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EXPERIMENTAL OCULAR AND NEUROSYPHILIS IN THE PRIMATE*†

BY

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The rabbit has been commonly used for experimental syphilis since Bertarelli (1906) first inoculated this species with *Treponema pallidum*. Three years earlier, Metchnikoff and Roux (1906) had succeeded in making the monkey the first animal successfully inoculated with the disease. However, but for isolated and notable exceptions (Neisser, 1906, 1908; Turner and Hollander, 1957), few subsequent studies of experimental syphilis have been performed in the primate. Because of interest in the clinical problem of sero-negative ocular and neurosyphilis (Smith, 1964, 1965; Smith, Singer, Moore, and Yobs, 1965; Smith and Taylor, 1965), here defined as late syphilis in which serum reagin tests are non-reactive but which can be detected by specific treponemal tests, a study of experimental syphilis in the primate was begun in this laboratory in 1963.

During the past 3 years, the owl monkey (*Aotus trivirgatus*) and the squirrel monkey (*Saimiri sciurea*) have been found to be especially suitable species for ophthalmological investigation (Smith, Singer, Reynolds, Moore, Yobs, and Clark, 1965; Taylor, Smith, and Singer, 1965). These small primates are inexpensive, hardy in nature, and safe as a laboratory animal. They possess obvious advantages over the rabbit in having fundi quite similar to man, and in not having laterally placed eyes, an important point when studying pupillary function. The owl monkey has larger eyes and has hence been preferred for anterior segment technical reasons, but the fundus of the squirrel monkey is more like that of man (Smith, Reynolds, Rane, and Justice, 1964). The purpose of this report is to document some interesting laboratory observations after the inoculation of virulent *T. pallidum* into various sites in these primate species.

Material and Methods

From 125 animals (58 rabbits, 51 owl monkeys, and 16 squirrel monkeys) studied to date, three owl monkeys and two squirrel monkeys have been selected for this report.

Before inoculation, each animal was examined and blood was drawn for the following serological tests:

(1) A reagin test—the Venereal Disease Research Laboratory test (VDRL),

(2) The treponema pallidum immobilization test (TPI),

(3) A new specific treponemal test—the fluorescent treponemal antibody absorbed (FTA-ABS) test (Hunter, Dealon, and Meyer, 1964; Smith and Taylor, 1965).

All serological tests here reported were performed at the Venereal Disease Research Laboratory in Atlanta, Georgia. Each of the animals was initially clinically healthy and serologically non-reactive. A suspension of virulent *T. pallidum* (Nichols strain) was prepared from the testicle of a rabbit* with an acute syphilitic orchitis for use as the inoculum. After excision of the testicle, it was finely minced and diluted with 4 ml. of 50 per cent. normal saline with 50 per cent. normal rabbit serum (the latter being non-reactive to all three of the serological tests cited). A dark-field count was done on the supernatant suspension and the number of treponemes present in the aliquots was thus estimated.

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The animals were anaesthetized with subcutaneous injections of 0·3 ml. of a 10 mg./ml. solution of phencyclidine hydrochloride* which produced a light anaesthesia with onset of action in 5 to 10 minutes and a duration of approximately 2 hours. Sterile tuberculin syringes with 25 or 27 needles were used for the inoculations. The volume of *T. pallidum* suspension used varied from 0·05 ml. for ocular injections to 0·1–0·2 ml. for other sites.

General physical and ocular examinations were done at appropriate intervals after inoculation. Significant clinical findings were documented by external and fundus photographs. Pupils were dilated for examination with cyclopentolate† and tropicamide‡. Sera were submitted periodically to the Venereal Disease Research Laboratory in Atlanta, Georgia, where the serological tests here reported (VDRL, TPI and FTA-ABS) were performed. All animals were kept in separate cages, fed only antibiotic-free commercial monkey chow, bananas, and water, and were at no time given any form of treatment.

Findings

(1) Primary Corneal Syphilis

Squirrel monkey 633 was inoculated on June 30, 1964, in the corneal stroma of the right eye with approximately 37,500 treponemes given in 0·05 ml. suspension. 0·05 ml. sterile serum/saline solution was inoculated into the left eye to serve as a control.

For the first 6 months after inoculation, periodic general physical and ocular examinations were negative in this monkey. At that time, however, condyloma-like lesions were noted on the scrotum (Fig. 1); these persisted for a time, but then spontaneously regressed. No other clinical abnormalities were noted during the next 10 months (Fig. 2), and 16 months after inoculation, the animal appeared in good general health, and the eyes were completely normal to external, biomicroscopic, and indirect ophthalmoscopic examinations. The serological data are summarized in Table I.

TABLE I

SEROLOGICAL DATA—SQUIRREL MONKEY 633

Date of Test	VDRL	TPI	FTA-ABS
June 30, 1964	NR	—	NR
August 3, 1964	NR	—	NR
December 12, 1964	NR	NR	WR
March 17, 1965	NR	WR	WR
June 2, 1965	NR	WR	R
September 7, 1965	NR	R	WR
October 25, 1965	NR	—	—

NR = Non-reactive, WR = Weakly reactive, R = Reactive, — = not done.

* Supplied through the courtesy of Parke, Davis, & Co. as Sernylan.
† Cyclogyl (Schieffelin & Co.).
‡ Mydriacyl (Alcon Laboratories, Inc.).

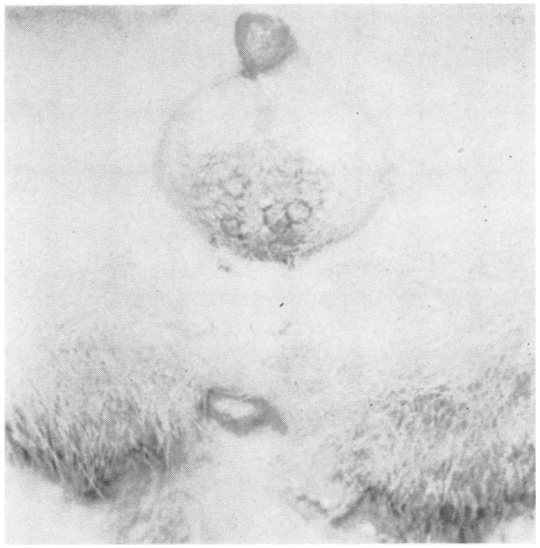

FIG. 1.—Squirrel monkey 633 on January 1, 1965. Condyloma-like lesions noted 6 months after inoculation.

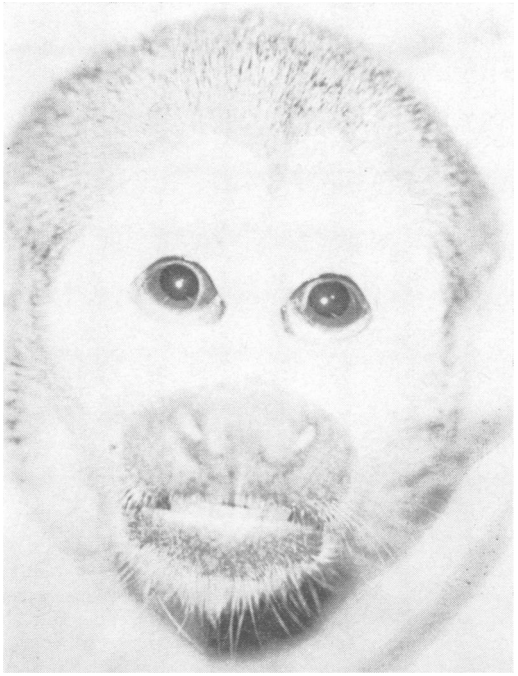

FIG. 2.—Squirrel monkey 633 on July 27, 1965. Clinically normal 16 months after infection.

Comment The VDRL test remained nonreactive in squirrel monkey 633 on seven occasions during 16 months' observation after the intracorneal inoculation of *Treponema pallidum*. However, the

FTA-ABS converted at 5 to 6 months and the TPI at 8 months after infection. As a rule, in these species, the FTA-ABS becomes reactive at 4 to 5 months after inoculation, the TPI at 6 to 8 months, and the VDRL at varying intervals thereafter. However, as seen in this animal, the VDRL may never become reactive. The point emphasized here is that had only a reagin test been performed, it would have been falsely concluded that the animal had NOT been infected, in view of the fact that no lesions occurred in over 16 months' observation. There is no doubt, however, from the specific treponemal tests, that serologically this animal has acquired syphilis. This is to our knowledge the first reported case of *corneal sero-negative syphilis in the primate.*

(2) Primary Vitreal Syphilis

Squirrel monkey 635 was inoculated on June 30, 1964, with approximately 37,500 treponemes given by inoculating 0·05 ml. suspension directly into the vitreous of the right eye. The left eye vitreous received 0·05 ml. of the sterile serum/saline solution. Except for minimal post-injection changes which cleared after one week, no abnormalities in either eye were found during the first 6 months' observation. However, during the following year, slight anisocoria was noted on occasion, the left pupil being the larger (Fig. 3).

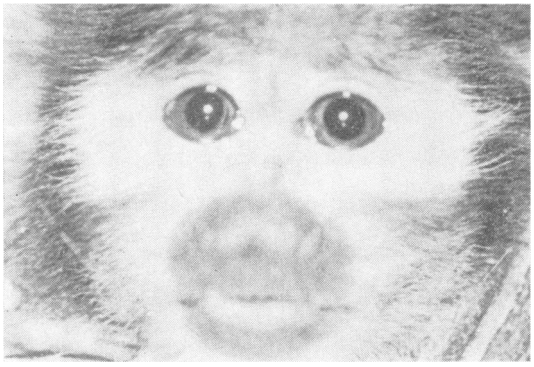

FIG. 3.—Squirrel monkey 635 on January 12, 1965. Subtle anisocoria with left pupil the larger.

Except for subtle anisocoria, examinations of this monkey were quite negative 16 months after infection. The serological data are summarized in Table II.

Comment This is the first reported case of *vitreal syphilis in the primate.* Serologically, by both non-specific and specific tests, the animal has syphilis. Clinically, however, it has never shown any notable signs of the disease. Except for moderate pupillary inequality, the eyes have revealed no evidence of infection, and the animal has been healthy throughout 16 months' observation.

TABLE II

SEROLOGICAL DATA—SQUIRREL MONKEY 635

Date of Test	VDRL	TPI	FTA-ABS
June 30, 1964	NR	—	NR
August 3, 1964	NR	—	qus
December 12, 1964	NR	R	R
February 1, 1965	R 1 dil	—	—
March 17, 1965	R 1 dil	—	WR
June 3, 1965	R 1 dil	—	—
September 7, 1965	WR	R	WR
October 25, 1965	NR	—	—
November 9, 1965	—	—	R

It is interesting to note that the VDRL became reactive at 7 months and spontaneously reverted to non-reactive 16 months after infection. This phenomenon of spontaneous reagin reversal has occurred not infrequently in the primates studied in this laboratory. It should be stressed that no animals have been treated and that all have been fed antibiotic-free commercial chow.

(3) Primary Carotid Syphilis

Owl monkey 135 was inoculated on February 25, 1965, in the left common carotid artery with approximately 600,000 *Treponema pallidum* in 0·3 ml. suspension. Subsequent periodical examinations in the following 8 months gave negative results (Fig. 4). However, 9 months after inoculation, indirect ophthalmoscopy revealed perivascular sheathing along the superior temporal artery in the left eye. The serological data are summarized in Table III.

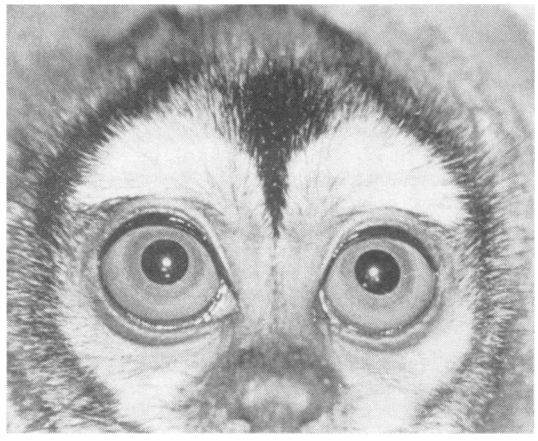

FIG. 4.—Owl monkey 135 on November 4, 1965. Normal after 8 month's observation.

TABLE III

SEROLOGICAL DATA—OWL MONKEY 135

Date of Test	VDRL	TPI	FTA-ABS
February 25, 1965	NR	—	NR
August 10, 1965	NR	NR	WR
October 25, 1965	WR 0 dils	R	R
December 31, 1965	—	—	R

Comment To our knowledge, direct carotid artery inoculation has been attempted only once in experimental syphilis in rabbits (by De Giorgio and Sterzi, 1938), and never before in primates. This animal (owl monkey 135) therefore represents the first case of carotid syphilis in the primate. Both the specific tests and the reagin test became reactive. Here again the specific test converted before the reagin test.

Perivasculitis is a well-known lesion in syphilis, and has been seen in several other primates in this laboratory.

(4) Primary Cisternal Syphilis

Owl monkey 153 was inoculated on February 2, 1965, with approximately 200,000 treponemes given in 0·1 ml. suspension by direct inoculation into the cisterna magna. Dr David Reynolds kindly performed the cisternal inoculations. The results of periodical examinations thereafter were negative (Fig. 5), until at 5 months definite anisocoria was noted, the left pupil being larger than the right. At 8 months both pupils were miotic (Fig. 6), and the right pupil was slightly irregular and reacted poorly to light. Ophthalmoscopy at that time revealed perivascular sheathing along the superior temporal artery in the left eye. On November 5, 1965, this monkey's blood was given intravenously *via* the ear vein to a normal rabbit as a passive transfer experiment, and 13 days later a large crusting lesion, which was dark-field positive on a direct smear, was noted on the rabbit's thigh. The serological data are recorded in Table IV.

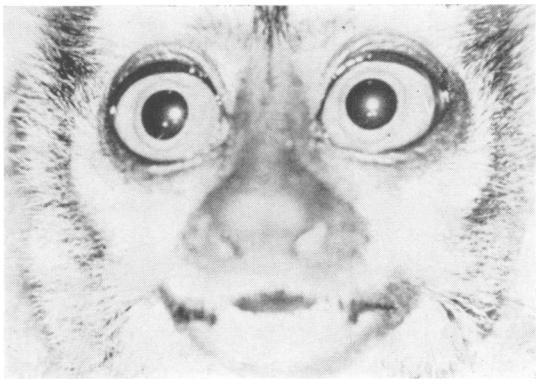

FIG. 5.—Owl monkey 153 on March 4, 1965. All tests negative for first 5 months after inoculation.

TABLE IV

SEROLOGICAL DATA—OWL MONKEY 153

Date of Test	VDRL	TPI	FTA-ABS
February 25, 1965	NR	—	NR
August 10, 1965	NR	NR	NR
October 25, 1965	NR	NR	NR
December 3, 1965	—	—	NR

FIG. 6.—Owl monkey 153 on November 4, 1965. Miotic pupils.

Comment This animal (owl monkey 153) has shown no serological evidence of syphilis during 8½ months' observation after direct inoculation of virulent *T. pallidum* into the subarachnoid space *via* the cisterna magna. Clinically, however, the animal developed abnormal pupils and perivascular sheathing in the fundi; i.e. it showed clinical signs without serological confirmation of infection. A definite diagnosis of syphilis could not be made in such circumstances, but since passive transfer of this animal's blood to a normal rabbit produced a definite darkfield-positive lesion the diagnosis of syphilis is substantiated in this instance.

(5) Primary Cisternal Syphilis

Owl monkey 93 was given 0·2 ml. *T. pallidum* suspension by injection into the cisterna magna on January 30, 1964. Serial examinations for the next 6 weeks gave negative results. On March 10, 1964, the left pupil dilated poorly to mydriatics and by May 14, 1964, definite anisocoria was observed, the left pupil being larger. Except for persistent anisocoria, all examinations gave normal results for the ensuing 6 months, and on November 25, 1964, the pupils were found to be equal in size. The animal remained clinically normal until 15 months after inoculation (May 6, 1965) when a fresh retinal haemorrhage was seen in the lower temporal quadrant of the left eye (Figs 7 and 8, opposite).

This spontaneously regressed during the next 2 weeks. 18 months after infection, anisocoria was again observed, the left pupil being the larger (Fig. 9, opposite). On October 14, 1965, 20½ months after infection, a second retinal haemorrhage was seen alongside a lower temporal arteriole in the left eye. Since the regression of this retinal

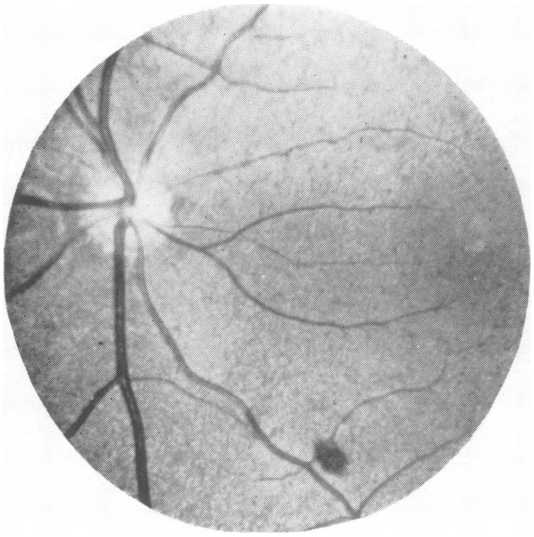

FIG. 7.—Owl monkey 93 on May 6, 1965. Retinal haemorrhage present in lower temporal quadrant in left eye.

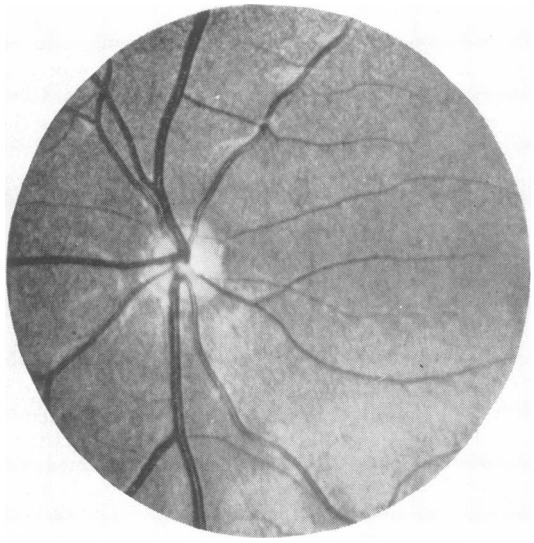

FIG. 8.—Owl monkey 93 on August 24, 1965. Left fundus now quite normal.

FIG. 9.—Owl monkey 93 on July 29, 1965. Anisocoria with left pupil the larger.

haemorrhage, the animal has remained clinically normal up to 24 months after infection. The serological results are tabulated in Table V.

Comment This animal developed both serological and clinical evidence of syphilis. Clinically, abnormal pupils and recurrent retinal haemorrhages occurred.

The development of retinal perivascular sheathing has been an interesting clinical finding in these animals.

TABLE V

SEROLOGICAL DATA—OWL MONKEY 93

Date of Test	VDRL	TPI	FTA-ABS
January 30, 1964	NR	NR	NR
April 10, 1964	NR	—	WR
May 19, 1964	NR	—	R
June 11, 1964	—	—	R
August 3, 1964	R 2 dils	R	R
September 11, 1964	R 2 dils	—	—
December 12, 1964	R 8 dils	R	WR
May 10, 1965	R4	—	—
September 27, 1965	R2	—	—
January 25, 1966	—	—	R

Discussion

The findings in five primates (two squirrel monkeys and three owl monkeys) inoculated with virulent *T. pallidum* into the cornea, vitreous, carotid artery, and cisterna magna (2) have been reviewed. Three of these represent the first reported examples of primary corneal sero-negative syphilis, primary vitreal syphilis, and primary common carotid syphilis in the primate. That these monkeys were infected was evidenced by conversion of both the TPI and FTA-ABS tests, which were repeatedly positive. The animal infected *via* the cornea was noteworthy because no clinical evidence of infection could be observed and the animal remained sero-negative to the reagin test during 18 months' observation. Similarly, the animal infected *via* the vitreous has remained clinically normal, but by the

results of all three serological tests it has in fact been infected. The animal infected *via* the carotid exhibited both clinical and serological evidence of infection. The first of the two animals inoculated *via* the cisterna magna (O-153) showed clinical signs of disease but with no serological confirmation whatsoever. The diagnosis was corroborated in this monkey, however, by passive transfer.

The second such animal (O-93) exhibited both serological and clinical evidence of infection.

These findings corroborate the previous important experiments of Turner and Hollander (1957) and also extend them because of availability of the TPI and the FTA-ABS tests. In the course of performing experiments to find an immunizing agent against syphilis, these authors obtained results which are of great interest and are strikingly similar to our own. They utilized two different primate species for their study—the *Macacus rhesus* and *Cerpithecus aetheopsis saaesis* (African green) monkeys. Ten animals (six rhesus and four African green) were inoculated with *T. pallidum* given intracutaneously in the thigh and also by scarification on the eybrow and in the preputial sac. In contrast to our findings, all but two of the African green monkeys developed transient darkfield-positive skin lesions within about 2 weeks. By the end of 3 months nearly all these lesions had disappeared. Serologically, about half of their animals became reactive to the Eagle flocculation test and half remained negative. Their findings are of such interest that their Tables VII and VIII are here reproduced as Tables VI (below) and VII (opposite).

Turner and Hollander (1957) performed cerebrospinal fluid examinations by cisternal puncture on their monkeys before inoculation and at various intervals after infection. They found that the cell count, Pandy reaction, and Wassermann reaction were essentially negative in all instances. Three of their animals died between the fifth and eighth months after inoculation, and the remaining seven were killed at 14 to 17 months after infection. *Post mortem* examination revealed no gross changes suggestive of syphilitic infection. At autopsy, suspensions of plasma, liver, and lymph nodes were removed and inoculated into the testes of two normal rabbits. In addition, emulsions of other tissues (heart muscle, cerebral cortex, spinal cord, etc.) were inoculated into rabbits. Their findings were summarized in their Table VIII.

These findings are very important and bear close scrutiny. Note that the blood from three monkeys, a suspension of liver from four of them, lymph nodes from five of them, and a brain and spinal cord emulsion from one, produced darkfield-positive lesions after inoculation into normal rabbits. Thus, passive transfer of these tissues yielded recovery of treponemes with darkfield-positive lesions from each of eight of the ten originally infected monkeys. It should be noted that their monkeys numbers 24, 27, 28, and 29 showed essentially sero-negative reactions throughout the entire course of the experimental period, and that two of these (28, 29) showed no lesions whatever at the inoculation sites, and yet that treponemes were recovered by animal inoculation repeatedly in their monkeys 24, 28, and

TABLE VI

DATA ON CLINICAL COURSE AND SEROLOGICAL REACTIONS (STS) AFTER INOCULATION WITH SYPHILIS TREPONEMES
From Turner and Hollander (1959), p. 51, Table VII

Species of Monkey	Monkey No.	First Lesions	Day of Incubation	Titre of Eagle Flocculation Test on Day indicated:								
				0	28	67	97	124	164	194	219	361
M. rhesus	19	Eyebrow	21	0	0	2	4	1	4	4	4	8
	20	Thigh	17	0	+–	0	4	8	4	8	8	4
	21	Thigh	12	0	0	0	0	1	1	1	1	died
	22	Thigh and eyebrow	14	0	0	4	2	died				
	23	Eyebrow	14	+–	0	0	+–	4	2	12	2	1
	24	Eyebrow	17	0	0	0	0	0	0	0	0	died
African Green	26	Eyebrow	12	+–	+–	2	8	8	2	4	4	2
	27	Eyebrow	21	0	0	0	0	0	0	0	+–	+–
	28	Negative	—	0	0	0	0	0	0	0	died	
	29	Negative	—	0	0	+–	0	0	0	0	died	

TABLE VII

ORGAN INFECTIVITY TESTS OF MONKEYS INOCULATED WITH SYPHILIS TREPONEMES AND RESULTS OF INOCULATIONS INTO PAIRS OF RABBITS

From Turner and Hollander (1957), p. 52, Table VIII

Species of Monkey	Monkey No.	Results of Infectivity Tests			
		Blood	Liver	Lymph Nodes	Other
M. rhesus	19	0, 0	+, +	0, died	Heart 0, 0 Brain 0, 0
	20	0, died	0, 0	0, 0	
	21	0, 0	+, 0, 0	0, +	
	22				
	23	+, 0	0, 0	+, +	
	24	+, +	+, +	0, 0	
African Green	26	0, 0	+, +	0, died	
	27	0, 0	0, 0	0, died	
	28	+, +	+, +		Brain Cord 0, 0
	29			+, 0	Brain Cord +, +

29. Of greatest interest were their two African green monkeys, 28 and 29. Monkey 28 showed no lesions after inoculation, had seven non-reactive Eagle flocculation tests in the ensuing 7 months, died at the 219th day, and had no gross changes of syphilis at autopsy; yet definite positive darkfield-lesions were found in rabbits inoculated with lymph nodes as well as emulsions of brain and spinal cord from this monkey in three of four rabbits inoculated.

Thus, Turner and Hollander (1957) found that four of ten inoculated monkeys were sero-negative to reagin testing. Three of these four animals showed treponemes on passive transfer, and two of the four had positive dark-field lesions. All four showed evidence of infection either by dark-field smear and/or passive transfer.

A comparison of Turner and Hollander's findings in the rhesus and African green monkeys with our findings in the owl and squirrel monkeys shows striking similarities. However, we had the advantage of being able to make the *ante mortem* diagnoses more easily because of the availability of specific treponemal tests (TPI and FTA-ABS). This evidence would lead us to disagree with their proposal that primates are unsuitable for experimental syphilis. On the contrary, these animals display many of the same clinical, serological, and pathological findings seen in patients. Examples of some of these findings are illustrated both in Turner and Hollander's excellent work and in the animals here presented. In two of our five animals here reported (Sq-635 and O-93), further confirmation of syphilitic infection was obtained by the finding of treponemes in the aqueous humour by means of the fluorescent antibody tissue stain (Yobs, Brown, and Hunter, 1964; Wells and Smith, in press). This will be the subject of another report.

Summary

The experimental and serological findings in two squirrel monkeys and three owl monkeys inoculated with virulent *Treponema pallidum* in the cornea, vitreous, carotid artery, and cisterna magna are presented. These are the first reported examples of primary corneal sero-negative syphilis, primary vitreal syphilis, and primary common carotid syphilis in the primate. Further laboratory confirmation of the clinical problem of sero-negative ocular and neurosyphilis is provided. These findings have now been documented experimentally in four different primate species.

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La neurosyphilis et la syphilis oculaire expérimentales chez les Primates

RÉSUMÉ

Les constations expérimentales et sériques chez deux sagouins et trois nyctipithèques dont la cornée, le corps vitré, l'artère carotide et la cisterna magna avaient été inoculés avec des tréponèmes virulents sont présentées. Ce sont les premiers exemples de la syphilis séro-négative primaire de la cornée, de la syphilis primaire du corps vitré, et de la syphilis primaire de l'artère carotide qui ont été rapportés chez les Primates. D'autres preuves provenant du laboratoire au sujet du problème clinique de la neurosyphilis et de la syphilis séro-négative oculaire sont fournies. Ces constatations expérimentales chez quatre différentes espèces de Primates ont jusqu'à présent été décrites.