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OBSERVATIONS OF THE PATHOGENESIS OF SYPHILIS IN *Aötus trivirgatus**

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Many reports of the study of experimental syphilis in primates other than man appeared early in the 20th century. We have not found an early report of the use of the owl monkey—*Aötus trivirgatus*—a small nocturnal primate native to South America. In a previously published cooperative study of ocular syphilis and neurosyphilis (Smith, Singer, Reynolds, Moore, Yobs, and Clark, 1965), this monkey was used as the animal model because of its phylogenetic proximity to man, its large eyes in relation to a diminutive body size, and its availability. Findings in that study, as well as the phylogenetic position of the *A. trivirgatus*, interested us in further investigation of experimental syphilis in the species. This is a report of our experience over some 3 years with 37 adult monkeys, twelve of which were infected with syphilis.

Inoculations were performed with one of two strains of *Treponema pallidum*: either the Nichols virulent strain, propagated in rabbits for more than 50 years, or a strain that we transferred directly from man to the monkey. The methods were those generally used in studies of experimental syphilis in animals. Monkeys and rabbits were inoculated intravenously, or in the testicle, the genital mucous membranes and adjacent skin, or in the shaved skin of the anterior and posterior torso. Inocula were chancre exudate, venous blood, aspirate of human or monkey inguinal lymph nodes, extract of excised inguinal or popliteal monkey nodes, or extract of syphilitic rabbit testes. Effects of inoculations were determined by regular clinical examinations; by microscopic examination of lesion exudates, lymph-node and testicular aspirates, and smears or sections of other organs; by serological testing; and by determination of the infectivity of blood, lymph nodes or lesion exudate on transfer to normal animals. In an attempt to observe the natural transmission of syphilis, heterosexual pairs of syphilitic and normal monkeys were housed together.

Four monkeys were inoculated in one testis, one with approximately 40,000 motile *T. pallida* and three with some $4 \cdot 7 \times 10^7$ organisms. Since the testicles of this primate are normally small and quite firm, we did not detect overt signs of testicular involvement by palpation. Scrotal inflammation and oedema with questionable induration of the inoculated testis developed in 2 to 7 days and persisted for from 1 to 3 weeks in each of the inoculated monkeys. Although scrotal involvement extended to the other side in varying degrees, the un-inoculated testes showed no palpable changes and were not aspirated. *T. pallidum* was demonstrated 5 days after inoculation by darkfield examination of testicular aspirates from two of the three that had received the larger inoculum, and in the third monkey 38 days after inoculation; in the interim, numerous specimens had been intensively examined with negative results. Although tissue-transfer procedures and treponemal serological tests proved that the animal which had received the smaller inoculum was infected, *T. pallidum* was not demonstrated microscopically in material from testicular aspirates. Other monkeys, inoculated into the testis with extracts of popliteal or inguinal lymph nodes from monkeys with syphilis of 6, 8, and 14 months' duration, were first darkfield positive 15, 29, and 29 days after inoculation. Their orchitis followed a similar course.

The period during which treponemes could be demonstrated microscopically from the testes of infected monkeys was not determined. Testicular aspirates from two monkeys inoculated at this site

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were still darkfield positive after 11 weeks of infection; at this time, palpable signs of orchitis had been absent for 8 weeks. No testicular tissues from syphilitic monkeys were transferred.

Spirochaetaemia in monkeys with syphilis of 6 months' duration was demonstrated by the infectivity of 0·1–1·0 ml. of their blood on inoculation into the testes of rabbits and monkeys. Spirochaetaemia was also demonstrated in one monkey with syphilis of 14 months' duration by the infectivity of 1·0 ml. of its venous blood when inoculated intravenously into another monkey.

Normally, inguinal lymph nodes are barely palpable in this species. After inoculation, these nodes generally enlarged during the second and third months (ranging up to 10 mm. in diameter) and tended to regress after a few months. Inguinal node aspirates from eight infected monkeys, examined at irregular intervals, were darkfield positive once or more during 2 to 24 months of infection.

27 darkfield-positive chancres were produced on the backs of seven monkeys by intracutaneous inoculations. Lesions first appeared as slightly erythematous pinpoints and developed into minute, usually scaly, macules 1–2 mm. in diameter, from which *T. pallidum* could be demonstrated. Some evolved into lesions generally resembling the chancres seen in man and in the rabbit, but they tended to be drier and less necrotic. Most chancres persisted as flat scaly areas for from several days to as long as 10 weeks. Others disappeared after as little as 3 or 4 days, leaving either a blanched scar or no discernible sign. Chancres were not routinely followed microscopically after they had been proved darkfield positive. Eight, including some which had almost completely regressed, were still darkfield positive 17–30 days after they were first found to be positive.

The effect of quantitated inocula of Nichols *T. pallidum* in the skin of the back of the rabbit is well documented. A method equating quantitated *T. pallidum* inocula with days of incubation to darkfield-positive lesions was published by Magnuson, Eagle, and Fleischman (1948). Tables I and II summarize results of three experiments in which we used this method to compare rates of reaction in *A. trivirgatus* and in rabbits. In two separate experiments (1 and 2), three rabbits and three monkeys were each inoculated intracutaneously at six sites on the back with 10-fold increments from the same suspensions of Nichols strain *T. pallidum* from the rabbit. From 2 to 200,000 organisms were inoculated per site. One (M-1) of the six monkeys inoculated at multiple sites developed only one darkfield-positive chancre; this occurred after 38 days' incubation at the site of the 200,000 *T. pallidum* inoculum. Two other monkeys (M-31 and M-35) either did not develop lesions from the inoculation of 2–200 *T. pallidum* or did so only after 63 to 90 days. Of the eighteen sites in each species injected with 2, 20, or 200 organisms, ten produced darkfield-positive chancres in the monkey after 21–90 days (average 44), compared with eleven chancres in the rabbit after 19–45 days (average 26). With the larger inocula, *i.e.* 2,000, 4,000, 20,000, and 200,000, seventeen of nineteen injections in the monkey produced chancres after 8–63 days (average 27), and lesions at eighteen of nineteen injection sites in the rabbit were darkfield positive after 8–14 days (average 10·5).

A different strain of *T. pallidum*, which will be described presently, was used in Experiment 3. A monkey (M-25) and a rabbit (No. 1627) were inoculated intracutaneously at one site on the back with 4,000 organisms from another monkey. The monkey became darkfield positive after 15 days, developing one of the largest chancres observed in monkeys

TABLE I

INTRACUTANEOUS INOCULATION OF 200,000 *T. PALLIDA* AT SIX SITES ON THE BACKS OF SIX RABBITS AND SIX MONKEYS

Post-inoculation Day No.	Monkey Lesions		Rabbit Lesions	
	Number	Average Diameter × Height (mm.)	Number	Average Diameter × Height (mm.)
10	3	5·0 × 1·5	6	9·1 × 1·5
13		Not measured	6	10·0 × 3·0
17	3	7·0 × 1·5	6	17·0 × 2·7
19		Not measured	6	17·0 × 2·5
24	3	6·0 × 1·0	6	17·0 × 3·0
31	4	7·0 × 1·0	4	13·0 × 1·5
38	5	6·0 × 0	3	13·0 × 2·7
44	2	6·0 × 1·0	3	16·0 × 3·0
52	2	5·0 × 1·0	4	15·0 × 3·0
59	2	6·0 × 0	4	15·0 × 3·0
66	2	5·0 × 0	3	20·0 × 3·7
73	2	4·0 × 0	2	24·0 × 5·0
Largest Lesion		12·0 × 2·0		30·0 × 6·0

which more closely resembled those seen in rabbits and man. The rabbit lesion was obviously infected by pyogenic organisms and *T. pallida* were not demonstrated in it; however, the animal's serum became reactive to treponemal tests.

Results with comparatively small quantitated intracutaneous injections of Nichols *T. pallidum* from the rabbit varied more widely in the *A. trivirgatus* than in the rabbit. Darkfield-positive lesions appeared 8–10 days after the inoculation of 200,000 organisms in three of six monkeys and all six rabbits. This inoculum produced darkfield-positive chancres in the other three monkeys on incubation days 19, 19, and 38. The inoculation of two *T. pallida* produced chancres in four of six monkeys after 21–66 days and in two of six rabbits after 27 and 45 days (Table II).

The six monkeys and three of the six rabbits inoculated intracutaneously at multiple sites in the back were also inoculated in the anterior skin with the same suspensions at the same times. Three sites were injected from the upper chest to the lower abdomen along each side, an inch from the midline. The lower abdominal skin of this monkey is thin, and the entire 0·2-ml. was not deposited within the dermis at every site in this area. Some of the suspension was observed to leak out, and no doubt some leaked or was injected subcutaneously. No lesions developed in monkeys from the 36 anterior injections of 2–200,000 *T. pallidum*, and only one was produced by the eighteen injections in rabbits. After 20-days' incubation, the 200,000-*T. pallidum* inoculum caused a small short-lived darkfield-positive lesion in the skin over the chest of one of the three rabbits (No. 2023).

Many syphilitic rabbits develop generalized lesions, particularly in the testes; in the shaved skin of the back, sides, and outer surfaces of the extremities;

TABLE II

QUANTITATED INTRACUTANEOUS INOCULATION OF *TREPONEMA PALLIDUM* IN THE BACKS OF RABBITS AND MONKEYS

	Experiment No.	Species	Animal No.	Incubation in Days: 200,000 Tp/Site	20,000 Tp/Site	4,000 Tp/Site	2,000 Tp/Site	200 Tp/Site	20 Tp/Site	2 Tp/Site
A Relation of Size of Inoculum to Development of Darkfield-positive Chancre	1	Monkey	M-1	38	*		*	*	*	*
			M-10	19	19		19	38	*	21
			M-11	19	19		19	21	32	21
		Rabbit	1445	10	10		14	19	24	*
			1475	10	10		10	19	24	*
			1492	10	10		12	24	24	*
	2	Monkey	M-31	10	63		63	*	63	*
			M-35	10	15		63	*	90	66
			M-36	8	13		38	41	*	45
		Rabbit	2021	8	10		13	*	*	45
			2022	8	10		13	24	24	27
			2023	8	10		13	31	*	*
	3	Monkey	M-25			15				
		Rabbit	1627			*				
B Proportion of Inoculations producing Darkfield-positive Lesions		Monkey	Incubation Days: Mean	17·3 ±11·2	26 ±21·0	15	40·4 ±21·6	33·3 ±10·8	61·2 ±29·0	38 ±21·7
			Range	8–38	13–63		19–63	21–41	32–90	21–65
			Sites Positive: Proportion	6/6	5/6	1/1	5/6	3/6	3/6	4/6
			Percentage	100	83·3	100	83·3	50·0	50·0	66·7
		Rabbit	Incubation Days: Mean	9 ±1·0	10 ±0	*	12·5 ±1·6	23·4 ±4·9	24 ±0	56 ±4·6
			Range	8–10	0		10–13	19–31	0	27–45
			Sites Positive: Proportion	6/6	6/6	0/1	6/6	5/6	4/6	2/6
			Percentage	100	100	0	100	83·3	66·7	33·3
		Magnuson Rabbit†	Incubation Days: Mean	14·3 ±4·6	17·1 ±9·0		26·5 ±11·4	26·7 ±8·0	31·7 ±10·6	34·9 ±9·1
			Range	8–24	7–56		14–62	17–64	20–64	25–56
			Sites Positive: Proportion	30/30	24/26		74/80	31/36	36/49	20/42
			Percentage	100	92·3		92·5	86·1	73·4	47·6

± = Standard deviation.
* = Not found to be darkfield-positive.
† Magnuson and others (1948).

in the facial bones; and in the long bones of the extremities (Brown and Pearce, 1921). Four of the seven infected rabbit controls in these experiments developed darkfield-positive secondary skin lesions. The entire torso of six of the seven monkeys infected intracutaneously was kept shaved from neck to groin for 6 months after inoculation. Only the left rear quadrant of the seventh monkey was kept shaved. Some monkeys developed heavy pigmentation over the back and sides. One (M-36) of the seven developed a darkfield-positive secondary lesion in a traumatic wound of the skin over the lower spine. No other lesions of the skin or mucous membranes were noted. Because of the minuteness of many of the primary lesions, it is possible that indistinct skin manifestations were overlooked. No other generalized lesions were noted by inspection of the skin or mucous membranes, or by palpation of the bony structures.

One adult female and three adult male monkeys were inoculated with infectious syphilitic material at the mucocutaneous junction of the prepuce or labia, either by injecting suspensions of *T. pallidum* in and under the mucous membranes or by rubbing the suspensions into scarified genital mucosa. The suspensions contained 10^7–10^8 virulent *T. pallida* per millilitre extracted from syphilitic rabbit testicles. The three males (M-31, M-35, and M-36) were also injected in other areas at the same time. All four of the animals were definitely infected. Injected and abraded areas generally healed immediately, as they did after the trauma of subsequent repeated collection of tissue fluids for microscopic examination. Examination of these fluids was negative. The female (M-39) developed no discernible surface lesion, but a suggestive area of very slight erythema was repeatedly darkfield negative; 2 months after inoculation, serum from this monkey was reactive to the FTA-ABS test, and the TPI test was weakly reactive.* All other subsequent treponemal tests of the serum were reactive, and lymph-node tissue transferred after 8 months infected rabbits.

To compare results from inoculations with *T. pallidum* from the current reservoir in man with those obtained with Nichols strain treponemes maintained in rabbits for half a century, a direct man-to-monkey transfer of treponemes was performed. Through the co-operation of Dr J. F. Hackney and Dr J. H. Tiedemann, a patient seen at the Fulton County Health Department Venereal Disease Clinic, Atlanta, Georgia, U.S.A., on July 21, 1964, was made available. Exudate from a primary penile chancre, estimated microscopically to contain approximately 40,000 motile *T. pallida*, was injected into one testis of an adult *A. trivirgatus* (M-13). A similar inoculation was made into a rabbit. The rabbit developed darkfield-positive orchitis after 36 days, which indicated that each inoculum contained at least 1–20,000 or more virulent *T. pallida* (Magnuson and others, 1948). The scrotum of the monkey was palpably affected—mildly thickened and slightly indurated—during the second and third post-inoculation weeks, but overt darkfield-positive orchitis did not develop. VDRL, FTA-ABS, and TPI tests became positive after 3–5 months. Venous blood and popliteal node extracts from this animal infected three other monkeys to which it was transferred.

The extract of an inguinal node from one of the "second generation" monkeys (M-28) (first monkey-to-monkey recipients of the recently-isolated strain), containing about 4,000 *T. pallida* per inoculum, was injected into the preputial membrane, the tip of the penis near the urethral orifice, and the skin of the lower left quadrant of the back of monkey M-25. As an inoculum control, a rabbit (No. 1627) was injected intracutaneously with this suspension in the same quadrant; however, the ensuing rabbit lesion was obviously contaminated with pyogens, and treponemes were not demonstrated from it. The rabbit was proved infected by serological and tissue-transfer procedures. The monkey skin injection produced an 8×2-mm. chancre by incubation day 8; this was repeatedly darkfield positive from the fifteenth day (Table II, Experiment 3). A small erythematous area developed near the end of the penis by day 8 and persisted for 25 days, ranging from barely discernible to 3–5 mm. in diameter. Numerous darkfield examinations of tissue fluids from the area during this period gave positive results only on incubation day 25. Spirochaetes morphologically resembling *T. pallidum* were again demonstrated in tissue fluids from the surface of the penis 53 days after inoculation when no abnormality was apparent. Serum from this monkey was reactive (VDRL, FTA-ABS, and TPI) 2 and 3 months after inoculation, and its inguinal node aspirate was darkfield positive when first examined 3 months after inoculation.

To study the natural transmission of syphilis in the *A. trivirgatus*, the infected female (M-39) and the male (M-25) with the darkfield-positive penile lesion and a chancre in the skin of the back were each housed with un-inoculated monkeys of the opposite sex for 14 and 7 months, respectively. The

*Fluorescent treponemal antibody-absorption (FTA-ABS) test and *Treponema pallidum* immobilization (TPI) test were all performed by Reagents, Testing and Evaluation Services, Venereal Disease Research Laboratory (VDRL).

monkeys seemed to enjoy companionship and, through playing, huddling, and grooming, the normal female was intimately exposed to the surface chancre of her cage mate. No sexual contact was observed. Spermatozoa were not demonstrated at periodical examinations of penile and vaginal washings. No lesions have developed in the uninoculated partners, and the serological responses of one were nonreactive for 7 months and of the other for 15 months after the start of exposure. Tissue transfers did not infect.

Histopathological examinations, including fluorescent-antibody (FA) staining and silver staining of lymph-node smears and sections, showed no spirochaetes in preparations from three non-infected monkeys and three infected for 3–17 months. *Post mortem* examinations were generally performed on syphilitic monkeys during latency, and there was little or no gross or microscopic evidence of syphilis. Stains for *T. pallidum* were negative.

Twelve *A. trivirgatus* monkeys with documented syphilis were observed for 6–28 months. Seven had been infected with the Nichols strain of *T. pallidum* from experimental rabbits and five with the recent man-to-monkey isolate described. Ten of the twelve developed primary lesions that were repeatedly darkfield positive. Nodes from one of the two monkeys that developed no demonstrable chancre infected on transfer, and both the blood and nodes from the other monkey infected recipient animals.

When received, all monkeys tested gave negative reactions in serological tests for syphilis: 37 monkeys to the VDRL test, 29 to the FTA-ABS, and 29 to the TPI test. No reactivity was detected in sera collected at intervals from non-infected monkeys during more than 2 years; 84 of these sera were tested by the VDRL, 63 by the FTA-ABS, and 37 by the TPI tests.

Results of periodical serum testing of the twelve syphilitic monkeys with these three tests are given in Table III. Once reactive, each of the tests has remained reactive until the time of writing. The reagin type of response, as detected by the VDRL test, ranged from no reactivity in proved syphilitic infection of 12 to 24 months' duration to early reactivity, increasing for several months and then declining. One monkey (M-28), first reactive at a titre of 1:4 2 months after inoculation, was reactive at 1:128 to 1:256 during the fifth to the ninth months of infection and reactive at 1:32 after one year of the disease. Another (M-11), which was repeatedly darkfield positive and FTA-ABS and TPI reactive after 3–24 months, was nonreactive to the VDRL test throughout the observations. This monkey's nodes were infectious 25 months after inoculation.

Sera from five of the twelve infected animals were nonreactive to the VDRL test after 6 months, as were five of ten survivors at 9 and 12 months. Sera from one monkey (M-34), after giving negative re-

TABL

REACTIVITY OF *AÖTUS TRIVIRGATUS* INFECTE
FLUORESCENT TREPONEMAL ANTIBODY-ABSORPTIO

Treponema pallidum		Nichols Strain																					
Source		Rabbit																					
Treponemes/ Monkey		$4{\cdot}7 \times 10^7$									$5{\cdot}0 \times 10^6$												
Monkey No.		M–1			M–10			M–11			M–31			M–35			M–36			M–39		
Test		FTA- VDRL	ABS	TPI	FTA- VDRL	ABS	TPI	FTA- VDRL	ABS	TPI	FTA- VDRL	ABS	TPI	FTA- VDRL	ABS	TPI	FTA- VDRL	ABS	TPI	FTA- VDRL	ABS	TP
Duration of Infection (mths)	0	N	N	ND	N	ND	ND	N	N	ND	N	N	ND	N	N	ND	N	N	N	N	N	ND
	1	N	N	ND	N	N	ND	N	N	N	N	N	ND	N	N	N	N	WR	N	N	WR	N
	2	N	R	N	N	N	N	N	WR	AC*	N	R	N	N	R	R	N	R	WR	N	R	WF
	3	N	R	R	N	WR	R	N	R	R	N	R	R	N	R	R	N	R	R	ND	R	R
	4	N	R	(R)	N	R	(R)	N	R	(R)	N	R	(R)	N	R	(R)	N	R	R	N	R	(R)
	5	N	(R)	(R)	R1	(R)	(R)	N	(R)	(R)	ND	(R)	(R)	ND	(R)	(R)	ND	(R)	(R)	ND	(R)	(R)
	6	N	R	(R)	R4	R	(R)	N	R	(R)	N	(R)	R	N	(R)	R	N	R	R	N	R	R
	7	ND	(R)	(R)		DIED		ND	(R)	(R)	N	R	(R)	N	R	(R)	N	R	R	N	(R)	(R)
	8	WR	(R)	R	—	—	—	N	(R)	R	N	(R)	(R)	N	(R)	(R)	N	(R)	(R)	N	(R)	R
	9	(R)	(R)	(R)	—	—	—	ND	(R)	(R)	N	(R)	(R)	N	(R)	(R)	N	R	R	N	(R)	(R)
	12	(R)	(R)	(R)	—	—	—	ND	(R)	(R)	N	(R)	(R)	N	(R)	(R)	N	(R)	(R)	N	(R)	(R)
	15	R8	(R)	R	—	—	—	N	(R)	R	—	—	—	—	—	—	—	—	—	—	—	—
	24	R128	R	R	—	—	—	N	R	R	—	—	—	—	—	—	—	—	—	—	—	—

AC* = Anticomplementary
— = Observations incomplete.
ND = Not done.
(R) = Not tested; previously reactive.

sults during each of the first 7 months of infection, became reactive at a titre of 1:8 after 8 months and at 1:128 after 9 months.

Another animal (M-1) gave nonreactive tests for 6 months, and was weakly reactive at 8 months; it was not tested again until the fifteenth month when it was reactive at eight dilutions. At 22 months the titre had reached 1:128. Serum from two of three monkeys was reactive at 15 months, and from one of two at 24 months. Results of the testing of three monkeys (M-1, M-10, M-11) 2 and 5 months after infection by the Kline standard, the Mazzini flocculation, and the unheated serum reagin (USR) tests* paralleled those of the VDRL test. All three monkeys were nonreactive in these tests at 2 months, as were two at 5 months. The only one of these three monkeys with serum reactive in the VDRL test at 5 months was also sero-positive in the Kline, Mazzini, and USR tests.

Treponemal immobilizing antibody was detected in three of the twelve infected monkeys as early as 2 months after inoculation (the sera being weakly reactive in two), and in all twelve by the fifth month. Of the twelve, the FTA-ABS test was reactive in three at one month, in ten at 2, and in twelve at 3; it was reactive in all sera tested 4 to 24 months after infection.

Examination of oral preparations showed treponemal forms resembling those common in the human mouth in all 37 *A. trivirgatus* monkeys examined. Some of the spiral forms seen in genital washings resembled *T. pallidum*. A large, motile fluke was sometimes present in preparations containing fresh blood. In monkeys in which the diagnosis of syphilis was not verifiable by other means, no spirochaete indistinguishable in life from *T. pallidum* was seen during microscopical examination of hundreds of samples of serum expressed from skin and genital mucosa, of node or testicular aspirates or extracts, of tears, aqueous, and vitreous fluids, and of many tissues obtained at autopsy.

*Kline standard, Mazzini flocculation, and USR tests performed by Reagents, Testing, and Evaluation Services, VDRL.

Discussion

The observations on twelve infected animals reported here indicate the potential value of the *A. trivirgatus* in the study of experimental syphilis. This primate was susceptible to infection with the Nichols virulent *T. pallidum* from the rabbit and with a strain from man. Its early responses parallel those of man and of other animals. The testis of this animal may be a less favourable reservoir for large numbers of treponemes than is the rabbit testis, or its small size and natural firmness, together with our inexperience with the species, may have prevented recognition of the signs of maximum involvement. The incidence and nature of early and generalized skin manifestations differed from those in man and the rabbit and varied widely in this small sample.

[I

'ITH *TREPONEMA PALLIDUM* IN THE VDRL,
ND *TREPONEMA PALLIDUM* IMMOBILIZATION TESTS

Human Strain													Proportion Reactive of all Twelve Monkeys*		
Man			M-13			M-13			M-13			M-28					
4 × 10⁴			Unknown			Unknown			Unknown			4 × 10⁴					
M-13			M-28			M-34			M-22			M-25					
FTA-			FTA-			FTA-			FTA-			FTA-					
VDRL	ABS	TPI	VDRL	ABS	TPI	VDRL	ABS	TPI	VDRL	ABS	TPI	VDRL	ABS	TPI	VDRL	FTA-ABS	TPI
N	N	ND	N	N	ND	N	N	ND	N	N	N	N	N	N	0/12	0/12	0/12
N	N	ND	N	N	ND	N	N	N	N	R	ND	ND	ND	ND	0/12	3/12	0/12
N	N	ND	R4	R	N	N	R	N	N	R	N	N	R	N	1/12	10/12	3/12
N	R	N	R8	R	R	N	R	R	N	R	N	R2	R	R	2/12	12/12	10/12
N	R	N	R8	R	(R)	N	R	(R)	N	R	N	R2	R	(R)	2/12	12/12	10/12
R16	(R)	R	R128	(R)	(R)	ND	(R)	(R)	R8	R	R	R4	R	(R)	5/12	12/12	12/12
R16	R	R	R256	R	R	N	R	R	R64	(R)	R	R4	(R)	(R)	5/12	12/12	12/12
(R)	(R)	(R)	R128	(R)	(R)	N	(R)	(R)	R64	R	(R)	R8	(R)	(R)	4/11	11/11	11/11
(R)	(R)	(R)	R256	(R)	(R)	R8	(R)	(R)	R16	R	(R)	—	—	—	5/10	10/10	10/10
(R)	(R)	(R)	R256	(R)	(R)	R128	(R)	(R)	R64	(R)	(R)	—	—	—	5/10	10/10	10/10
(R)	(R)	(R)	R32	(R)	(R)	R8	(R)	(R)	R16	(R)	(R)	—	—	—	5/10	10/10	10/10
R16	R	R	—	—	—	—	—	—	—	—	—	—	—	—	2/3	3/3	3/3
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1/2	2/2	2/2

* Figures represent cumulative total reactive, since animals remained reactive once converted.

Varied response is typical of syphilis, particularly when small numbers of treponemes are introduced experimentally. The nature of this monkey, the pigmentation of the skin of its back, and particularly the minuteness of early lesions and the bizarre characteristics of some of the skin manifestations may have influenced the days-to-darkfield-positivity parameter. The lack of obvious response of the skin of the anterior torso to the injection of up to 200,000 *T. pallida* should be investigated. Since the six monkeys inoculated in this area were also inoculated at other sites at the same time, the possibility of infection without chancre formation could not be studied. A single inoculation in the labia at the mucocutaneous border infected M-39 without producing any sign at the point of injection.

Extraneous spirochaetes morphologically resembling *T. pallidum* with characteristic motility have not been reported in lymph tissue in man. The microscopical demonstration of motile *T. pallida* in inguinal lymph-node aspirates or biopsies constitutes proof of infection in man, as it should in monkeys in which pre-inoculation treponemal serological tests have given negative results.

Garcia (1933a,b) reported that some of a group of Philippine monkeys infected with yaws or with syphilis remained sero-negative to the Wassermann reaction and Kahn test for many months.Turner and Hollander (1957) found the Eagle flocculation test reactive at low titre in approximately one-half of a group of six rhesus and four African green monkeys inoculated with large numbers of Nichols *T. pallida*. Niel and Fribourg-Blanc (1964) reported one of two baboons to be sero-negative to the VDRL test 51 days after inoculation with syphilis, by which time both had become sero-positive to the TPI test. In recent studies by Smith and others (1965) and Taylor, Singer, Yobs, and Smith (1965), seven *A. trivirgatus* monkeys with syphilis of 6 months' duration were sero-positive to the FTA-ABS and TPI tests or infectious by node transfer, but three were sero-negative to the VDRL test. Recipients were infected by nodes transferred from two of their monkeys, six or seven months after inoculation, at which time the donors were still sero-negative to the VDRL, FTA-ABS, and TPI tests.

A. trivirgatus apparently does not produce cardiolipin antibody with the regularity or rapidity seen in the rabbit or in man. It should be noted, however, that in the present study one of the five (M-28) inoculated with the recent human isolate was sero-positive to the VDRL test at a titre of 1:4 2 months after infection and at titres of 1:128 to 1:256 after 5–9 months. This appears to be individual variation, as another (M-34) inoculated with this strain of *T. pallidum* was first sero-positive to the VDRL test after 8 months.

Treponemal antibodies as detected in the standard TPI test generally appeared earlier in the twelve monkeys than in the rabbit. Sera from ten were reactive after 3 months, and all were sero-positive after 5 months. The FTA-ABS test was reactive in three after 1 month, in ten after 2 months, and in all twelve after 3 months. The monkeys that were slowest to develop TPI antibody were two of the five inoculated with the recently-isolated strain. Variations in the development of reactivity occur in man and the rabbit, and the rate of rise in antibody-titre in the rabbit is somewhat related to the number of organisms inoculated. Three of the monkeys (M-1, M-10, and M-11) received approximately $5 \cdot 6 \times 10^7$ *T. pallida*, three (M-31, M-35 and M-36) approximately 5×10^5, and the others only a few thousand each. As in other species, treponemal antibody was detected earlier by the FTA-ABS test than by the TPI test.

The first monkey-to-monkey subtransfer of the man-to-monkey strain of *T. pallidum* produced a skin chancre equal to the largest of 26 caused by the Nichols treponemes from rabbits. It also produced one penile lesion from one inoculation, compared to no genital lesions in the four monkeys inoculated with the Nichols strain. Controlled data that are sufficient for statistical analysis would be necessary to demonstrate differences in strains of *T. pallidum*. Experimental syphilis has been followed for one year in eleven monkeys, and for 2 years in three. Nine survive and are being observed for the development of late manifestations.

The weights of the forty *A. trivirgatus* monkeys ranged from 500 to 1040 g. (average 850). The monkeys were maintained under standard laboratory conditions at 70 to 75°F. Procuring healthy animals with stamina to withstand the rigours of shipping (especially cold weather) and acclimatization in the laboratory is a problem. Three of forty (7·5 per cent.) died during their first 3 weeks in the laboratory, and nine more within a month of arrival after being subjected to the stress of restraint, anaesthesia, injection, collection of blood and spinal fluid specimens, and the like. However, this species is certainly simpler to house and manipulate than the large sub-human primates.

Conclusions

A. trivirgatus is susceptible to infection with *T. pallidum*, developing symptomatic disease with primary and secondary lesions and anti-treponemal antibodies. Some of these responses may not be as

rapid or as prominent as are those seen in the rabbit and in man, but generally their courses run parallel. The species apparently shows a varied reagin response and an early specific anti-treponemal antibody response. Infection in *A. trivirgatus* has not been observed long enough to ascertain whether late manifestations of syphilis develop or to determine the duration of the serological response. As an additional subject for the experimental investigation of syphilis, this monkey offers the advantages of primate phylogeny with smaller size and easier manipulation than the larger sub-human primates.

Summary

Certain observations on twelve *Aötus trivirgatus* monkeys experimentally infected with Nichols strain or with a recently-isolated human strain of *Treponema pallidum* are reported. Primary syphilomata were produced in the skin of the back, in the testis, and on the penis. Results of quantitated intracutaneous inocula varied more widely than in the rabbit. Only one secondary skin lesion was noted. Spirochaetaemia and lymph-node involvement were demonstrated by tissue transfer. Lesions were not produced by intracutaneous inoculation of up to 200,000 *T. pallida* in the anterior torso or the genital area.

Treponemal antibodies were detected in all infected animals after 1 to 5 months. VDRL reactivity varied, with seven of twelve monkeys remaining sero-negative after 6 months' infection and one of two after 2 years' infection. Animals becoming seropositive by a given test remained so by that test during observation for as long as 2 years. A strain of *T. pallidum* transferred directly from man was experimentally established in the monkey.

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Des observations sur la pathogénèse de la syphilis chez les nyctipithèques (*Aötus trivirgatus*)

RÉSUMÉ

Certaines observations sur 12 singes *Aötus trivirgatus* infectés expérimentalement par la souche Nichols ou par une souche humaine de *Treponema pallidum* isolée récemment sont rapportées. Des syphilomes primaires ont été produits dans la peau du dos, dans les testicules et sur la verge. Les résultats des différentes quantités d'inocula injectés dans la peau ont varié d'une façon plus grande que chez le lapin. Une seule lésion cutanée secondaire avait été notée. La spirochétémie et la complication des ganglions lymphatiques ont été demontrées par le transfert de tissus. Des lésions n'avaient pas été produites dans le torse antérieur ou dans la région génitale par l'inoculation intra-dermique jusqu'à 200,000 *T. pallida.*

Les anticorps du tréponème ont été décelés chez tous les animaux infectés après un à cinq mois. Les réactions VDRL ont varié, sept des douze singes restants séronégatifs après une infection datant de six mois, et un des deux singes après une infection de 2 ans. Les animaux devenant séro-positifs par un test specifié sont restés ainsi pendant une période d'observation qui a duré aussi longtemps que 2 ans; Une souche de *T. pallidum* transférée directement de l'homme a été transmise expérimentalement au singe.