# RECIPROCAL EFFECTS OF CONCOMITANT INFECTIONS.

## I. The Influence of Vaccinia on the Reaction to Infection with Experimental Syphilis.

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The existence of two infectious diseases concurrently in the same individual is now well recognized as not infrequent, and it has been pointed out, among other interesting observations, that the symptoms of each disease may be more severe than when either occurs alone. Probably the infections which most often develop concomitantly or approximately so, are the acute infections of childhood, as for example, scarlet fever and diphtheria, or measles and diphtheria. In other infectious conditions, an intercurrent or complicating infection is a relatively common occurrence, and again, in such circumstances there may be an intensification of symptoms of one or both diseases, as for instance in tuberculosis and syphilis, or measles and tuberculosis. The unfavorable effect of influenza on certain patients with syphilis was shown during the epidemic of 1918 and 1919 in which there were many examples of an abrupt and serious change in a previously mild syphilis following an attack of influenza. On the other hand, a pre-existing disease may be favorably influenced for a longer or shorter time by an intercurrent infectious process, as for example, certain types of psychoses including general paresis, or even cases of malignant disease.

No satisfactory explanation of the various effects observed in these circumstances has been made, for the lack of accurate information regarding their precise nature has not permitted any but the most general statements. It is surprising, in view of the modification of symptoms which may occur and the significance which such alterations undoubtedly have from the standpoint of the host's reaction to disease, that the subject has received comparatively little experimental investigation.

Our interest in concomitant diseases arose first during our study of experimental syphilis and yaws, particularly with reference to superinoculation and crossed inoculation, and later, in connection with a transplantable malignant neoplasm of the rabbit which developed in a syphilitic animal. More recently our attention was again directed to this subject as a field for experimental investigation from observations of a strain of *Treponema pallidum* obtained from a rabbit found to be also infected with vaccine virus. It became evident that this virus was being transferred with the syphilitic inocula. The syphilis which developed in rabbits inoculated with this material was unusually mild, and this finding was wholly unexpected since this particular strain has been carried in rabbits for many years and is reported to be capable of inducing a severe disease. The possibility that the accompanying vaccinal infection contributed to these unforeseen results was naturally considered, but the acceptance of such an explanation obviously required experimental evidence obtained under properly controlled conditions. To this end, several series of experiments were undertaken in which was studied the influence of a vaccinal infection upon the reaction to syphilis induced by a strain of *T. pallidum* uncontaminated with vaccine virus. An extension of the investigation comprised experiments dealing with the reaction to *T. pallidum* of rabbits immune to vaccine virus.

The present paper contains the results of experiments in which one of the two commonly used routes of syphilitic inoculation was employed, namely, the intratesticular; the vaccinal inoculations were made intracutaneously on the side of the body. Subsequent papers contain the results of experiments in which *T. pallidum* and vaccine virus were inoculated in the same testicle, with observations on the syphilitic reaction of rabbits immune to vaccine virus and with the results of the intracutaneous route of both syphilitic and vaccinal inoculations. A summary of the results obtained has already appeared (1).

## EXPERIMENTAL.

### *Materials and Method.*

Three experiments are reported on the course of syphilitic infection in rabbits inoculated simultaneously with vaccine virus and with the Nichols strain of

*T. pallidum*. For comparison, other series were inoculated only with *T. pallidum*. The Noguchi strain of vaccine virus which was used was obtained from a fresh testicular lesion; its virulence was controlled by inoculation in normal rabbits using the intracutaneous and intratesticular routes as well as applications to scarified skin areas.

The experimental animals were inoculated intracutaneously (0.2 cc.) and on a scarified skin area (0.2 cc.) with vaccine virus and immediately thereafter with *T. pallidum* injected into the right testicle. The emulsions used were prepared from actively growing testicular lesions and contained from 1 to 3 spirochetes to the microscopic field; each animal received 0.2 cc.

The rabbits employed were young adult male animals approximately 8 months of age. They were placed in individual cages and were divided into 2 groups as nearly comparable as possible with respect to age, breed and weight. In each experiment, the control group, that is the group inoculated only with *T. pallidum*, comprised 10 rabbits; in the first and second experiments there were 5, and in the last, 10 animals in the vaccinated group.

The dates of inoculations were as follows: November 10, 1926, January 13 and February 14, 1927. The periods of observation were from 3 to 5 months, but for the purpose of comparing the results of the 3 experiments one with another, the data obtained during the first 3 months following inoculation have been used.

In these experiments special attention has been given to the time and frequency of occurrence and to the duration of successive phases of the reaction to syphilitic infection with a view to reducing the comparison of results to as quantitative a basis as possible. The particular conditions chosen for comparison were, (1) the incubation time of primary lesions, (2) the time and frequency of the occurrence of a critical edema in the inoculated testicle, (3) the time and frequency of occurrence of lesions in the uninoculated testicle (metastatic orchitis), (4) the time and frequency of occurrence of generalized lesions in the skin and mucous membranes, bones or eyes, (5) the number of foci affected by such lesions, (6) the proportion of animals that showed complete healing of all lesions during the 3 months observation period.

It should be noted, in discussing time relations of the various reactive phenomena, that there is a basic tendency toward the preservation of a uniform interval of time between the occurrence of successive reactions in syphilitic rabbits. With the Nichols strain of *T. pallidum* as carried in our laboratory, the reaction interval is approximately 2 weeks. In these experiments, as stated above, the Nichols strain was used, but the particular line from which the present substrain, as it may be termed, was derived had been transferred under somewhat different conditions from those employed with the parent strain. It was not known at the time this work was started, however, that the infection induced by this substrain would differ in any essential respect from that of the parent strain, but the results of these and other experiments have shown certain peculiar features of the syphilitic reaction, notably a delay in the development of a metastatic orchitis and an un-

usually early appearance of generalized lesions. These peculiarities in the behavior of the strain must be taken into account in analyzing the results obtained.

The methods employed for recording results require little explanation. The term "focal distribution" or "focal incidence" as applied to generalized lesions refers to the number of discrete foci at which lesions developed as determined by actual count. The figures for actual distribution are the mean values for those animals of a group that actually developed generalized lesions, while the figures for relative distribution give the results in terms of the entire group. This distinction is made in order to permit comparison of the extent of the lesions irrespective of the number of animals affected and at the same time to avoid any erroneous impression that might arise from the chance occurrence of an occasional case of severe syphilis in any group of animals.

## *Results.*

The results of the experiments are recorded in Tables I, II and III and in Text-figs. 1 and 2. It will be noted that the figures given in

TABLE I.

*Incidence of the Various Phenomena of the Syphilitic Infection and the Focal Distribution of Generalized Lesions.*

| Experiment | Animal group | No. of rabbits | Primary orchitis | Edema of inoculated testicle | Metastatic orchitis | Generalized lesions | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Incidence | Focal distribution | Focal distribution |
| | | | *per cent* | *per cent* | *per cent* | *per cent* | *actual* | *relative* |
| I | C | 10 | 100.0 | 60.0 | 100.0 | 100.0 | 9.8 | 9.8 |
| | V V | 5 | 100.0 | 80.0 | 100.0 | 100.0 | 22.2 | 22.2 |
| II | C | 10 | 100.0 | 50.0 | 90.0 | 70.0 | 7.0 | 4.9 |
| | V V | 5 | 100.0 | 40.0 | 80.0 | 100.0 | 14.4 | 14.4 |
| III | C | 10 | 100.0 | 70.0 | 90.0 | 90.0 | 8.4 | 7.6 |
| | V V | 10 | 100.0 | 44.4* | 88.8 | 100.0 | 16.3 | 16.3 |
| Mean values | C | 30 | 100.0 | 60.0 | 93.3 | 86.7 | 8.6 | 7.4 |
| | V V | 20 | 100.0 | 52.6 | 89.5 | 100.0 | 17.4 | 17.4 |

C = controls; V V = animals inoculated with vaccine virus.

* One animal in this group which developed edema died shortly thereafter; its inclusion would bring the incidence of edema to 50.0 per cent.

Tables I and II and in the text-figures represent group values. In Text-fig. 1, the detailed results are presented graphically in 3 charts in order to show the entire sequence of events from the development of the first or primary lesions to the appearance of the last generalized lesions; the abscissæ represent the time after inoculation in days. These curves also illustrate an important feature of the syphilitic reaction, namely, that while these events occur successively, they also overlap each other to some extent. In order to simplify the reading of the charts, a division of the curves has been made at the 35th day

TABLE II.

*Mean Time of Occurrence of the Various Phenomena of the Syphilitic Infection as Estimated in Days from the Date of Inoculation.*

| Experiment | Animal group | Primary orchitis | Edema of inoculated testicle | Metastatic orchitis | Generalized lesions | | |
|---|---|---|---|---|---|---|---|
| | | | | | First | Last | Mean of all |
| | | *days* | *days* | *days* | *days* | *days* | *days* |
| I | C | 12.8 | 31.0 | 46.5 | 48.0 | 78.8 | 62.8 |
| | V V | 12.4 | 23.5 | 40.4 | 41.2 | 95.0 | 62.7 |
| II | C | 22.9 | 40.4 | 61.8 | 62.0 | 80.4 | 67.4 |
| | V V | 23.6 | 31.0 | 58.5 | 63.4 | 87.4 | 75.9 |
| III | C | 12.2 | 33.9 | 45.3 | 47.6 | 67.6 | 55.0 |
| | V V | 12.0 | 25.3 | 43.0 | 43.4 | 80.1 | 59.6 |
| Mean values | C | 15.8 | 34.7 | 51.0 | 51.6 | 75.1 | 61.0 |
| | V V | 15.7 | 25.7 | 45.9 | 48.1 | 88.6 | 64.0 |

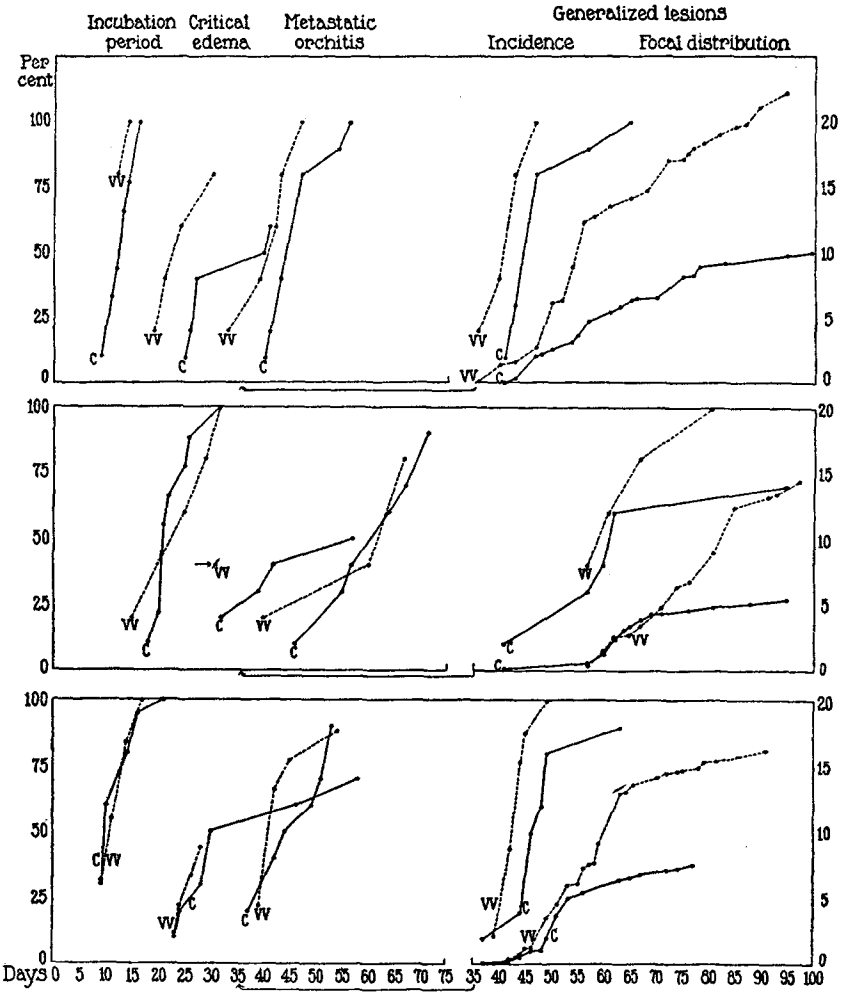
as indicated by arrows, and the curves representing the incidence and focal distribution rates of generalized lesions have been separated from the others. The time of appearance or the distribution of generalized lesions is shown by the curves in Text-fig. 2 in which a time interval of 4 days has been used. Table III contains the total number of generalized lesions. In the following discussion, mean values have been used for the most part since the results of the individual experiments are in essential agreement one with another.

TABLE III.

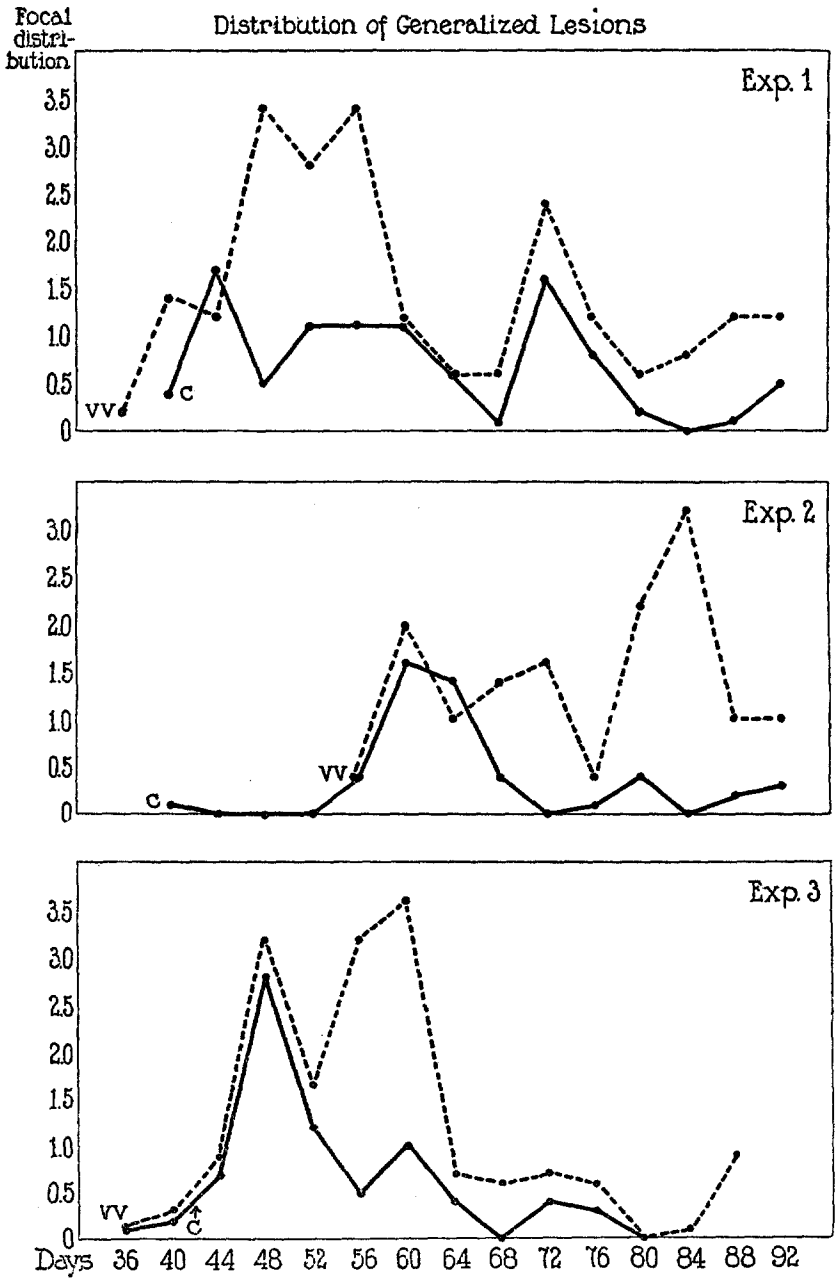
*Time of Appearance of the Generalized Lesions as Estimated from the Date of Inoculation.*

| Time interval | Experiment I | | Experiment II | | Experiment III | |
|---|---|---|---|---|---|---|
| | No. of lesions | | No. of lesions | | No. of lesions | |
| | C (10 rabbits) | V V (5 rabbits) | C (10 rabbits) | V V (5 rabbits) | C (10 rabbits) | V V (9 rabbits) |
| *days* | | | | | | |
| 35 | 0 | 1 | 0 | 0 | 0 | 0 |
| 37 | 0 | 0 | 0 | 0 | 1 | 1 |
| 39 | 0 | 6 | 0 | 0 | 0 | 0 |
| 41 | 1 | 0 | 1 | 0 | 2 | 3 |
| 43 | 3 | 1 | 0 | 0 | 3 | 4 |
| 45 | 0 | 0 | 0 | 0 | 4 | 4 |
| 47 | 19 | 6 | 0 | 0 | 1 | 0 |
| 49 | 3 | 17 | 0 | 0 | 9 | 20 |
| 51 | 0 | 1 | 0 | 0 | 18 | 9 |
| 53 | 6 | 13 | 0 | 0 | 12 | 13 |
| 55 | 5 | 16 | 0 | 0 | 5 | 13 |
| 57 | 11 | 1 | 4 | 2 | 0 | 3 |
| 59 | 0 | 0 | 7 | 5 | 0 | 14 |
| 61 | 7 | 6 | 9 | 6 | 0 | 0 |
| 63 | 4 | 0 | 7 | 0 | 10 | 34 |
| 65 | 6 | 3 | 3 | 1 | 1 | 5 |
| 67 | 0 | 3 | 4 | 3 | 3 | 0 |
| 69 | 1 | 0 | 3 | 0 | 0 | 5 |
| 71 | 0 | 0 | 1 | 7 | 3 | 4 |
| 73 | 0 | 11 | 0 | 8 | 1 | 1 |
| 75 | 16 | 3 | 1 | 0 | 0 | 1 |
| 77 | 8 | 2 | 0 | 2 | 3 | 1 |
| 79 | 0 | 2 | 0 | 10 | 0 | 4 |
| 81 | 0 | 3 | 4 | 11 | 0 | 0 |
| 83 | 2 | 0 | 0 | 0 | 0 | 1 |
| 85 | 0 | 3 | 0 | 17 | 0 | 0 |
| 87 | 0 | 1 | 2 | 0 | 0 | 2 |
| 89 | 1 | 6 | 0 | 4 | 0 | 0 |
| 91 | 5 | 6 | 3 | 6 | 0 | 5 |
| Total | 98 | 111 | 49 | 72 | 76 | 147 |

Influence of Concomitant Vaccinal Infection on Reaction to Syphilis




TEXT-FIG 1.


Distribution of Generalized Lesions
Focal distribution
Exp. 1
Exp. 2
Exp. 3
VV
C
Days 36 40 44 48 52 56 60 64 68 72 76 80 84 88 92

TEXT-FIG 2.

## DISCUSSION.

The purpose of this investigation was to determine whether a concomitant vaccinal infection would influence the reaction to experimental syphilis. It should be kept in mind that in the experiments reported in the present paper, the inoculation of both viruses was made at the same time but at different sites—the vaccine virus intradermally on the side of the body and *T. pallidum* in one testicle. The results obtained have been analyzed in terms of various reactive phenomena in accordance with the general principles that govern the evolution of syphilitic infection (2, 3) and in the following discussion these phenomena are taken up in the order of their occurrence, so that the picture of the disease as it developed may be more readily visualized.

*Incidence of Primary Lesions and Incubation Period.*—All rabbits in these experiments developed primary lesions (Table I; Text-fig. 1), and there was practically no difference in the length of the mean incubation period between the vaccinated and control groups, that is 15.7 and 15.8 days (Table II). It will be noted that all incubation periods for the first and third experiments were essentially the same, 12.0, 12.2, 12.4 and 12.8 days, while those of the second were prolonged to 22.9 days in the case of the controls and to 23.6 days for the vaccinated group. As will be seen later, the magnitude of these values are indicative of the general character of the disease that prevailed, that is, it was much less severe in the second than in the first or third experiment.

It is evident from these findings that the concomitant intradermal inoculation of vaccine virus did not influence either the incidence of the primary syphilitic reaction or the time of its development as determined clinically by palpation.

*Critical Edema.*—While the occurrence of a critical edema in an inoculated testicle or in association with other syphilitic lesions is a variable phenomenon, it has an important significance as indicating an intense reaction; it usually marks the end of a local reaction, either temporary or final.

The mean values of the incidence of edema in the inoculated testicle were essentially the same for the controls and the vaccinated animals,

that is 60.0 and 52.6 per cent respectively, but the incidence in individual experiments was variable (Table I and Text-fig. 1). In the first experiment in which the disease was very severe there was a higher frequency in the vaccinated than in the control groups, but this order was reversed in the third experiment with a disease of somewhat less severity, while in the second in which a relatively mild syphilis prevailed, there was little difference between the 2 groups.

The time at which edema occurred, however, indicates very clearly the more prompt reaction of the vaccinated animals as shown by the mean values calculated from the time of inoculation, that is 28.6 for the vaccinated and 34.7 days for the control group (Table II). The results of individual experiments were similar, the difference in time between vaccinated and control groups being 7.5, 9.4 and 8.6 days respectively. In addition, it should be pointed out that there was a closer agreement in the time of development of edema among individual animals of each vaccinated group than among the controls as shown by the curves of Text-fig. 1. In the control groups, there were 2 rabbits each in the first and third and 1 in the second experiment in which an edema of the inoculated testicle was considerably delayed. And although the group and mean time values are obviously influenced by these particular animals, still their omission does not disturb the order of group values as shown by the following figures:

| Experiment | "Corrected" controls | | Vaccinated | |
|---|---|---|---|---|
| | No. of rabbits | Edema | No. of rabbits | Edema |
| | | *days* | | *days* |
| I | 4 | 26.3 | 4 | 23.5 |
| II | 4 | 36.3 | 2 | 31.0 |
| III | 5 | 26.8 | 4 | 25.3 |
| Mean values | 13 | 29.5 | 10 | 25.7 |

An idea of the progress of the reaction is also obtained by estimating the time of appearance of edema from the incubation period of the primary lesion instead of from the date of inoculation, as has been done in the table at top of following page.

*From Incubation.*

| Experiment | All controls | "Corrected" controls | All vaccinated rabbits |
|---|---|---|---|
| | *days* | *days* | *days* |
| I | 18.2 | 13.5 | 11.1 |
| II | 17.5 | 13.4 | 7.4 |
| III | 21.7 | 14.6 | 13.3 |
| Mean values | 18.9 | 13.7 | 10.0 |

A comparison of these values brings out very clearly the fact that there was a more prompt reaction to the syphilitic infection in the vaccinated than in the control rabbits irrespective of whether all controls are included in the comparison or only those animals in which the development of edema was not delayed.

As has already been mentioned, the usual time interval between the occurrence of successive reactions with highly virulent strains of *T. pallidum* is 2 weeks. The above table shows that with respect to the interval between the incubation period of the primary lesion and the critical edema, the majority of controls conformed to this value (13.7 days), while it was somewhat lengthened in the case of all controls (18.9 days). With the vaccinated animals, on the other hand, this interval was shortened to the unusually short time of 10.0 days.

The essential point to be remembered with respect to the phenomenon of critical edema is the earlier time in which this reaction occurred in the vaccinated groups as compared with the controls of these experiments, bearing in mind that this interval in the controls was not shorter than that usually seen in rabbits infected with the Nichols strain.

*Metastatic Orchitis.*—One of the most characteristic and regular phenomena of the syphilitic reaction is the occurrence of a metastatic orchitis in the uninoculated testicle which with a disease of well marked severity develops approximately 6 weeks after inoculation.

The incidence of a metastatic orchitis in the control and vaccinated groups of the present experiments was the same, as is shown in Table

I and Text-fig. 1. It occurred in all animals of both groups in the first experiment in which a disease of marked severity prevailed, while it was not detected in 1 animal each of the vaccinated and control groups of the second and third experiments in which the disease was less pronounced.

With respect to the time at which a metastatic orchitis developed, the vaccinated groups showed a more prompt response (Table II). The mean values reckoned from the day of inoculation were 51.0 days for the controls and 45.9 days for the vaccinated animals. These mean values are higher than those ordinarily seen with strains of high virulence, owing largely to the prolonged reaction time observed in the second experiment. But in this experiment as well as in the others, an orchitis of the uninoculated testicle occurred earlier in the vaccinated than in the control groups, the difference in time being 3.1 days in the second, 6.1 days in the first and 2.3 days in the third experiment. Moreover, as shown in Text-fig. 1 it uniformly occurred sooner in the vaccinated group than in the controls in the first experiment, and so too with most of the animals in the third experiment. In the second experiment, however, as might be expected with a mild disease, the lesions developed irregularly with no definitely defined group difference.

The results bearing on the development of a metastatic orchitis with respect to the time values of the previous reactions, that is to say, the incubation period of the primary lesion and the critical edema are shown by the following figures:

*From the Incubation Period.*

| Experiment | Controls | Vaccinated rabbits |
|---|---|---|
| | *days* | *days* |
| I | 33.3 | 28.0 |
| II | 38.9 | 34.9 |
| III | 33.1 | 31.0 |
| Mean values | 35.2 | 30.2 |

*From the Critical Edema.*

| Experiment | Controls | "Corrected" controls | Vaccinated rabbits |
|---|---|---|---|
| | *days* | *days* | *days* |
| I | 15.2 | 20.2 | 16.9 |
| II | 21.4 | 25.5 | 27.5 |
| III | 11.4 | 18.5 | 17.7 |
| Mean values | 16.3 | 21.7 | 20.3 |

With the Nichols strain, the interval between the development of a metastatic orchitis and the incubation period of the primary orchitis is usually from 4 to 5 weeks. In the case of the first and third vaccinated groups as well as with the mean value for the 3 groups this interval was actually or approximately 4 weeks, while the average intervals for the individual control groups as well as the mean value were approximately a week longer. Comparisons based upon the interval between the occurrence of the critical edema and the development of the metastatic orchitis are less satisfactory with group than with individual animals because of the variability in the incidence and time of occurrence of this phenomenon. In the present instance, if the comparison includes all control animals that developed edema, the interval is considerably shortened, owing to the delayed edema of 5 rabbits, and the resulting mean and individual group values are consequently smaller than the corresponding ones for the vaccinated animals. But with the omission of these 5 rabbits ("corrected" controls), this interval between edema and metastatic orchitis is slightly shorter in the vaccinated than in the control groups of the first and third experiments. It is slightly longer in the second experiment, a discrepancy which may be explained by the difference in the number of animals in the control (corrected) group which developed edema—that is 4 as contrasted with 2 in the vaccinated group. Such small differences, however, are of interest only in that they are in agreement with other results.

From the above analyses of the results obtained in connection with the incubation period of the metastatic orchitis with reference to the time of inoculation as well as from the time relations between its oc-

currence and that of preceding reactive phenomena, it is clear that the reaction to the syphilitic infection as measured by the phenomena of a metastatic orchitis was more prompt in rabbits which were vaccinated with vaccine virus coincidently with the inoculation of *T. pallidum* than in control animals. It will be recalled that a similar result was obtained in connection with the preceding phenomenon of a critical edema.

*Generalized Lesions.*—The phenomenon of generalized lesions is the most indicative single basis for estimating the character of the reaction to syphilitic infection. The occurrence or non-occurrence of generalized lesions, the time of their appearance, and the duration of the period of active development, their number, extent, character and persistence are essential features of this phase of the disease which can be employed in analyzing its character. The disease exhibits a well recognized variability in all these respects, but it is usually comparatively constant under a given set of conditions.

In the present experiments, a large number of control animals developed generalized lesions, 86.7 per cent, but this was surpassed by the vaccinated groups with the unusually high incidence of 100.0 per cent (Table I). As has been mentioned before, the disease in the first experiment was very severe, in the second, comparatively mild, while in the third it was quite severe, and these differences are indicated by the incidence of generalized lesions in the control groups, namely, I, 100.0 per cent; II, 70.0 per cent; III, 90.0 per cent (Table I), but irrespective of these variations, generalized lesions occurred in every vaccinated rabbit.

In addition, an extremely large number of lesions developed in the vaccinated animals, the mean actual and relative distribution rates for the vaccinated groups being 17.4 as compared with control rates of 8.6 and 7.0 (Table I). Results of similar orders of magnitude were obtained in individual experiments. The extent of the differences between the vaccinated and control groups in this respect is better appreciated by estimating the focal distribution rates of the vaccinated groups in percentage terms of control values, as has been done in the table at top of following page.

| Experiment | Actual rate of control values | Relative rate of control values |
|---|---|---|
| | *per cent* | *per cent* |
| I | +122.4 | +122.4 |
| II | +132.9 | +230.6 |
| III | +94.1 | +114.5 |
| Mean values | +102.3 | +148.6 |

These figures demonstrate in a striking manner that the so called generalized phase of the syphilitic infection as measured by the actual number of lesions detected was much more pronounced in the animals which received an intracutaneous injection of vaccine virus at the time of intratesticular inoculation with *T. pallidum* than in the controls.

The time at which generalized lesions developed is another point of comparison in which the vaccinated rabbits differed from the controls. Reckoned from the day of inoculation, as has been done in the analyses summarized in Table II, the mean time of the appearance of the first generalized lesions in the vaccinated groups antedated that of the controls by 3.5 days, while the mean time of the last lesions to develop was 14.5 days later in the vaccinated than in the control animals. These differences as well as those obtaining in individual experiments are shown in the following table in which a minus or a plus sign indicates a shorter or a longer time than that for the controls.

*Difference in Time of Appearance of Generalized Lesions of Vaccinated Groups as Compared with Controls.*

| Experiment | First lesion | Last lesion |
|---|---|---|
| | *days* | *days* |
| I | −6.8 | +17.2 |
| II | +1.4<br>(−2.1)* | +7.0 |
| III | −4.2 | +12.5 |
| Mean values | −3.5<br>(−3.9)* | +13.5 |

* Values obtained by omitting one control animal with a single precociously early lesion.

Although the differences with respect to the last lesions are more striking than those for the first, still the first differences demonstrate the significant fact that the reaction time of the vaccinated groups in regard to another phenomenon of the syphilitic infection, that is to say, the development of generalized lesions, continued to be in advance of that of normal animals as was seen with the earlier phenomena of critical edema and metastatic orchitis. It should be mentioned that the corrected figure for the time difference in the second experiment is a fairer expression of the group picture than the uncorrected, as will be seen by referring to Text-fig. 1. That the severity of the disease prevailing in different experiments is an important factor in influencing the extent or range of the results obtained is well illustrated by the relative magnitudes of the values for the first and last lesions in the above table, the highest values being found in the first experiment in which the most severe disease prevailed, while the lowest occurred in the second with a comparatively mild disease.

From what has been said regarding the time of appearance of the first and last generalized lesions, it is obvious that the period of active development of this important phase of the disease was considerably longer in the case of the rabbits which received vaccine virus. The extent of these differences is strikingly brought out by the following comparison:

*Period of Active Development of Generalized Lesions.*

| Experiments | Controls | Vaccinated | Increase of vaccinated groups | | |
|---|---|---|---|---|---|
| | *days* | *days* | *days* | | *per cent* |
| I | 30.8 | 53.8 | +23.0 | or | 74.8 |
| II | 18.4 | 24.0 | + 5.6 | or | 30.5 |
| | (14.9)* | | (+9.1)* | or | (61.0)* |
| III | 20.0 | 36.7 | +16.7 | or | 83.5 |
| Mean values | 23.5 | 40.5 | +17.0 | or | 72.3 |
| | (23.1)* | | (+17.4)* | or | (75.3)* |

* These values were obtained by omitting 1 animal with a precocious lesion, as explained in the foregoing text.

These figures show that in each experiment the period of active development of generalized lesions was much longer in the vaccinated

than in the control animals. The percentage increases in time of the first and third experiments, 74.8 and 83.5 per cent, are of the same order of magnitude as the mean value of 72.3 per cent. In the case of the second experiment, the increase of 30.5 per cent is considerably smaller, but the corrected figure of 61.0 per cent is comparable to the others; and as has already been mentioned, the corrected figure is more representative of the group as a whole.

In round numbers, the mean value of the duration of activity as regards generalized manifestations was 3 weeks in the case of the controls and 6 weeks in the case of the vaccinated animals. There was comparatively little difference between the 2 groups, however, in regard to the mean time of appearance for *all* generalized lesions reckoned from the day of inoculation, that is 61 and 64 days for the controls and vaccinated groups respectively (Table II); and a similar result is obtained if the interval is estimated from the incubation time of the primary orchitis. But if the comparison is made from the time of the reactive phenomenon immediately preceding the appearance of generalized lesions, namely, the development of a metastatic orchitis, the difference between the 2 groups is well shown. The results of these 2 analyses appear in the following tabulations:

*From Primary Orchitis to Mean Time for All Generalized Lesions.*

| Experiment | Controls | Vaccinated |
|---|---|---|
| | *days* | *days* |
| I | 50.0 | 50.3 |
| II | 44.5 | 52.1 |
| III | 42.8 | 47.6 |
| Mean | 45.8 | 50.0 |

*From Metastatic Orchitis to Mean Time for All Generalized Lesions.*

| Experiment | Controls | Vaccinated |
|---|---|---|
| | *days* | *days* |
| I | 16.3 | 22.3 |
| II | 5.6 | 17.2 |
| III | 9.7 | 16.6 |
| Mean | 10.5 | 18.7 |

Both these comparisons of time reactions show the existence of a longer interval between the date of the previous reactive phenomena and the mean time of appearance of all lesions in the case of the vaccinated animals as compared with the controls, but the difference is greater with respect to the phenomenon immediately preceding the development of generalized lesions, namely, the metastatic orchitis. From the various analyses of the results already presented, it is evident that this second comparison is more indicative of the general character of the disease prevailing in the 2 groups than in the first, and it illustrates one of the biological principles governing syphilitic infection, namely, that the character of any reaction is closely connected and, indeed, is particularly dependent upon the nature of the preceding reaction. In addition, the foregoing tables bring out the fact that, as regards these time relations, the vaccinated groups were more uniform than the controls, and this was to be expected under the conditions of relatively uniform disease severity prevailing in the vaccinated animals.

Neither of these comparisons involving the mean time of appearance for *all* generalized lesions, however, shows a marked divergence between control and vaccinated groups, and this is largely due to the small difference between these mean values. The differences that exist in the actual distribution of lesions in the vaccinated as compared with the control animals, however, are shown in the distribution curves of the times at which new lesions appeared (Text-fig. 2). A time interval of 4 days has been chosen in plotting these curves to obviate some of the irregularities associated with shorter periods, as shown in Table III which lists the total number of lesions in 2 day intervals. The relative rather than the actual number of lesions has been used because of the difference in number of animals in the groups of the first 2 experiments, so that in addition to the time distribution of lesions, the height of the curves gives an idea of the severity of the disease as measured by the numerical distribution of lesions in the order of their appearance.

Although the 3 sets of curves show certain dissimilar features due largely to the different character of the disease prevailing in individual experiments, those representing the vaccinated animals are in remarkable agreement as regards their general levels which are much higher

throughout than the control curves. For the most part, the fluctuations of the two curves in the first and third experiments, in which the disease was pronounced, occur in the same time interval, showing that in general the reaction of the vaccinated group had not been altered as regards the time of appearance of generalized lesions, but the much greater height of the curve for the vaccinated animals shows that the intensity of the reaction was profoundly affected as measured by the number of lesions which developed. There were three outstanding exceptions, however, to the synchronous time reaction of the vaccinated animals as compared with the controls. In the first experiment in which the most severe disease prevailed, the first generalized lesions appeared earlier in the vaccinated than in the control animals. In the second experiment many lesions developed in the vaccinated rabbits during the last 4 weeks of the observation period, while there were extremely few among the controls, and in the third, lesions were developing in the vaccinated animals when the period of observation was discontinued, although no lesions had appeared in the controls for the preceding 2 weeks.

Without analyzing these curves in detail, it is apparent that with a syphilitic disease of varying degrees of severity, it has been possible to increase the numerical distribution of generalized lesions and to prolong the period of active development of lesions over and beyond that of normal animals by means of an intracutaneous inoculation with vaccine virus made at the time of an intratesticular inoculation with *T. pallidum*. The general schedule of the times at which lesions first appeared, however, was not appreciably disturbed except in the experiment in which the disease of the control animals was mild, and this exception should be especially noted. The two curves of the second experiment are similar in their first portion, the peak of both occurring in the 60 day interval. The control curve then falls to the base line (72nd day) and practically continues there, while there are two high peaks in the vaccinated curve occurring in the 72nd and 84th day intervals, and at no time does it reach the base line. Furthermore, the second of these two peaks is higher than the first, showing that a greater number of lesions first appeared late in the course of the disease. Such an occurrence is unusual and indicates that earlier reactions were not sufficient to prevent the development of additional

and numerous late lesions; in the case of the control animals, as shown by the curve, an apparently similar early reaction was sufficient. In the third experiment, the second peak of the vaccinated curve closely follows the first, and apparently the reaction associated with such a shower of lesions was sufficient to prevent the later development of numerous lesions, although it should be noted that at its end, the curve rises abruptly to an appreciable height indicating that a number of generalized lesions were continuing to appear. In the first experiment, the curve of the vaccinated animals is a marked exaggeration of the control curve. It will be noted that the peak of the control curve in the 72nd day interval follows a long period (from the 40th day) in which many generalized lesions developed continuously at a fairly uniform rate and that there was not an early and relatively short period of marked eruptive activity which ordinarily would not be followed by successive periods of lesion development.

*Recovery.*—The last phase of the syphilitic reaction to be considered is that of complete resolution and healing of all lesions within the 3 months period of observation. Under ordinary circumstances, certain rabbits show no lesions by this time; in others residual lesions, usually of the genitalia, remain, while some animals continue to show active generalized manifestations. In the first and second experiments, the resolution of lesions was delayed, owing in one instance to the severity of the infection, and in the other to the late development of lesions and the consequent prolongation of the time of resolution and healing as reckoned from the day of inoculation. The condition of the lesions at the end of the observation period, therefore, has been described as follows: healed, not healed and active. The comparison of the vaccinated and control animals upon this basis gives the results, expressed in per cent, as shown on following page.

These figures show that the vaccinated animals differed markedly from the controls in the final phase of the syphilitic reaction, and furthermore, that the character of the difference was in accord with what was observed in earlier phases of the reaction. In the great majority of vaccinated animals, 89.5 per cent, the reaction to the syphilitic infection had not ceased at the end of the observation period, for lesions not only continued to be active, but as has already been mentioned, fresh lesions were developing in many animals. On the

other hand, only 26.7 per cent of the controls showed a comparable state of lesion activity, the majority of the animals having entered upon the phase of resolution.

*Final Condition of the Lesions.*

| Experiment | | Healed | Not healed | Active |
|---|---|---|---|---|
| | | *per cent* | *per cent* | *per cent* |
| I | Controls | 10.0 | 50.0 | 40.0 |
| | Vaccinated | | | 100.0 |
| II | Controls | | 60.0 | 40.0 |
| | Vaccinated | | 20.0 | 80.0 |
| III | Controls | | 100.0 | |
| | Vaccinated | | 11.2 | 88.8 |
| Mean values | Controls | 3.3 | 70.0 | 26.7 |
| | Vaccinated | | 10.5 | 89.5 |

At this point, something should be said of the general character of the lesions. It is well recognized that in a group of 5 or 10 rabbits, there is an individual animal variation as regards size, consistency and destructiveness of both genital and generalized lesions, which features are, of course, associated with the duration and degree of active development and persistence of lesions. In the present experiment, variations in these respects occurred in individual rabbits of both the control and the vaccinated groups, but in general, it may be said that both the genital and generalized lesions of the vaccinated animals were larger, more destructive and persisted longer than those of the controls. This difference was particularly striking in the case of scrotal chancres, and of bone and periosteal granulomata.

The disease which was observed in this investigation presented certain peculiar and uncommon features, especially of time reactions and of lesion type, which should be referred to, as they must be kept in mind in appraising the experimental results obtained. For example, the development of the metastatic orchitis was delayed while generalized lesions appeared unusually early, so that the two reactions occurred within a few days of each other instead of being separated by the usual interval of approximately 2 weeks. As a matter of fact,

in approximately one half of the total number of both the control and vaccinated animals, the appearance of generalized lesions preceded the detection of the metastatic orchitis by a few days, or both reactions were observed to occur on the same day as is shown in the following table:

*Appearance of Generalized Lesions in Relation to Metastatic Orchitis.*

| Controls | Earlier | Same day | Total |
|---|---|---|---|
| | *per cent* | *per cent* | *per cent* |
| I | 40.0 | 20.0 | 60.0 |
| II | 30.0 | 10.0 | 40.0 |
| III | 40.0 | 10.0 | 50.0 |
| Mean values | 36.7 | 13.3 | 50.0 |
| Vaccinated | | | |
| I | 20.0 | 20.0 | 40.0 |
| II | 20.0 | | 20.0 |
| III | 33.3 | 33.3 | 66.7 |
| Mean values | 31.6 | 21.1 | 52.7 |

These figures, as well as the time intervals between the development of the metastatic orchitis and the appearance of the first generalized lesions (Table II) indicate that an intracutaneous inoculation of vaccine virus coincident with the intratesticular inoculation of *T. pallidum* did not affect this peculiar feature of the disease.

The infection was further characterized by an unusually high preponderance of bone lesions and a relatively low number of skin lesions. Up to a certain point, such a distribution is to be expected, for numerous and severe cutaneous manifestations do not usually develop in animals in which previous bone involvement has been extensive. But this relationship does not obtain in cases of very severe or malignant syphilis which are characterized by numerous extensive and persistent lesions of both bone and skin and frequently of other tissues as well. In the first experiment, there were many instances of syphilis which could be called malignant judging from the character of genital and bone lesions, and in the second, there were several which approached this state, but nevertheless, there were comparatively few cutaneous manifestations, other than those of the

scrota which are not usually included among generalized or secondary lesions. This aspect of the infection is illustrated by the following table:

*Relation of Skin and Bone Lesions.*

| Experiment | | Total Relative No. | Bone Relative No. | Skin | |
|---|---|---|---|---|---|
| | | | | Relative No. | Per cent of total |
| I | Controls | 9.8 | 9.1 | 0.7 | 7.14 |
| | Vaccinated | 22.2 | 20.0 | 2.2 | 9.09 |
| II | Controls | 4.9 | 3.6 | 1.3 | 26.53 |
| | Vaccinated | 14.4 | 14.0 | 0.4 | 2.77 |
| III | Controls | 7.6 | 7.5 | 0.1 | 1.32 |
| | Vaccinated | 16.3 | 15.6 | 0.7 | 4.29 |
| Mean values | Controls | 7.4 | 6.7 | 0.7 | 9.44 |
| | Vaccinated | 17.4 | 16.4 | 1.0 | 5.75 |

The relatively large proportion of skin lesions in the control animals of the second experiment is apparently out of harmony with the findings in both groups of the first and third series. However, if the values for individual experiments are tabulated in the order of the severity of disease, the situation becomes somewhat clearer:

| Experiment | Controls | Vaccinated |
|---|---|---|
| | *per cent* | *per cent* |
| I | 7.14 | 9.09 |
| III | 1.32 | 4.29 |
| II | 26.53 | 2.77 |

This arrangement shows that for all vaccinated groups and for the first and third control groups, the proportion of skin to total secondary lesions follows a consistent numerical relation as regards the different levels of disease severity in the 3 experiments, and furthermore, that in the second experiment in which the infection was less marked than in the others, the proportion of skin to total lesions was not disturbed in the vaccinated animals. In the second control group, on the other hand, there was a relatively large number of cutaneous

manifestations but these were preceded by comparatively few bone lesions, a finding which was absent in the corresponding vaccinated group as is shown in the following table:

*Relative Number of Bone Lesions.*

| Experiment | Controls | Vaccinated |
|---|---|---|
| I | 9.1 | 20.0 |
| III | 7.5 | 15.6 |
| II | 3.6 | 14.0 |

It is evident from these figures that the numerical relationships of bone and skin lesions in the second vaccinated and control groups were in harmony with what is known of this phase of syphilitic infection, and the seeming contradiction of the second vaccinated group with respect to the number of skin lesions as compared with the corresponding control group is in reality in accord with the preservation of disease type and severity on the part of the vaccinated animals.

Still other evidence of the influence induced by vaccinal inoculation upon the course of the syphilitic disease is furnished by the distribution curves of generalized lesions (Text-fig. 2). The shape of the curves representing the control groups illustrate the general relations that obtain under ordinary conditions with respect to the numerical distribution of secondary lesions, that is, the early development of numerous lesions is not usually followed by the successive appearance of many others (see curves of Experiments II and III), while severe syphilis is characterized by the continued appearance of lesions for a prolonged period, a fact exemplified in the control group of the first experiment. The curves illustrating the distribution of lesions in all vaccinated groups show that this relationship was interfered with, for despite the large number of lesions that appeared early in the period of generalized manifestations, numerous lesions continued to develop in these animals.

It is evident from the results obtained in these experiments that intracutaneous inoculation with vaccine virus at the time of intratesticular inoculation of *T. pallidum* was associated with a profound disturbance in the syphilitic reaction, particularly as regards the rela-

tive frequency of occurrence of various reactive phenomena, the altered time relationships of these manifestations and in the relative failure on the part of one phenomenon to prevent or delay the development of successive manifestations. As measured by the various criteria selected for comparison, the efficiency of reaction of the vaccinated animals was unquestionably lowered with the result that the infection pursued a severe and uninterruped course.

The coexistence of two or even three and four various infections has been attributed to an "increased susceptibility" on the part of the patient consequent upon the severity of the primary disease and in attempts to explain the gravity of these cases, the rapidity with which the diseases follow one another has been frequently invoked. In the present experiments, the syphilitic and vaccinal inoculations were made simultaneously. It must be remembered though that the reaction to vaccine virus develops more promptly than the syphilitic reaction as judged from gross manifestations although it is known that changes do occur at the site of inoculation and probably elsewhere within a few days after the injection of *T. pallidum.*

The present results indicate, nevertheless, that vaccination did not bring about a state of "increased susceptibility" to the syphilitic infection, if one means by this term an increased liability to infection as indicated by a shortening of the incubation period of the primary lesion. But the findings amply demonstrate that it did markedly affect the syphilitic reaction, and since the effect was evident not only early but late in the disease, it is evident that the disorganization of the reaction was profound. The fact that it was evident for so long a time, as well as certain peculiar changes in the reaction such as the shortened time interval between successive phenomena, the marked increase in the number of secondary lesions and their general character suggest that the general alteration was of the nature of a decreased resistance on the part of the animals.

From knowledge already available, it is clear that the syphilitic reaction involves many factors that are essentially expressions of functional activity which, in some instances at least, appear to be related to changes in physical constitution. It is not unlikely that the differences brought out in the present experiments are the result of similar changes.

In conclusion, it may be pointed out that the study of experimental coexistent or concomitant infections is of value as a means of investigating the general problem of susceptibility and resistance to disease. Hitherto, this study has been confined almost entirely to individual diseases.

## SUMMARY.

Experiments are reported in which was studied the effect of a concomitant infection, vaccinia, upon the syphilitic reaction of rabbits. Vaccine virus was inoculated intracutaneously on the side of the body at the time of intratesticular inoculation with *Treponema pallidum*.

The results showed clearly that the vaccination caused a profound disturbance in the syphilitic reaction, the ensuing syphilis being extremely severe. From an analysis of various features of the reaction, it appeared that the factor of host resistance was primarily concerned in the effects observed.

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