) of 42 normal rabbits. Similarly, of 24 rabbit sera tested in Atlanta during the same 3-year period, six were weakly reactive and eighteen nonreactive. Thus, six (25 per cent.) of 24 rabbits tested in another laboratory showed some VDRL reactivity. Sato (cited by Mulzer, 1927) found in 25 rabbits that six (24 per cent.) were positive to the Meinicke-Trubungsreaktion and the Sachs-Georgi reaction.

Although the age and sex of the rabbits have, in the past, been considered as possible causes of variations (Fried and Orlov, 1932), to our knowledge, the difference in reactivity between albino and pigmented rabbits has not previously been described. Data obtained in the past 3 years merit emphasis since they represent 93 per cent. of the available total of rabbits of which the pigmentary characteristics were noted. Of 117 albino rabbits tested, the VDRL test was weakly reactive in 15.4 per cent. and reactive in 3.4 per cent., so that 18.8 per cent. of albino rabbits showed some VDRL reactivity. However, of 145 pigmented rabbits tested, 29 per cent. gave weakly reactive and 9.6 per cent. reactive VDRL test results, so that 38.6 per cent. of pigmented rabbits showed some VDRL reactivity. It is evident that the VDRL test shows some degree of reactivity twice as often in pigmented rabbits as in albino rabbits. This observation appears important in selecting animals for the laboratory study of syphilis. A recent report by Clark, Yobs, and Artley (1968) confirmed the usefulness of the owl monkey (*Aotus trivirgatus*) for experimental syphilis, and documented the fact that normal members of this species show nonreactive serological tests for syphilis. The possible significance of the observation here reported in explaining reactive serum reagin tests in normal rabbits is such that other investigators are urged to study the relationship between pigmentation and serum reagin response in normal rabbits and to report their results.

Summary

Serum VDRL tests were performed on 262 normal rabbits. Some degree of reactivity was found in 18.8 per cent. of 117 albino rabbits. The comparable finding in 145 pigmented rabbits was 38.6 per cent. The significance of the fact that serum reagin reactivity was twice as frequent in normal pigmented rabbits as in normal albino rabbits is discussed.

REFERENCES

- BERTARELLI, E. (1906). *Zbl. Bakt.*, I. Abt. Orig., **41**, 320.
- CLARK, J. W., Jr., YOBS, A. R., and ARTLEY, C. W. (1968). *Brit. J. vener. Dis.*, **44**, 208.
- FRIED, S. M., and ORLOV, S. S. (1932). *Arch. Derm. Syph. (Chicago)*, **25**, 893.
- KOLMER, J. A., and CASSELMAN, A. J. (1913). *J. med. Res.*, **28**, 369.
- MULZER, P. (1927). In "Morphologie und Biologie der *Spirochaeta pallida*. Experimentelle Syphilis", ed. E. Hoffman, E. Hofmann, and P. Mulzer, in "Handbuch der Haut- und Geschlechtskrankheiten", ed. J. Jadassohn, band 15, teil 1, p. 306. Springer, Berlin.
- NOGUCHI, H. (1921). *J. Amer. med. Ass.*, **77**, 2052.
- PANNU, J. S., ROSENBERG, M. A., ISRAEL, C. W., and SMITH, J. L. (1967). *Brit. J. vener. Dis.*, **43**, 114.
- SMITH, J. L., and ISRAEL, C. W. (1968). *Ibid.*, **44**, 109.
- and PESETSKY, B. R. (1967). *Ibid.*, **43**, 117.
- , SINGER, J. A. REYNOLDS, D. H., MOORE, M. B., Jr., YOBS, A. R., and CLARK, J. W., Jr. (1965). *Ibid.*, **41**, 15.
- TAYLOR, W. H., SMITH, J. L., and SINGER, J. A. (1965). *Amer. J. Ophthal.*, **60**, 1093.

La réaction de VDRL chez les lapins sains: albinos et pigmentés

RÉSUMÉ

Des réactions de VDRL furent effectuées sur le sérum de 262 lapins sains. Un certain degré de réactivité fut constaté chez 18,8 pour cent de 117 lapins albinos. Il en fut de même chez 38,6 pour cent de 143 lapins pigmentés. La signification de cette réactivité réaginique du sérum, deux fois plus fréquente chez les lapins sains pigmentés que chez les lapins sains albinos, est discutée.