STUDIES ON MEASLES.

III. ACQUIRED IMMUNITY FOLLOWING EXPERIMENTAL MEASLES.

BY FRANCIS G. BLAKE, M.D., AND JAMES D. TRASK, JR., M.D.

(From the Hospital of The Rockefeller Institute for Medical Research.)

(Received for publication, February 17, 1921.)

It has been shown in a preceding paper1 that monkeys (Macacus rhesus) inoculated intratracheally with unfiltered or filtered nasopharyngeal washings from patients in the prodromal or early eruptive stage of measles exhibit an illness which closely resembles measles in man in its course and symptomatology. It has furthermore been shown2 that the lesions which develop in the skin and buccal mucous membrane during the course of the infection in monkeys present essentially the same histologic picture that is found in the corresponding lesions of human measles. The experimental infection has been successfully transmitted1 from monkey to monkey with the development of the same group of symptoms and pathologic lesions in the passage animals. These fundamental points of similarity between measles in man and the experimental disease in monkeys would appear sufficient to warrant the application of the term “experimental measles” to the latter condition. It has, nevertheless, seemed desirable to determine whether further points of resemblance between the two might not be shown.

Since an apparently permanent immunity against reinfection characteristically follows one attack of measles, the same phenomenon should hold true with respect to the experimental disease if the two conditions are to be regarded as similar. Furthermore, an acquired immunity, if present, should theoretically be efficient against a virus of heterologous source as well as against that of homologous origin, since there is little clinical evidence to show that one attack of measles fails to confer an immunity that is effective against all subsequent

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

2 Blake, F. G., and Trask, J. D., Jr., J. Exp. Med., 1921, xxxiii, 413.
exposures. Authentic reports of repeated attacks of measles in the same individual are so few as to be negligible in this connection. In order to test the validity of the foregoing assumptions a series of reinoculation experiments in monkeys which had recovered from a previous attack of experimental measles has been carried out as described below.

EXPERIMENTAL.

Six monkeys which had previously been inoculated with nasopharyngeal washings from cases of measles and had recovered from the ensuing attack of the experimental disease have been subjected to reinoculation with material containing the virus of measles (Table I). In five instances virus of heterologous source was used, in one the homologous virus. In two monkeys the material, consisting of the supernatant fluid from an 0.85 per cent salt solution emulsion of the skin and buccal mucosa of a monkey killed on the 4th day of experimental measles, was injected intratracheally. In four monkeys whole blood withdrawn from a monkey on the 3rd day of experimental measles was injected intravenously. The intervals elapsing between recovery from the preceding experimental measles and the time of reinoculation varied from 12 to 254 days. None of the six monkeys following reinoculation showed any evidence of infection with the virus of measles, while the control normal monkeys, inoculated at the same time with equivalent amounts of the same material, developed the characteristic symptoms and pathologic lesions of the experimental disease. The protocols follow.

Experiment 1.—June 8, 1920. Monkeys 6, 9, and 19 were injected intratracheally at 12.15 p.m., 12.30 p.m., and 12.45 p.m., respectively, each with 10 cc. of the unfiltered supernatant fluid of an 0.85 per cent salt solution tissue emulsion (Virus MC. 3). The emulsion had been prepared from the minced and ground skin and buccal mucosa of Monkey 16, which was killed on the 4th day of experimental measles, about 24 hours after the first appearance of the exanthem. Monkey 6 had been inoculated Apr. 9, 1920, with pooled, filtered nasopharyngeal washings (Virus AM) from two sisters with measles and had recovered from the ensuing experimental measles on Apr. 23, 46 days before reinoculation. Monkey 9 had been inoculated May 12, 1920, with filtered nasopharyngeal washings (Virus

For a detailed description of the first attack of experimental measles in these animals see Blake and Trask.¹
MC) from a patient with measles and had recovered from the ensuing attack of experimental measles on May 27, 1920, 12 days before reinoculation. Monkey 19 was a normal monkey and served as a control.

**TABLE I.**

*Immunity Following Experimental Measles.*

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>First inoculation.</th>
<th>Second inoculation.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Date.</td>
<td>Virus.</td>
</tr>
<tr>
<td>6</td>
<td>1920</td>
<td>Apr. 9 AM</td>
</tr>
<tr>
<td>19 (control)</td>
<td>2 Mar. 24</td>
<td>RG</td>
</tr>
<tr>
<td>3</td>
<td>“ 24</td>
<td>RK</td>
</tr>
<tr>
<td>5</td>
<td>Apr. 9</td>
<td>AM</td>
</tr>
</tbody>
</table>

* The figure indicates the number of monkeys through which the virus had been passed.

Monkeys 6 and 9 showed no evidence of measles during 21 days observation. There were no conjunctivitis, no enanthem, and no exanthem, the eyes, buccal mucosa, and skin remaining normal in appearance throughout this period. Monkey 19 after an incubation period of 6 days developed the characteristic symptoms.
of the experimental disease. On the 7th day a few discrete hyperemic spots appeared on the labial mucous membranes. On the 8th day the animal was listless; the conjunctiva were injected; fresh Koplik spots had appeared on the mucous membrane of the cheeks. On the 9th day there was a well developed, confluent enanthem on the mucous membrane of the lips, gums, and cheeks. On the 10th day a few discrete, red maculopapules appeared about the lips, on the chin, and behind the ears. The animal was killed and the infection was successfully transmitted to two other monkeys. Histologic sections of the labial mucosa and tongue show the typical lesions of measles. The endothelial cells of the capillary walls are greatly swollen. There are a marked accumulation of endothelial leucocytes and some serous exudate about the capillaries, especially in the papille. In the stratified epithelium of the labial mucosa are many small foci (Koplik spots) showing endothelial leucocytes, serous exudate, and beginning necrosis of the epithelial cells. In some of these the process is more advanced and there is maceration of the epithelium, with shallow ulceration and secondary invasion by polymorphonuclear leucocytes. A few similar foci are seen in the epithelium of the tongue. There is also a more diffuse infiltration of the epithelium by endothelial leucocytes.

Experiment 2.—Dec. 15, 1920. Monkeys 2, 3, 5, 8, and 46 were injected intravenously in turn, each with 5 cc. of citrated whole blood (Virus JJ. 5) withdrawn from Monkey 36 on the 3rd day of experimental measles about 6 hours after the first appearance of the exanthem.

Monkey 2 had been inoculated Mar. 24, 1920, with nasopharyngeal washings (Virus RG) from a patient with measles and had recovered from the ensuing attack of experimental measles on Apr. 5, 254 days before reinoculation.

Monkey 3 had been inoculated Mar. 24, 1920, with nasopharyngeal washings (Virus RK) from a patient with measles and had recovered from the ensuing attack of experimental measles on Apr. 11, 248 days before reinoculation.

Monkey 5 had been inoculated Apr. 9, 1920, with pooled nasopharyngeal washings (Virus AM) from two cases of measles and had recovered from the ensuing attack of experimental measles on Apr. 23, 236 days before reinoculation.

Monkey 8 had been inoculated May 12, 1920, with nasopharyngeal washings (Virus MC) from a case of measles and had recovered from the ensuing attack of experimental measles on May 28, 201 days before reinoculation.

Monkey 46, normal, served as a control.

Monkeys 2, 3, 5, and 8 showed no evidence of infection during 21 days observation. They were well and active throughout this period. There were no conjunctivitis, no enanthem, and no exanthem. Monkey 46, after an incubation period of 3 days, developed the characteristic symptoms of measles. On the 4th day three Koplik spots appeared on the mucous membrane of the upper lip. On the 5th day the conjunctiva were injected. On the 6th day a cluster of fresh Koplik spots was present on the mucous membrane of the lower lip. The animal was drowsy and listless. On the 7th day there were confluent patches of hyperemic enanthem studded with minute white specks on the labial mucosa. A few
red maculopapules appeared on the lower abdomen and inner surfaces of the thighs. By the 10th day there was a thick, red, maculopapular exanthem on the face, neck, chest, abdomen, and legs. By the 12th day the exanthem had faded; the exanthem was fading and showed fine branny desquamation. By the 14th day the animal had recovered except for slight remaining desquamation. Blood cultures on the 5th, 6th, and 7th days showed no growth. The infection was successfully transmitted from this animal to another monkey by means of blood withdrawn on the 5th, 6th, and 7th days. A section of skin excised from the thigh on the 10th day shows the characteristic histologic picture of measles. About the capillaries and small veins in the upper layers of the corium there is a marked accumulation of endothelial leucocytes. Occasionally one is seen in mitosis. A few polymorphonuclear leucocytes are also present. Focal accumulations of endothelial leucocytes with vacuolation and necrosis of epithelial cells are seen in the epithelium of many of the hair sheaths and sebaceous glands. The epidermis shows vacuolation and necrosis of the cells of the Malpighian layer in minute foci. These areas are invaded by endothelial leucocytes. In the cornified layer are occasional, small, deeply staining plaques with the remains of minute vesicles beneath them.

DISCUSSION.

The result of the foregoing experiments shows that one attack of experimental measles confers an apparently complete immunity against reinfection with measles for at least a considerable period. In all probability this immunity is permanent. In Experiment 1 it should be noted that Monkeys 6 and 9 were originally inoculated with filtered (Berkfeld N) nasopharyngeal washings. Their subsequent immunity, therefore, not only provides additional evidence of the similarity between human measles and the experimental disease but also tends to confirm the filterable nature of the virus. The strain of virus used in the reinoculation of Monkey 6 was of different origin from the strain with which this animal was originally inoculated, while with Monkey 9 the same strain of virus was employed in both the first and second inoculations. Since there was no apparent difference in the immunity of the two animals it would seem probable that the immunity provided by one attack of experimental measles is as efficient against a heterologous virus as against the homologous one. This is further supported by the result of the second experiment, in which four monkeys originally inoculated with strains of virus from four different sources exhibited a complete immunity against reinfection with a virus obtained from a still different source.

4 This was done under ether anesthesia.
This result, as has been pointed out, was to be expected. Furthermore, it would suggest the probability that all strains of measles virus are of homologous nature in as far as their property of stimulating immunity is concerned, a fact which, of course, might readily be predicated from clinical observation.

The results of the two experiments, although they do not provide an explanation of the mechanism of acquired immunity against measles, nevertheless suggest certain possibilities. The course of measles, itself, in conjunction with evidence already presented concerning the infectivity of the blood in the experimental disease, leaves little reason to doubt that the virus gains access to the blood by way of the respiratory mucous membrane and is subsequently distributed by the blood stream to the skin and buccal mucosa where it sets up the characteristic lesions of the disease. It is conceivable that the process of immunity against reinfection might reside in the respiratory mucous membrane which, in the immune animal, would present a barrier to invasion of the body by measles virus. While this supposition might serve as a possible explanation of the immunity exhibited by Monkeys 6 and 9 which were reinoculated by the intratracheal route, it is obviously inadequate in the case of the second experiment in which all the monkeys were inoculated intravenously. Since these animals showed an apparently complete immunity, it is clear that the immunity is not solely, if at all, dependent upon a possible barrier offered by the respiratory mucous membrane of the immune animal. That it is a function of the body tissues or fluids would seem more probable. Whether the immunity is humoral or cellular or both, however, only further experiment can determine.

SUMMARY.

It is shown that monkeys which have recovered from experimental measles are immune to reinfection with the virus of measles irrespective of whether the virus is of homologous or heterologous origin. In this respect experimental measles in the monkey corresponds with measles as observed in human beings, and the result is the same whether the virus is inoculated on the respiratory mucous membrane or is injected intravenously.

CONCLUSION.

Experimental measles in the monkey, like measles in man, is followed by an acquired immunity against the disease.