of leprosy research and leprosy clinical work, and will not have any real guidance for the future development of leprosy control. So I trust that what I have said now will prick some of your consciences, if you haven't joined, and like the recruiting sergeant I say please line up and join.

Dr. Mason. I do not feel that the next speaker needs any introduction whatever. Dr. Binford will discuss "The inoculation of human leprosy in the chimpanzee."

The Inoculation of Human Leprosy in the Chimpanzee

Initiation of a Long-Term Project

Chapman H. Binford, M.D. 1

Mild local injections detectable only microscopically have been produced in the foot pads (1) and ears of mice and hamsters (2) by the inoculation of human leprosy bacilli. The period of observation possible for transmission experiments in these short-lived rodents rarely exceeds two years. During this period macroscopic lesions and dissemination have not been seen. Even if these rodents were as susceptible as man to \( M. \) leprae, it is doubtful if results would be different, because in man the usual incubation period is thought to be from three to five years (2).

In recognition of the need for infecting an animal with a life span long enough to study the pathogenesis of leprosy, the Technical Committee on Pathology and Experimental transmission at the Eighth International Conference on Leprology at Rio de Janeiro, in 1963 (4), made a formal recommendation that a long range program for the inoculation of a colony of chimpanzees he undertaken.

The biologic similarity of the chimpanzee to man obviously makes the use of this animal highly desirable in leprosy transmission experiments. Only a few reports, however, of the inoculation of chimpanzees with \( M. \) leprae have been made. Marchoux and Bourret (5), in 1907, inoculated a chimpanzee under the skin of the ear with a freshly excised nodule of leprosy tissue, but the animal died 98 days after inoculation. Nicotie and Blainot (6), in 1911, reported the results of eight inoculations of fresh lepromas over a period of three months into a chimpanzee. One of these inoculations, made under the skin of an eyebrow, was followed by a small nodule and several satellite nodules, which persisted about two weeks and then regressed. No further details were given of the results of this experiment.

Encouragement for the use of the chimpanzee in the transmission of leprosy has been furnished in recent years by one investigation. Gunders (7), in Liberia, reported, in 1958, the results of intravenous inoculation of a young chimpanzee with the leprosy bacillus. Within 11 months the animal exhibited numerous nodules over the hands and feet, which on microscopic examination bore some resemblance to human leprosy. After three months the nodules subsided, but the report gave no further follow up of the animal.

It has now been possible, through the cooperation of Dr. Arthur J. Riopelle and his staff at the Tulane University Delta Regional Primate Center, Covington, Louisiana, to begin a five year experiment on the transmission of \( M. \) leprae to the chimpanzee. The Board of Trustees of the Leonard Wood Memorial authorized the purchase of 13 chimpanzees, and agreed to meet the cost of their daily maintenance. Plans were made for the microbiologic and histopathologic studies to be carried out at the Armed
Forces Institute of Pathology in the laboratory for the transmission of *M. leprae* to animals.

Dr. J. P. Wiersema, the co-investigator, and I, in planning this project, have met in consultation with Dr. J. H. Hanks and his staff, Baltimore, and with Dr. Charles C. Shepard and his associate Dr. C. F. F. Hillen, Atlanta. By correspondence we have consulted with Dr. R. J. W. Rees, London, Dr. W. F. Kirchheimer, Carville, and Dr. M. F. Shaffer, New Orleans. Dr. Orlando M. Daumy and Dr. Hans F. Smetsma, Members of the Staff at the Delta Primate Center, are cooperating in this experiment.

It was agreed that information gained in transmission experiments in the mouse and hamster indicated that the cooler parts of the animal should be used, and that some of the inoculations should be intradermal. It was agreed also that the sites inoculated should be those that would be easily accessible for biopsy, so that follow-up studies could be made regularly. In the first experiments it was decided to inoculate 5,000 bacilli at each site, a dose Dr. Shepard has used successfully in mouse foot pad experiments. Dr. Shepard [7] has reported that if 10-100 solid-staining organisms are included in the inoculated dose, "takes" will be produced consistently. Dr. Shepard's experience also indicated that multiple sites could be used without any untoward effects on the outcome. Because of the encouraging results that have been obtained by Gunders, it was agreed that in some animals intravenous inoculation should be used.

There was considerable discussion concerning testing the chimpanzees with lepromin before inoculation, but in view of the possibility that lepromin might produce some antigenic effect it was decided not to test them, but to attempt to obtain some information on the lepromin reactivity of chimpanzees by making tests, if possible, on chimpanzees that were not on leprosy experiments.

Lepromatous skin specimens from untreated cases, transported in thermos bottles packed with wet ice, were obtained from Dr. Jose G. Tolentino, Cebu, Philippines, through the cooperation of Dr. Antonio C. Jovellanos, Chief of the Eversley Childs Sanitarium, and Dr. S. J. Bueno de Mesquita, Research Leprologist, Paramaribo, Suriname. Histopathologic studies on skin specimens used for inoculation were made at the Armed Forces Institute of Pathology.

On 26 February 1965, five chimpanzees were inoculated with inoculum prepared by Dr. Charles Shepard. Two animals were inoculated on 26 March 1965, and on 6 May 1965 six were inoculated with material prepared by Dr. Wiersema. Multiple sites, viz., ears, brows, fingers, and nose were inoculated intracutaneously in seven of the animals and intravenous infections were made in six. Bacilli were counted in each inoculum and an estimate of the percentage of solid bacilli was made. The sites of intracutaneous inoculation were identified by tat-
too marks. All inocula were cultured for acid-fast and other bacteria. In the animals given intradermal inoculations by injection an area on the posterior surface of one ear was scarified with a dry electric tattoo needle. Undiluted inoculum was rubbed into the scarified skin.

Small punch biopsies, perhaps at intervals of six months, will be made of the skin sites inoculated, even in the absence of visible skin lesions. In some animals repeated inoculations will be made.

By use of an electric thermometer, temperature records were made on all chimpanzees immediately after the induction of anesthesia (Sernyl). Table 1 gives the record for one animal.

SUMMARY
A five year cooperative project designed to transmit leprosy to a colony of 13 chimpanzees has been started. The inocula consisted of suspensions of M. leprae from skin lesions of untreated patients with lepromatous leprosy. Intravenous, intracutaneous, and scarification inoculation methods have been used. The results will be followed by periodic biopsy examinations.

REFERENCES

Dr. Mason. Thank you, Dr. Binford. I wish there could be some discussion on this because I am sure there are many people who would like to ask questions, but as Dr. Binford said, that can be done outside the meeting. I shall now turn the chair over to Dr. Frank B. Johnson, Chief of the Histotechnology Branch of the Armed Forces Institute of Pathology.

Dr. Johnson. Nestling in the hills above Caracas, is a unique research institution. Dr. S. C. Chang and I had the opportunity of visiting there about three years ago to see some of the work of the next speaker. Dr. Tamotsu Imaeda was originally a dermatologist in Kyoto, where he learned electron microscopy. Later he moved to the Venezuelan Institute for Scientific Investigation (IVIC). Dr. Imaeda will speak on "Electron microscopy: Approach to leprosy research."