

EXPERIMENTAL STUDIES ON TRANSMISSION OF HUMAN LEPROSY TO MONKEYS

I. SYMPTOMATIC STUDY

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Despite the work of many investigators, it has been impossible as yet to transmit human leprosy to experimental animals or to grow cultures of the bacillus. It would be extremely helpful to our understanding of the disease if we could produce the infection in animals.

Since 1941 I have been working on this problem, using the Taiwan monkey, *Macacus cyclopis* (Swinhoe), persisting in spite of various difficulties. The work was halted by World War II, but of late it has been continued, and I have now obtained results approaching those desired. I wish to describe here my methods and results. This is not a report of success in experimental infection of animal with human leprosy, but one of findings encountered in the experiment, which it is hoped can be extended.

PROCEDURE

Healthy monkeys were bought in southern Taiwan, in and near the towns of Chia-yi, Taitung, and Hengchun. They were observed for at least one month, and no signs of leprosy were seen. They were fed daily with rice, pumpkin, grass, and fruits in season. Information about the monkeys used in this experiment is given in Table 1, except No. 7, a 2-kilogram male which was set aside as a control on August 23, 1941, and which died a year later, and Nos. 13 and 15, both of which died within a few days after inoculation by the third method (first group).

Three systems of inoculation were employed during different periods. For convenience, these may be called the fluid-material injection method, the once-repeated implantation method, and the multiple, long-term implantation method. The lepromas used in this work were skin nodules excised from patients aged from 20 to 40 years.

Injection of fluid material.—This inoculation material was prepared by the method of Ota and Nitto (¹). Preparation (a) contained 0.3 gm. of diatomaceous earth and 0.3 gm. of Sudan III in 30 ml. of distilled water; preparation (b) was a suspension of 3 gm. of leproma material in 30 ml. of 0.9 per cent saline. In preparing this suspension the lepromas were cut into bits with scissors, ground in a mortar, and suspended in saline; the suspension was then filtered through gauze. For the injections, 1.0 ml. of the former and 1.5 ml. of the latter were mixed in the syringe just prior to use. The mixture was injected into the left femoralis muscle of each of the first four monkeys (Table 1), on only one occasion (May 17, 1941).

Once-repeated implantations.—On two occasions, August 23 and October 21, 1941, two pieces of human leproma each as large as two or three grains of rice were implanted under the skin of the back in the first four monkeys (Nos. 1 to 4), and in two others (Nos. 5 and 6). Monkey No. 7 received no implantations in order that it might serve as a control.

Frequent, long-term implantations.—In these series of inoculations, subcutaneous implantations were made every two or three weeks until the animals died. The first

group so treated totaled 16 monkeys, Nos. 5 and 6 and Nos. 8 to 21, the first inoculations being made between May 6, 1942 and April 20, 1948. What is here called the second group, because previously inoculated by the other methods, were Monkeys 1 to 3, first put on this third inoculation routine on May 6, 1942. Besides the leproma

TABLE 1.—Data on the various inoculations of the several monkeys used.^a

No.	Sex	Body wt. kgm.	Inoculations			Date of death	Duration, months	Clinical signs ^c
			Method ^b	Date of first	Number given			
1	M	4.5	Injection	5/17/41	1	12/25/42	19	Yes
			Duo-plants	8/23/41	2			
			Multi-plants	5/ 6/42	18			
2	M	4.5	Injection	5/17/41	1	12/ 1/42	18	Yes
			Duo-plants	8/23/41	2			
			Multi-plants	5/ 6/42	16			
3	F	3.7	Injection	5/17/41	1	1/28/43	20	Yes
			Duo-plants	8/23/41	2			
			Multi-plants	5/ 6/42	22			
4	F	3.0	Injection	5/17/41	1	3/31/42	10	No
			Duo-plants	8/23/41	2			
5	M	5.0	Duo-plants	8/23/41	2	4/15/44	32	Yes
			Multi-plants	5/ 6/42	49			
6	F	5.0	Duo-plants	8/23/41	2	5/16/42	9	No
			Multi-plants	5/ 6/42	1			
8	M	2.0	Multi-plants	5/ 6/42	21	1/14/43	8	Yes
9	M	3.0	Multi-plants	2/18/43	45	5/ 8/45	27	No
10	M	2.0	Multi-plants	2/18/43	5	4/21/43	2	No
11	M	2.5	Multi-plants	2/18/43	39	10/22/44	20	Yes
12	F	2.5	Multi-plants	2/18/43	43	12/ 5/44	22	No
14	M	1.5	Multi-plants	2/28/43	18	11/ 8/43	8	No
16	F	3.5	Multi-plants	3/ 4/43	14	9/23/43	7	No
17	M	2.0	Multi-plants	3/ 4/43	42	1/ 2/45	22	Yes
18	M	2.5	Multi-plants	3/ 4/43	43	2/ 5/45	23	No
19	M	1.8	Multi-plants	2/21/48	6	10/12/48	8	No
20	F	3.5	Multi-plants	4/20/48	2	3/ 5/49	11	No
21	F	4.0	Multi-plants	4/20/48	2	10/11/48	6	No

^a Monkeys 7, 13 and 15 do not appear in this table, No. 7 because it was merely an uninoculated control, Nos. 13 and 15 because they died a few days after the first inoculations (implants).

^b In this column "injection" means the intramuscular injection (Method 1); "duo-plants" means the original "once-repeated" implantations (Method 2); and "multi-plants" refers to the frequent, long-term implantations (Method 3).

^c Referring to the development of clinical signs suggestive of leprosy.

implants, these three animals received, only once, intramuscular injections of 0.1 per cent mercuric bichloride, 0.5 ml. per kgm. of body weight, for the purpose of reducing resistance. All of these monkeys received regular physical examinations.

RESULTS

Injection of fluid material.—After the intramuscular injections (the first four monkeys, May 17, 1941), there was local swelling which also affected the neighboring lymph nodes. This reaction disappeared in 4 to

5 weeks. Aside from the swelling and its abatement, nothing particularly noteworthy was observed during the three-month period of observation before the first implantation was made (second method, August 23rd).

Repeated implantations.—The monkeys were observed for about 8½ months after the second leproma implantations (October 21st). Leptra bacilli were not found in the nasal mucus or in testicle punctures. However, bacilli were present in all of the nodular tumors caused by the implantations, and occasionally globi were found. These tumors developed only at the sites of implantation. At first they were papules, but these enlarged and ulcerated, the ulcers having a purulent discharge. After absorption, the ulcers finally healed with scar formation. There was no reaction other than that at the site of the implantation in any of the six monkeys. The control monkey (No. 7) remained healthy.

Frequent, long-term implantations.—I. The first group: From the 16 monkeys of this group I choose three notable examples to describe the developments.

MONKEY No. 5. Leproma implantations were made on August 23 and October 21, 1941, on May 6, 1942, and thereafter to a total of 49 such inoculations. The first sign suggestive of leprosy infection was noted on September 22, 1942, as a slight flexion affecting the third finger of the left hand. Within three weeks (on October 12) the affected finger was markedly flexed, and on the right hand the second finger was slightly flexed. Although the left foot was held in the normal position, there was slight flexion of the third and fourth toes. One month later, on November 15, in the left hand the third finger was flexed notably and the fourth finger was slightly flexed. The second finger on the right hand was bent towards the palm, and the third and fourth fingers were flexed as in Fig. 1. On December 9 both hands and the left foot had the marked abnormalities shown in Fig. 2. This monkey died on April 15, 1944.

MONKEY No. 8. The first implantation was made on May 6, 1942, and as shown in Fig. 3, which was made on July 15, a large ulcer developed at the site of the fourth implantation, made on June 11. The ulcer was surrounded by a ring of nodular little tumors. On September 22 the left second finger was flexed, and the right third, fourth, and fifth fingers were bent inwards. The axillary and thoracic lymph nodes were swollen on October 2, and within the scrotum near the right testis there was a soft tumor about the size of a bean. On December 1 the left second finger was affected. The right antebrachium was depilated and the third, fourth, and fifth fingers were bent slightly inwards, as shown in Fig. 4. On January 14, 1943, when this monkey died, there were numerous tumors of the lymph nodes (Fig. 5).

MONKEY No. 11. This animal received the same treatment as did No. 8. It responded similarly, and had numerous tumors which enlarged, growing for more than three months. The lymph nodes in the axillary, thoracic, and inguinal regions were greatly swollen (Fig. 6). This monkey died on October 22, 1944.

II. The second group: On May 6, 1942, Monkeys Nos. 1, 2, and 3 were injected with 0.1 per cent mercuric bichloride intramuscularly, and the frequent implantation of leprous tissue was commenced. The bichloride injections were not repeated.

MONKEY No. 1. This animal never developed any marked flexion of the dactylia, but as shown in Figs. 7 and 8 several conspicuous tumors appeared in places close

to the sites of implantation. The axillary and thoracic lymph nodes swelled to the size of a fresh pea, and were easily seen beneath the skin. This monkey died on December 25, 1942.

MONKEY No. 2. An abnormality was first observed in this animal on June 11, 1942, the left third and fourth toes showing slight flexion, as seen in Fig. 9. One month later, on July 15, the left flexion had increased (Fig. 10) and the second, third, and fourth toes were involved. The right second and third toes were slightly flexed. However, as can be seen, the fingers and hands were still normal. By September 22 all of the right and left toes were affected, and the hands and fingers were slightly flexed. The right fourth toe was swollen and showed signs of necrosis. On November 2, 1942 the hands were flexed, as can be seen in Fig. 11. A purulent discharge from the right fourth toe had healed with scar formation. Brachydactylia and depilation were present in this toe (Fig. 11). This monkey died on December 1, 1942.

MONKEY No. 3. This animal died on January 28, 1943. It had also once received mercury sublimate and thereafter frequent implantations of lepromas. The lymph nodes in the axillary, thoracic, and inguinal regions were greatly swollen. The viscera also showed abnormalities (Fig. 12).

During the examinations of these experimental monkeys I found malaria plasmodia in Nos. 1 to 6 and 8 to 11. Therefore, the transmission of human leprosy to monkeys which have malaria is an interesting problem for investigation.

CONCLUSIONS

Suggestive signs of human leprosy infection can be induced in Taiwan monkeys. This has been done by implantation of leproma nodules excised from active cases. To cause the spreading of lesions and their development in places apart from the sites of implantation, it is necessary to make many frequent implantations, over long periods of time.

CONCLUSIONES

En los monos de Formosa pueden producirse signos sugestivos de infección leprosa humana. Esto se hizo por medio del implante de nódulos lepromatosos excindidos de casos activos. Para lograr la difusión de las lesiones y su aparición en zonas apartadas de los sitios de implante, es necesario ejecutar muchos implantes frecuentes, durante períodos prolongados de tiempo.

ACKNOWLEDGMENT

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REFERENCE

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DESCRIPTION OF PLATES

PLATE (1)

FIG. 1. Monkey No. 5, of Group I of the long-term series. Third left finger flexed, and fourth one slightly flexed. Second right finger flexed toward the palm, third and fourth fingers slightly flexed. November 15, 1952.

FIG. 2. The same monkey, showing deformities of hands on December 9, 1942.

FIG. 3. Monkey No. 8, of Group I of the long-term series. Nodular tumors around the edge of an ulcer occurring at the site of the fourth implantation, done on June 11, 1942; photographed July 15.

FIG. 4. The same monkey, six months later. Third and fourth fingers of right hand slightly flexed, the second finger of the left hand markedly so. December 1, 1942.

FIG. 5. The same monkey, showing numerous nodular tumors on the left breast. January 14, 1943.

FIG. 6. Monkey No. 11, of Group I of the long-term series. Numerous tumors on the back. June 14, 1943.

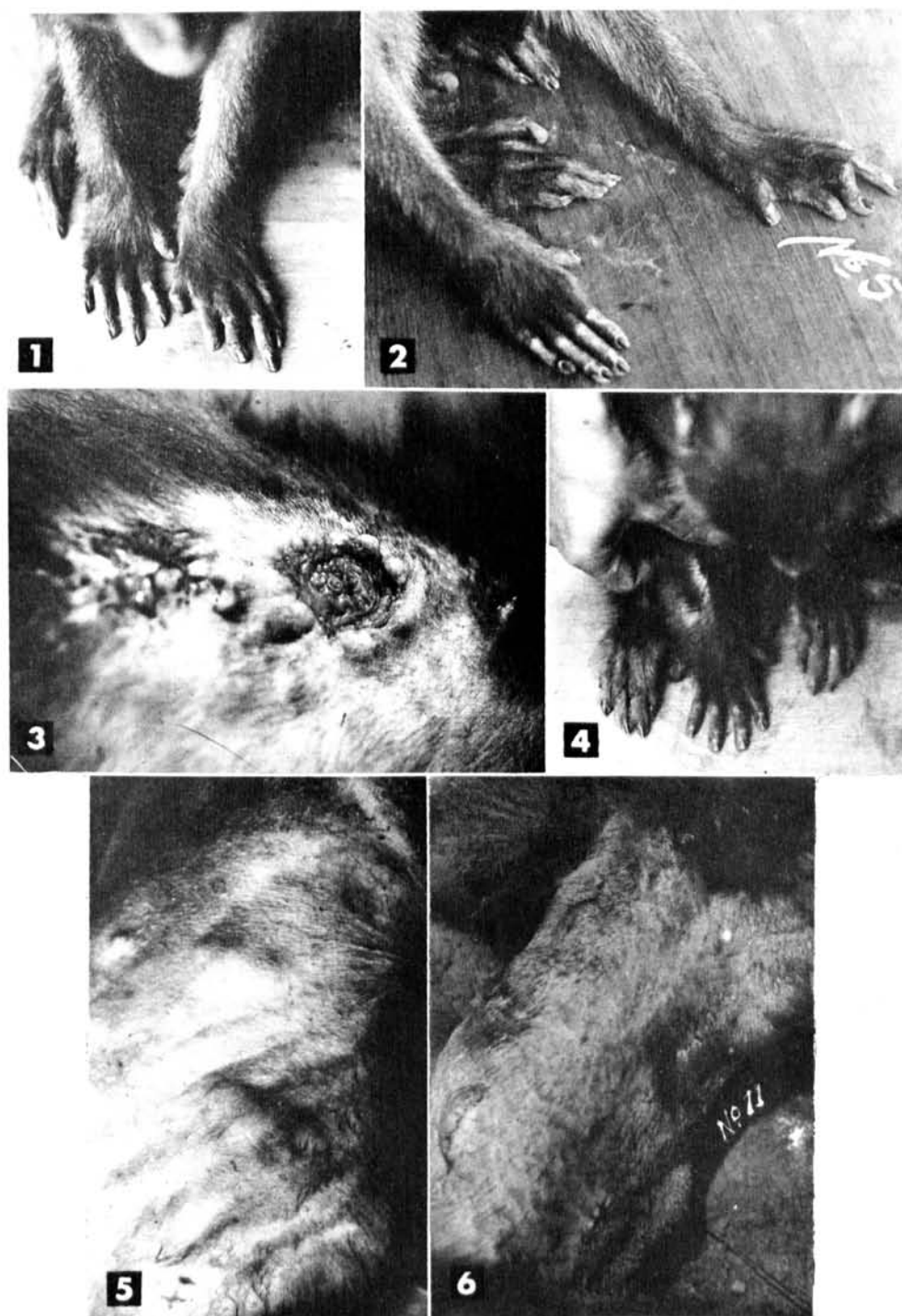


PLATE 1.

PLATE (2)

FIGS. 7 and 8. Monkey No. 1, of Group II of the long-term series. Showing tumors over the right shoulder (Fig. 7) and loss of hair and presence of tumors on the back. December 9, 1942.

FIG. 9. Monkey No. 2, of Group II of the long-term series. Abnormalities of the third and fourth toes of the left foot. June 6, 1942.

FIG. 10. The same monkey, showing the second, third and fourth left toes flexed, and slight flexion of the second and third right toes. July 7, 1942.

FIG. 11. The same monkey four months later. Deformity of all limbs, the right toes being especially noteworthy. November 11, 1942.

FIG. 12. Monkey No. 3, of Group II of the long-term series. Note tumors in the inguinal regions. The viscera also showed abnormalities. January 28, 1943.

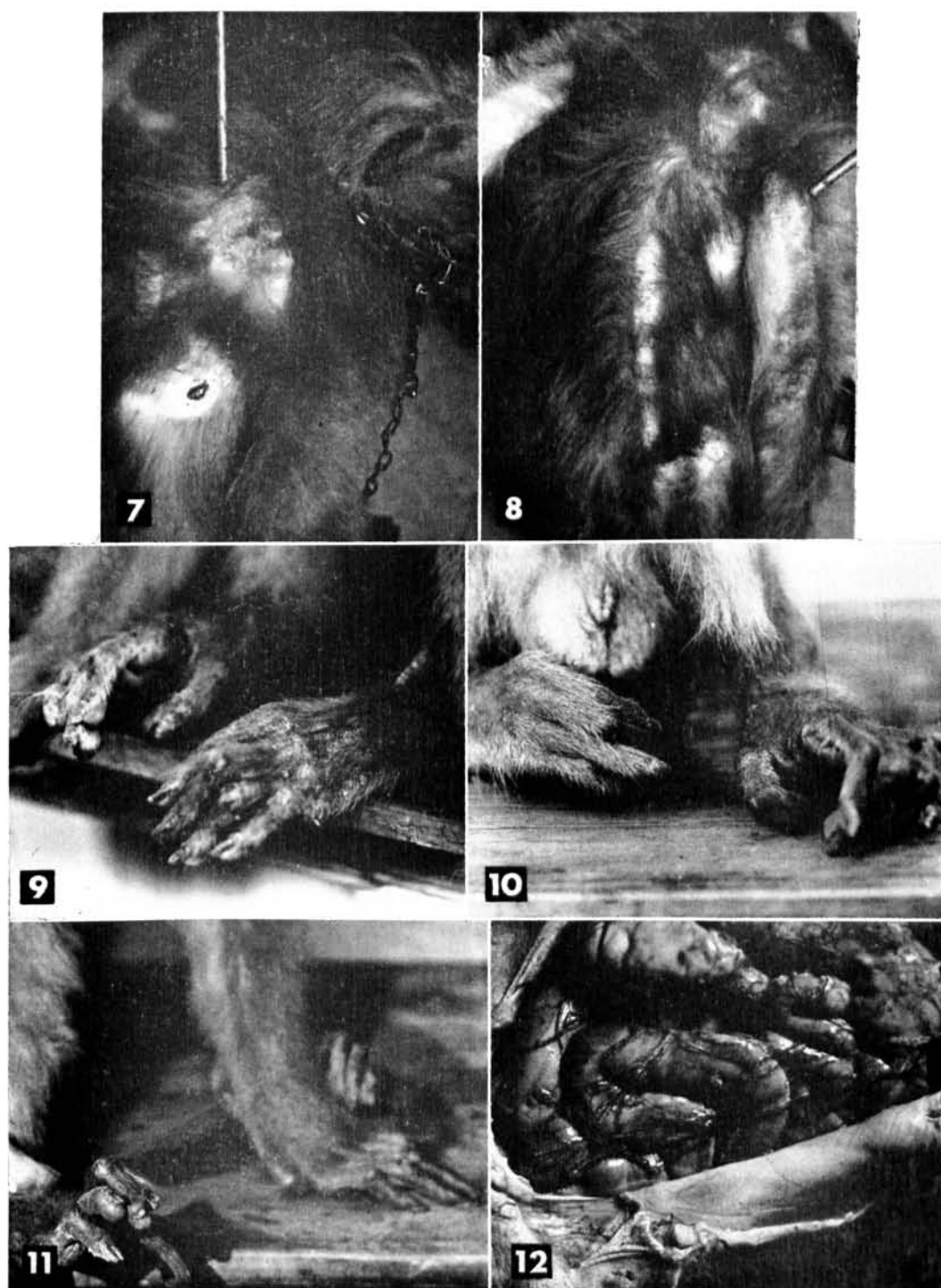


PLATE 2.