INOCULATION EXPERIMENTS OF HUMAN AND RAT LEPRA IN THE HEN[1]

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Of our inoculation experiments on fowl with cultures of different acid-fast bacilli, and with material from human and rat leprosy, only the results of the latter work are reported here since they proved to be more favorable than we had expected.

INOCULATION EXPERIMENTS WITH RAT LEPROSY

For material for the inoculations with rat leprosy we used emulsions from subcutaneous nodules and also from the livers and spleens of white rats which had been infected by inoculation with material from two different spontaneously leprous rats, one of which came from the laboratory of Dr. Asami, at Kobe, and the other from the Dermatology Clinic of the Imperial University of Tokyo. Ten-gram lots of the tissues, rich in bacilli, were finely triturated, suspended in 10 cc. of physiologic salt solution, and filtered through gauze. Eight chickens (6 cocks and 2 hens), most of which weighed about 350 grams though some weighed 1,500 grams, were injected in the muscles of the breast with 2 to 5 cc. of this emulsion. Each animal was inoculated two or three times, at intervals of from 3 to 6 months. In several of them an intraperitoneal inoculation of 2 cc. of emulsion was made simultaneously.

Except one cock, which died during the course of the experiment probably through accident, the animals were killed from six to eight months after the first inoculation. Most of them had become thin and looked somewhat poorly nourished. The macroscopic and microscopic findings are as follows:

Injection site.—The changes in the breast muscles were striking. Immediately upon removal of the skin there was noted here

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and there a yellowish color of the surface of the muscle. As the layers of muscle were removed one by one it was seen that the orange-yellow coloration was distributed quite extensively. There were also found several sharply circumscribed indurations of the same color, varying in size from that of a sparrow's egg to that of a plum. On the incised surface of these indurations larger and smaller orange-yellow masses could be seen, which lay as cords or layers between the usual brownish-red muscle fibers. Associated with them were thicker and thinner yellowish- or grayish-white cords of connective tissue.

The orange-yellow masses were viscous and had an oily appearance. Microscopically they were composed of colossal numbers of acid-fast bacilli and a relatively small amount of fatty substance. Culture experiments with this material on ordinary nutrient media, including that of Petragnani, were all unsuccessful. On the other hand, we invariably succeeded in producing leprosy in white rats by inoculations of this material. It appears, therefore, that rat leprosy bacilli can be kept living in the muscles of the chicken, and that they probably multiply there.

Histologically this orange-yellow induration is a specific granulation tissue, located beside the destroyed muscle tissue. The foci are elongated or irregularly shaped, and are frequently surrounded by a zone of connective tissue. Here and there, running through the granulation tissue, are the thick cords of connective tissue which macroscopically looked yellowish- or grayish-white. The granulation tissue is composed principally of bacillus-free epithelioid cells and larger lepra cells filled with bacilli; some of the lepra cells also contain fat globules. Giant and plasma cells are only seldom observed. There are also smaller centers of infiltration which are composed almost exclusively of small round cells, some of which also contain bacilli. Most of the blood vessels in these granulation tissues show thickened and more or less degenerated walls, in which, however, no bacilli are found. The regional lymph nodes are not swollen.

Lungs.—In general the lungs show only slight changes. In isolated cases one observes accumulations of small round and epithelioid cells, in which acid-fast bacilli are also found.

Liver.—Although the liver macroscopically does not show any characteristic change, histologically quite important signs of inflammation can be found. There are, indeed, small foci composed of round and epithelioid cells; these are present through-

out the whole organ, especially in the interlobular tissues and around the central veins, and often even between the liver cells. These foci have a histological structure that is almost the same as that in the muscles, except that here they are still further developed. Bacilli may be detected at times in epithelioid cells.

Spleen.—The spleen, also, does not show any striking macroscopic change, except that the cut surface is more grayish-white than reddish. Microscopically marked hyperemia and focal accumulation of round cells may be seen. Acid-fast bacilli were found in the spleen of only one cock.

Kidneys.—No recognizable macroscopical change. Histologically, small foci of round cells are scattered here and there, and degenerative changes of the epithelium of the uriniferous tubules are observed. Epithelioid cells are also associated with the accumulations of round cells. In the kidney of one cock especially there were formed relatively large nodules, with epithelioid cells in which sparse acid-fast bacilli could be found.

Testicles.—In general this organ does not show any recognizable change, either macroscopically or microscopically. In one cock only there was atrophy of both testicles.

Ovary.—Isolated rounded foci of round cells were found in an ovary of one hen.

INOCULATION EXPERIMENTS WITH HUMAN LEPROSY

A leprosy nodule the size of the tip of the little finger was finely emulsified in 10 cc. of physiologic salt solution. A cock of about 350 grams body weight was injected with 2 cc. of this emulsion, and another of about 1,500 grams was given 5 cc., into the muscles of the breast on both sides. Two months later each cock was given a second injection under almost the same conditions. Three months after the last injection they were killed.

The macroscopic and microscopic findings in the breast muscles and also in the internal organs were almost the same as in the chickens that were treated with rat leprosy material. The muscles showed quite extensive areas of orange-yellow color. In these places there were found colossal numbers of acid-fast organisms which could not be other than human leprosy bacilli, since they could not be cultivated on ordinary nutrient media, or on Petragnani's media which is very suitable for tubercle bacilli. Inoculation of white rats with a part of the affected muscle tissue did not produce any symptom of rat leprosy.

Histologically the orange-yellow part of the breast muscle, as in the birds inoculated with rat lepra material, was a granuloma composed of lepra cells containing bacilli and also free epithelioid cells and smaller round cells. The histological changes in the liver, spleen and kidneys were also analogous to those in the chickens inoculated with rat leprosy material. The changes in the liver were especially striking, there being rounded foci of infiltration composed of round cells and scarce epithelioid cells; these were found in the interlobular connective tissues, around the central veins, and also between the liver cells. Leprosy bacilli were also detected in the epithelioid cells. The testicles of both cocks showed no noteworthy change.

DISCUSSION

From the experimental results described it can be concluded that the human and rat lepra bacilli, injected in the breast of chickens, not only remain alive there for from six to eight months but that they probably also multiply. To determine if both kinds of bacilli are capable of producing a general infection in chickens a relatively short observation time is not sufficient. Yet it is to be emphasized, especially in connection with inoculation experiments with human leprosy, that among those experimental animals that have as yet been used, most of which remain without any reaction on inoculation of such material, the chicken shows the most characteristic macroscopic and microscopic changes.

ADDENDUM

These experiments have been repeated by Masaro Sato, of the Dermato-Urologic Clinic of the Imperial University at Tokyo, and by Yoshimasa Watanabe and Nobu Nonaka, of the Kitasato Institute for Infectious Diseases.

In a communication presented to the 37th Congress of the Japanese Dermatological Society, held in Sendai on May 7 and 8, 1937, M. Sato reported on inoculation experiments with rat leprosy material with cocks and squirrels. Four squirrels, inoculated subcutaneously, showed no change worthy of mentioning except a passing local induration at the site of the injection. On the other hand in five cocks to which the material was inoculated in the comb (and also in the muscles of the breast) there was formed an induration in the comb after one month, which developed after three months into quite large nodules. These nodules, however, showed no further tendency to enlarge.

On the 165th day after the inoculation 3 cocks were killed and the changes examined critically. Each comb contained a hard, bean-sized nodule the center of which was caseous. Colossal numbers of acid-fast bacilli were found not only in the caseous center, but also in the granulation tissue around it. Histologically this granulation tissue was for the most part composed of epithelioid cells with and without vacuoles, with which a number of giant cells were associated. All these cells contained numerous bacilli. With the exception of the necrosis of the central part, the structure of the granuloma was almost identical with that seen in spontaneous rat leprosy. At the site of injection in the muscles of the breast the skin was loosely adherent to the muscle fascia. Under the fascia there was found a plain grayish-white hard mass which, histologically, was a granulation tissue with central necrosis, just like that in the nodules of the comb. Numerous bacilli could also be detected therein. Bacilli were further found in large numbers in the liver and lungs of a cock. The reporter was of the opinion that the chicken, at least in the first inoculation-generation (passage inoculations in chicken's have not yet been made), is a susceptible animal for rat leprosy.

Watanabe and Nonaka state [*Tokyo Izi Sinsi* (1937) No. 3042, July 24] that of the common laboratory animals they consider the chicken to be the most suitable for inoculation with human leprosy. Emulsions of freshly removed leprosy nodules were injected two or three times into the breast muscles at intervals of 2 to 4 weeks. Contrary to our findings the authors observed almost no macroscopic changes, or only little nodules, in the breast muscles that had been injected with the emulsion, whereas in the internal organs, especially in the liver, miliary nodules were almost always produced. Histologically these lesions consisted of a granuloma composed of epithelioid cells, histiocytes and lymphocytes surrounding a large central focus of necrosi. These authors conducted similar experiments on six chickens with rat lepra material and have confirmed always the characteristic small nodule formation, with bacillary content, at the site of injection (muscles of the breast) without being able to detect any macroscopic or microscopic changes in the internal organs.