Part III.

STUDIES UPON THE IMMUNITY REACTIONS OF THE MONKEY AFTER INOCULATION WITH VACCINE OR WITH VARIOLA VIRUS.

1. On the immunity reactions of the monkey after inoculation of the skin with vaccine or with variola virus.
2. On the effects of the locus of inoculation upon the development of the immunity.
3. On the time of development of the immunity after inoculation of the skin with vaccine or with variola virus.

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1. ON THE IMMUNITY REACTIONS OF THE MONKEY (*Macacus cynomologus*) AFTER INOCULATION OF THE SKIN WITH VACCINE OR WITH VARIOLA VIRUS.

In this section we propose to bring together certain observations upon the immunity following the development of vaccinal or variolous lesions upon the skin of the monkey. The data bears upon the general problems of immunity to vaccinia and to variola, and we will show certain differences in the immunity reactions of the monkey to the two sorts of virus which throw light upon the general question of the inter-relationships of the two diseases.

The experiments which form the basis of this section were in part those detailed in other papers of this series, and in part experiments performed with special reference to the problems here treated.

The technic used for inoculation was that described in previous papers of this series. The diagnosis of the results of the second inoculations was based upon the descriptions already given of the specific lesions of vaccinia and variola inoculata in the monkey. We have been guided wholly by the naked eye appearances. In our experience we have rarely been in doubt as to the specificity of a vaccinal or

variolous skin lesion in the monkey. In the few instances where we could not feel certain of the diagnosis the experiment has been ruled out.

EXPERIMENTS.

1. Vaccination of the skin after successful vaccination of the skin.

Thirteen monkeys were selected for this experiment. Each animal had had a typical vaccinal lesion of the skin of the abdomen from inoculation with virus No. 1, 148, 236, 246, or 251. Twenty-two days after, the animals were vaccinated on the skin of the abdomen with virus No. 148. All these attempted revaccinations resulted negatively. At the site of the second inoculation there was only the usual slight reaction which follows a scratch. The scratches healed as if no virus had been used.

2. Variolation of the skin after successful vaccination of the skin.

Six monkeys which had had typical lesions on the skin of the abdomen as the result of inoculation with vaccine virus No. 1, 251, 236, 246, or 148, were selected for this experiment. Each animal was variolated on the skin of the abdomen with virus No. 52 or 200. No reaction followed the second inoculation. An interval of from thirty-eight to fifty-eight days elapsed between the two inoculations.

3. Vaccination of the skin after variolation of the skin.

The results obtained in this series are best shown by presenting a number of the experiments in detail.

No. 114. — Adult male, M. cynomologus. Variolated on the skin of the abdomen with virus No. 167 (vesicle contents). Animal developed a typical variola inoculata including a general exanthem. On the thirty-seventh day after the variolous inoculation the monkey was vaccinated on the skin of the abdomen with virus No. 148. At the site of inoculation there developed a lesion which had all the characteristics of a vaccine process. The lesions only differed from primary vaccinations in the extent of the process and in the indefiniteness of the vesiculation. The lesions

passed through a definite evolution and healed spontaneously. We had no hesitation in diagnosing the lesions as specific but atypical reactions.

The experiment was repeated on two other monkeys of the same species with identical results and need not be given in detail.

No 153. — Adult male, M. cynomologus. The monkey was variolated in a number of places on the skin of the abdomen with virus No. 167 (vesicle contents). A typical primary lesion developed, which, however, was not so extensive as in the other animals inoculated at the same time with the same virus. No exanthem was noted.

On the tenth day of the experiment the animal was vaccinated on the skin of the abdomen with virus No. 148. An atypical reaction resulted, similar to that seen in the monkeys described above.

On the twenty-fifth day the monkey was vaccinated on the skin of the abdomen with virus No. 1. An atypical lesion was again produced.

No. 141. — Adult male, M. cynomologus. Variolated on skin of abdomen with virus No. 167 (vesicle contents). Variola inoculata with exanthem developed. Twenty-eight days after the variolation the animal was vaccinated on the abdomen wlth virus No. 1. No reaction followed the vaccination.

Two other monkeys were shown to be immune to vaccination of the skin, with virus No. 1, nineteen days after a variolation on the abdomen with virus No. 167 (pustule contents dried with lycopodium), which had been followed by a typical primary lesion but no general exanthem.

4. Variolation of the skin after variola inoculata.

This experiment was only tried in one instance and showed the monkey to be immune, after a variola inoculata, to a second skin inoculation with variola virus.

SUMMARY.

1. Vaccination of the skin in thirteen monkeys protected against subsequent vaccination of the skin.

2. A vaccination of the skin in six monkeys protected against subsequent variolation of the skin.

3. A variolation of the skin in three monkeys protected against subsequent vaccination of the skin. In the case of three monkeys the following vaccination yielded a positive, though an abortive, reaction. Another monkey showed an

abortive reaction with two successive vaccinations which were subsequent to a variolation.

4. Variolation of the skin in one monkey protected against a subsequent variolation of the skin.

5. The time which elapsed between the first and second inoculation in these monkeys varied between ten and fifty-eight days.

6. Three of the monkeys which were shown to be susceptible to vaccination after successful variolation were tested thirty-seven days after the inoculation. The three animals of the same series that were refractory to the vaccination after variola inoculata were tested twenty-eight days after the primary inoculation. The animal which did not seem to acquire an immunity to inoculation (No. 153) was tested on the tenth and twenty-fifth days, that is, previous to the date in which complete immunity was shown to exist in three monkeys, and to the date in which three were shown not to be immune to a second inoculation.

DISCUSSION. — The results of our inoculations conform to the general law that vaccination and variolation confer an immunity upon the affected animal to subsequent infection with vaccine and variola virus. The results of similar inoculations in man, which were performed in the early days of vaccination, seem to have yielded more constant results than we have obtained in monkeys. The immunity conferred by a vaccine lesion of the skin of the monkey is complete against later inoculation with vaccine and variola virus.

The conclusions are not so definite in primary variolation. In a certain proportion of animals a complete immunity to vaccination on the skin has been produced by a previous variola inoculata, but an equal number show only a diminished susceptibility to the vaccine virus. These observations agree with those made by Roger and Wiel on Macacus monkeys in which substantially the same phenomenon was noted.

In seeking for an explanation of this partial immunity conferred by variola inoculata against vaccination of the skin, we might refer it simply to a dying out of the immunity, for we

find the completely immune animals were re-inoculated on the twenty-eighth day of the experiment, while the animals showing partial immunity were tested on the thirty-seventh day, but this is contradicted by the single animal which was shown to react by a specific process to three successive inoculations, with an interval of ten and fifteen days, with variola and vaccine virus (experiment No. 153).

The explanation that there is a qualitative difference in the reaction of this species of animal to the two viruses is not borne out by experimental inoculation. The inoculation of variola virus affords partial protection in a certain percentage of cases, and absolute protection in others to subsequent vaccination.

A third possibility lies in a hypothetical quantitative difference in the immune substance called forth by the two sorts of virus.

It seems evident that the immunity which the animal presents to the skin inoculation must depend upon certain properties of the individual conferred upon it by the disease which follows the first inoculation. The weight of evidence is in favor of the immunity being due to a bactericidal or germicidal property resident in the blood serum (Sternberg and Reed, Béclère, Chambon and Menard). If such be the case the animals in which complete immunity to vaccinia follows variolation, and in which complete immunity to variolation follows vaccination, indicate that the immune property of the serum of the inoculated animal, whether vaccination or variolation be practised, is identical. We then would expect to find simply a quantitative difference in this germicidal property of the sera of the animals depending upon the character of the virus used for inoculation. The reason that variola inoculata in the monkey always protects against an inoculation with variola virus, if confirmed by more experimental evidence than we present, would be that this species is less favorable to the development of the variola than to the development of the vaccine contagium, in that the former fails to develop in monkeys protected by previous variolation, whereas the latter develops and produces a lesion.

That immunity resulting from inoculation of the monkey with variola virus is less efficient than that resulting from vaccination is apparent from the fact that vaccination protects against both subsequent variolation and vaccination, while variolation protects against subsequent variolation and only partially against subsequent vaccination.

At the present time technical difficulties prevent the putting of the quantitative aspects of this hypothesis to the test of experiment.

CONCLUSIONS.

1. A vaccine lesion on the skin of the monkey (M. cynomologus) confers upon the animal an immunity to subsequent inoculation of the skin with vaccine or with variola virus.

2. A variolous lesion on the skin of the monkey (M. cynomologus) protects the animal against subsequent inoculation of the skin with variola virus, but does not, in all cases, protect against later inoculation with vaccine virus.

3. The failure of variola inoculata in the monkey to protect against subsequent skin inoculation with vaccine virus depends upon the fact that this species of animal produces a smaller amount of the germicidal substance necessary to inhibit a second inoculation after variolation than it does after vaccination.

2. ON THE INFLUENCE OF THE LOCUS OF INOCULATION UPON THE DEVELOPMENT OF THE IMMUNITY IN VARIOLA AND VACCINIA IN THE MONKEY (*Macacus cynomologus*).

INTRODUCTION. — In the preceding section we have studied the immunity reactions of the monkey to inoculation of the skin with variola and with vaccine virus, and have brought out certain differences in the immunity produced by the two viruses. In this section we will detail experiments which bear upon the general problem of the immunity reactions of the monkey to vaccine and to variola virus from a somewhat different point of view.

In testing the immunity of rabbits after skin and after corneal inoculations with vaccine virus *Dr. R. L. Thompson obtained results which tended to show a difference in the degree of immunity depending on the locus chosen for the primary vaccination. As we were more favorably situated as regards facilities and animals for experimentation we decided to continue this line of work on monkeys. We have extended the scope of the experiments so as to include both the study of the relative immunity produced by vaccination and variolation of the skin, cornea, and mucous membranes.

EXPERIMENTS. — (*a.*) Vaccination of the cornea after vaccination of the skin. This experiment was performed upon five monkeys (M. cynomologus). Each animal had had a typical vaccinal lesion on the skin as a result of an inoculation with virus No. 1, 148, 236, or 251. Each animal was tested twenty-two days after the first inoculation by vaccination of the skin with virus No. 148. The cornea was vaccinated with virus No. 148 on the twenty-ninth day after the initial skin vaccination. The animals were killed after

*These experiments were carried on in the Pathological Laboratory of the Boston City Hospital under the direction of Dr. W. T. Councilman. Owing to the impossibility, at the time, of carrying out the research on lines extensive enough to yield definite conclusions, the results were not published.

forty-eight hours, and paraffin sections of the corneas were examined microscopically.

Four of the monkeys showed no specific lesion on the cornea. One showed a typical vaccinal keratitis with proliferation of the epithelium and the presence of numerous Cytoryctes.

(*b.*) Variolation of the cornea after variola inoculata from skin inoculation. This series consisted of five monkeys which had had a typical primary lesion on the abdomen following inoculation with variola virus No. 167 (disk). Three of these animals had developed a general exanthem.

On the twenty-fourth day of the experiment each monkey was inoculated on the cornea with variola virus No. 167 (vesicle contents). The animals were killed after seventy-two hours and the corneas studied microscopically. Each animal presented a typical variolous keratitis at the site of inoculation, and Cytoryctes were present in large numbers.

(*c.*) Variolation of the skin after variolous keratitis. This experiment was performed on a single animal. The cornea was inoculated with variola virus No. 200. A typical lesion developed. Eighteen days after the corneal variolation, variola virus No. 252 was inoculated on the skin of the abdomen without producing a lesion. The skin inoculation was repeated on the forty-first day with variola virus No. 307, and again no reaction followed.

(*d.*) Vaccination of the skin after variolation of the mucous membrane of the palate. The five monkeys employed for this experiment had developed a typical variolous lesion on the soft palate following variolation with virus No. 167 (vesicle contents). Each animal was vaccinated on the skin with virus No. 148, on the twenty-first day of the experiment. In every animal a typical vaccine lesion developed at the site of inoculation.

(*e.*) Variolation of the skin after variolation of the mucous membrane of the palate. Three monkeys were selected that had shown a typical variolous lesion on the soft palate after inoculation with virus No. 307. Eighteen days after the initial inoculation the animals were variolated

on the abdomen with virus No. 307. Two of the monkeys showed no reaction to the skin inoculation, while one yielded a typical primary lesion, but no exanthem developed.

(*f.*) Vaccination of the skin after variolation of the mucous membrane of the lip and nose. One monkey was inoculated in this way. Typical variolous lesions developed on the inner side of the lip and on the nasal septum after inoculation with variola virus No. 167 (vesicle contents). The monkey was vaccinated on the abdomen with virus No. 148 and No. 1 on the seventeenth and forty-first days, respectively. No lesion developed after either vaccination.

(*g.*) Vaccination on the skin of the temple after vaccination of the abdomen. Two monkeys that had had a typical vaccine lesion on the abdomen were later shown to be refractory to vaccination on the temple.

SUMMARY.

(*a.*) A vaccine lesion of the skin protects against subsequent inoculation of the cornea with vaccine virus, but the protection is not complete.

(*b.*) A variola lesion of the skin does not protect against ubsequent inoculation of the cornea with variola virus.

(*c.*) In one monkey a variola lesion on the cornea protected against subsequent inoculation of the skin with variola virus.

(*d.*) A variola lesion on the mucous membrane of the palate does not protect against subsequent inoculation of the skin with vaccine virus.

(*e.*) A variola lesion on the mucous membrane of the palate does not protect completely against subsequent inoculation of the skin with variola virus.

(*f.*) A variola lesion on the mucous membrane of the lip and nose protected, in one instance, against subsequent inoculation of the skin with vaccine virus.

(*g.*) A vaccine lesion on the skin of the abdomen protected against subsequent inoculation of the skin of the temple with vaccine virus.

DISCUSSION. — The summary of our experiments in this section demonstrates that the immunity produced by a variolation on the mucous membrane is lower than that produced by a variolation on skin.

We have already shown that the immunity produced by a variolation on the skin is lower than that following a vaccination on the skin, and we find that this is emphasized by the results of inoculations of the cornea after vaccination and variolation of the skin. The fact that even vaccination of the skin does not completely protect against subsequent corneal inoculation with vaccine virus illustrates our point with regard to the quantitative relation of the two immunities.

In interpreting the results of these inoculations, the following factors must be considered, viz.:

a. The locus of the initial inoculation.

b. The virus used in the initial inoculation.

c. The locus of the second inoculation.

d. The virus used in the second inoculation.

Bearing these factors in mind we see that loci chosen for initial inoculations bear the following relation to the resulting immunity:

The immunity conferred by skin locus is greater than that of the cornea, and that of the cornea is greater than that of the mucous membrane. The immunity conferred by vaccine is greater than that conferred by variola as we have already indicated in the preceding section.

The influence of the locus of the second inoculation can only be estimated in regard to the cornea and skin when we find that the immunity conditioned by the initial inoculation is less efficacious when the cornea is chosen than when the skin is the site of the second inoculation.

The influence of the sort of virus used in the second inoculation upon the test seems to indicate that the vaccine virus is more potent than the variola virus in that it may produce a lesion in an animal in which there is complete protection to inoculation with variola.

We find, in short, that the skin is a relatively more efficacious locus than the cornea, and the latter locus is more

efficacious than the mucous membrane in immunity production. The immunizing power of vaccine virus is higher than that of variola virus.

If we interpret the experiments above in the light of the hypothesis elaborated in the previous section, and keep in mind the physical conditions at the various loci of inoculation we feel that the phenomena observed are quite consistent.

A variolation of the cornea after skin variolation succeeds because the total amount of immune substance present in the individual is relatively small, owing to the character of the virus used in the initial inoculation, and because the physical conditions on the cornea do not favor a free mixing of the immune-bearing plasma with the inoculated virus.

In the case of a skin vaccinated monkey a vaccination of the cornea only rarely succeeds because the relatively large amount of immune substance present, even under the adverse physical conditions in the cornea, usually is sufficient to produce a germicidal effect upon the inoculated virus. We feel that the fact of an occasional animal yielding a positive reaction to such a second inoculation only emphasizes our view that the phenomenon is a quantitative one.

In the case of the mucous membrane the physical conditions doubtless factor largely in its low immunizing power as a locus of initial inoculation. Study of the lesion on the mucous membrane shows that almost from the first an open wound is present at the site of inoculation. This condition would favor the discharge of toxin and products of degeneration of the organism, and would be unfavorable for the production of an immune substance which resulted from the reaction of the host cells.

The high potentiality of the skin for immunity production as a locus of the initial inoculation is in sharp contrast with that of the mucous membrane, and the physical conditions are in keeping with the interpretation given above. In a skin lesion the greater part of the products of the lesion must be absorbed and go to produce the general immunity.

It seems probable from the histological study of the specific lesion in the nose that that locus would stand nearer to the skin than the lip or palate in its potentiality for immunity production. We feel that our data are insufficient for generalization on this point.

CONCLUSIONS.

1. The degree of protection conferred by a vaccinal or variolous lesion on the monkey (M. cynomologus) is conditioned by the locus chosen for inoculation as well as by which virus is employed.

2. The varying degree of immunity production which follows the development of vaccinal or variolous lesions at different loci of inoculation is dependent upon the physical conditions there present.

3. The outcome of an inoculation of an animal which has had a variolous or vaccinious lesion depends upon the locus and upon the virus employed in the second inoculation, as well as upon the locus and upon the virus employed in the first inoculation.

3. ON THE TIME OF DEVELOPMENT OF THE IMMUNITY AFTER INOCULATION OF THE SKIN OF THE MONKEY WITH VACCINE AND WITH VARIOLA VIRUS.

INTRODUCTION. — The following experiments were planned to show what interval elapses between the inoculation of the monkey's skin with vaccine or variola virus and the development of an immunity inhibiting further inoculations. The results of these experiments bear upon the general problem of the diseases, and particularly upon that of the causation of the exanthem in variola inoculata. The experiments are arranged in three series, as follows:

(*a.*) Daily inoculation of the skin with vaccine virus.

Five monkeys (M. cynomologus) were selected and each received upon the skin of the abdomen a single vaccination, daily, for nine days The development of each lesion was observed, and objective descriptions recorded from day to day. Vaccine virus No. 148 was employed.

No. 108. — The lesions from vaccinations performed on the second, third, fifth, sixth, and seventh days of the experiment showed a typical development. Those from inoculations on the first and fourth day were abortive in character, vesiculation not being complete. The inoculations done on the eighth and ninth day were entirely negative.

No. 109. — The first, second, third, fourth, and sixth vaccinations were positive, while no reaction followed those on the fifth, seventh, eighth, and ninth days of the experiment.

No. 110. — The first to the fifth vaccinations, inclusive, resulted positively, while those on the four succeeding days were not followed by a reaction.

No. 111. — The first to the fifth vaccinations, inclusive, yielded a typical vaccine process, while the remaining four were without result.

No. 112. — The first, second, third vaccinations were negative, the fourth was abortive, and the fifth, sixth, seventh, eighth, and ninth were negative.

(*b.*) Daily inoculation of the skin with variola virus.

Eight monkeys (M. cynomologus) were used in this series of experiments. Each animal received a single inoculation with variola virus daily. Variola virus No. 167 (vesicle contents), No. 199, or No. 200, was employed, and after the final inoculation the sample was tested by inoculation upon the skin of a fresh monkey and shown to be still potent.

No. 136. — The inoculations on the first, second, third, fourth, and fifth days of the experiment yielded typical lesions. The fifth, sixth, and seventh inoculations were not followed by a lesion. A general exanthem developed on the ninth day of the experiment.

No. 137. — The first to the fourth inoculations, inclusive, were positive, while the remainder were negative. An exanthem appeared on the ninth day.

No. 138. — The first four inoculations were positive, while the last three were negative. An exanthem developed on the eighth and ninth days.

No. 213. — The first, second, third, and fourth inoculations were positive, while the fifth, sixth, and seventh were negative. On the seventh, eighth, and ninth days a general exanthem was observed.

No. 214. — The inoculations on the second and third days yielded positive, those on the first and fourth abortive, and those on the fifth, sixth, and seventh negative reactions. No general exanthem developed.

No. 215. — The first four inoculations were positive, the fifth was questionable, the sixth and seventh were negative. No exanthem developed.

No. 116. — The first, second, and third inoculations were positive, the fourth was questionable, the fifth, sixth, and seventh were negative. A general exanthem appeared on the eighth and ninth day.

No. 117. — The first four inoculations were positive, the fifth was questionable, the sixth and seventh were negative. An exanthem appeared on the ninth day.

(*c.*) Simultaneous inoculation with vaccine and variola virus.

Five monkeys were selected and inoculated on the left groin with vaccine virus No. 1, and on the right side of the chest with variola virus No. 200.

Four animals reacted typically to both inoculations, and two of these developed an exanthem on the ninth day of the experiment. The fifth monkey reacted typically to the vaccine virus, but did not show a process at the site of the inoculation with variola virus or develop an exanthem.

SUMMARY.

1. In two monkeys daily inoculations with vaccine virus ceased to produce a positive lesion five days after the first inoculation.

2. Three monkeys, similarly vaccinated, failed to react six days after the first successful inoculation.

3. In four monkeys, inoculated daily with variola virus, positive reactions were not obtained four days after the first inoculation.

4. In two monkeys, inoculated daily with variola virus, an abortive lesion followed the inoculation done four days after the first inoculation, and in two animals a similar lesion developed from an inoculation performed three days after the primary inoculation.

5. Six of the eight monkeys subjected to daily inoculations with variola virus developed an exanthem. The eruption was first present on the ninth day in two, on the eighth day in three, and on the seventh day of the experiment in one.

6. The interval between the last successful daily inoculation (counting abortive lesions as positive) and the appearance of the exanthem was five days in two monkeys, four days in three, and three days in one.

7. Four monkeys reacted to both vaccine and variola virus, simultaneously inoculated, by the development of typical lesions which developed apparently without influencing one another. Two of these animals developed a general exanthem on the ninth day of the experiment.

DISCUSSION. — A comparison of the results of daily inoculation of the skin with vaccine virus with the results following similar inoculation with variola virus shows that there is a distinct difference in the time of onset of the immunity.

In the experiments where vaccine was used the refractoriness to skin inoculation, if judged by the day on which the first unsuccessful inoculation was performed, appeared, on an average, during the seventh day of the experiment. That is to say, the seventh daily inoculation, which was performed six days after the first successful insertion, fails to show a specific reaction.

In the series where variola virus was employed this refractoriness to reinoculation appeared, on an average, during the fifth day of the experiment.

Without committing ourselves to the exact date of onset of the immunity, we may yet assert from this that it is of earlier development in variola inoculata than in vaccinia. The determination of the exact day of development of an

immunity to subsequent skin inoculation cannot be accurately determined by this procedure. In studying the evolution of a vaccine or a variola lesion on the skin we see that an interval of from seventy-two to ninety-six hours intervenes between the inoculation and the appearance of a process diagnosable by the naked eye.

That this incubation period is apparent rather than real, as shown by microscopic study of sections from the inoculation sites, does not help us in this connection. By the methods adopted in these experiments we are in doubt for three days as to the outcome of our inoculation. At any time during this period the development of the lesion may be checked by the onset of the immunity. We see then that the fact that the inoculations on the first four days of an experiment result in a diagnosable lesion, while those on the fifth day and on succeeding days do not develop to a diagnosable condition, does not fix the date of onset of immunity at the fifth day. Obviously an inoculation done on the fifth day might not appear in the records of the experiment as a positive reaction if the immunity developed even two days later, as the lesion would be inhibited before recognizable.

We must conclude, therefore, that the onset of the immunity is not before the date of the last successful inoculation, and may be as much as three days later. Applying this conclusion to our experiments, where daily inoculation was practised, we see that the development of immunity to reinoculation of the skin with vaccine virus may manifest itself at any time between the sixth and the eleventh day, and to reinoculation with variola virus between the fifth and the eighth day. These figures are arrived at by selecting the days of the earliest unsuccessful inoculation for the earliest date, and the day of the latest unsuccessful inoculation plus three for the latest date. These limits, while wide, are as narrow as we believe to be warranted by the method of experimentation adopted.

The appearance of a general exanthem in the animals, inoculated daily with variola virus, from three to five days

after the last successful insertion of virus on the skin, seems at first sight difficult to explain. If we allow for a three-day interval between the invasion of the skin and the appearance of the eruptive lesion we see that this brings the date of invasion, and hence the period of intravascular transit of the organisms, to within the limits set for the onset of the immunity. The number of organisms which go to produce a regular eruptive lesion are undoubtedly very much less than those introduced in an inoculation of the skin with virus. As the growth of the lesion depends upon the multiplication of the organisms, it is probable that the interval between invasion of the skin and the appearance of the exanthem lesion is longer than that between inoculation of the skin with virus and the diagnosable stage of the primary lesion. We must conceive of the organisms which are to produce the exanthem as passing from the primary lesion to the skin before the date of onset of the immunity. By comparing the dates we can readily understand how the organisms might make this intravascular journey before the immunity developed. Another explanation lies in the possibility that phagocytes act as carriers and as protectors of the organism from the immune plasma. The development of an exanthem is therefore quite consistent with our conclusions with regard to the time of onset of the immunity. The brief evolution and abortive development of the lesion of the exanthem is what might be expected in an animal which had already developed such a germicidal power in its plasma that its presence in the inoculation wound, and in the subsequent exudate stream of inflammatory origin, inactivated the virus introduced at the site of a skin inoculation.

In the lesion developing spontaneously on the skin the immune plasma doubtless does not have as free access to the organisms as is the case where the virus is mixed in a scratch with fresh drawn blood serum.

The phenomenon of an exanthem in variola inoculata and its absence in vaccinia is not explained by these experiments. Had it proved that the general immunity was of notably later development in variola inoculata the exanthem producing

quality of variola virus would have been readily explained. The reverse, however, seems to be the case, and we have to seek further for the explanation of this fundamental difference between the two viruses.

Simultaneous variolation and vaccination of the monkeys shows that the synchronous development of a vaccine lesion has no effect upon the appearance of the exanthem of variola inoculata. The fact that vaccination, on or about the date of exposure to smallpox, inhibits the production of clinical types of variola vera characterized by an exanthem emphasizes the difference between the diseases variola vera of man and variola inoculata of monkeys.

In a previous section we have shown that the immunity potential of the mucous membrane is low. In variola vera it seems exceedingly probable that the atrium of infection and the site of the primary lesion is on a mucous membrane. If such be the case we would expect that little if any immunity would develop as a result of the evolution of this lesion, and the organisms that seek the skin to produce the exanthem would develop in a practically unimmunized animal This agrees with the course of the exanthem in a typical variola vera in man. In other cases of smallpox the exanthem shows an evolution very like that in variola inoculata (variola abortives), or is absent (variola sine exanthem), and in these we conceive of the organisms which go to form the exanthem as acting against a more or less fully developed immunity. This condition of immunity might be dependent upon an early development of the general immunity arising from the primary pock, and be conditioned by its locus. In any case a vaccination on the skin at the time of exposure produces an immunity which develops before the exanthem, probably killing the organisms in transit from the protopustule to the skin, and so inhibiting the eruption.

This suggests an explanation of the failure of all attempts to abort the exanthem in variola vera by the injection of what certainly were highly germicidal sera (Béclère and others).

It is evident that at the time when the case is a fully

declared smallpox and is put under treatment the organisms are inaccessible to the serum. To be effective the serum would have to be given before the disease had advanced to a diagnosable state. Such sera might be useful in cases where a patient, well advanced in smallpox, is discovered in an unvaccinated family. The unprotected ones in contact with such a case will probably be in the incubation stage of smallpox, and might be protected from a variola vera with exanthem by injections of serum.

CONCLUSIONS.

1. The immunity which accompanies the development of a vaccine lesion on the skin of a monkey becomes manifest between the sixth and eleventh day.

2. After a variola lesion of the skin the immunity appears between the fifth and eighth day.

3. The organisms which produce this exanthem in variola inoculata in the monkey pass from the point of inoculation to the skin before the onset of the general immunity.

4. The development of an exanthem in variola inoculata in the monkey is not dependent upon a late development of the immunity reaction of the animal.

5. The use of variolicidal sera is indicated only in cases where it can be administered during the incubation stage of the disease.