RELATION OF VARICELLA TO HERPES ZOSTER.

II. CLINICAL AND EXPERIMENTAL OBSERVATIONS.

BY T. M. RIVERS, M.D., AND L. A. ELDRIDGE, JR., M.D.

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

(Received for publication, February 13, 1929.)

In the preceding paper (1) the problem relating to the identity of the causal agents of varicella and herpes zoster was stated. The mere occurrence of varicella in an individual following exposure to herpes zoster might easily be explained upon coincidence. Certain other observations, however, dealing with complement fixation work, inoculation experiments, and protection tests conducted with convalescent sera deserve serious consideration.

Netter (2) and Netter and Urbain (3), de Lange (4), and others, because of their results with complement fixation tests, contend that the etiological agents of zoster and varicella are quite frequently identical. Lauda and Silberstern (5), however, were unable to confirm these observations. Kundratitz (6) and Lipschütz and Kundratitz (7) inoculated 28 children under 5 years of age with material from herpes zoster patients in the hope of immunizing them against chicken-pox. In 17 of the children a local reaction, characterized by papules and vesicles, was observed at the point of inoculation. The children were subsequently found to be immune to varicella. These writers also claim that injections of convalescent herpes zoster serum will protect exposed children against varicella. Lauda and Stöhr (8) inoculated 54 children with material from 17 cases of herpes zoster, and in none of them did they observe a local reaction at the site of the inoculation. Three of the inoculated children and 3 other uninoculated infants who came in contact with some of the zoster patients developed typical chicken-pox 2 or 3 weeks after exposure. These workers were unable to demonstrate that convalescent zoster serum protects against varicella and conclude from the results of their work that the majority of cases of zoster are not caused by the virus of chicken-pox.

Chicken-pox is a highly contagious disease, but for a long time doubt existed in regard to its inoculability. A great deal of this doubt arose from the fact that most of the experimental inoculations were made during epidemics of varicella and exposures to the disease under natural conditions could not absolutely be eliminated. Nevertheless, in spite of these difficulties, it has been shown (9) that
varicella is inoculable. Successful experiments, however, are not as constant as one might suppose when considering the extremely contagious nature of the disease. Evidence is accumulating in favor of the idea that zoster also is caused by a virus, but the question as to its inoculability in humans is still moot.

If it were possible without difficulty to produce varicella and zoster in animals, the relation of the two diseases would be settled quickly. It is very doubtful if the virus that causes the majority of cases of zoster has been propagated in experimental animals. With one exception this is also true in regard to varicella. Certain kinds of monkeys (Cercopithecus sabaeus and Cercopithecus lalandi) are susceptible to the virus of chicken-pox. Even in these animals the results are irregular and the evidence of infection consists of the occurrence of acidophilic nuclear inclusions in affected cells of inoculated testicles. These inclusions, however, do not occur in controls and their appearance is regularly prevented by convalescent varicella serum (10, 11). In view of the regularity with which convalescent varicella serum prevents these inclusions in testicles inoculated with chicken-pox virus, it seemed likely that information regarding the identity of varicella and zoster might be obtained by conducting neutralization tests with varicella virus and convalescent zoster serum. It is with observations concerning these experiments that the present paper deals.

Methods and Materials.

Monkeys Employed.—Young male green monkeys (Cercopithecus sabaeus) were used. Animals in which spermatogenesis had been established were discarded.

Neutralization Tests.—Emulsified papules and vesicles collected from varicella patients, usually within the first 72 hours of the disease, were used as virus. Non-immune serum was secured from chicken-pox patients during the first 72 hours of disease. Immune varicella serum was obtained from convalescent patients 14 to 22 days after the appearance of the rash. Immune zoster serum was collected from convalescent patients 23 to 28 days after the appearance of the eruption.

The papules and vesicles were excised under aseptic conditions and emulsified by grinding in a mortar moistened with Locke's solution. Sand was not used. The emulsified material was taken up in 0.5 to 2.0 cc. of Locke's solution and portions of it were mixed as desired with equal amounts of non-immune serum, immune varicella serum, or immune herpes zoster serum. Measured amounts (0.25 cc.) of the mixtures were then injected into the testicles of monkeys. More than 45 minutes never elapsed between the collection of the varicella virus and its injection into the animals.
Removal and Examination of Testicles.—It was previously shown (10, 11) that nuclear inclusions are present in the testicles of green monkeys on the 5th and 6th days after inoculation with chicken-pox. Consequently, in the experiments reported at the present time, the monkeys were castrated\(^1\) on the 5th or 6th day following inoculation. The testicles removed for histological studies were fixed in Zenker’s fluid, sectioned, and stained with eosin and methylene blue. A careful search for eosin-staining nuclear inclusions was made in numerous sections of each testicle. Details concerning the tinctorial reactions of the inclusions are given by Tyzzer (12), Lipschütz (13), and Goodpasture (14).

Results.—Testicles in which nuclear inclusions were found were considered infected with varicella virus. If no inclusions were found, infection was considered not to have occurred. In this type of work, positive results are naturally much more significant than are negative ones.

EXPERIMENTAL.

In the study of the relation of varicella to herpes zoster 4 neutralization experiments were performed.\(^2\) A detailed account of each follows.

Experiment I.

The first experiment was conducted in order to determine whether the serum of an adult, who had had chicken-pox in childhood, would neutralize varicella virus.

Serum was collected from J., 35 years of age, who had chicken-pox when 5 years old. Non-immune serum was obtained from W. R. during the first 48 hours after the appearance of the varicella rash. Virus in the form of 4 varicella vesicles was secured from B. The serum to be tested, from J., was mixed with an equal amount of virus, and then 0.25 cc. of the mixture was injected into each testicle of Monkey 1. Non-immune serum, W. R., was also mixed with virus, and 0.25 cc. of the mixture was injected into each testicle of Monkey 2. 5 days later the testicles were removed and examined in the usual manner for the presence of acidophilic nuclear inclusions. They were found in the testicles of both monkeys.

\(^1\) All operative procedures were conducted under anesthesia.

\(^2\) Because of the scarcity of green monkeys, control tests with convalescent varicella serum were at times omitted. In fact, it is not essential to use this control with each experiment, inasmuch as convalescent varicella serum has never failed to prevent the appearance of inclusions in testicles inoculated with varicella virus (11).
The results of the above experiment indicated to us that the serum from an individual who had had varicella many years previously did not contain sufficient antibodies to prevent the appearance of inclusions in testicles inoculated with chicken-pox virus. Consequently it was deemed safe in this work to use convalescent zoster serum obtained from individuals who a number of years earlier in life had passed through an attack of varicella.

Experiment II.

In this experiment an attempt was made to ascertain whether the serum from a case of so called idiopathic herpes zoster was capable of neutralizing varicella virus.

C. S., male, white, 16 years old. No history of having had chicken-pox or zoster previously. No exposure recently to either disease. Patient seen on the first day of the zoster eruption which was distributed over the right sacral and iliac regions; serum collected. Lesions healed slowly. Serum again collected 23 days after onset of the disease.

In the neutralization tests, 4 monkeys were used. Virus, 9 lesions, was collected from 3 varicella patients (G. K., G. M., H. B.). Non-immune serum (W. R.), immune serum (M. W.), and serum collected from C. S. on the 1st and 23d days after the appearance of the zoster eruption were employed. Virus and non-immune serum were injected into Monkey 3; virus and convalescent varicella serum into Monkey 4; virus and early zoster serum into Monkey 5; virus and convalescent zoster serum into Monkey 6. 6 days after the inoculations, the testicles were removed, fixed, sectioned, and stained. Upon examination no inclusions were found in any of the sections.

In view of the negative results in all the monkeys, the experiment was repeated. At this time, however, only non-immune serum (W. R.) and convalescent zoster serum (C. S.) were used. The virus, 6 varicella vesicles, was obtained from A. M. and W. McK. Virus and non-immune serum was injected into Monkey 7; virus and convalescent zoster serum into Monkey 8. 5 days later the testicles were removed and examined in the usual manner. In sections from the testicles of Monkey 7 numerous nuclear inclusions were found, while none were seen in those from Monkey 8.

From the results of this experiment one might conclude that the convalescent zoster serum neutralized the varicella virus. Monkeys, however, display a certain amount of irregularity in their susceptibility to chicken-pox virus. Consequently one set of negative findings is not highly significant.
Experiment III.

The following test was made to determine whether the serum from a patient who developed herpes zoster following the administration of salvarsan would neutralize chicken-pox virus.

M. R., female, colored, age 36. No history of previous attacks of chicken-pox or herpes zoster. No known recent exposure to either disease. +++ Wassermann. On Jan. 13, 1927, at the Vanderbilt clinic, the patient received her first intravenous injection of salvarsan. 24 hours later a burning sensation was felt over the left shoulder, and 48 hours following the treatment blisters were seen over the left side of neck and left shoulder. Patient was seen at the Rockefeller Hospital for the first time on Jan. 21 and was found to have typical herpes zoster. The lesions healed slowly. Convalescent serum was collected on Feb. 11.

Virus, 4 fresh vesicles, was collected from a varicella patient on the 3d day of eruption. Virus and non-immune serum (W. R.) were injected into Monkey 9; convalescent zoster serum (M. R.) and virus were inoculated into Monkey 10. 5 days after the injections the testicles were removed and examined for the presence of acidophilic nuclear inclusion. In the testicles of both animals many were found.

In this experiment it seems that the convalescent zoster serum did not neutralize the chicken-pox virus.

Experiment IV.

The experiment described below is the most important one in the series. In it was used serum from a convalescent zoster patient who had had varicella 7½ years previously and from whom a sister appeared to have contracted chicken-pox through exposure to her during the attack of zoster.

M. B., female, white, age 8. No history of previous attacks of zoster. Had chicken-pox when 6 months old. No recent exposure to varicella or zoster. Attends a public school. On Oct. 24, 1926, the patient developed blisters over the right side of the body. She was not quarantined and came in contact with relatives, classmates, and playmates. On Nov. 1, she was seen at the Vanderbilt clinic and referred to the Rockefeller Hospital. At that time the patient presented the picture of typical herpes zoster. The eruption was on the right side of the body over an area supplied by the 9th and 10th thoracic nerves. The child was not admitted to the hospital, but was seen from time to time in the clinic. Dr. E. visited the home on several occasions and a nurse observed the children in the patient's class at school. The lesions on the child healed slowly, and convalescent serum was obtained on Nov. 20, 26 days after the appearance of the eruption.
V. B., age 3, a sister who had never had varicella or zoster was constantly exposed to the patient. On Nov. 13, 19 days after the appearance of zoster in M. B., a chicken-pox eruption was observed on V. B. The child was admitted to the Rockefeller Hospital and after passing through a typical attack of varicella was discharged on Nov. 27. Just prior to discharge convalescent serum was obtained.

The parents said that V. B. had not been exposed to varicella, yet she developed the disease 19 days after exposure to herpes zoster in her sister. This, then, is an excellent example of chicken-pox developing in a child after exposure to herpes zoster. Many such examples have been reported and have been adduced as evidence of the identity of the two diseases. Upon pursuing the matter further, however, it was found that M. B.'s class at school consisted of 33 children, 21 of whom had never had varicella. All of them were exposed to M. B., yet not a case of chicken-pox developed as a result of the exposure.

Four monkeys were used in the neutralization tests. The virus consisted of 12 vesicles removed from 3 varicella patients (J. R., W. R., V. D.). Non-immune serum (W. R.), chicken-pox convalescent serum (M. W.), convalescent serum from M. B., and convalescent serum from V. B. were respectively mixed with equal amounts of virus. 0.25 cc. of the mixtures were then injected into the testicles of monkeys; virus and non-immune serum into Monkey 11, virus and convalescent varicella serum into Monkey 12, virus and convalescent serum from M. B. into Monkey 13, virus and convalescent serum from V. B. into Monkey 14. 5 days after the injections the testicles were removed and examined in the usual way for the presence of nuclear inclusions. Sections from Monkeys 11 and 13 showed numerous inclusions, while those from Monkeys 12 and 14 revealed none.

The results of the experiment indicate that the non-immune serum and the serum from the child (M. B.) convalescent after zoster did not neutralize varicella virus, while convalescent varicella serum of extraneous derivation and convalescent serum from the sister (V. B.) who had just had chicken-pox did inhibit its action. In view of these findings, together with the failure of varicella to develop in any of the classmates of the child (M. B.) who had zoster, one is justified in concluding that the relation of chicken-pox to zoster in the two sisters, V. B. and M. B., was apparent rather than real.

A summary of the experiments dealing with the neutralization of varicella virus by convalescent herpes zoster serum is given in Table I. An analysis of the results reveals that, under the conditions of the tests, sera from individuals who had had varicella 7½ to 30 years previously did not neutralize varicella virus. This fact does not imply that these individuals were susceptible to chicken-pox and that their sera did not possess a certain amount of neutralizing power for varicella virus.
virus. The experiments also indicate that 2 of 3 convalescent herpes zoster sera did not neutralize varicella virus while in each of 2 in-

TABLE I.
Summary of Neutralization Experiments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Monkey</th>
<th>Inoculum</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Varicella virus + non-immune serum</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>&quot; + serum from adult who had varicella 30 years previously</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>&quot; + non-immune serum</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>&quot; + convalescent varicella serum</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>&quot; + serum from patient early in course of herpes zoster</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>&quot; + serum from patient convalescent from herpes zoster</td>
<td>-</td>
</tr>
<tr>
<td>2 Repeat</td>
<td>7</td>
<td>&quot; + non-immune serum</td>
<td>+</td>
</tr>
<tr>
<td>2 Repeat</td>
<td>8</td>
<td>&quot; + convalescent herpes zoster serum</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>&quot; + non-immune serum</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>&quot; + convalescent herpes zoster serum</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>&quot; + non-immune serum</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>&quot; + convalescent varicella serum</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>&quot; + convalescent herpes zoster serum from an individual (M. B.) who had had varicella 7½ years previously</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>&quot; + convalescent varicella serum (V. B.)</td>
<td>-</td>
</tr>
</tbody>
</table>

Monkeys inoculated in testicles.

+ indicates infection by virus, determined by presence of nuclear inclusions in sections of inoculated testicles.

- indicates no infection by virus, determined by absence of nuclear inclusions in sections of inoculated testicles.

stances\(^3\) convalescent chicken-pox serum did neutralize it. An adequate explanation of why varicella virus appeared to be neutralized by one of the convalescent zoster sera is lacking. It is certainly not

\(^3\) Convalescent varicella serum regularly prevents the occurrence of nuclear inclusions in monkeys' testicles inoculated with varicella virus (11).
safe to conclude, even though the experiment was repeated, that the herpes zoster in that case was caused by varicella virus. From the experiments reported in this paper and those previously recorded in regard to varicella in monkeys (10, 11), one is justified in concluding that herpes zoster is not always produced by varicella virus. Such is the case even in instances where clinical observations might lead one to suspect that the causal agents of the two diseases are identical.

**DISCUSSION.**

Many investigators believe that certain cases of zoster are caused by a specific virus. Instances, however, of “recurring zoster,” of zoster following mechanical or drug injuries to nerves, and of zoster occurring in patients with tabes, tuberculosis of the spine, and cord tumors have led numerous observers to consider that this type of the disease is due not to an infectious agent, but to trophic changes in the skin incident to nerve injury. Regardless of the conditions under which herpes zoster occurs, the character of the lesions has induced a number of workers familiar with virus diseases to entertain the idea that it is caused by some kind of virus.

The question as to whether zoster is caused by one or by several viruses naturally arises. The pathological changes observed in zoster lesions are strikingly similar to those seen in herpes simplex and varicella. In fact, lesions from these three diseases cannot be differentially diagnosed one from another by means of the microscope. As a group, however, they can be separated from other skin lesions occurring in man. This is made possible by the occurrence of acidophilic bodies in the nuclei of affected cells. From records of work dealing with the identity of herpes simplex and herpes zoster (15) it seems unlikely that the etiological agents concerned in the majority of the cases of these two diseases are identical.

It now remains to consider the relation of zoster to chicken-pox. The studies presented in the preceding paper (1), the experiments recorded in the present one, and the fact that an attack of herpes zoster as a rule does not protect against varicella and *vice versa* (16, 17, 18, 22) seem to indicate that the majority of the cases of the two diseases is not caused by the same virus. There is no good reason, however, why one should say that the virus of chicken-pox cannot under
certain conditions produce localized lesions clinically indistinguishable from herpes zoster. It probably does, but only rarely.

The conditions under which the virus of varicella causes a disease clinically similar to herpes zoster are probably dependent upon the rôle injury or irritation plays in the localization of many viruses. The viruses of measles (19) and varicella (20) localize in areas of irritated skin as is evidenced by a marked increase in the number of lesions appearing in such areas. Therefore, in hospitals where large numbers of tuberculous children and syphilitic infants undergoing treatment with arsenicals are cared for, a few cases of clinical zoster may occur during an epidemic of varicella. In all probability, under these conditions, some if not all of the so called zoster is caused by chickenpox virus. Consequently it would be better to diagnose such cases as varicella with an abnormal localization of the rash.

It is not likely that trophic changes incident to nerve injury alone or to drug poisoning alone, e.g., with arsenic, are capable of producing herpes zoster. If such were the case, the disease should be seen more often under these conditions. The fact that viruses tend to localize in irritated tissues may also account for the relation that has been observed to exist between certain cases of herpes zoster and nerve injury due to chemical, physical, or disease-producing agents. Whether the virus localizes primarily in ganglia and subsequently travels by way of nerves to the skin or whether the localization is only in the skin, the site being determined by circulatory and other changes incident to the injury of nerves supplying the region (21), is not definitely known. There is no reason why the primary localization of the virus may not occur in either place. The presence or absence of pain in zoster may in some instances be dependent upon the place at which the virus localizes.

Since in the majority of cases of herpes zoster the disease is caused neither by the virus of herpes simplex nor by the virus of varicella, is there any evidence at present that a special virus is concerned in its production? There is no direct proof that such a virus exists, yet the indirect evidence is suggestive. In the first place, the pathological picture presented by zoster lesions is one that has not been shown to occur in the absence of some kind of virus. Although zoster is not, as a rule, highly contagious, it breeds true when occur-
ring epidemically, i.e., zoster gives rise to zoster (15, 22). Finally, in the majority of instances one attack of zoster confers an immunity against a second attack of the disease but not against an infection with herpes simplex and varicella viruses.

SUMMARY.

Experiments and clinical observations dealing with the identity of the viruses of varicella and herpes zoster were presented. The results indicate that the etiological agents concerned with these two diseases are in the majority of instances not identical.

BIBLIOGRAPHY.