A REVIEW OF RECENT ANIMAL INOCULATION STUDIES WITH HUMAN AND MURINE LEPROSY BACILLI

TADAYASU TANIMURA AND SHINJI NISHIMURA
Department of Dermatology, Medical School and Leprosy Laboratory, Research Institute of Microbial Diseases, Osaka University
Osaka, Japan

It is not necessary to dwell on the difficulty of transmission of leprosy to laboratory animals, or on the importance that in the interest of prophylaxis and treatment of leprosy, fundamental experiments to accomplish that end should not be abandoned. Since 1941, Yukichi Satani, Tadayasu Tanimura, Hosaku Sakurai, Mineo Tamura, Shinji Nishimura, Eifuku Tadedatsu and Ichiro Fukuda (1-12) of our institute have carried on such studies, with the cooperation of Yasuji Nojima and other workers of the Oshima Seisho-En national leprosarium. The work has involved inoculations of human and murine leprosy bacilli into fowls, white rats, guinea-pigs, rabbits, and recently golden hamsters. The results obtained are summarized in this paper.

SUSCEPTIBILITY OF FOWLS TO HUMAN AND MURINE BACILLI

The interesting report of Masao Ota and Shuichi Nitto (16, 17) of positive results of inoculations of fowls with the human leprosy bacillus together with histiocyte-stimulating substances led us to attempt similar experiments.

We used both human and murine leprosy bacilli, and also as controls the human tubercle bacillus, BCG, a nonpathogenic acid-fast (No. 16), also called a pseudomurine bacillus.[1] Large doses of these bacilli were injected deep into one side of the chest muscles of fowls, either living or heat-killed, alone or mixed with histiocyte-stimulating substances such as trypan blue, diatomaceous earth or potassium iodide. The pectoral muscle on the other side was left intact for comparison. One or two fowls with each kind of inoculum were autopsied every month for a period of five months, and successive inoculations were made with material from these fowls to others.

[1] This culture was isolated from a rat in Osaka in 1942 and has been carried on since then in our laboratory. The term "pseudomurine bacillus" is used to designate a kind which Nishimura reported in 1938 (15). Out of 7,800 house and wild rats examined, 70 were found to have acid-fast microorganisms in skin, muscle, or lymph-node lesions. Those with skin lesions, and some of those with only lymph-node lesions, were proved by inoculation to be true rat leprosy. From certain of the other lymph node lesions acid-fasts were obtained in cultures, and these proved nonpathogenic.

Other groups received injections of human and murine bacilli three times, and were kept under observation for from 1 to 1½ years. The results were judged from the anatomical and histological findings, and by inoculation of white rats in the case of the murine bacillus and by cultures on Petragnani's medium with the other acid-fasts after fowl passage.

Results.—Both the human and murine bacilli give rise to macroscopic findings such as swelling, congestion, infiltration, granulation tissue, and yellowish pseudo-membrane in the site of inoculation, and to microscopic changes like intense cell infiltration in the interstitial tissue, atrophy of muscle fibers, multiplication of phagocytic cells, and follicles of epithelioid cells loaded with bacilli. At first glance these histological pictures (Figs. 1 and 3) reminded us of human or murine leprosy lesions, but on more detailed examination they could not be recognized as leprous changes because of the absence of the fat-containing cell vacuoles of lepromatous leprosy and of Langhans' giant cells containing bacilli usually observed in murine leprosy. The macroscopic and microscopic changes diminished in intensity as the days passed, until they returned to sound tissue (Figs. 2 and 4), and the bacilli were decreased in numbers by the fifth month. The murine bacillus had lost its infectivity by the thirtieth day, when inoculated back to white rats. Lesions and bacilli could not be found in every case at the second or the third generation of successive inoculations, although in one instance they reached the fourth generation.

The results of injection of living bacilli with histiocyte-stimulating substances added or of heat-killed vaccine showed no essential gross or microscopic differences from those due to living bacilli, although the former produced stronger inflammatory reactions than the latter. Mobilization of histiocytes and multiplication of bacilli, human and murine, within the cells, caused by Ota's stimulating substances, could not be seen. The nonpathogenic acid-fast bacillus, the tubercle bacillus, and the BCG bacillus caused similar lesions, including bacilli, also with marked reparative changes by the fifth month. The nonpathogenic bacillus could barely be recovered on media by the second month of fowl passage, and later the results were negative.

The repeated inoculation of human and murine bacilli, under observation for long periods, produced a somewhat intense local lesion, but the longer the period of observation the more marked was the healing process, without multiplication of bacilli or metastases in other organs.

In short, neither the human nor the murine bacillus infected

fowls, and the histiocyte-stimulating substances failed to alter the cell properties which prevent growth and multiplication of bacilli. The lesions caused by all kinds of the inoculated bacilli were essentially the same granulation-inflammation, the differences of intensity of reaction depending upon the kind of bacillus. Moreover, from the fact that the lesion underwent a definite reparative process it must be concluded that this change is a temporary reactional picture and quite different from the specific human or murine leprosy.

TISSUE REACTION AND FATE OF THE BACILLI IN SUSCEPTIBLE AND RESISTANT ANIMALS

The results just related led us to conclude that human and murine leprosy bacilli cannot multiply in the tissue cells of fowls, and that animals of the rodent class, such as white rats, guinea-pigs and rabbits, should offer these bacilli more favorable media for multiplication. It is conceivable that the susceptibility of white rats and mice to the murine bacillus is due to a fact that these animals have a greater tendency to produce leprous change than any other animals. However, many experiments have shown that the human leprosy bacillus infects only the human being, and that the murine bacillus attacks only the rat family. Hence we made further experiments to investigate to what degree the changes caused by inoculation of the human bacillus into white rats might resemble those of human leprosy, and to observe the tissue reaction against the human and murine bacilli in susceptible or naturally immune animals, such as guinea-pigs and rabbits which are zoologically related to rats and mice.

The experiment was carried out as follows: The lepromas with human and murine bacilli were diluted 100 times. The No. 16 acid-fast was used in the concentration of 2 mgm. per cubic centimeter. These suspensions were injected subcutaneously, in doses of 0.2 cc., in three places on the back of each animal. The animals were sacrificed serially after from one to five months and examined histologically, with vital staining by trypan blue.

Results.—In white rats inoculated with the murine bacillus and examined after from 1 to 5 days almost no macroscopic lesions occurred at the inoculated sites, although histological examination revealed numerous polymorphonuclear leucocytes and monocytes which phagocytosed bacilli. After more than a week there was proliferation of fibroblasts and diminution of the numbers of polymorphonuclears, while innumerable monocytes and lymphocytes had appeared. Bacilli were found in these cells, but diminution of their numbers was striking. More

than two weeks later, inflammatory cells were scarce, fibrocytes had increased, and epithelioid cells were congregated into follicles in which masses of bacilli were observed. Much later the bacilli multiplied and filled the epithelioid cells and fibrocytes. Granulomatous lesions consisting of these cells appeared, together with Langhans' giant cells which included bacilli. It was noted that in this case epithelioid cells stuffed with bacilli did not present a picture of nucleus collapse, although later the whole cells underwent necrosis. On staining with trypan blue, the cells including bacilli appeared to have the power always of phagocytosing dye granules, which became stained with them.

The first tissue reaction provoked in white rats by inoculation with the human bacillus was the same as seen with the murine bacillus, and the phagocytic cells were of the same kinds. As the condition progressed, however, the bacilli in cells were disappearing, and epithelioid cells underwent necrosis. The lesion was enveloped with lymphocytes and proliferating phagocytes all around, and the central necrosed part was absorbed.

The sites inoculated with the nonpathogenic acid-fast showed similar changes, although the inflammatory reaction was more severe than that caused by the leprosy bacilli, and necrosis of the phagocytic cells appeared earlier.

In guinea-pigs the tissue reaction against human and murine bacilli was similar to what was seen in the white rats, but more intense. All wandering and phagocytic cells were of the same kinds. Multiplication of bacilli was not observed.

In the case of inoculation of human and murine bacilli into rabbits, a much stronger inflammatory reaction was observed. Infiltration of polymorphonuclear leucocytes and lymphocytes, proliferation of fibrocytes, and nodule formation by epithelioid cells were also found. The inoculated bacilli were phagocytosed in these cells, but they were modified in morphology and disappeared in the course of the process. What interested us in the rabbit experiment was the occurrence of large, rough masses of murine bacilli which suggested chestnut burrs in appearance, inlaid in surrounding cell groups but not contained in cells. This fact is suggestive of the existence of some germicidal power in rabbits, which is stronger than that in white rats or guinea-pigs.

In summary, in the susceptible white rats leucocytes killed and digested murine leprosy bacilli, perishing themselves, while histiocytes and connective tissue cells allowed the bacilli to

multiply in them, the cells maintaining their vital functions and forming giant cells by fission. In other words, there was a symbiosis of the bacilli and certain kinds of cells. Both the polymorphonuclear leucocytes and the histiocytes of guinea-pigs killed the bacilli, and did not allow their proliferation. This phenomenon explains the difference between the animals which are naturally susceptible to the murine leprosy bacillus and those which are immune to it. The rabbits exhibited a stronger bactericidal effect than the guinea-pigs. With the human bacillus inoculations, similar tissue reactions occurred in white rats, guinea-pigs and rabbits. Bacilli were taken up by polymorphonuclear leucocytes, monocytes and other phagocytic cells, and nodules of epithelioid cells were built up. The same findings were seen after the inoculation of the nonpathogenic acid-fast, but in this case inflammation was more severe. Comparing the different experimental animals, rabbits produced the most intense reaction, and white rats the mildest.

AFFINITY OF THE OCULAR TISSUE FOR THE HUMAN AND MURINE BACILLI

Many studies have shown that only certain living tissue cells of susceptible animals offer the appropriate conditions for multiplication of the human and murine leprosy bacilli. The fact that these bacilli can multiply only in living cells is interestingly similar to the conditions required for proliferation of the viruses and rickettsias. But, as is well known, the various organs of an animal have different susceptibilities, and the endothelial cells of Descemet's membrane are the most easily infected. We therefore inoculated human and murine leprosy bacilli—the former of which has an affinity for the ocular tissue in the natural infection, while the latter has not—into eyes of animals to determine whether the ocular tissue can supply an adequate medium for their multiplication, without regard to the race specificity of susceptible or immune animals, and whether traumas like wounds can influence the specificity.

The experiment was carried out as follows: Doses of 0.1 cc., 0.05 cc. and 0.03 cc. of suspensions of human and murine lepromas, 1:20 dilutions, were injected into the anterior chamber by the cornea-puncture method. To judge the results, animals were killed serially after from 30 to 60 days and the eyeballs were extirpated. Smears and sections were obtained from the aqueous humor, corneal endothelium, iris, vitreous, crystalline lens and retina.

Results.—With regard to the inoculations of the murine bacillus in rabbits' eyes, in spite of the large doses inoculated the

reactional inflammation disappeared in a comparatively short time. In the corneal endothelium bacilli were observed for a longer period, but they were thin in form and stained poorly and could not be recognized as newly multiplied bacilli. On histological examination, thickening of the supporting connective tissue of the iris and masses of phagocytosed bacilli were seen. This change had some resemblance to the murine lesion, although in comparison with the findings in white rats, to be described, the intensity was slight. In eyes which had been subjected to contusion, the reactional inflammation was more intense but there was no favorable effect with respect to multiplication of the bacilli.

In guinea-pigs, both the human and the murine bacilli were used. In the animals inoculated with the murine bacillus, infiltration of inflammatory cells and changes in the cornea were slight, although the iris showed a strong reaction, with proliferation of fibrocytes and phagocytes loaded with masses of bacilli. Histologic sections revealed that the pigment cells of the iris contained bacilli. Inoculation of the human bacillus produced similar pictures, with proliferation of the cells of the interstitial tissue or the iris, but multiplication of bacilli was not observed.

Both the human and murine bacillus were also used in white rats. The murine bacillus provoked a somewhat intense infiltration of inflammatory cells. At first the number of bacilli was small, but it increased later. In sections, granulation tissue appeared in the anterior chamber after a month, and scattered in it there were follicles of epithelioid cells loaded with innumerable bacilli. After two months this change became so severe that the eyeball was occupied with the bacillus-laden murine lepra cells, and mixed with them were some Langhans' giant cells. This picture was typical, quite different from that seen in rabbits or guinea-pigs. After the inoculation of the human bacillus, there was first an inflammatory reaction, and later proliferation of connective tissue and follicle-formation were observed in the cornea and iris, similar to what was seen in rabbits and guinea-pigs. Bacilli and, sometimes, imperfect lepra cells were found in these follicles, although there was no evidence of multiplication of the bacilli.

To sum up, the endothelium of Descemet's membrane of rabbits, guinea-pigs and white rats was not a favorable medium for the multiplication of either the human or the murine bacillus. In all animals the supporting connective of the iris was the

most sensitive, and here were produced tubercle-like changes, but only in the white rats inoculated with the murine bacillus did the ocular tissue permit multiplication of bacilli and progressive proliferation of granulation tissue. It appears that the human bacillus, which frequently affects and seems to have some affinity for the ocular tissue of man, produces no lesions in this tissue of other animals. On the other hand, the murine bacillus in white rats, although quite indifferent to the ocular tissue in ordinarily infected animals, produced marked reaction and multiplied in this tissue, especially the mesodermal elements—this animal being the one susceptible to this bacillus. These results lead to the conclusion that the affinity of the human bacillus for the ocular tissue does not exist without regard to the specific susceptibility of the animal race, and that it cannot be easily influenced by stimuli like trauma.

SUSCEPTIBILITY OF THE GOLDEN HAMSTER TO THE MURINE BACILLUS

The golden hamster, *Mesocricetus auratus*, is classified among the rodentia, rat family, having an equal position with the genera *Rattus* and *Mus*. This animal being more akin to rats than to rabbits or guinea-pigs, it seemed possible that it would prove susceptible to the murine leprosy bacillus. In 1927, Muir and Henderson (14) reported that the Chinese hamster was not susceptible, whereas in 1937 Balfour (13) reported positive results with the golden hamster. As we have recently been supplied with this animal, experiments with it have been made.

The material was a saline suspension of leproma of murine leprosy bacillus, Kumamoto strain. Fifteen hamsters weighing 70-80 gm. each were divided into three groups, 9 animals being injected subcutaneously with 0.5 cc. of the suspension, 3 intraperitoneally with the same amount, and 3 into the testis with 0.2 cc.

First generation.—The group inoculated subcutaneously showed lepromas at the inoculation sites after 2 to 3 months. These lepromas were somewhat hyperemic compared with those of white rats. The inguinal and axillary lymph nodes were also hyperemic and swollen, although no necrosis was observed, and the number of bacilli was smaller than in lymph nodes of infected rats. Granules composed of bacilli were stained dark red and observed clearly.

In the intraperitoneal group the liver enlarged to two times the normal size and the spleen to five times normal after four months, and they contained as many bacilli as are found in

lepromas of white rats. Lepra cells loaded with bacilli appeared around venous capillaries in the liver (Figs. 5 and 6) and in the peripheral zones of the spleen follicles.

The group which had received bacilli in the testis showed atrophic granulation after 3 to 5 months. In sections, innumerable bacilli were found in this granulation tissue, and the interstitial tissue was filled with proliferating lepra cells. The spermatic ducts underwent atrophy.

Second generation.—A suspension was made of the liver of an animal of the intraperitoneally injected group and was used for subcutaneous inoculation of other hamsters. All animals so inoculated developed lepromas at the injection sites by the third month.

Third generation.—A suspension of subcutaneous lepromas of the second generation was inoculated into two groups of three hamsters each, intraperitoneally and subcutaneously. All animals of both groups showed appreciable lesions.

Inoculation of white rats after hamster passage.—A liver suspension of the intraperitoneal group of the first generation was diluted 20, 100, 10,000 and 100,000 times, and 0.5 cc. of each suspension was injected subcutaneously into ten young white rats. All of these animals developed palpable lepromas after two to three months.

By the positive results of successive inoculations of golden hamsters, and of inoculation of white rats after hamster passage, it has been confirmed that these hamsters are susceptible to murine leprosy. This fact, however, does not signify an extension of race specificity, but it is at least of some use to have a wider field for animal experimentation, and to get different lesion pictures and bacillus forms.

SUMMARY AND CONCLUSIONS

During the past ten years we have carried out animal experiments with leprosy, using the human and murine leprosy bacilli, to determine the results of fowl inoculations, the tissue reactions and the properties of the bacilli at the inoculated sites of white rats, guinea-pigs and rabbits, the affinity of the ocular tissue of these animals to these bacilli, and the susceptibility of hamsters to the murine bacillus. The results of these experiments were as follows:

1. In animals inoculated with human and murine leprosy bacilli, infection cannot be established without the condition that the tissue cells have the specific property of taking in the

bacilli, allowing them to multiply by symbiosis in the histiocytes, and other cells of the same origin, without disturbing the metabolism of these cells. It is known that this specificity is so narrow that the human bacillus finds only the human being to be the favorable host, and the murine bacillus is limited in this respect to rats, mice and hamsters. Our attempts to alter the constitution of animals did not succeed in changing this strict specificity. The ocular tissue, which has an affinity for many pathogenic organisms, could not react indifferently to the race specificity. It is an interesting fact, however, that white rats show a slower tissue reaction against the acid-fasts than any of the other animals, and have a tendency to produce granulation lesions.

2. As for the properties of the murine bacillus in the host, those which are phagocytosed by polymorphonuclear leucocytes are digested and perish by the bactericidal action of these cells. On the other hand, bacilli taken up by histiocytes and other cells of the same origin assimilate some part of the cell cytoplasm, growing slowly, while the metabolic products of the bacilli do not injure the cell, but make up for some part of the cell stroma to maintain the vital condition. These phenomena can be interpreted as a temporary symbiosis, but extreme multiplication of the bacilli leads to an upset of the balance of metabolism and the cells perish. An imaginable reason that the human and murine leprosy bacilli can carry on symbiosis in certain cells of animals is that the assimilation of both cellular and bacillary components are in close correlation, just as a key and its hole. When these phenomena are explained chemically, the problem of animal transmission of leprosy will be settled.

3. The success of inoculation of hamsters with the murine leprosy bacillus confirms the existence of a strict specificity of this bacillus to the tissue cells of the rat family, and broadens the scope of animal experimentation by giving more materials for observation of this disease.

ABSTRACTO

Este es un resúmen de trabajos reacionados con inoculaciones de bacilos de lepra humana y murina en varios animales bajo condiciones experimentales distintas, usando otros mycobacterias como controles.

Se trató de confirmar sin éxito los reportes de Ota y Nitto que lesiones se pueden producer y transmitir en serie, en el músculo pectoral de aves, cuando se usan ciertos estimulantes histiocitarios. Se inocularon ratas blancas, conejos y cobayos por vías subcutáneas e intraoculares, y las reacciones tisulares fueron estudiadas.

En éstos animales, en ambas regiones, todas las mycobacterias causan primero una reacción inflamatoria aguda, variable, seguida de cambios exudativos que al fín y al cabo demuestran regresión en la mayoría. Aún en las ratas blancas, los bacilos murinos que son fagocitados por polimorfonucleares son destruídos, pero los que son fagocitados por otras células produjeron las reacciones granulomatosas características.

La susceptibilidad del "golden hamster" al bacilo murino fué confirmada. Este hecho no significa una más amplia susceptibilidad de especies a éste bacilo, debido a la relación del "hamster" con las ratas y los ratoncillos, pero sí ensancha el campo de experimentación en animales.

Se dá énfasis a la gran especificidad tanto del bacilo humano como el murino, la cual no se afecta ni aún alterando las condiciones tisulares por medio de aditivos en los experimentos en aves, o por trauma en las inoculaciones oculares. Se apunta el hecho que los bacilos viven en una especie de simbiosis con las células del huésped. Cuando se pueda explicar éste condición, se sugiere, el problema de la transmisión de la lepra en animales será dilucidado.

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DESCRIPTION OF PLATE

PLATE (9)

FIG. 1. Fowl, chest muscle, one month after inoculation with human leprosy bacilli. Nodular granulomatous lesions.

FIG. 2. The same, after five months. Fusion of the nodular lesions and marked proliferation of connective tissue.

(*Note*: The lesion produced by the inoculation of the pseudomurine bacilli presented, after one month, a nodular granulomatous picture very similar to that produced by the human leprosy bacillus shown in Fig 1.)

FIG. 3. Fowl, chest muscle, one month after inoculation with emulsion of rat leprosy bacilli. Nodular granulomatous lesions.

FIG. 4. The same, after five months. Disappearance of lesions and proliferation of connective tissue.

FIG. 5. Hamster, liver, four months after intraperitoneal inoculation with rat leprosy bacilli. Murine lepra cells around the central veins and throughout the liver lobule.

FIG. 6. The same specimen, demonstrating the multiplication of the bacilli.

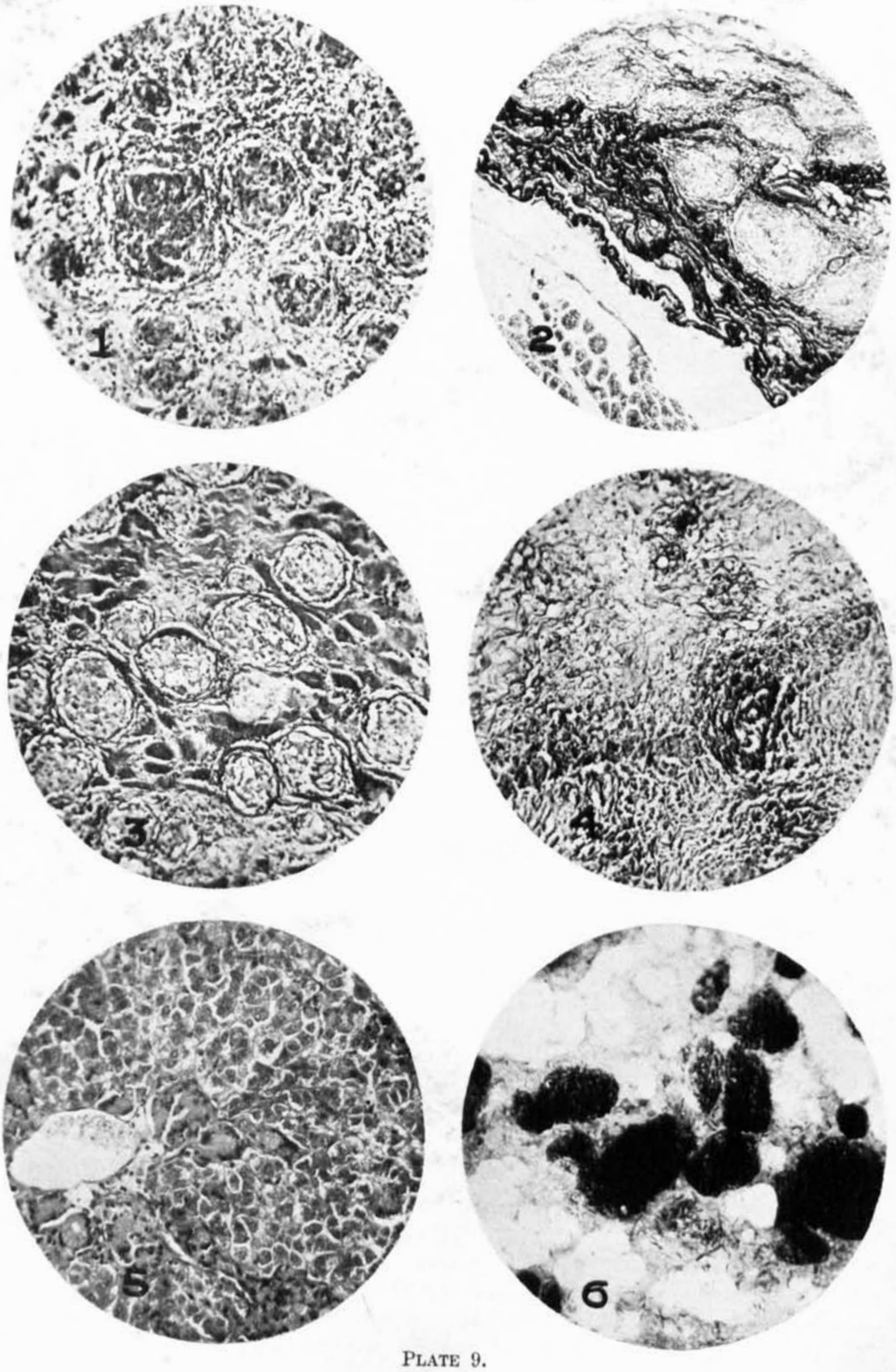

PLATE 9.