STUDIES ON MEASLES.

II. SYMPTOMATOLOGY AND PATHOLOGY IN MONKEYS EXPERIMENTALLY INFECTED.

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PLATES 37 TO 41.

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It has already been shown¹ that a group of symptoms closely resembling those of measles is readily induced in monkeys by inoculation with the nasopharyngeal secretions of patients in the pre-eruptive or early eruptive stages of measles, and that the virus inducing this reaction is readily transmitted from monkey to monkey by inoculation with blood or tissue emulsions from monkeys experimentally infected. In this paper the symptoms and course of the reaction and the histology of the lesions of the skin, labial mucous membrane, and tongue will be described.

Symptomatology.

Incubation Period.—In monkeys inoculated on the mucous membranes of the respiratory tract the incubation period is remarkably constant, being 7 days in the majority of animals. It may vary, however, from 6 to 10 days. Thus in sixteen monkeys inoculated on the respiratory mucous membrane, the incubation period was 6 days in four, 7 days in nine, 8 days in one, 9 days in one, and 10 days in one. During this period the animal appears well and exhibits no recognizable symptoms. The incubation period after intravenous inoculation is apparently somewhat shorter (4 days), but enough animals have not been inoculated by this method to warrant a definite statement.

¹ Blake, F. G., and Trask, J. D., Jr., *J. Exp. Med.*, 1921, xxxiii, 385.

Onset and Course.—The onset is indicated by listlessness, loss of appetite, drowsiness, and diminution in the total leucocyte count. There may be a sharp rise in temperature, but this is not constant. The conjunctivæ become injected, and small, discrete, hyperemic macules appear on the labial mucous membrane. During the following 2 or 3 days these increase in number and may eventually coalesce to form a diffuse, hyperemic enanthem on the mucous membrane of the lips and cheeks. From 1 to 5 days after onset a red, maculopapular rash appears on the skin, usually coming out first on the face. The rash increases and may eventually spread to the skin of the neck, arms, chest, abdomen, and thighs, reaching its height in 2 or 3 days. By this time the enanthem is fading. The exanthem in turn progressively fades, and by 6 to 10 days after onset all symptoms have disappeared.

Conjunctivitis.—An acute catarrhal conjunctivitis is exhibited by nearly all animals during the period of the reaction. This conspicuously involves the inner canthus and tarsal conjunctiva, the bulbar conjunctiva remaining little if at all affected. It varies from a moderate congestion of the vessels to a diffuse hyperemia. In the more severe cases there are increased muroid secretion, increased lacrimation, and definite photophobia. Moderate edema of the lids may be present. Purulent inflammation does not occur.

Enanthem.—Coincident with or shortly following the onset of the reaction a definite and characteristic enanthem appears. This begins with the development of one or more discrete, red spots on the labial mucous membrane. These spots usually occur only on the labial mucosa, but may occasionally be present on the inside of the cheeks and on the gums. They do not occur on other parts of the buccal mucosa. They present the appearance of discrete, bright red, slightly elevated, lusterless macules, 1 to 2 mm. in diameter. Occasionally they show a minute, bluish white center. They occur either singly or in small groups of two or three. Their number progressively increases during the first 2 or 3 days of the reaction. By the end of this time there may be ten to fifteen in all. From this point the enanthem pursues one of two courses. In the milder infections the spots now begin to fade and in 1 to 2 days have disappeared. In more severe infections, however, the spots coalesce to form a more

diffuse, red, slightly granular rash covering large areas of the labial mucous membrane with intervening areas of pale pink mucosa which appear to be normal. In still severer infections the entire labial mucous membrane and the inside of the cheeks finally become covered with a diffuse, bright red, granular rash studded with numerous minute, white, slightly depressed specks. The enanthem now begins to fade and rapidly disappears in the course of 2 to 3 days.

Exanthem.—From 1 to 5 days, usually on the 3rd or 4th day, after onset a characteristic exanthem appears. It generally comes out first on the face, especially about the eyes, the corners of the mouth, and on the cheeks, but may also appear approximately at the same time on other parts of the body, particularly on the inner surface of the thighs. It begins with a few, discrete, red maculopapules occurring either singly or in small groups. The individual maculopapules are 2 to 3 mm. in diameter, fade out gradually at their margins into the surrounding normal skin, and are frequently located about a hair follicle. They disappear on pressure and are never hemorrhagic. There is no diffuse erythema of the intervening skin, which appears normal. The further progress of the rash shows the characteristic evolution of the measles exanthem, though the rash itself rarely becomes as widespread or as thick as it commonly does in man. Generally in the course of 2 or 3 days the rash comes out progressively on the skin of the neck, chest, lower abdomen, and the inner surfaces of the upper arms and thighs. While it is usually limited to these less hairy areas of the skin, it may occasionally be sparsely present on the scalp, shoulders, back, forearms, and posterior surface of the legs. During this period the individual maculopapules tend to increase in size, but they never become sufficiently large or thickly aggregated to produce a confluent rash. Having reached its height the rash begins to fade and disappears in the course of 2 to 5 days. This sometimes takes place without noticeable desquamation, but there frequently is a fine, branny desquamation of the skin over the central portion of the maculopapules. Occasionally a slight, yellowish brown pigmentation may persist for 2 or 3 days after the complete fading out of the hyperemia. While the foregoing description presents a composite picture of the exanthem, the extent of the rash and the number of maculopapules vary considerably in individual animals.

Thus it may vary from a comparatively sparse exanthem limited to the face or face and inner surfaces of the thighs, to a widespread, moderately thick exanthem involving the skin of the face, neck, trunk, and extremities. The character of the individual maculopapules, however, remains constant and typical.

Fever.—There is the widest possible variation in febrile reaction. The temperature may rise abruptly at onset, even to as high as 105–106°F. On the other hand, the disease may begin without fever and remain so throughout. When fever occurs it may persist only during the prodromal stage before the appearance of the exanthem or may continue throughout the course of the disease. It may be distinctly remittent in type or fairly well sustained. In an occasional animal the preliminary rise at onset is followed by a return to normal and a distinct secondary rise. In these cases the temperature curve closely resembles that of measles in man.

Leucopenia.—A definite diminution in the total leucocyte count consistently occurs coincident with the reaction. This usually begins 1 or 2 days before the onset of symptoms and persists for several days until the height of the exanthem is reached. At this time the leucocytes begin to increase and gradually return in the course of 3 days to a week to their former level. The degree of leucopenia varies somewhat in different animals, but it is nearly always well defined and may be very marked, many animals showing counts as low as 4,000 to 9,000 cells per c.mm. over a period of several consecutive days. No significant change in the leucocyte count which may be attributed to the virus of measles appears to occur during the earlier part of the incubation period. Animals inoculated with unfiltered nasopharyngeal washings usually show a leucocytosis for 24 to 48 hours after inoculation, but this is undoubtedly due to the effect of other organisms, since it does not occur in animals inoculated with filtered washings in which other organisms than the virus of measles are presumably not present. It has not as yet been determined whether the diminution in leucocytes is due to a greater reduction in one type of leucocyte as compared with other types.

Other Symptoms.—The general symptoms of malaise, such as loss of appetite and activity, are usually moderate in degree, but may occasionally be quite marked. They begin with the onset of the

reaction and persist until the exanthem begins to fade. A very definite drowsiness is often present during the first 2 or 3 days of the disease. Another symptom of not infrequent occurrence is a moderate diarrhea during the earlier part of the reaction. The entire absence of symptoms of rhinitis and bronchitis should be noted.

Microscopic Pathology of Skin, Labial Mucous Membrane, and Tongue.

The tissues available for study consisted of small pieces of skin excised,² during life from twelve monkeys during the period of the exanthem and pieces of skin, labial mucous membrane, and tongue obtained at autopsy from seven monkeys killed for passage of the virus. The tissues were fixed in Zenker's fluid and stained with alum-hematoxylin and eosin. A composite picture of the lesions will be given.

Skin.—Lesions are present both in the corium and in the epidermis. The reaction in the corium (Figs. 1 to 6) is definite and typical. It consists of swelling and proliferation of the endothelial cells lining the capillaries and smaller veins, accumulation of endothelial leucocytes³ about the capillaries (Figs. 2, 3, and 5), and active multiplication of these emigrated leucocytes. In addition there is a moderate exudation of serum into the pericapillary tissue (Figs. 1 and 3). In the earlier lesions a very few eosinophils, polymorphonuclear leucocytes, and lymphocytes may also be present in the pericapillary exudate. Diapedesis of red blood corpuscles has not been seen. The endothelial cells of the capillary walls appear swollen; their cytoplasm is finely granular. Occasionally one is seen in mitosis (Fig. 1). The emigrated endothelial leucocytes are young and active. Their nuclei are frequently lobulated. In early cases these leucocytes are in active mitosis (Figs. 1, 2, and 4), as many as three to five mitotic cells sometimes being present about a capillary. Occasionally they

² This was always done under anesthesia.

³ The term endothelial leucocyte is used for the sake of convenience in comparing the lesions in monkeys with the lesions of human measles as recently described by Mallory and Medlar.⁵ The authors, however, do not wish to commit themselves to the opinion that the wandering mononuclear phagocytes are derived solely from endothelial cells and not in part from other fixed tissue cells of mesenchymal origin.

show phagocytosis of polymorphonuclear leucocytes and lymphocytes. In later lesions the exudation of serum is less marked, mitotic cells are no longer present, and polymorphonuclear leucocytes are not seen.

These lesions are chiefly in the upper part of the corium and not infrequently lie close to the hair sheaths and sebaceous glands. Although the exudate is primarily pericapillary in location, it also extends into and involves the epithelium of the epidermis, hair sheaths, and sebaceous glands when the capillary lesions lie closely adjacent to these structures.

The lesions in the epidermis (Figs. 6 to 9) are less numerous and conspicuous than those in the corium. In early cases there are minute foci of serous exudate in the epidermis leading to swelling and vacuolation of the epithelial cells of the Malpighian layer (Figs. 6 and 7). The nuclei of these cells are distorted and may appear crescent-shaped and pycnotic. The serum sometimes accumulates under the cornified layer, producing minute vesicles (Figs. 8 and 9). There is slight infiltration of these foci with endothelial leucocytes. Occasionally a more diffuse infiltration of the epidermis with endothelial leucocytes takes place, but this is never very conspicuous. These early exudative lesions quickly give way to retrograde changes. The epithelial cells either singly or in minute clumps show necrosis, the serous exudate disappears, and minute, thickened plaques are present in or beneath the cornified layer.

Lesions similar to those in the epidermis are seen in the hair sheaths and in the sebaceous glands when they lie close to a capillary lesion. Infiltration with endothelial leucocytes is apt to be more conspicuous than in the epidermal lesions and may result in the appearance of minute pustules in these structures.

Labial Mucous Membrane.—The lesions in the labial mucous membrane (Figs. 10 to 13) are essentially the same as those occurring in the skin. There are swelling and proliferation of the capillary endothelium (Fig. 10), exudation of serum, and migration of endothelial leucocytes into the pericapillary tissue (Fig. 10), and multiplication of the emigrated leucocytes. A very few polymorphonuclear leucocytes, eosinophils, and lymphocytes are also present in early lesions. Invasion of the epithelium is similar to that of the epidermis, but

usually more marked. It consists of small foci of serum and endothelial leucocytes beneath the stratified epithelium with vacuolation and necrosis of the epithelial cells (Figs. 11 and 12). Diffuse infiltration of the epithelium by endothelial leucocytes is sometimes quite marked. With the progress of the lesion the stratified epithelium covering these minute pustules macerates and sloughs off leaving minute shallow ulcerations on the surface (Fig. 13). At this stage the minute lesions in the epithelium not infrequently show a considerable number of polymorphonuclear leucocytes, presumably due to secondary infection.

Tongue.—The lesions in the mucous membrane of the tongue (Fig. 14) are identical with those in the labial mucosa. The capillaries supplying the papillæ show swelling and proliferation of the endothelium, there are exudation of serum and endothelial leucocytes into the stroma, and invasion of the epithelium by serum and endothelial leucocytes with the formation of minute pustules beneath the stratified layers.

DISCUSSION.

That the symptoms observed in monkeys experimentally inoculated with material containing the virus of measles closely resemble those of human measles seems evident. The incubation period, the conjunctivitis, the enanthem, the exanthem, and the leucopenia closely parallel the similar characteristic features of measles in man. While it is true that the average incubation period in monkeys is 3 to 4 days shorter than it is in man, this might reasonably be expected in view of the presumably large amount of virus inoculated by intratracheal injection. At least it would seem probable that a much larger amount of virus than that which serves to transmit measles from man to man in the natural spread of the disease, is present in 5 to 10 cc. (the amount used) of nasopharyngeal washings from a patient or of tissue emulsion from an infected monkey. If this is so, and there is no evident reason for believing that it is not, the somewhat shorter incubation period is not surprising. The conjunctivitis in monkeys as in man is catarrhal rather than purulent in character, and like that of the human disease it is not infrequently accompanied by definite photophobia and increased lacrimation.

The early, discrete macules on the buccal mucous membrane correspond in time of appearance and in distribution with Koplik spots and differ from them only in the inconstant presence of the characteristic minute, bluish white center. The further evolution of the rash on the buccal mucous membrane is essentially identical with the evolution of the measles enanthem. The exanthem in time of appearance, in the character of the individual lesions, and in evolution likewise closely parallels the exanthem of measles and differs from it only in that it is usually more sparse and less widespread. In fact, there would appear to be only one conspicuous difference between the experimental disease and human measles; namely, the absence of respiratory symptoms in the experimental infection. While this difference cannot be explained at present, it would not appear to constitute a valid reason for rejecting the belief that the reaction observed in experimentally infected monkeys is caused by the virus of measles.

Further and even more conclusive evidence is found in the character of the lesions in the skin and in the buccal mucous membrane. These lesions have been carefully studied in cases of human measles by Ewing⁴ and more recently by Mallory and Medlar.⁵

According to the latter authors the lesions in the skin are "due to a proliferative and exudative reaction in and around a small network of capillaries in the upper part of the corium. The reaction consists of occasional mitoses in the lining endothelial cells; of emigration of endothelial leucocytes, and of an active proliferation of them around the vessels; of a very slight emigration of polymorphonuclear leucocytes and lymphocytes; and of phagocytosis of them by the endothelial leucocytes.

"The exudation of serum and endothelial leucocytes at first is active, and passes to the adjoining epidermis, hair sheaths and sebaceous glands. It often collects in small foci, forming minute vesicles and pustules. The epithelial cells involved in the exudation undergo necrosis. By the time the exanthem is clearly evident, these minute lesions in the epidermis are already beginning to dry up, and later desquamate as scales. In the meantime the endothelial leucocytes in the corium continue to proliferate and accumulate around the blood vessels for two to four days, and then gradually disappear.

"Koplik spots correspond exactly to the minute early lesions of the epidermis,

⁴ Ewing, J., *J. Infect. Dis.*, 1909, vi, 1.

⁵ Mallory, F. B., and Medlar, E. M., *J. Med. Research*, 1920, xli, 327.

but instead of drying up they tend to macerate, and may terminate in erosions or, if secondarily infected, in ulcerations."

It is clear from the foregoing description that the lesions of the skin and buccal mucosa in human measles are exactly like those found in the skin and buccal mucous membrane of the experimental infection induced in monkeys.⁶

SUMMARY AND CONCLUSIONS.

The symptomatology of the reaction induced in monkeys by inoculation with material containing the virus of measles is described. The symptoms and course of this reaction closely parallel those of human measles.

The microscopic pathology of the lesions of the skin and buccal mucous membrane of monkeys experimentally infected with the virus of measles is also described. These lesions are essentially identical with the corresponding lesions of measles in man.

EXPLANATION OF PLATES.

PLATE 37.

FIG. 1. Early lesion in the corium showing mitosis of an endothelial cell lining a capillary, and pericapillary exudate of serum and endothelial leucocytes, one of which is in mitosis. $\times 1,000$.

FIG. 2. Endothelial leucocytes accumulated about a capillary in the corium; one is shown in mitosis. $\times 1,000$.

PLATE 38.

FIG. 3. Early lesion about a small vein in the corium showing an exudate of serum and endothelial leucocytes. $\times 1,000$.

FIG. 4. Two endothelial leucocytes in mitosis near a capillary in the corium. $\times 1,000$.

FIG. 5. Exudate of serum and endothelial leucocytes in the corium. $\times 500$.

⁶ We wish to express our gratitude to Dr. Mallory for being so kind as to examine histological sections of the skin and buccal mucosa from the infected monkeys. We are also indebted to him for permission to quote his opinion that the lesions in the monkeys present essentially the same histological picture that is found in the corresponding lesions of measles in man.

PLATE 39.

FIG. 6. Accumulation of endothelial leucocytes about capillaries and small veins in the corium; focal infiltration of the epidermis with serum and endothelial leucocytes. $\times 240$.

FIG. 7. Early lesion in the epidermis showing invasion of the Malpighian layer by endothelial leucocytes and beginning vacuolation. $\times 1,000$.

PLATE 40.

FIG. 8. Early vesicle formation in the epidermis. $\times 240$.

FIG. 9. Vesicle in the epidermis with beginning plaque formation in the cornified layer. $\times 240$.

FIG. 10. Accumulation of endothelial leucocytes near two capillaries in the labial mucosa; swelling of the capillary endothelium. $\times 1,000$.

PLATE 41.

FIG. 11. Koplik spot in the labial mucosa, very early stage; focal infiltration of the epithelium with serum and endothelial leucocytes. $\times 1,000$.

FIG. 12. Koplik spot in the labial mucosa, early stage; focal accumulation of serum and endothelial leucocytes in the epithelium (pustule formation); degeneration and necrosis of epithelial cells. $\times 240$.

FIG. 13. Koplik spot in the labial mucosa, late stage; necrosis and maceration of the epithelium with erosion of the surface. $\times 240$.

FIG. 14. Diffuse infiltration of the epithelium of the tongue with endothelial leucocytes. $\times 240$.

(Blake and Trask: Studies on measles. II.)

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