16105

A Search for *Treponema pallidum* in the Lymph Nodes of the Syphilitic Mouse.*

BORIS GUEFT. (Introduced by Paul D. Rosahn.)

From the Laboratories of the New Britain, Conn., General Hospital, and the Department of Pathology, Yale University School of Medicine.

In the course of an investigation of experimental mouse syphilis, it was found important to be certain that all the mice inoculated with syphilis actually became infected and harbored the *Treponema pallidum*. As Kolle and Schlossberger[1] first showed in 1926, symptoms do not appear despite an infection which persists throughout the life of the mouse. One method of ascertaining the presence of infection is to subinoculate mouse tissues into rabbit testicle. A darkfield positive syphiloma will usually develop after 20 to 80 days if the inoculated tissues contained viable *Treponema pallidum* in amounts sufficient to infect. This is a cumbersome method and the question was raised whether the treponemes might be easily found by darkfield search of excised node tissue, as the nodes are known to be highly virulent. Also, Jahnel and Prigge[2] demonstrated the presence of *Treponema pallidum* in the lymph nodes of the infected mouse by using silver impregnation methods.

Levaditi and Rousset-Chabaud[3] examined skin biopsies of syphilitic mice impregnated by Stroesco's silver method, to exclude noninfected mice from therapy trials. However, this method uses fixed tissue and the *Treponema pallidum* may be confused with the *Spirillum morsus muris* or other spirochetes often found in the asymptomatic mice. Two observers have reported finding the *Treponema* in nodes by darkfield examination. Kato[4] was the first in 1931 and Bessemans and his co-workers Van Haelst and De Moor have repeatedly found and counted the treponemes in mouse lymph nodes[5,6] after extremely laborious searches.

Kato reported finding the *Treponema pallidum* frequently in the iliac nodes of mice inoculated intrascrotally one to 40 days previously with rabbit chancre tissue. His method was to divide the node into 5 fragments, emulsify each fragment in a drop of normal saline, and search each fragment with the darkfield microscope, examining 50 fields per fragment, or a total of 250 fields per node. His results are given in Table I.

Van Haelst[6] found it necessary to examine from 500 to 3,000 darkfields of node tissue emulsified in such a way as to allow enumeration of the treponemes seen. He found that almost all nodes examined, from 56 to 425 days after infection, contained visible characteristic treponemes. Karrenberg[7] attempted to repeat Kato's findings and reported failure in a very complete review of mouse syphilis. He mentioned seeing "spirochetoid" movements but no treponemes. The apparent ease with which Kato found treponemes led to the present attempt to use his method. It was hoped that examination of an excised mouse lymph node would suffice to establish the presence of syphilitic infection in the mouse, and that rabbit inoculations of control mice

* This report represents work performed under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the New Britain General Hospital.

1 Kolle, W., and Schlossberger, H., *Deutsche Med. Wchnschr.*, 1926, **52**, 1245.

2 Jahnel, F., and Prigge, R., *Deutsche Med. Wchnschr.*, 1929, **55**, 694.

3 Levaditi, C., and Rousset-Chabaud, D., *Bull. Acad. de Méd. Paris*, 1941, **124**, 176.

4 Kato, N., *Bull. Soc. Japon. de Syph.*, 1931, **6**, 21.

5 Bessemans, A., and De Moor, A., *Bull. Soc. Franc. de Dermat. et Syph.*, 1937, **4**, 483.

6 Van Haelst, J., *C. R. Soc. de Biol.*, 1933, **113**, 1535.

7 Karrenberg, C. L., *Arch. f. Dermat. u. Syph.*, 1932, **165**, 585.

TABLE I.
Results Obtained by Kato[3] in Darkfielding Nodes of Syphilitic Mice from One to 43 Days After Infection.

Days duration syphilitic infection	Treponemes in 250 darkfields per node	Days duration syphilitic infection	Treponemes in 250 darkfields per node
1	0	23	2
2	3	24	6
3	0	25	2
4	19	26	4
5	0	27	5
6	0	28	2
7	0	30	0
8	0	32	5
9	0	33	4
10	0	34	2
11	10	35	0
14	9	36	0
15	1	37	0
16	3	38	0
18	3	39	0
20	3	40	0
21	8	41	1
22	9	43	0

Total No. of mice, 36; total No. of Positive mice, 20.

would be only rarely necessary.

In this experiment, white mice were inoculated subcutaneously or intraperitoneally with Nichols rabbit strain of *Treponema pallidum*. Each mouse received 0.2 ml of rabbit chancre-emulsion containing more than 4 active treponemes per darkfield. A control rabbit inoculated with 0.5 ml of similar virus intratesticularly later developed a characteristic darkfield positive lesion.

The mice were kept in an air conditioned room at 65-70°F. They were housed in wire mesh cages with 4 to 10 mice per cage and were fed a proprietary food.† During the periods of observation, 45 and 90 days, unequivocal lesions of the mice were not observed. Forty-five and 90 days after infection, groups of mice were sacrificed and various organs from each mouse were emulsified in normal saline and inoculated into the testes of normal rabbits kept under similar conditions to the mice. The rabbits were examined at least twice weekly for the development of testicular lesions. Suspicious lesions were punctured and darkfielded. If characteristic motile treponemes were seen in the dark field, it was considered adequate proof that the mouse whose tissues had been subinoculated was infected with syphilis.

† Purina Laboratory Chow.

At the time these mice were sacrificed, iliac, axillary, or popliteal lymph nodes were dissected out and examined by Kato's method. The node was placed in a sterile petri dish and divided into 5 parts with a sharp scalpel. Each part was in turn placed in a drop of normal saline solution on a clean slide and emulsified by crushing and rotary movements of the flat surface of the scalpel blade. A cover slip was then placed on this emulsion, and 50 darkfields examined. A Spencer darkfield microscope with parabaloid condenser and built-in light was used. The objective was an oil immersion achromat with a numerical aperture usually of 0.8 being used together with a 10x ocular. Three observers examined the preparations. These all had considerable experience in recognition of the living *Treponema pallidum* by darkfield examination. All suspicious treponema-like objects were re-examined by the author.

The results as shown in Table II were completely negative. Because these results were obtained in an experiment mainly designed to study the biology of syphilis in the mouse, certain limitations existed which deserve discussion.

Actually, of the 36 mice employed, only 15 animals had darkfield examinations of a lymph node with simultaneous injections

TABLE II.
Comparison of the Results Obtained by Darkfielding Syphilitic Mouse Lymph Nodes with the Results Obtained by Inoculating Rabbit Testes with Mouse Tissues.

No. of mice	Route of infection	Duration of infection	Results of mouse organ* inoculation into rabbit testis		Results of mouse node inoculation into rabbit testis		Darkfield study of node†
5	Subcutaneous	45 days	4/5	Positive	—		Negative
11	”	45 ”	—		11/11	Positive	”
4	Intraperitoneal	45 ”	4/4	Positive	3/4	”	”
6	”	45 ”	4/6	”	—		”
10	”	90 ”	9/10	”	—		”

* Either skin, blood, brain, spleen, or node, or a combination of these organs.
† Usually iliac node, rarely axillary or popliteal.

of other nodes into rabbit testes. Of these 15, none was infected intrascrotally, as Kato had done. Four of these 15 were infected by intraperitoneal injection, which is tantamount to intrascrotal injections in the mouse, which has free communication between the scrotum and the peritoneal cavity. It may seem from these statements that Kato's procedure was not duplicated. However, the important fact that 14 of the above 15 mice had chancrigenic nodes (for rabbits) suggests that the *Treponema pallidum* was present in the node that was simultaneously darkfielded. In addition, it is known from the work of many others[8] that once any syphilitic mouse organ is shown virulent for rabbit testis, it is extremely likely that lymph nodes of the same mouse also contain the syphilitic virus. From this last statement, it follows that the 33 mice with positive rabbit subinoculation tests most likely had nodes containing the *Treponema pallidum*, and that Kato's procedure of performing darkfields was inadequate for demonstrating the organism. The existence of an invisible form of the syphilitic virus is not considered in this discussion.

Summary. 1. Attempts to use dark field examinations of the lymph nodes of mice as proofs of syphilitic infection 45 and 90 days after inoculation have failed, using the method employed successfully by Kato.

2. This failure to find the *Treponema pallidum* in lymph nodes by Kato's method confirms Karrenberg's studies.

3. More exhaustive methods of searching for the treponeme, such as those of Van Haelst, appear to be necessary.

8 Gueft, B., and Rosahn, P. D., *Am. J. Syph., Gon. and Ven. Dis.*, in press.