STUDIES UPON EXPERIMENTAL MEASLES.

II. THE ENANThEMATOUS, EXANTHEMATOUS, PYREXIAL, AND LEUCOCYTIC SYNDROME PRODUCED IN THE RABBIT BY INTRAVENOUS INOCULATION OF BLOOD FROM CASES OF HUMAN MEASLES.

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In a previous paper we reported the successful propagation of the virus of measles in the guinea pig, the criteria of transmission being the constant occurrence of leucopenia, pyrexia, and nephritis following, after a definite incubation period, the intracardiac injection of blood from cases of human measles. For this host, however, no well defined rash, or exanthem, was noted. In the absence of any definite cutaneous reaction, the symptoms observed, although striking, did not permit of the conclusion that the identical clinical picture of the human infection had been reproduced. Therefore, the purpose of the present series of experiments was not only to determine whether the virus of measles could be propagated in the rabbit by direct transfer of infected blood from man, but also to ascertain whether in this animal the experimental infection would induce the characteristic syndrome of the human disease.

While we were engaged upon the experiments herein reported there appeared the observations of Nevin and Bittman upon the effect of the virus of measles on the rabbit. These authors record the appearance of enanthem, exanthem, and pyrexia in a great proportion of rabbits infected by the intracirculatory injection of

citrated blood from cases of human measles. Hardie\textsuperscript{4} also reports similar observations in which the rash appeared upon the shaved skin of rabbits on an average of 36 hours after the circulatory introduction of citrated blood from cases of measles.

**EXPERIMENTAL.**

Defibrinated human blood obtained from measles cases at the stage of temperature height was used for inoculation. The interval between collection of infected material and animal inoculation was never more than 1 hour, and during this intervening period the defibrinated blood was kept at a temperature of 37.5°C. Injections in rabbits were made into the marginal ear vein in a majority of cases, although in a few instances the intracirculatory introduction of infected material was accomplished by cardiopuncture.

**Experiment 1 (Preliminary).**—The divergence of opinion regarding the normal variations in temperature and number of circulating leucocytes in rabbits made it necessary for us to determine these factors in order to establish a basis for the experiments. Twelve healthy animals were isolated, and their rectal temperatures and leucocytic counts determined daily for a period of 37 days. Text-fig. 1 represents the normal variations observed.

The results show that the average temperature of the rabbits was 101°F. In the experimental animal of this species we regarded as pyrexia only the reading about 103°F. The white elements averaged 13,000. Leucocytic counts below 9,000 are considered as evidence of leucopenia.

**Experiment 2.**—Here the purpose was to determine whether the virus of human measles could be directly carried from man to rabbit, and could induce in this host the clinical picture of the human infection. Blood from a typical case of human measles in the eruptive stage of the disease was employed as the inoculation material. Twenty rabbits comprised the number used in the experiment, sixteen being inoculated and four held as controls. The inoculated animals each received 2 cc. of human defibrinated virus blood injected into the marginal ear vein. Of the control animals two were isolated and injected intravenously with 3 cc. of normal human blood collected under aseptic conditions; the remaining two were not injected but left in the cage with the inoculated animals with the view of determining whether the infection

\textsuperscript{4} Hardie, M., Essais de transmission expérimentale de la rougeole au lapin. Constatation d'un érythème sur la peau rasée, Compt. rend. Soc. biol., 1921, lxxxiv, 968.
could be transmitted through contact. Cultures made from the blood containing virus before inoculation remained sterile for ordinary bacteria. Of the sixteen rabbits receiving the blood from cases of measles, seven showed, after a period of approximately 5 days, reactions indicative of specific infection. There was a sharp rise in temperature of 2-3°F., beginning on or about the 5th day after inoculation in the majority of animals, with a coincident fall in the total number of circulating leucocytes. In some rabbits the rise in temperature did not appear before the 10th day. As a rule, the temperature remained high with slight variations for 2 to 4 days. During the pyrexial stage four animals developed an exanthem in the form of a dif-

**TEXT-Fig. 1.** Composite temperature and leucocytic charts of twelve normal rabbits.

**TEXT-Fig. 2.** Typical reaction in the rabbit to blood containing the virus of measles.
fuse macular rash followed by the appearance of discrete papules over the neck and chest. At about the same time there were noted several sharply defined smaller erythematous patches on the buccal mucous membrane, resembling in the main the so called Koplik spots. The maculopapular eruption began to fade after 48 hours though it remained visible for 72 hours as a brownish discoloration. Branny or scaly desquamation invariably followed the rash. While only four of the seven reacting animals of this series presented a well defined rash, the leucocytic and temperature reactions in the remaining three were of sufficient significance to lead to the conclusion that the virus of human measles was capable of infecting the rabbit without giving rise to the cutaneous lesion. On the other hand, the appearance of an exanthem in the inoculated animal, coincident with the prominent and constant signs of pyrexia, leucopenia, coryza, etc., permits of the conclusion that the rabbit affords an experimental host in which the virus of measles produces the same clinical picture as observed in man. Text-fig. 2 graphically represents the typical reaction induced in the rabbit.

Experiment 3.—In order to determine the effects of reinoculation upon previously reacting animals, four rabbits of Experiment 2 which had shown typical exanthemata, leucocytic and pyrexial reactions were reinoculated, after complete recovery, with 2 cc. of blood from human and guinea pig measles cases. Two animals were used for the human virus, and two for the guinea pig virus, the blood containing the virus in both instances being obtained at the height of the reaction. In none of these animals were there noted any further changes in the temperature or cell count, nor the appearance of exanthemata. This would seem to prove that infection establishes an immunity (Text-fig. 3).

Experiment 4.—The purpose of this experiment was to continue the propagation of the virus by passage from rabbit to rabbit with the view also of increasing its virulence through repeated passage. Blood from animals reacting to the direct transfer of human measles virus was injected intravenously in 1 cc. quantities into twelve rabbits. In the animals of the second series the reaction was identical with that seen in the direct transfer animals. The proportion of reactions, however, was greater than with those of Experiment 2. Eleven of the rabbits reacted, nine of them showing exanthemata and enanthemata. After an incubation period of 3 to 5 days there occurred a rather abrupt rise in temperature of 1-4°F. and a coincident slight fall in the leucocytes. The temperature elevation continued usually for 24 to 36 hours in the majority of the animals; in the others it lasted with remission for 3 to 4 days. In the latter the leucocytes always showed the greatest decrease (well marked leucopenia). Furthermore, it may be said that the exanthem occurred in the animals with the more pronounced symptoms. In all, eight transfers from rabbit to rabbit were carried out, thus transmitting the virus through the eighth generation. In this experiment it is noteworthy that a number of rabbits died presumably as result of the virus, death occurring more frequently in animals of the later transfers. Such animals were visibly sick during the period of maximum reaction. Autopsies revealed grave nephritic changes, not unlike those reported by us for the measles-infected
reacted to a previous infection.

TEXT-FIG. 3. Effects of inoculation, with blood containing the virus of measles, of a rabbit which had
The kidneys were swollen, cloudy, and often infiltrated with hemorrhages. No noteworthy lesions were demonstrable in other internal organs, other than that common for toxemia. In no instance was pneumonia observed, nor were the ordinary pathogenic bacteria ever isolated from the heart's blood or internal organs of these fatal cases. This is of unusual interest because in fatal cases of human measles, pneumonia and septicemia almost invariably occupy the clinical field at death. While mortality is attributed in the human to secondary invaders, these seem to play no part in the experimental disease for rabbits and guinea pigs, the virus being capable per se of destroying the host. Text-fig. 4 expresses graphically the results of this experiment.

Text-fig. 4. The continued reaction in the rabbit through successive generations, and the cross-reaction through the rabbit and guinea pig.

Experiment 5.—Since it was found that the rabbit as well as the guinea pig reacts in a characteristic manner to direct intravenous injections of human blood from cases of measles a series of experiments was carried out to determine what effects would be induced by transmission of the virus from rabbit to guinea pig and vice versa. Accordingly, one set of twelve guinea pigs received intracardially 1 cc. of fresh defibrinated rabbit blood obtained from an infected animal at the height of the reaction. Another set of twelve rabbits was inoculated intravenously with 1 cc. of fresh defibrinated blood secured from an infected guinea pig at the time of maximum pyrexia. Typical reactions were secured in 75 per cent of the cross-inoculated guinea pigs and in ten of the twelve rabbits.
DISCUSSION AND SUMMARY.

An active transmissible virus exists in the blood of measles patients during the eruptive stage of the disease. This virus produces in rabbits after intravenous injection a specific reaction analogous in all essential features to that of the human infection. Following a definite incubation period of from 2 to 5 days the animals infected show pyrexial, leucocytic, and cutaneous alterations. Fully 90 per cent of such inoculated rabbits react in a remarkable manner. The earliest constant symptom of the infection is a rise in temperature, which on the average occurs 4 days after inoculation and most probably marks the end of the incubation period. Concomitantly with this temperature rise there is a diminution in the total number of circulating leucocytes. This decrease in the number of white blood elements may be relative or may appear in the form of a well defined leucopenia. The most striking objective signs are the coryza, conjunctival injection, exanthemata, and exanthemata. The mucous membrane lesions are similar in their physical appearance to the so called Koplik spots seen in man. They occur on the buccal side of the oral cavity ranging in number from two to eight discrete hemorrhagic areas with paler centers. They appear as a rule coincidentally with the temperature rise or shortly thereafter. The exanthematous lesions though occurring only in about 40 per cent of the infected animals complete the clinical syndrome in this particular experimental host. The rash may appear as early as the 3rd and as late as the 7th day after inoculation. In its early stage it is of the macular variety, appearing as a diffuse eruption which later develops into a more papular type of lesion. At this time the cutaneous manifestations appear as slightly raised, flattened, purplish red, discrete areas in the skin of the face, neck, chest, and abdomen.

Repeated passage of the virus of measles through the rabbit seems to increase its virulence. A number of animals infected with such passage virus succumb in the fourth and subsequent generations, undoubtedly as the direct result of the action of the specific excitant, as in none of the animals was there cultural evidence of secondary intercurrent infection. In the animals dying presumably as a result of the specific virus grave nephritic changes were evident. It is a
noteworthy fact that the pneumonia so common in fatal cases of human measles was not evident in any of the experimental animals. We believe this to be of considerable significance, especially in elucidating the direct etiological factor of the fatal pneumonias so often present in human measles cases. Apparently such infections in man can be explained purely on the basis of the destruction of normal defense barriers by the specific excitant of the infectious disease, and the lack of host resistance to the ordinary pyogenic microorganism.