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THE SERIAL TRANSMISSION OF HUMAN LEPROSY IN
FOWLS, CONTINUED FOR SEVEN GENERATIONS*

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The fact that the fowl is the animal best suited for transmission experiments with human and rat leprosy materials was discovered by chance by one of us (M.O.). Some time ago he happened to encounter two human cases of infection of *Mycobacterium avium*, and made an experiment with the cultures of this bacterium to infect the fowl. He then inoculated, together with several other kinds of acid-fast bacilli, the materials of human leprosy and rat leprosy into the same animal, and realized the usefulness of the fowl for this purpose. A report on this work, by M. Ota and S. Sato, was sent to the Cairo conference, and one published in this country has been reprinted in this periodical (3).

If a simple emulsion prepared from a human leproma is injected into the breast muscle of a fowl, certain definite changes occur though they are rarely remarkable. It was with difficulty that in any case passage inoculations with the lesions so produced were accomplished for more than two or three generations. We therefore devised other means to obtain better results with this animal. It was finally found that when the leproma emulsion is injected in admixture with siliceous sinter, trypan blue and potassium iodide, it is possible to obtain positive results without exception; i.e., leprosy can be transmitted to the fowl.

*This article consists of a summarization of work previously reported by the same authors in Japan, most fully in two articles in the *Japanese Journal of Experimental Medicine* 18 (1940) 327-344 and 345-351. The first of them, entitled *Durch sieben Passagen hindurch ohne Ausnahme gelungene Uebertragungen von menschlicher Lepra bei Hühnern*, reviews the work done along this line in Japan and gives details of the experiments; it is illustrated by two plates, one in colors. The second article, which also gives more details than the present one and is illustrated by a plate showing skin reactions, is entitled *Ueber Mitsudache Reaktion, angestellt mit einem Antigen aus leprösen Gewebe von mit menschlicher Lepra infizierten Hühnern*.
—EDITOR.

The original leproma emulsion was made with 1 gm. of leproma tissue, finely emulsified under aseptic precautions with enough physiologic saline solution to make 10 cc. Each fowl was inoculated with the following "standard mixed emulsion."

Leproma emulsion.....	5.0 cc.
Kieselgur.....	0.05 gm.
Trypan blue.....	0.05 gm.
Potassium iodide.....	0.05 gm.

The mixture was filtered through gauze and filter paper, and consequently the leprosy bacilli which it finally contained were much reduced in number, in comparison with the original material. The injections were made into the deepest layer of the fowl's small pectoral muscle on the right side, using a syringe fitted with a needle suited to allow the emulsion to pass.

As a control test, a paper-filtered mixture of the following composition, designated the "standard stimulus mixture," was injected similarly into the pectoral muscle of the other side.

Kieselgur.....	0.05 gm.
Trypan blue.....	0.05 gm.
Potassium iodide.....	0.05 gm.
Physiologic saline solution.....	5.0 cc.

The first experiment was performed in May, 1939, with 36 male fowls, of which 21 have been killed and examined. These autopsies were made at intervals varying between 20 days and 12 months after inoculation. In every instance we found a characteristic change in the muscle at the site of inoculation.

Passage inoculations have been carried out as follows: The second generation (inoculated July 17, 1939) was transferred to the third (inoculation October 2), which in turn gave further generations: the fourth (inoculation November 2), the fifth (inoculation December 8), the sixth (inoculation February 4, 1940), the seventh (inoculation March 14). Two to six fowls of each of these generations were killed and examined, with the finding that every fowl, without exception, revealed positive signs of infection. Female fowls were used from the second generation on. The results of these experiments will be published in detail elsewhere (2);* only a brief description of them will be given here.

MACROSCOPIC CHANGES

When the skin of the inoculated fowl is removed there appears a flat, smooth fascia through which, on the inoculated side, the

* In press at the time this report was prepared; see reference.

changes affecting the deeper levels may be recognized. On dissecting the muscle fascia, as well as the great pectoral muscle, the fascial layer between the great and small pectoral muscles comes to sight, and the observer is struck with the features characteristic of the specific affection.

These changes appear as orange-yellow streaks or spots, or they form fat-like scars in the form of yellowish-white stripes. On the surface of the small pectoral muscle, gross lesions appear as areas of the same orange-yellow color; the surface is markedly viscous and rather soft in consistence. Incision of these parts of the small pectoral muscle reveals the fact that the coloration extends down to the deepest part of the muscle. Those parts of the muscle tissue without such spots or infiltrations are of a turbid grayish-yellow color throughout. For several weeks, in some cases even after the lapse of several months, traces of trypan blue are sometimes seen between the fasciae, as well as in the muscle tissue. They appear as small foci or infiltrations of deep blue color. In the large external pectoral muscle, especially on its surface facing the small pectoral muscle, the orange-yellow spots are sometimes noticed.

On the contrary, in the breast muscles of the control side, after the lapse of twenty days—or at the latest one or two months after the inoculation—such changes are no longer seen. Only minor remains of trypan blue may sometimes be observed, in thin streaks.

Smears made from the orange-yellow infiltrations described, stained by the Ziehl-Neelsen or Pooman method, reveal on microscopic examination remarkable numbers of acid-fast rods. In most cases they are seen clustering within the vacuoles of large cells.

Besides the breast muscle of the inoculated side, the liver often shows changes. Macroscopically the lesions appear as yellow nodules or spots on the surface of the organ. Here, however, acid-fast bacilli are rarely found, and then only in quite small numbers. With regard to the spleen, usually there is no change at all, and foci with the bacilli are even fewer than in the liver. In no case have changes occurred in the lungs, testicles or ovaries. Swelling of lymph nodes has very rarely been observed.

MICROSCOPIC CHANGES

Sections of tissue taken from the orange-yellow areas in the pectoral muscle show that the lesions are granulomata. The

muscle fibers have begun to undergo degenerative changes, staining unevenly and undergoing disintegration into fragments. The connective tissue between the fibers is increased, and there is notable perivascular cellular infiltration. In the central part of the granuloma the muscle fibers have almost completely disappeared, and in the connective tissue there appear large numbers of round masses which in some cases are compact and in others not, sometimes isolated and sometimes connected with each other.

These formations are composed of large cells with vacuoles which seem to correspond to the lepra cells in human tissues. Within these round formations large numbers of acid-fast rods are found in masses. Often they appear in the granular form, which cannot be stained easily. On the other hand, there may also be observed small groups of lepra cells, not attaining the distinctive appearance described but lying scattered or in collections.

These formations are closely surrounded by infiltrative cells. These cells consist chiefly of two kinds: (a) small round cells with narrow protoplasm, of which it is doubtful whether or not all are lymphocytes; and (b) histiocytes with a broader protoplasm, from which, it is suspected, the lepra cells are formed. Small conglomerates of siliceous sinter are observed either within or outside of the formations described. Probably these aggregations induce the accumulation of the histiocytes, which afford the lepra bacilli a suitable place to lodge and propagate.

The bacilli found are neither tubercle bacilli nor any other ordinary kind of acid-fast bacillus. This is shown by the fact that guinea-pigs inoculated with the granuloma containing them never develop tuberculous changes, and from the fact that in cultures, even on Petraghani's medium, no growth takes place.

MODIFICATIONS OF THE INOCULATION METHOD

If any of the constituents of the standard stimulus mixture is used alone with the leprosy material for injection, no better results can be expected than with the whole mixture. Particularly poor results are obtained if trypan blue alone is used with the leprosy material. A mixture of siliceous sinter and potassium iodide gives far better results than does trypan blue alone.

An injection of Sudan III and the leproma emulsion causes a remarkable change in the muscle at the site of inoculation, but that change disappears in two or three months without leaving any trace of infection. On the contrary, if siliceous sinter and Sudan

III are used with the leproma emulsion, the results are scarcely inferior to those obtained with the standard mixture.

EXPERIMENTS WITH OTHER ANIMALS

The standard mixed emulsion, when injected into the muscles on the back or the abdomen of a rabbit, may form a leproma at the place. However, the muscular layer of the rabbit is so thin that further development of the leproma is quite limited.

As regards the white mouse, every attempt failed to obtain signs of affection with human leprosy material, even when the standard emulsion was injected intraperitoneally. On January 17th, however, we injected into white mice an emulsion made with the standard mixture and a leproma that had developed in the breast muscle of a female fowl of the fifth passage generation, and there resulted changes of high degree. For instance, in one mouse killed on July 15th there was a small ulceration of the skin where the injection had been made, and a number of acid-fast rods were detected from the lesion. The inguinal lymph nodes on both sides were swollen to the size of a rice grain or even of a small pea, and large numbers of bacilli were found in them. The liver was remarkably enlarged, and in it, as well as in the kidneys—which showed no evident change—the presence of bacilli was ascertained. In another white mouse bacilli were found in the spleen as well. These experiments with the white mouse are still under way. It would be more convenient to use this animal than any other in experimentation with regard to the chemotherapeusis of leprosy. Similar results have been obtained with the rat; experiments with the guinea-pig are in progress.

In experiments of simple inoculation of the leproma into Chinese hamster (*Cricetulus griseus* Milne-Edward, 1867), no satisfactory results have been obtained.

EXPERIMENTS WITH THE TUBERCLE BACILLUS

Tubercle bacilli of the human type, injected together with our standard stimulus mixture into the breast muscle of fowl, can produce a change similar to that caused by the bacilli of human leprosy. In this case the color of the granuloma is lemon-yellow rather than orange. Acid-fast bacilli are also found in abundance, but the mode of distribution in the tissue is different from that of the human leprosy bacilli. A brief report of this experiment will be made shortly by Dr. Pelicano-Argentine, of our laboratory.

THE MITSUDA REACTION WITH INOCULUM PREPARED FROM
LEPROTIC TISSUE OF THE FOWL

The value of the so-called "Mitsuda reaction" was first investigated by Yoshinobu Hayashi (1918), Kensuke Mitsuda (1919), and Fumio Hayashi (1930). If an emulsion of a boiled human leproma (or a boiled suspension of bacilli obtained from leprous nodules) is injected intracutaneously a positive reaction is observed in cases of *lepra maculosa* and *lepra nervorum*, and also in nonlepers, while with *lepra tuberosa* the reaction is negative in a majority of cases (1). The value of this reaction is generally accepted by leprologists in Japan. It has been pointed out by F. Hayashi that it may find an application in ascertaining the identity as leprosy bacilli of organisms from cultures used in this way.

Following Hayashi's method, we have made such preparations from the leprous granuloma of the breast muscle, and also from the livers and spleens of fowls to which leprosy had been transmitted. These preparations have been applied in cases of nodular and neuromacular leprosy, as well as to nonlepers, to test the outcome of intracutaneous reaction. As regards the preparations made from the granuloma of the breast muscle, the results are in accordance with those of the Mitsuda reaction with the usual antigen, used as a control on the same individuals at the same time. A preparation made from the liver of a fowl of the fifth generation also gave similar reactions. The antigen made from the liver of a fowl of the first experimental generation, however, produced far weaker reactions. As regards the inocula made from the spleen, one of that organ from a fowl of the first generation produced no reactions, while one from a fowl of the fifth generation caused positive but very faint reactions. It is concluded that the antigen made from the granuloma of a fowl which contained the bacilli in abundance causes the same reaction as that made from the human leproma.

REFERENCES

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