

EXPERIMENTAL OCULAR SYPHILIS AND NEUROSYPHILIS*†

BY

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The challenge in clinical syphilis to-day is the detection of the disease in the sero-negative patient. The magnitude of this problem is evident when one considers that over 100 cases of ocular syphilis and neurosyphilis have been encountered in this institution within the past year (Smith, 1964; Smith and Moore, 1964; Smith and Taylor, 1965). Of paramount importance is the fact that the majority of these patients were non-reactive to the standard reagin tests for syphilis. Diagnosis was established by the following criteria: (1) history of infection and inadequate treatment; (2) clinical signs of the disease—as optic atrophy, pupillary changes, and the like—and (3) reactive *Treponema pallidum* immobilization (TPI) and/or fluorescent treponemal antibody absorbed (FTA-ABS) tests on the peripheral blood (Deacon, Falcone, and Harris, 1957; Deacon, Freeman, and Harris, 1960; Deacon and Hunter, 1962).

A combined clinical and experimental study was therefore undertaken in collaboration with the Venereal Disease Research Laboratory of the US Public Health Service in Atlanta, Georgia. The purposes of this project were as follows: (1) to study sero-negative syphilis, both in the human and in the experimental animal; (2) to investigate primary neurosyphilis (induced by injecting *T. pallidum* directly into the subarachnoid space in the primate); (3) to study ocular syphilis and neurosyphilis in the rabbit and monkey—using more recent clinical techniques (such as fluorescein fundus photography), and correlated with both standard reagin tests (VDRL) and more exacting (TPI and FTA-ABS) serological methods; (4) to attempt recovery of virulent *T. pallidum* from sero-negative patients and

animals. This report presents the findings in the experimental animals noted during the first year of this study.

Material and Methods

A primate, the owl monkey (*Aotus trivirgatus*), was chosen because of species proximity to man and because of previous experience with this animal (Smith and Singer, 1965a, b). The rabbit was also used both as a control to assess virulence of treponemes and because of the large body of data available in the older literature concerning experimental syphilis in this species.

Pigmented and albino rabbits weighing approximately 5 lb. (2.3 kg.) and owl monkeys weighing approximately 1½ lb. (0.69 kg.) were housed in rooms at constant temperature and humidity. They were fed standard rabbit and monkey food and were allowed water as desired.

Rabbits with acute testicular syphilomas were transported from the Venereal Disease Research Laboratories at Atlanta, Georgia, to Miami. The syphilomas were removed at the height of the inflammatory response for use in these experiments. The testicular tissue was finely minced and mixed with a 50–50 serum saline solution (the latter being non-reactive to both VDRL and TPI tests). The resulting emulsion was centrifuged to remove tissue particles and the supernate was used for inoculations.

It was elected to inoculate virulent *Treponema pallidum* directly into the cisterna magna of five owl monkeys and five rabbits. This was done in order to induce primary neurosyphilis. Ocular syphilis was investigated by inoculating treponemes into varying ocular sites (anterior chamber, cornea, vitreous cavity, and optic nerve). Seven other normal owl monkeys and seven other rabbits were used for the ocular injections.

The monkeys were anaesthetized with subcutaneous injections of phencyclidine hydrochloride||. It was found that 0.2 to 0.4 ml. of a 10 mg./ml. solution of this medication would induce light anaesthesia with onset within five minutes after injection and lasting for one to two hours. It was necessary on occasion to repeat these

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|| Supplied as Sernylan (10 mg./ml.) through the courtesy of Parke Davis and Company.

dosages to keep the monkeys properly sedated. In the rabbits 0.7 to 1 ml. of pentobarbital (50 mg./ml.) was given intravenously for anaesthesia. All ocular injections were given using tuberculin syringes and short gauge 30 needles. Cisternal injections were performed with gauge 20 paediatric lumbar puncture needles. The volume of *T. pallidum* suspension used for ocular injections was 0.01 to 0.05 ml., while 0.2 to 0.4 ml. was used for the intracisternal inoculations. All eyes were examined daily using focal illumination and loupes. Biomicroscopic and indirect ophthalmoscopic examinations were performed at appropriate intervals. Significant ocular and physical changes were documented by external, fundus, and fluorescein fundus photographs. Pupils were dilated for examination with 1 per cent. cyclopentolate hydrochloride*. This drug not only provided better mydriasis than did atropine in the rabbits, but possessed the additional advantage of shorter duration of action, so that pupillary responses could be evaluated within 36 hours after ophthalmoscopy.

Sera were submitted from all animals for both quantitative VDRL and TPI testing. All the serological studies were performed at the Venereal Disease Research Laboratories in Atlanta. Proof of infection with *T. pallidum* was attempted by passive transfer technique on some of the

animals six months after inoculation. This was done by excising popliteal lymph nodes from selected rabbits and monkeys, and injecting freshly emulsified suspensions of this tissue into the backs of normal rabbits which were then observed for the appearance of dark-field positive lesions.

Results

The data, first for rabbit and then for monkey, will be presented as outlined: (1) intracisternal inoculation; (2) ocular routes—cornea, anterior chamber, vitreous, and optic nerve.

Rabbit

(1) Intracisternal Inoculation (Table I)

Five rabbits were inoculated with treponemes in the cisterna magna. The rabbits appeared healthy and the eyes remained white, quiet, and developed no lesions during the ensuing two months. In spite of these negative findings the serological tests for syphilis performed two to three months after injection showed that all the animals in the group had reactive VDRL tests.

Hair and Skin Findings.—A consistent finding during the third month was a change in the texture

* Cyclogyl (Schieffelin & Co.).

TABLE I
RABBIT: INTRACISTERNAL INOCULATION

Rabbit	Month							Comment
	First	Second	Third	Fourth	Fifth	Sixth	Seventh and Eighth	
R50		Sluggish pupils	Alopecia Snuffles Anisocoria VDRL R4	Alopecia Snuffles Anisocoria	Alopecia Snuffles Anisocoria	Dead VDRL R4 TPI R Lymph node		Generalized syphilis
R51			Alopecia Snuffles Anisocoria VDRL R2	..	Alopecia Snuffles Interstitial keratitis Tears dark-field +	Alopecia Snuffles Iris atrophy VDRL R4 TPI R Lymph node +	Alopecia etc.	Generalized syphilis Iris atrophy
R52			Alopecia Snuffles Head tilt Left hemiparesis VDRL R4	..	Alopecia Snuffles Anisocoria	Alopecia Snuffles Iris atrophy Koeppel nodules VDRL R4 TPI R Lymph node +	Dead	Generalized syphilis Uveitis
R53		VDRL R8	Alopecia Anisocoria	Alopecia etc. VDRL R4 TPI R	Alopecia etc.	Generalized syphilis
R54			VDRL R32		Alopecia	Alopecia Iris atrophy VDRL R2 TPI R	Dead	Generalized syphilis Iris atrophy

VDRL WR = weakly reactive; VDRL R4 = reactive 1:4, etc.

and colour of the fur, giving it an unkempt appearance (Figs 1, 2, and 3). A very typical "moth-eaten" alopecia starting around the eyes and ears and then involving the back, nape of the neck, and paws was also noted (Fig. 4). Mucous membrane lesions of the nose and conjunctiva were found after three to four months. These changes were very striking and presented an almost identical picture in all the animals inoculated in the cisterna magna. The findings were similar to those reported over 40 years ago in the classic study by Brown and Pearce (1920a).



FIG. 1.—Rabbit five months after intracisternal inoculation with *T. pallidum*. Note unkempt hair, alopecia.

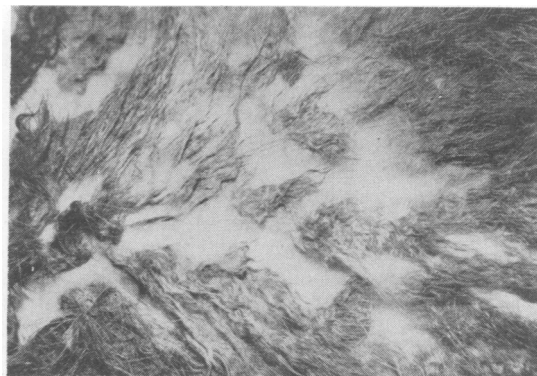


FIG. 3.—Rabbit three months after intracisternal inoculation with *T. pallidum*. Note abnormal hair texture.



FIG. 2.—Rabbit three months after intracisternal inoculation with *T. pallidum*.



FIG. 4.—Rabbit three months after intracisternal inoculation with *T. pallidum*. Note the periocular alopecia.

Ocular Findings.—Iris atrophy, without bio-microscopic evidence of uveitis, was noted in two of the five animals during the sixth month following inoculation in the cisterna magna. The iris atrophy tended to start in the upper nasal quadrant, spread in a symmetrical fashion, and involve both eyes equally. In the pigmented animal the iris atrophy was best visualized by retro-illumination. The pupils were quite variable in size from one examination to the

next. In this regard, anisocoria is more difficult to assess in the rabbit with laterally situated eyes than in the monkey, where both eyes can be observed simultaneously.

Keratitis developed in the right eye of one animal (R51) inoculated in the cisterna magna. The tears of this eye were dark-field positive at five months.

Serological Response.—During the third and sixth months the VDRL tests were reactive in dilutions from 1:2 to 1:32 on all five animals in this group.

TPI tests performed during the sixth month were also reactive in these five rabbits. As will be noted later, this was in marked contrast to the serological response following intra-ocular injections in the rabbit and the sero-reactions in monkeys.

Lymph Node Transplants.—Popliteal lymph nodes were excised at 161 days from three rabbits inoculated in the cisterna magna. The nodes were emulsified

with 50–50 serum saline solution and promptly inoculated intracutaneously on the backs of normal (VDRL and TPI non-reactive) rabbits. These rabbits were transported to the Venereal Disease Research Laboratories for observation. Within one month two out of the three lymph node inoculation sites were dark-field positive for *T. pallidum*.

(2) Ocular Routes (Table II)

Because of interest in interstitial keratitis, uveitis, chorioretinitis, and optic nerve lesions it was elected to inoculate *T. pallidum* into the cornea, anterior chamber, vitreous, and optic nerve.

The corneal stroma of both eyes of two rabbits was injected with treponemes. One animal (R55) died four days later. A careful autopsy failed to reveal any significant lesions. The other rabbit (R58) began to develop the typical “moth-eaten” alopecia after four months. The eyes, however, remained normal for eight months. VDRL and TPI tests remained non-reactive, and one attempt at lymph node transfer was unsuccessful.

The anterior chambers of the eyes of two rabbits were injected with *T. pallidum*. One (R56) received treponemes in the anterior chamber of the right eye

and a similar injection of saline in the left. The other animal (R57) received treponemes in the anterior chambers of both. No ocular lesions developed in the first rabbit during the next eight months, but the quantitative VDRL titre was 1:2 during the third month, 1:5 during the sixth month, and 1:2 at eight months. In the other rabbit a few tufts of vessels were seen at the upper limbus of both eyes at six weeks. By four months, kerato-uveitis with Koeppe nodules was apparent in both eyes and the VDRL test was reactive. The fundi were normal, revealing that the reaction was primarily an anterior uveitis. One week later the animal developed a head tilt, ataxia, and hemiparesis. The rabbit died on day 133 of the experiment. The histo-pathological studies will be the subject of another report.

Intravitreal inoculations were given to two rabbits. One of these (R59) received treponemes in the vitreous of the right eye and saline in the left. In this animal no ocular or systemic lesions developed, and serological tests for syphilis have remained non-reactive. The other rabbit (R60) was injected with *T. pallidum* intravitreally in both eyes. By two months alopecia was present. In this regard, it is of interest that at three months the animal was sero-negative but

TABLE II
RABBIT: OCULAR ROUTES

Rabbit	Route of Inoculation	Month							Comment
		First	Second	Third	Fourth	Fifth	Sixth	Seventh and Eighth	
R55	Cornea OU <i>T. pallidum</i>	Dead							
R56	Anterior chamber RE <i>T. pallidum</i> LE saline			VDRL R2			VDRL R5 TPI—	VDRL R2 TPI—	No lesions
R57	Anterior chamber OU <i>T. pallidum</i>		Blood vessels at upper limbus OU	VDRL R2	Kerato-uveitis OU Head tilt	Nystagmus Anisocoria Hemiparesis Dead			
R58	Cornea OU <i>T. pallidum</i>			VDRL—		Alopecia	Alopecia VDRL— TPI— Lymph node—	Alopecia VDRL—	
R59	Vitreous RE <i>T. pallidum</i> LE saline			VDRL—			VDRL— TPI— Lymph node—	VDRL—	No lesions
R60	Vitreous OU <i>T. pallidum</i>		Alopecia	Alopecia VDRL—	Alopecia	Alopecia Snuffles	Dead VDRL WR TPI— Lymph node—		No ocular lesions
R61	Optic nerve RE <i>T. pallidum</i> LE saline			VDRL—	Dead				No lesions seen

the VDRL test was weakly reactive during the sixth month. The animal died on day 166—five days after an unsuccessful attempt to demonstrate *T. pallidum* by lymph node transfer.

One rabbit (R61) was injected with *T. pallidum* in the optic nerve of the right eye and saline in the left. The serological tests for syphilis remained non-reactive, and no physical findings had been noted when the animal died during the fourth month.

Monkey

(1) Intracisternal Inoculation (Table III)

Five owl monkeys were inoculated with *T. pallidum* in the cisterna magna. The animals remained

essentially negative to examination for approximately 10 weeks when pupillary changes were noted.

Anisocoria was a frequent finding, making its appearance in the second to third months after inoculation. Pupillary inequalities were extremely variable from one examination to the next.

At nine weeks one animal (O50) developed ptosis and mydriasis—signs of third nerve paresis (Figs 5 and 6). At 12 weeks alopecia was noted above the right eye and both optic disks were pale. It was not until during the sixth month, however, that the serological test for syphilis became reactive. Another animal (O52) developed right-sided weakness during the second month and died several days later.



FIG. 5.—Owl monkey nine weeks after intracisternal inoculation with *T. pallidum*. Note dilated right pupil.



FIG. 6.—Owl monkey nine weeks after intracisternal inoculation with *T. pallidum*. Note ptosis of right lid and dilated right pupil.

TABLE III
MONKEY: INTRACISTERNAL INOCULATION

Monkey	Month							Comment
	First	Second	Third	Fourth	Fifth	Sixth	Seventh and Eighth	
O50			Ptosis, RE Dilated right pupil (variable) VDRL --	Alopecia above RE Optic atrophy VDRL --		Dead VDRL WR		Right III nerve paresis Optic atrophy
O51	Cisternal tap -- 26 WBC (15% polys, 85% monos.)	Anisocoria	Anisocoria VDRL --	Anisocoria VDRL -	Flame haemorrhage LE White exudate LE	New flame haemorrhage VDRL -- TPI R	VDRL WR TPI R	Fundus lesion Rising VDRL titre
O52		Right-sided paresis	Dead					
O53			Alopecia on occiput. Dead					
O54	Vertical nystagmus		Anisocoria VDRL --	Anisocoria VDRL R4	Anisocoria	Ulcer on right heel VDRL R1 TPI R Lymph node+	VDRL R4 TPI R	Mal perforans ulcer

At five months flame haemorrhages and an exudate appeared in the left eye of one monkey (O51) inoculated in the cisterna magna. Serological studies from this animal revealed non-reactive VDRL tests during the third and fourth months. The TPI test was reactive when tested during the sixth month when the VDRL test was still non-reactive. At seven months the VDRL test became weakly reactive.

Vertical nystagmus was seen in one monkey (O54) at two weeks. Anisocoria was seen at three months and by six months a mal perforans-like ulcer was noted on the right heel. During the third month the VDRL was non-reactive, but during the fourth month it was reactive 1:4. In the sixth month the TPI was reactive, and a popliteal lymph node inoculated intracutaneously on the back of a rabbit produced dark-field positive lesions.

(2) Ocular Routes (Table IV)

In an attempt to produce interstitial keratitis, *T. pallidum* was injected into the corneal stroma of three eyes in two monkeys. Saline was given as a control in one eye. A small infiltrate around the injection sites cleared during the first two weeks. One monkey (O55) remained sero-negative and died in the fourth month. No significant lesions were seen at necropsy. The other animal (O56) showed a weakly reactive VDRL during the third and ninth months, but was non-reactive during the seventh month. A TPI test during the seventh month was non-reactive.

Anterior chamber inoculations were made in two

monkeys. One animal (O57) was injected with treponemes in the right eye and with saline in the left. The other animal (O58) received treponemes in the anterior chambers of both eyes. Anisocoria was noted during the second month in both animals. TPI and VDRL tests remained non-reactive for seven months. Although no anterior uveitis was observed, at six months the first animal (O57) developed a sub-hyaloid haemorrhage and a large exudate in the control eye (Figs 7, 8, and 9). This occurred on day

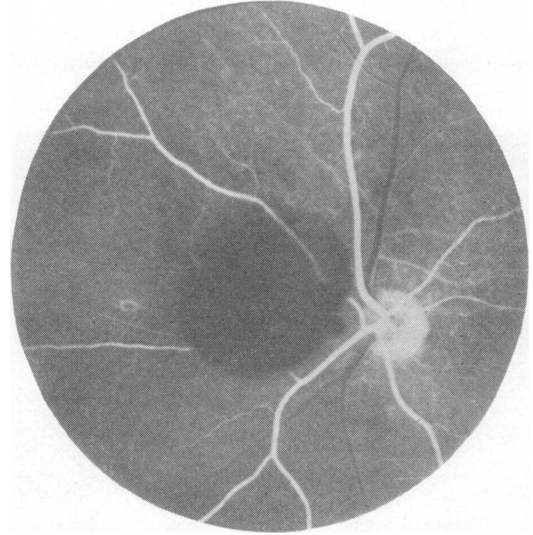


FIG. 8.—Owl monkey (O57). Fluorescein fundus photograph of sub-hyaloid haemorrhage. Animal held upside down.

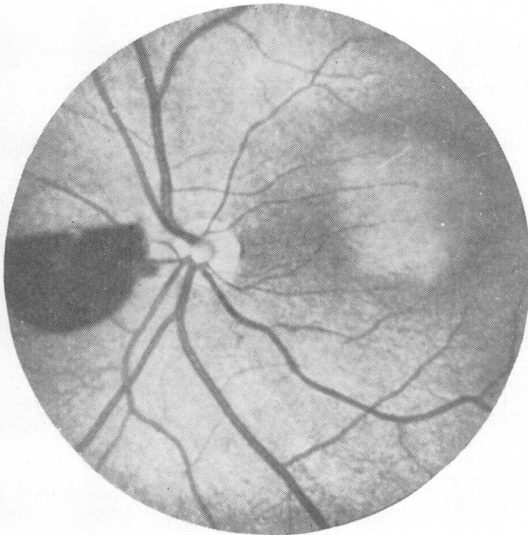


FIG. 7.—Owl monkey (O57). Left (control) eye 162 days after injection of *T. pallidum* in anterior chamber of right eye. Note sub-hyaloid haemorrhage and exudate.

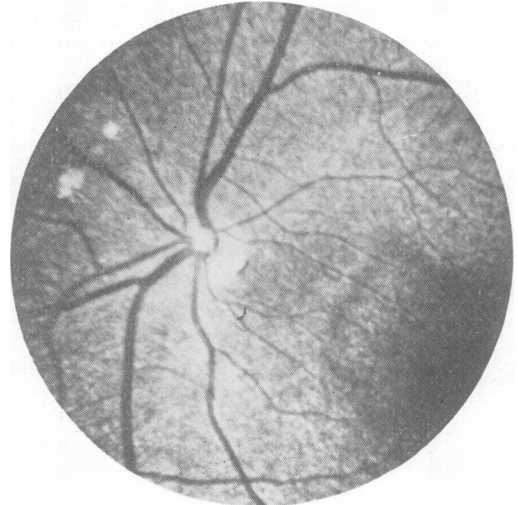


FIG. 9.—Owl monkey (O57). Taken three weeks after Fig. 8. Note absorption of sub-hyaloid haemorrhage.

162, a week after a diagnostic intracisternal tap. During the sixth month a popliteal lymph node was excised and inoculated intracutaneously on the back of a rabbit. Virulent *T. pallida* were subsequently demonstrated in the lesion that developed from the node of this sero-negative monkey.

The other monkey (O58) is also of great interest. During the sixth month a few small haemorrhages developed in the fundus around the posterior pole of the right eye. Although this monkey remained sero-negative to VDRL and TPI tests for over six months, a popliteal lymph node excised during the sixth month and inoculated on the back of a rabbit contained virulent treponemes. Both of these sero-negative monkeys had active infection as shown by lymph node transplant.

Two monkeys inoculated with *T. pallidum* in the vitreous developed endophthalmitis and died during the first week. This was apparently due to a secondary pyogenic organism.

One monkey (O61) was inoculated with treponemes in the optic nerves of both eyes. Both disks became pale during the first month (Figs 10 and 11). VDRL and TPI tests remained non-reactive for over seven months. An attempt at lymph node transfer during the sixth month was unsuccessful. It is difficult to differentiate the effects of trauma from syphilis as the cause of this optic atrophy.

Discussion

The number of reported cases of primary and secondary syphilis in the USA has risen from 6,323 in 1957 to 20,540 in 1962 (USPHS, 1963). The number of cases of syphilis of all stages reported during 1962 was about 124,000 (Moore, 1963). If added to this are the unreported cases and those that remain undetected because of non-reactive serological tests, the total number becomes alarming. This rising incidence prompted a renewed interest in experimental syphilis.

TABLE IV
MONKEY: OCULAR ROUTES

Monkey	Route of Inoculation	Month							Comment
		First	Second	Third	Fourth	Fifth	Sixth	Seventh and Eighth	
O55	Cornea RE <i>T. pallidum</i> LE saline	Infiltrate at injection site		VDRL -	Alopecia above both eyes Dead				
O56	Cornea OU <i>T. pallidum</i>	"		VDRL WR -			VDRL WR - TPI -	VDRL -	
O57	Anterior chamber RE <i>T. pallidum</i> LE saline		Anisocoria	Anisocoria VDRL -	Anisocoria VDRL -	Anisocoria	Sub-hyaloid haemorrhage and exudate LE VDRL - TPI - Lymph node +	VDRL -	Negative serology Positive transfer
O58	Anterior chamber OU <i>T. pallidum</i>		"	"	"	"	Small haemorrhage RE VDRL - TPI - Lymph node +	Dead	"
O59	Vitreous RE <i>T. pallidum</i> LE saline	Endophthalmitis Dead							Secondary infection
O60	Vitreous OU <i>T. pallidum</i>	"							"
O61	Optic nerve OU <i>T. pallidum</i>	Pale disks	Pale disks	Pale disks Sluggish pupils VDRL -	Pale disks Sluggish pupils VDRL -	Pale disks	Pale disks VDRL - TPI - Lymph node -	Pale disks VDRL -	Trauma vs. <i>T. pallidum</i> as cause of pale disks

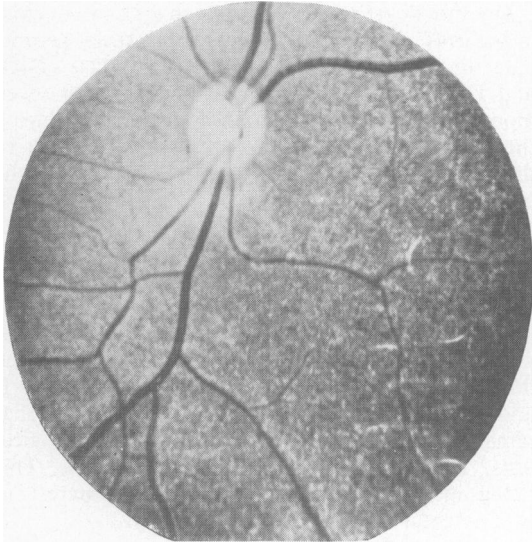


FIG. 10.—Owl monkey (O61). Right eye third month after injection of *T. pallidum* in optic nerve. Note pale optic disks.

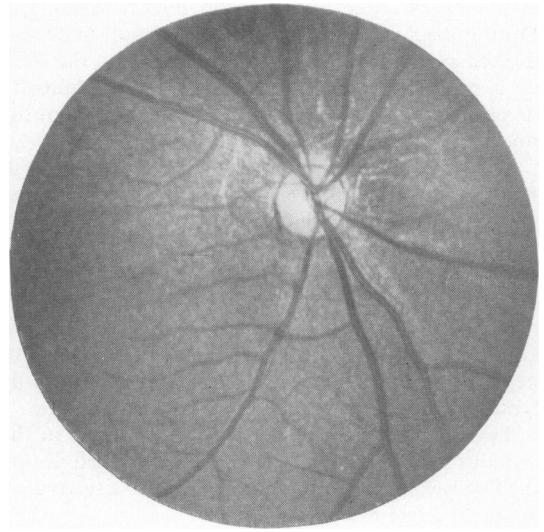


FIG. 11.—Normal owl monkey. Compare disks with Fig. 10.

T. pallidum was identified as the causative agent of syphilis by Schaudinn and Hoffmann (1905). The following year Bertarelli (1906) successfully inoculated the rabbit with *T. pallidum*. The natural course of experimental syphilis in this animal was beautifully delineated by Brown and Pearce (1920a-f, 1921a, b). The rabbit has retained its position as the principal laboratory animal for the study of syphilis. Since our interest in this study was mainly in syphilis of the eye and nervous system of the primate, we elected to inoculate *T. pallidum* directly in the central nervous system and eye in both monkeys and rabbits. All the rabbits inoculated intracisternally developed clinical signs of generalized syphilis as manifested by alopecia, anisocoria, and reactive VDRL and TPI tests. Iris atrophy without uveitis was quite common. The rabbit developing the most marked interstitial keratitis was inoculated in the cisterna magna, not the cornea. In the monkey, optic atrophy, third nerve paresis, anisocoria, and retinal haemorrhages and exudates were produced. In general, serological response in the owl monkey is much less marked than in the rabbit.

One of the most striking findings and the one with the greatest clinical significance is the successful demonstration of dark-field positive lesions in rabbits inoculated with popliteal lymph nodes from monkeys previously injected with *T. pallidum* yet non-reactive to both VDRL and TPI tests.

Turner and Hollander (1957) reported similar findings with *Macacuscus rhesus* and *Cerpethecus*

aetheopsis (African green) monkeys. One of his monkeys remained sero-negative for 361 days, yet suspensions of liver and blood yielded positive results on transfer to rabbits. Another monkey never developed a primary lesion at the inoculation site, remained sero-negative for 219 days, and yet blood and lymph node suspensions yielded positive results on transfer to rabbits. Sero-negative infectious relapse has been produced in rabbits as well. Nelson (1952) treated a group of syphilitic rabbits with small doses of penicillin one month after inoculation. The VDRL titres fell to zero within three months after treatment. In marked contrast, the TPI tests, although initially falling, again rose to pre-treatment levels. Moreover, treponemes were demonstrated by positive lymph node transfer in these VDRL negative animals after five months. A study of sero-negative syphilis in humans is now in progress.

Summary

The natural course of experimental primary ocular syphilis and neurosyphilis in the rabbit and in the owl monkey is presented. Virulent treponemes have been recovered from the lymph nodes of sero-negative primates previously inoculated intracocularly with *T. pallidum*.

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Syphilis oculaire et neurosyphilis experimentales

RÉSUMÉ

On décrit l'évolution naturelle d'une syphilis oculaire et d'une neurosyphilis expérimentales chez le lapin et le singe "chouette". On trouva des tréponèmes virulents dans les ganglions lymphatiques de primates auparavant inoculés avec le tréponème pallidum par voie oculaire.