Brit. J. vener. Dis. (1968), **44**, 267.

LATE SYPHILIS IN THE PRIMATE*†

BY

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Recent studies on ocular and neurosyphilis (Smith, Singer, Reynolds, Moore, Yobs, and Clark, 1965; Wells and Smith, 1967; Smith and Israel, 1968) have renewed interest in the primate as an experimental model for treponemal disease. Metchnikoff and Roux (1906) initially studied syphilis in the primate, but investigators soon turned to the rabbit. A partial explanation for this was that some workers felt that the monkey did not develop late syphilitic lesions similar to those seen in man (Turner and Hollander, 1957). The present paper documents the occurrence of a large, crusted late syphiloderm in an owl monkey (*Aötus trivirgatus*) infected with virulent *Treponema pallidum* obtained from a human chancre, and presents the histopathological features of this interesting lesion. The animal also developed non-granulomatous iridocyclitis, and *T. pallidum* organisms were demonstrated in the aqueous humour of the eye. To our knowledge, this is the first report concerned with late syphilis in the experimental primate.

On September 22, 1966, a healthy owl monkey (O-LCH-2) was inoculated in the right femoral vein with 0·2 ml. serum expressed from a penile chancre of a 23-year-old male Negro. Darkfield examination of the chancre material revealed numerous *T. pallida* of typical morphology and motility. Opportunity for making fresh inoculations from this typical human chancre was provided by Dr Elgin of the Dade County Health Department. The monkey was housed in a separate cage and fed commercial monkey chow and water as desired. Serial clinical and serological examinations were performed thereafter, and during over 18 months of observation, the animal was given no medication. The serological data are presented in Table I. Suffice it to say here, that unequivocal sero-conversion was documented in this animal, reactive VDRL, TPI, and FTA-ABS tests being obtained. The FTA-ABS test converted at 4 months and the VDRL test at 8 months after inoculation.

TABLE I

SEROLOGICAL REACTIONS IN OWL MONKEY LCH-2

Date	VDRL	FTA-ABS	TPI
21-9-66	NR	NR	
22-9-66	date of inoculation		
5-10-66	NR	NR	
17-11-66	NR	NR	
12-1-67	NR	1+R	
17-2-67		1+R	
23-3-67	NR	1−2+R	
1-5-67	R	2+R	
1-6-67 (aqueous OU)	NR	NR	
21-9-67	R	2−3+R	
21-9-67 (aqueous OS)	WR	1-2+R	
5-12-67	R (4 dils)	2−3+R	R
28-2-68	R	3+R	

NR = non-reactive. WR = weakly reactive.

4½ months after inoculation, the monkey was found to have pupillary inequality and biomicroscopy revealed a non-granulomatous iridocyclitis with cells and flare in the right eye. Paracentesis of the anterior chamber of the right eye on February 9, 1967, revealed a darkfield negative aqueous. 2 months later the iritis had cleared, both eyes were white and quiet, and the pupils were equal in size (Fig. 1).

FIG. 1.—O-LCH-2 on April 6, 1967; 7 months after inoculation. Pupils are equal and eyes are white and quiet after first bout of iridocyclitis in right eye.

* Received for publication April 17, 1968.

† This work was supported in part by grants 5 RO 1 CC 00144-03, Communicable Disease Center; 5 RO 1 NB 06308-02, NB 05277-08; and 1 RO 1 NB 06574-02, National Institute of Neurological Diseases and Blindness.

‡ Special Fellowship 2-F11-NB-1633-02 VSN, National Institute of Neurological Diseases and Blindness.

§ Special Fellowship 1-F11-NB-1792-01 VSN, National Institute of Neurological Diseases and Blindness.

10 months after inoculation, a large, crusted lesion was evident on the monkey's back at the base of the tail. Fig. 2 shows this lesion on July 6, 1967, just before a biopsy was performed. A smear of the biopsy was darkfield positive. Microscopic examination revealed marked acanthosis of the epidermis, with scattered areas of inflammatory cells in the dermis. These infiltrates contained both polymorphonuclears and plasma cells. Krajian silver stains revealed numerous spirochaetes in the lesion.

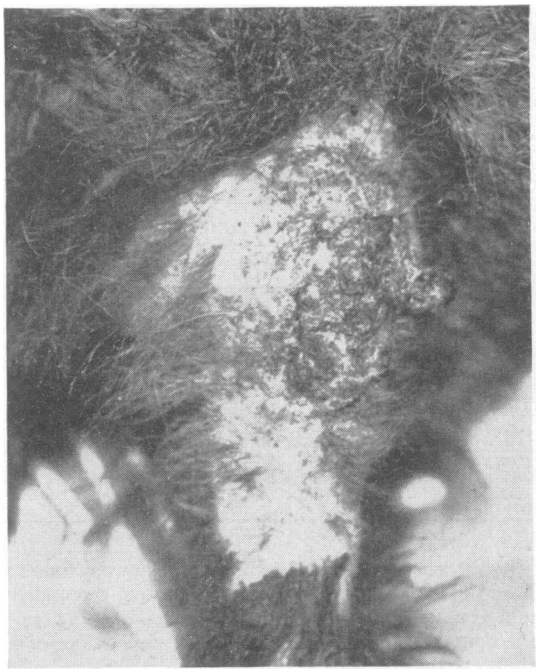


FIG. 2.—Skin lesion on base of monkey's back on July 6, 1967; 10 months after intravenous inoculation.

FIG. 3.—O-LCH-2 on September 21, 1967; one year after inoculation. Note anisocoria and beginning of cataract in left eye.

One year after inoculation, the left pupil was larger than the right, and multiple opacities were seen in the left lens (Fig. 3). Biomicroscopy revealed 2+ cells and flare in the left eye. Paracentesis of the anterior chamber of the left eye on September 21, 1967, revealed numerous spirochaetes in the aqueous humour on darkfield microscopy and these glowed brilliantly under ultraviolet microscopy with the fluorescent antibody stain for *Treponema pallidum* (Wells and Smith, 1967b, c) (Fig. 4 and colour plate C and D). An FTA-ABS test on the aqueous humour of the left eye was non-reactive on June 1, 1967, but was 1–2+ reactive on September 21, 1967.

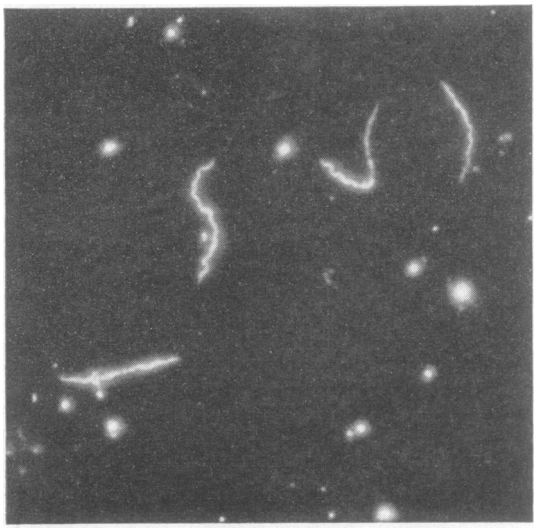
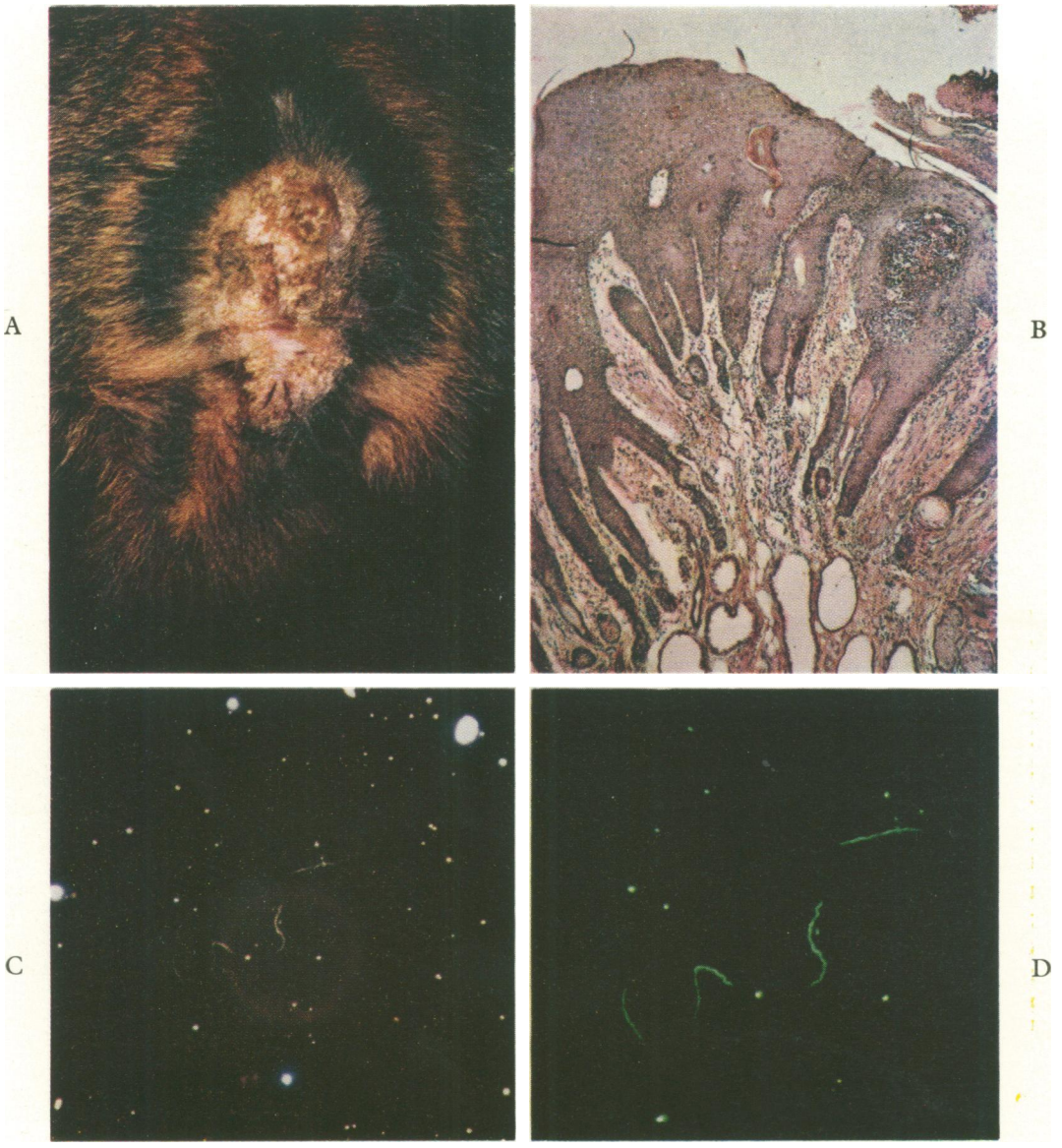


FIG. 4.—Oil immersion darkfield photomicrograph of treponemes found in aqueous humour of left eye of O-LCH-2 on September 21, 1967.

During the next 8 months, the lesion on the base of the tail continued to grow slowly. The appearance on February 28, 1968, 18 months after infection is shown in Plate A. A surrounding zone of alopecia was evident. Repeat biopsy was then performed and this was again darkfield positive. Histopathological examination revealed hyperkeratosis, parakeratosis, and acanthosis with scattered micro-abscesses (Fig. 5; Plate B). A biopsy of an identical site from a control owl monkey is shown in Fig. 6 for comparison. The marked difference in thickness of the epidermis is apparent. The dermis of the syphilitic lesion revealed many foci of plasma cells and lymphocytes, especially around the base of the rete pegs (Fig. 7). One of these areas is shown at a higher magnification in Fig. 8 to illustrate the cartwheel nuclei of the plasma cells. No epithelioid or giant cells were seen and there was no caseation necrosis. The arterioles, although moderately hypertrophied, showed no notable perivascular inflammatory reaction. Krajian silver stains again revealed numerous spirochaetes throughout the lesion (Fig. 9).



A.—Skin lesion on back of owl monkey O-LCH-2 on February 28, 1968; 18 months after inoculation.

B.—Haematoxylin and eosin photomicrograph of lesion seen in A. Biopsy taken February 28, 1968.

C.—Darkfield photomicrograph of treponemes found in aqueous humour of left eye of O-LCH-2 in paracentesis on September 21, 1967. Tungsten light illumination.

D.—Fluorescent antibody tissue stain for *Treponema pallidum*, showing same field as C. The organisms can be seen much more easily by this technique.

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Discussion

That the monkey described in this paper had syphilis is incontrovertible. The relevant data are listed in Table II. The development of uveitis with the presence of spirochaetes in the aqueous humour further attests to the generalized syphilitic involvement. The classification of the stage of disease and

TABLE II

FINDINGS DOCUMENTING THE DIAGNOSIS OF SYPHILIS IN OWL MONKEY O-LCH-2

1. Animal inoculated intravenously with serum expressed from human chancre showing myriads of typical *T. pallidum* organisms on darkfield examination.
2. Serum VDRL conversion from non-reactive to reactive.
3. Rise in serum VDRL titre.
4. Serum FTA-ABS conversion from non-reactive to reactive.
5. Rise in serum FTA-ABS titre.
6. Reactive serum TPI test.
7. Conversion of aqueous humour VDRL test.
8. Conversion of aqueous humour FTA-ABS test.
9. Clinical course of disease showing late cutaneous and ocular manifestations.
10. Histopathology of skin lesion in monkey showing plasma cell infiltration.
11. Positive darkfield examination of skin lesion on two occasions.
12. Spirochaetes demonstrated by Krajian silver stain of skin lesion on two occasions.
13. Treponemes demonstrated to stain with fluorescein tagged anti-*Treponema pallidum* globulin under ultraviolet microscopy.

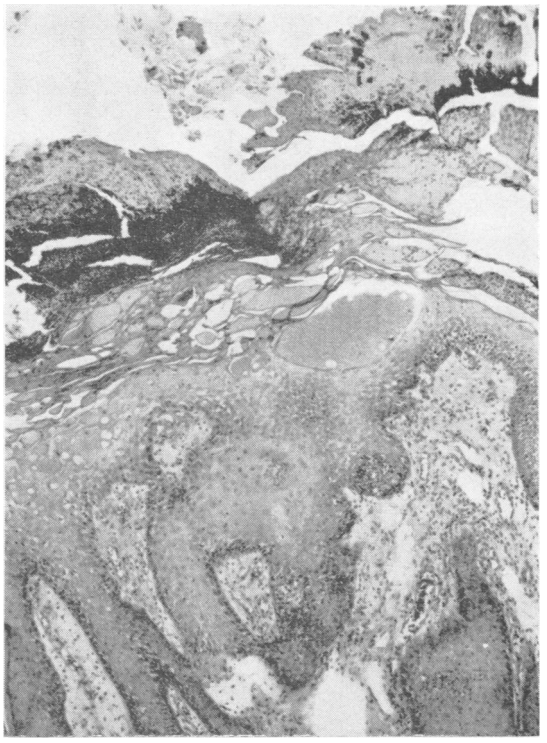

FIG. 5.—Haematoxylin and eosin photomicrograph of biopsy of skin lesion on back of O-LCH-2, February 28, 1968; 18 months after inoculation.

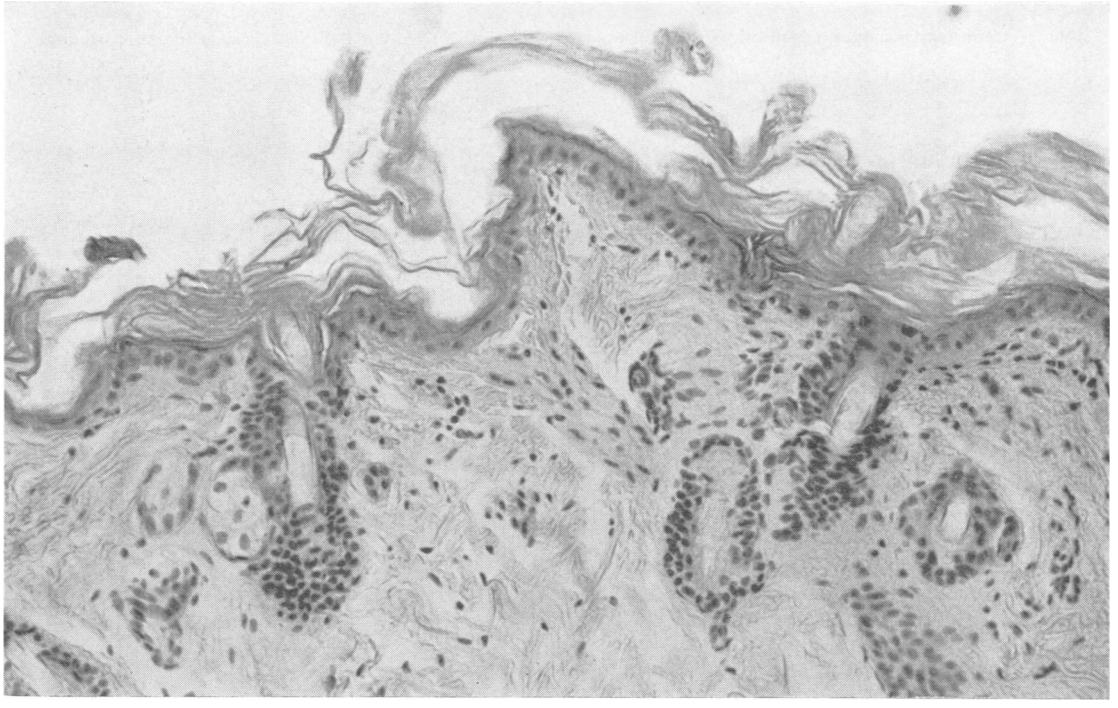

FIG. 6.—Haematoxylin and eosin photomicrograph of skin biopsy made at identical site on a control owl monkey, for comparison with Fig. 5.

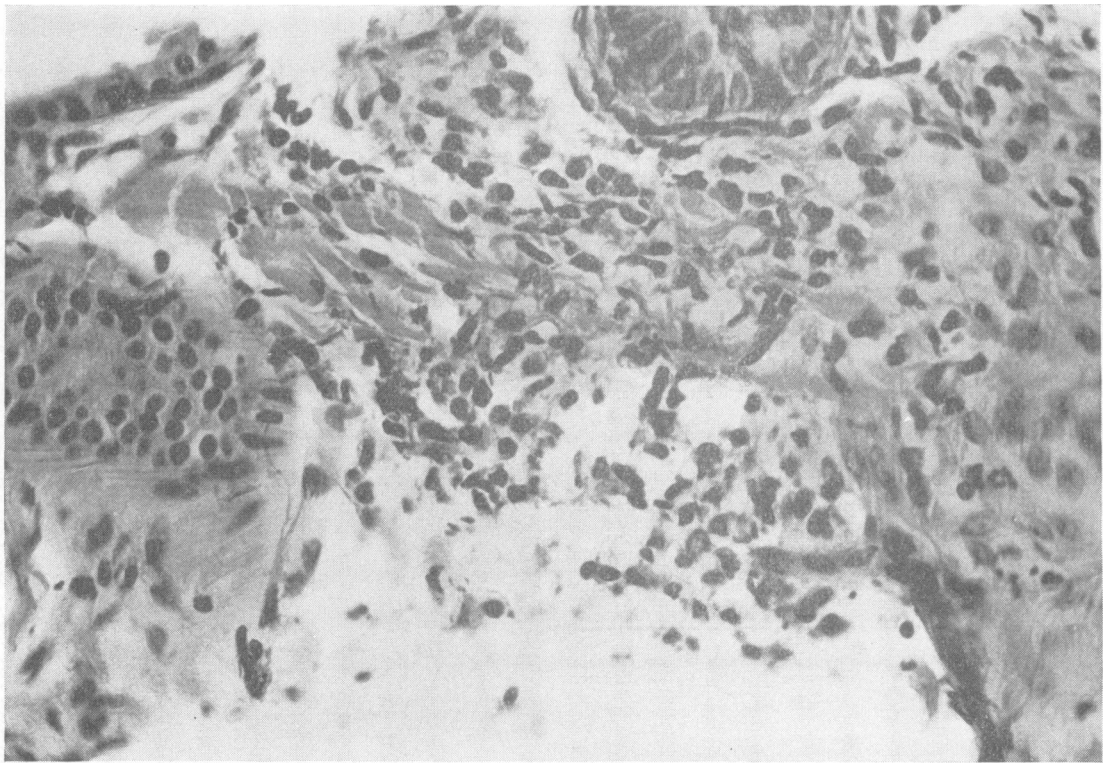

FIG. 7.—Haematoxylin and eosin photomicrograph of biopsy of skin lesion of O-LCH-2 to show inflammation at base of rete pegs.

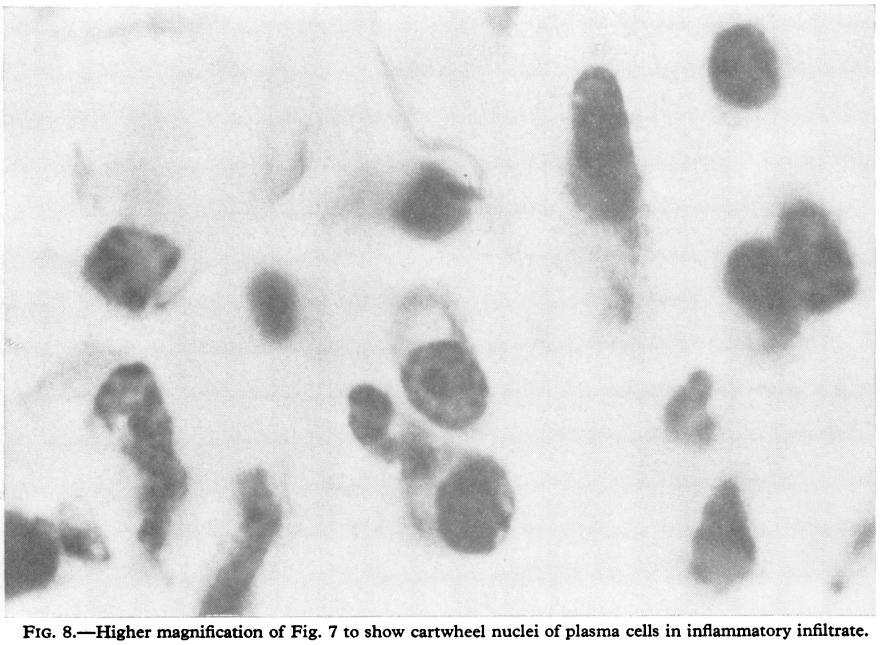

FIG. 8.—Higher magnification of Fig. 7 to show cartwheel nuclei of plasma cells in inflammatory infiltrate.

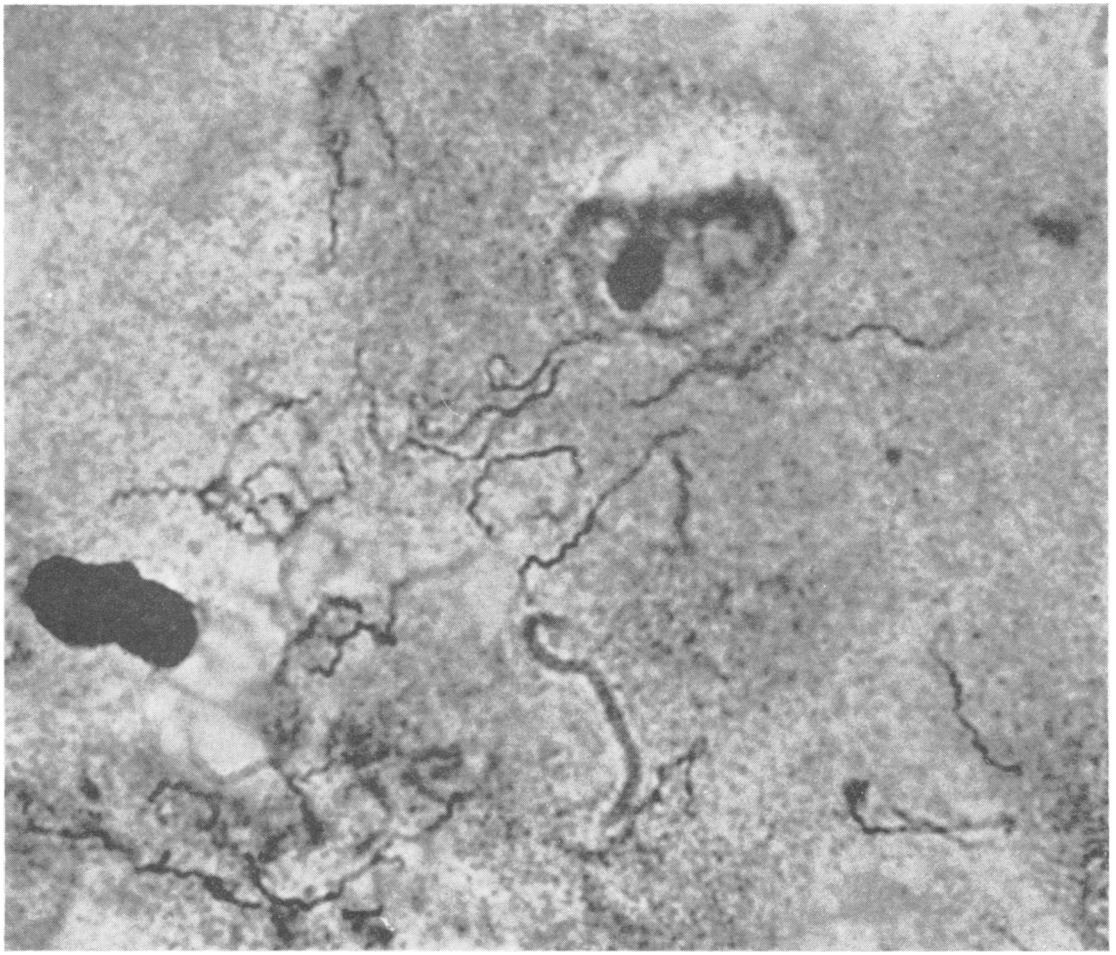

FIG. 9.—Oil immersion photomicrograph of Krajian silver stain to demonstrate spirochaetes in biopsy of skin lesion of O-LCH-2, February 28, 1968.

the type of the cutaneous syphilitic lesion, however, is more open to discussion.

The skin lesion reported here may perhaps be best classified as a "late syphiloderm". Its appearance, induration, indolence, and solitary nature are characteristics of the tertiary syphilid in the human. The ten basic physical characteristics of late syphilids as cited by Stokes, Beerman, and Ingraham (1944) are listed in Table III. The lesion cannot, however, be called a gumma or a granuloma. It contained no epithelioid cells, giant cells, or caseation necrosis. Its relatively benign inflammatory response, comprised of plasma cells and lymphocytes, coexisting with great numbers of treponemes, more closely resembles the secondary lesion in the human (Montgomery, 1967). Sections of the lesion were forwarded to the Armed Forces Institute of Pathology and were reviewed there by Dr Lorenz Zimmerman and Dr Elson Helwig, who interpreted the lesion as histopathologically consistent with a condyloma latum,

TABLE III

TEN BASIC PHYSICAL CHARACTERISTICS OF LATE SYPHILIDS
(Stokes, Beerman, and Ingraham, 1944)

1. *Solitary character;* or at least the presence of few lesions.
2. *Asymmetry;* though by no means invariable.
3. *Induration;* deep palpable infiltration.
4. *Indolence;* a relatively low-grade inflammatory process.
5. *Arciform configuration;* borders polycyclic or forming segments of circles, both in the individual lesion and in the configuration of a group of lesions.
6. *Sharp margination of lesions;* in ulcers, "punched out" appearance.
7. *Tissue destruction* and replacement with or without ulceration.
8. *Tendency to central or one-sided healing* with peripheral extension.
9. *Scar formation;* superficial atrophic (thin and wrinkled), noncontractile. The scar retains the arciform configuration of the original lesion.
10. *Peripheral hyperpigmentation* of a rather persistent type.

but the fact that the lesion had been observed as slowly progressing for over 8 months speaks against its being secondary syphilis on clinical grounds.

The presence of a gross skin lesion and recurrent uveitis in a primate over 18 months after infection raises the question of the natural life span in this species. To our knowledge, the exact duration of the natural life span in *Aötus trivirgatus* is unknown. Owl monkeys have been kept under observation in this laboratory for over 5 years and appear to show external changes of ageing. Dr Leo Rane, who has kept marmoset monkeys, a species of comparable size, under observation for over 7 years, has stated that the life span of the marmoset is thought to be approximately 12 years. If the life span of the owl monkey is roughly similar, the development of a skin lesion nearly a year after infection, and its presence nearly 2 years after inoculation, might correspond to a period of time covering one-sixth of the animal's life span, a period of perhaps 10 to 12 years in man. The term "late syphiloderm", therefore, seems appropriate for the lesion here reported, since it is difficult to state categorically whether it is a late secondary or an early tertiary manifestation, and this term has no histological prerequisites. The important point is not that the lesion corresponds exactly to late syphilis in the human, but that it is a late lesion of a type previously unreported in this species.

In a long-term study of experimental syphilis in the primate in this laboratory, the disease has been demonstrated in owl monkeys and squirrel monkeys inoculated with *Treponema pallidum* into the cornea, vitreous, cisterna magna, carotid artery, and skin (Wells and Smith, 1967a). Treponemes have been recovered from the aqueous humour of a monkey 8 months after intradermal inoculation with human cerebrospinal fluid (Smith and Israel, 1968). This monkey developed a darkfield positive crusted lesion on the nose 10 months after inoculation. In a series of over 100 owl and squirrel monkeys inoculated with virulent Nichols strain of *T. pallidum*, there have been no instances of skin lesions as striking as the one here reported. The development of this lesion raises the question whether the LCH strain of *T. pallidum*, or organisms obtained from a fresh human chancre, are more virulent for the primate than the Nichols strain which has been maintained by serial passage in the rabbit for over 40 years.

In their studies of syphilis in the primate, Turner and Hollander (1957) gave intracutaneous inoculations of Nichols strain *T. pallidum* to African green and rhesus monkeys. These authors felt that the monkey was not suitable for this work, but they did demonstrate passive transfer of infection to rabbits from the tissues of these primates, and the importance of that observation has been cited in a previous report (Wells and Smith, 1967a). Two recent studies have given impetus to research in this field. Fribourg-Blanc, Niel, and Mollaret (1963, 1966) found a treponeme with the morphological and immunological characteristics of *Treponema pallidum* occurring in the Cynocephalus monkey from Guinea in the natural state. Kuhn, Brown, and Falcone (1968) studied the serological reactions of twelve species of subhuman primates, and found reactive FTA-ABS tests in 39 of 250 chimpanzees, approximately 15 per cent. Thivolet, Sepetjian, and Guerraz (1968) recently confirmed the presence of antitreponemal antibodies in Cynocephalus monkeys from West Africa, but they noted that these antibodies had never been discovered in Asian monkeys. The importance of these observations in primates is evident. Routine VDRL and FTA-ABS tests are done in our laboratory on all animals before any inoculations, and no owl monkey obtained from commercial sources and tested before inoculation has been found to be reactive to the FTA-ABS test. For several years our laboratory has been engaged in the long-term study of ocular and neurosyphilis in the primate, as we feel that the monkey reproduces more faithfully than any other laboratory animal both the clinical course and the serological reactions seen in human disease. The documentation of the late syphiloderm reported here further strengthens this belief, and should provide impetus for additional investigation of the treponematoses using primate species.

Summary

This report records the occurrence of a late syphiloderm in an owl monkey (*Aötus trivirgatus*) infected with virulent *Treponema pallidum* obtained from a human chancre. The animal also developed uveitis, and *T. pallidum* organisms were demonstrated in the aqueous humour of the affected eye. The significance of the occurrence of late manifestations of syphilis in the experimental primate is discussed.

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La syphilis tardive chez les Primates

RÉSUMÉ

Ce rapport consigne l'apparition d'un syphiloderme tardif chez un nyctipithèque (*Aötus trivirgatus*) infecté par le *Tréponema pallidum* virulent obtenu d'un chancre humain. L'animal a aussi développé une uvéite et des *T. pallida* ont été demontrés dans l'humeur aqueuse de l'œil affecté. La signification de l'apparition des manifestations tardives de la syphilis chez le primate expérimental est discutée.