AN EXPERIMENTAL STUDY OF MEASLES IN MONKEYS.*

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The first report of the production of experimental measles dates back to 1852 when Mayr was able to prove the infectivity of the buccal and nasal secretions in man, producing measles in human subjects. In 1898 Chavigny reported the appearance of what he thought was measles in a monkey, which had been in close contact with his keeper during the early stages of an attack of measles.

Josias in 1898, stimulated by this report, allowed monkeys to play about a measles ward for a period of six months, but was never able to detect any signs of measles in the monkeys. Apparently, this negative attempt delayed further animal experimentations until 1905, when Hektoen produced measles in man by injecting blood from a human case early in the eruptive stage. In 1910 Anderson and Goldberger attempted the production of measles in monkeys by the intra-peritoneal injection of blood from a case of measles, getting what at that time they thought negative results, though upon later analysis they concluded that one of their monkeys had shown a characteristic rise in temperature on the eleventh day. Their second attempt was also apparently negative except for a rise in temperature. Their third attempt succeeded somewhat better since one of their monkeys besides the temperature rise showed signs of a sparse papular eruption on the face, brow and chin, with a diffuse erythema of the brow and lids; five days later a browny scaling was noted. Their fourth case, however, proved beyond a doubt that the blood from a case at the end of about fourteen to twenty

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hours after the appearance of the skin eruption could produce experimental measles in the monkey. They have since carried on the virus from this case through six generations of monkeys, showing that virus can retain its pathogenicity for a considerable length of time when grown exclusively in monkeys. They were unable to grow the virus on artificial media. Their inoculations were given intravenously, intracerebrally, and intraperitoneally with about equal success. Their positive evidence of measles consisted in a more or less definite rise of temperature four to seven days after inoculation, coughing, sneezing, and the appearance of a more or less typical papular rash.

Hektoen and Eggers have recently repeated the research of Anderson and Goldberger on experimental measles in the monkey and have made a special study of the leucocytes. They conclude that "Macacus rhesus is susceptible to a mild kind of measles if injected with the virus of human measles present in the blood . . ." and that "the leucocytes appear to behave very much as they do in human measles; that is to say, that preceded by a more or less distinct leucocytosis there appears a leukopenia of variable degree in what would correspond in a general way to the latter part of the pre-eruptive and the early part of the eruptive periods." They add: "In our animals this leucopenia involved principally the neutrophils, the lymphocytes being relatively somewhat increased."

Our experiments were undertaken to study further the production of measles in the monkey, especially with reference to the blood picture changes. We obtained our blood from a case of beginning measles, about six hours before the appearance of the skin eruption and about thirty-six hours after the initial rise in temperature, at a time when the child was known to have been exposed to measles in the orthopedic ward of the Children's Hospital. Blood was withdrawn from one of the veins at the elbow under strict sterile precautions and within an hour thereafter was injected as follows into two monkeys: No. 112 received two cubic

centimeters of clear blood serum from the clot intracerebrally and three cubic centimeters intraperitoneally, and No. 113 received five cubic centimeters of serum plus blood cells obtained by vigorously shaking the tube containing the original blood specimen, two cubic centimeters of this blood cell serum suspension being injected intracerebrally and three cubic centimeters intraperitoneally.

The first signs of illness occurred in monkey No. 112 towards the end of the sixth day and consisted of listlessness, shivering, and hurried respirations unaccompanied by any appreciable rise in temperature. At the close of the tenth day both monkeys showed characteristic evidence of measles in the form of definite Koplik spots on the buccal mucous membranes. These spots in one monkey we were fortunate enough to have verified by Dr. English from the South Department of the Boston City Hospital. Late on the ninth day after inoculation and at a time when monkey No. 112 was decidedly sick we drew blood and inoculated monkey No. 115 with two cubic centimeters of the serum intracerebrally and three cubic centimeters subcutaneously. This monkey showed a similar periodicity of symptoms, Koplik spots appearing as in the first two monkeys on the tenth day. More than forty-eight hours after the appearance of the Koplik spots, monkey No. 113 was bled and from his blood serum No. 116 was inoculated intracerebrally with two cubic centimeters of the serum and subcutaneously with three cubic centimeters more. This monkey gave no certain evidence of having contracted measles.

All of the monkeys successfully inoculated showed at some time during the acute stage of the disease a transient erythema of the face and forehead but never any more characteristic eruption. The blood picture, however, taken in conjunction with the appearance of the Koplik spots and the invariable absence of both leucopenia and Koplik spots in the monkeys examined from time to time as controls proves without doubt that our monkeys were successfully inoculated with measles virus.

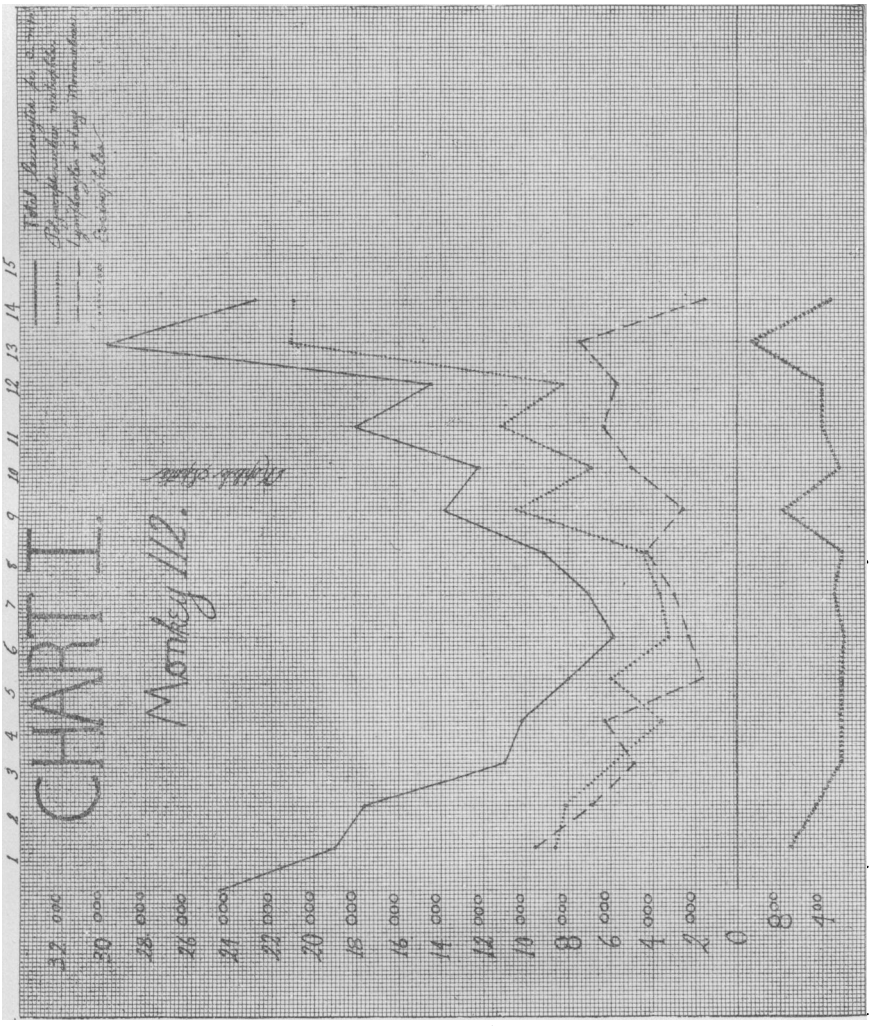
The blood used in making our counts was obtained by

puncture of the marginal vein of the ear. The leucocyte count was made immediately and cover-glass smears were prepared for further study at our convenience. Except on Saturdays and Sundays, when the hour was considerably earlier, blood was drawn each afternoon at approximately 6 P.M.

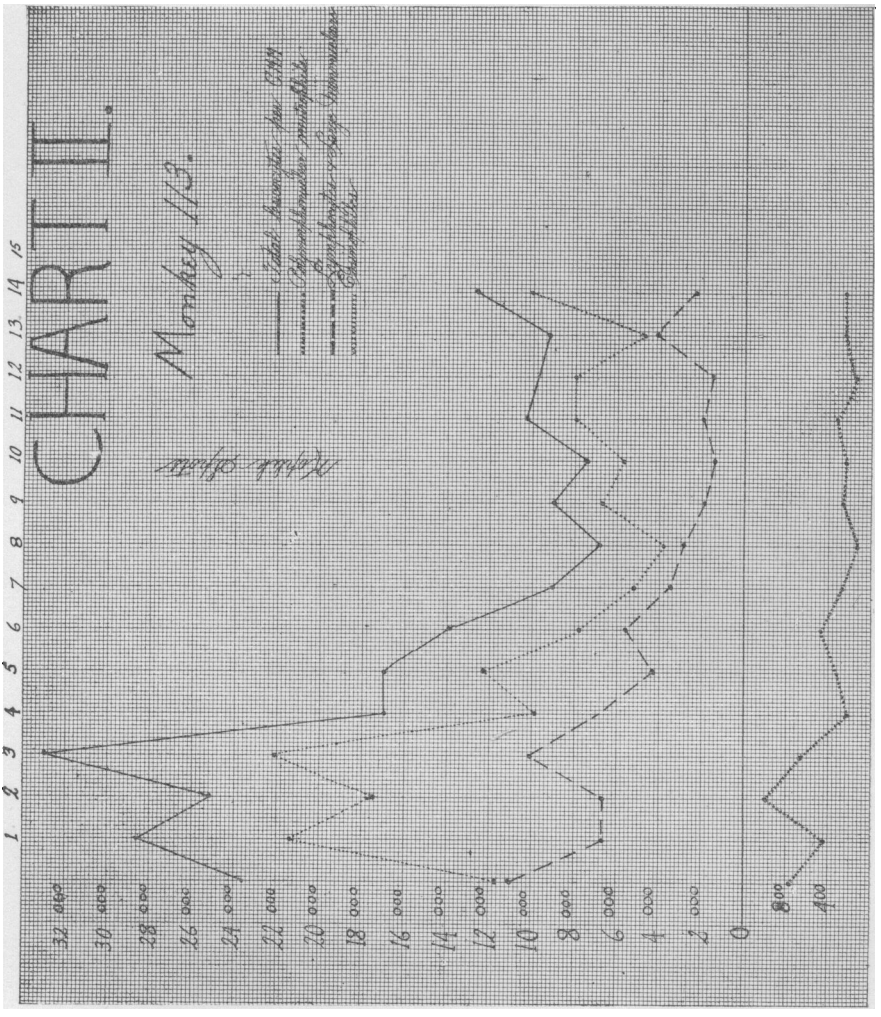
For differential counts Wright's modification of Leishmann's blood stain was used and five hundred or more cells counted. As pointed out by Hektoen and Eggers, differentiation between large lymphocytes and large mononuclear leucocytes is not always easy in the monkey's blood and we attempted to classify with large mononuclear leucocytes all those lymphocytes which exceeded in diameter twice that of a red corpuscle.

This arbitrary rule of division proved unsatisfactory, consequently in our discussion we group together large mononuclear leucocytes and lymphocytes both large and small. To simplify our results as much as possible we have reduced all percentages to number of corpuscles per cubic millimeter and to facilitate comparison we have treated in like fashion the tables given by Hektoen and Eggers from their two most typical cases. These tabulations are presented graphically in the form of charts.

Unfortunately the temperature varied so much in all our monkeys that whatever temperature reaction may have occurred is not manifest. However, in the three monkeys which we succeeded in inoculating with measles, Koplik spots were found in considerable numbers toward the close of the tenth day. We observed no maculopapular eruption, though conjunctivitis occurred in one monkey on the day following



etherization. In every instance the number of leucocytes fell rapidly, either from the first day, as in monkey No. 112, or after a transient leucocytosis, as in monkeys Nos. 113 and



115. The two charts which we have prepared from Hektoen and Eggers' tables show more strikingly than do our tables the mononuclear character of the early leucocytosis, though in one of our monkeys (No. 115) we got an identical

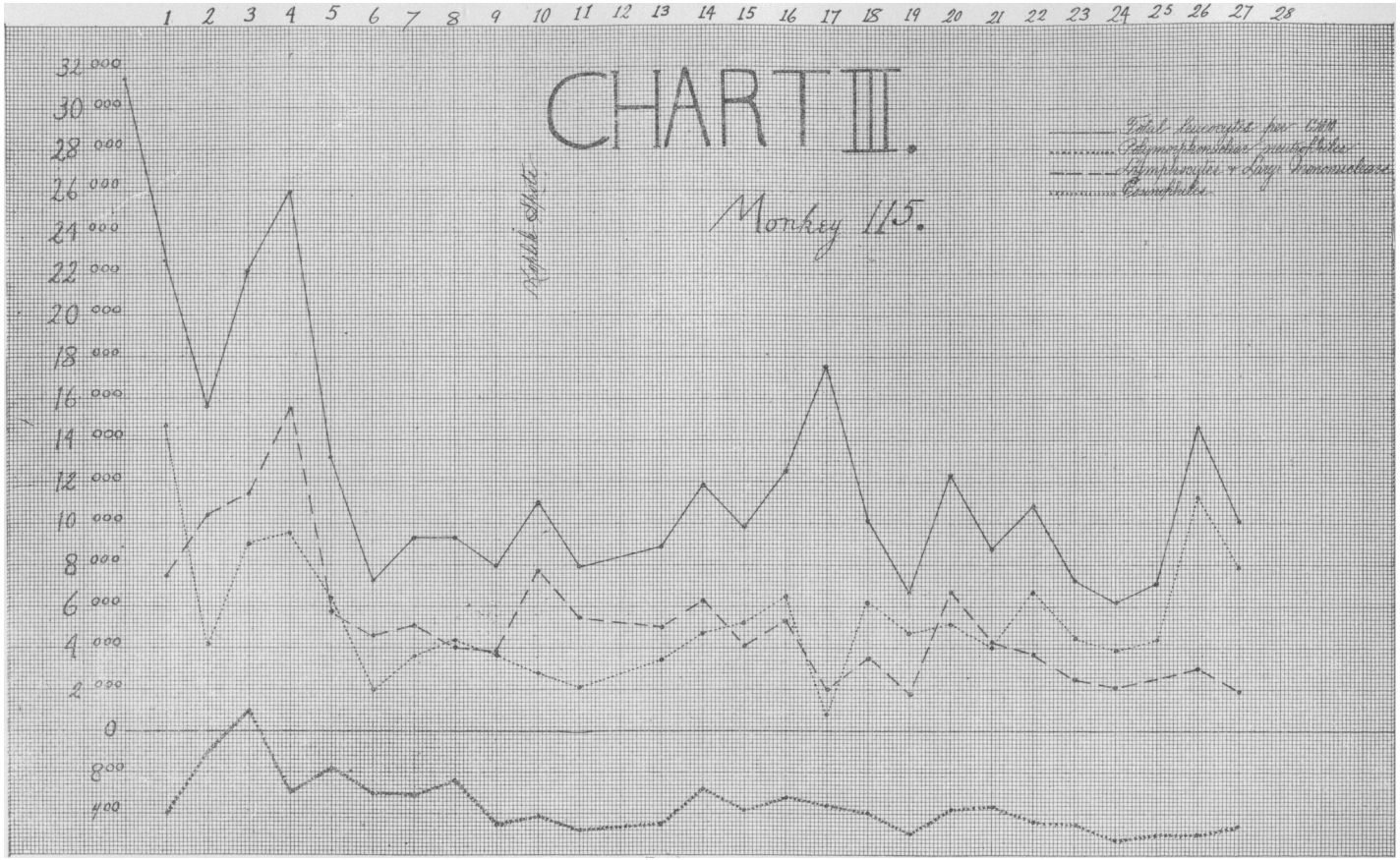
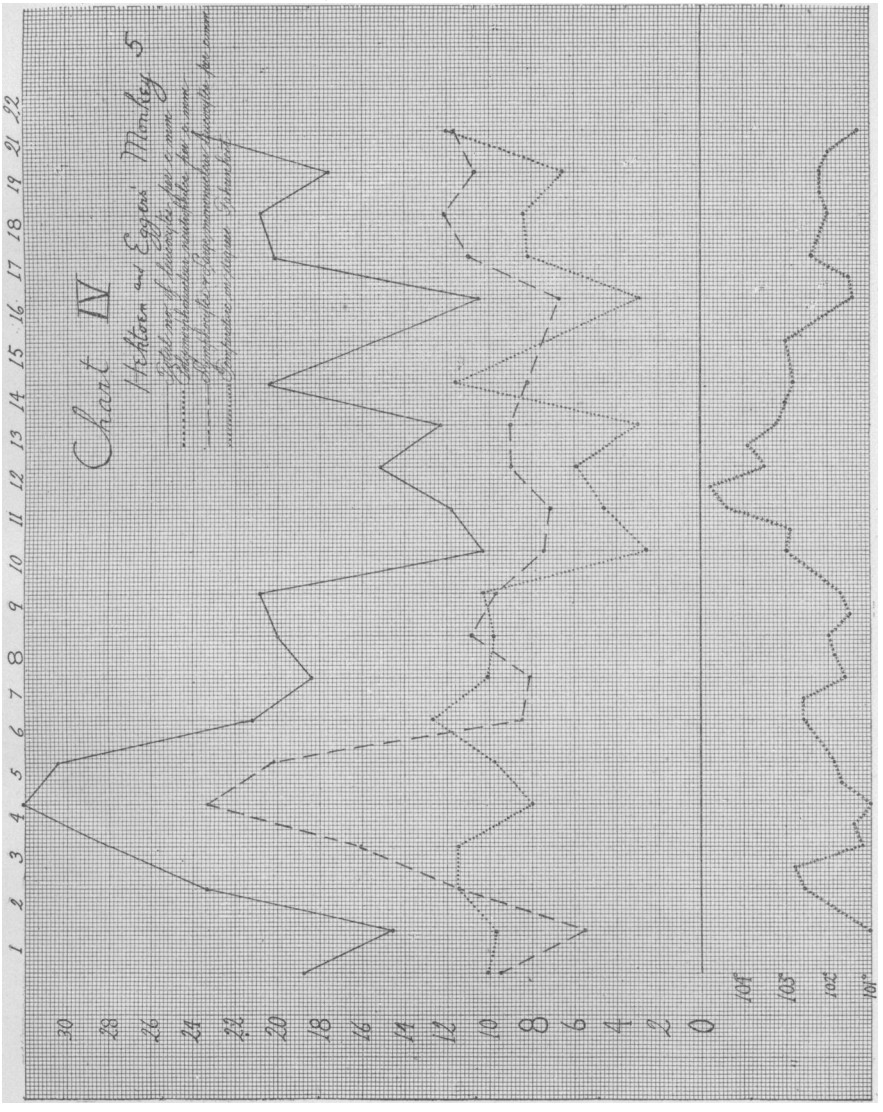


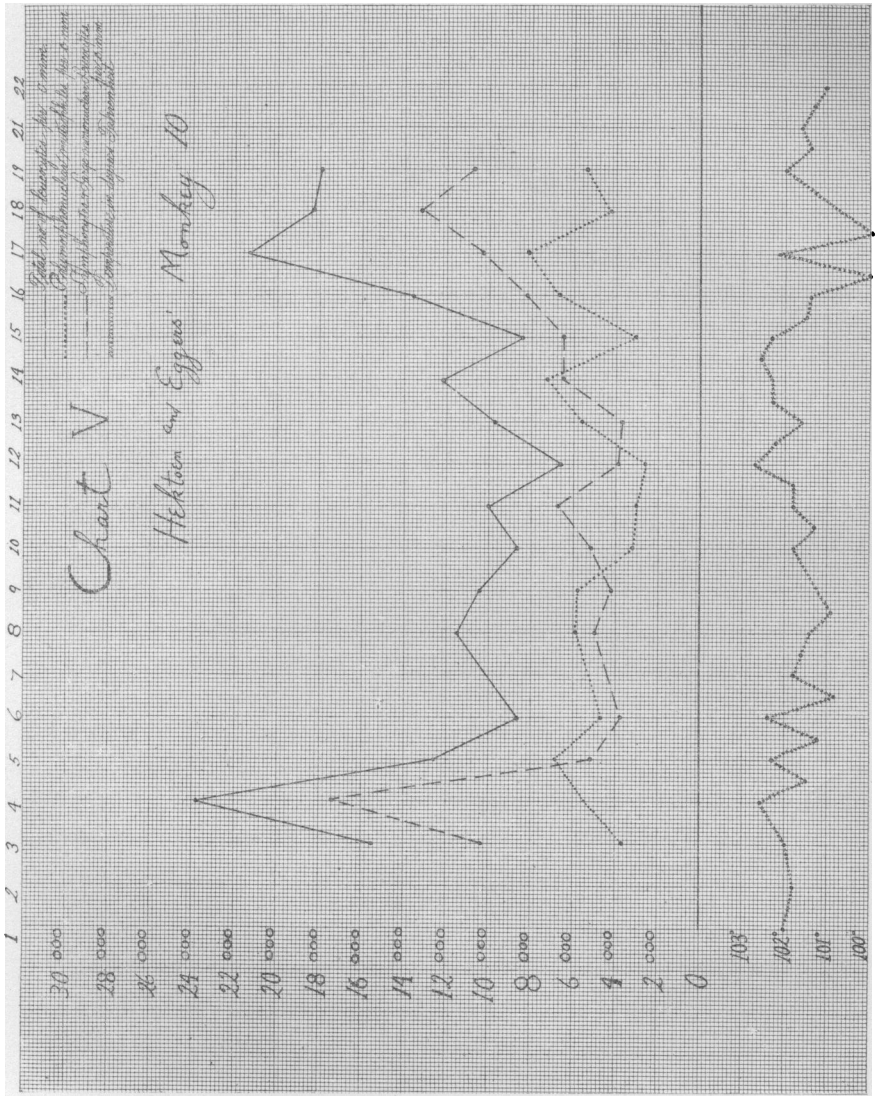
CHART III.
Monkey 115.
Total leucocytes per c.mm.
Polymorphonuclear neutrophiles
Lymphocytes + Large Mononuclears
Eosinophiles
Days after Inoculation
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
32 000
30 000
28 000
26 000
24 000
22 000
20 000
18 000
16 000
14 000
12 000
10 000
8 000
6 000
4 000
2 000
0
800
400

reaction. It is worthy of note that in our other two monkeys we had a slight increase in the number of lymphocytes and large mononuclear leucocytes at the end of the third day, which corresponds exactly in time with the reaction just described, although the picture is obscured in the one instance (monkey No. 113) by a marked increase in the number of polymorphonuclear neutrophils, and in the other (monkey No. 112) by a coincident fall in the total number of leucocytes.

No final explanation can be offered for these variations in the character of the reaction, but it should be noted that certain differences in technic and experimental conditions did occur. In the first place we used blood serum to inoculate monkeys Nos. 112 and 115 instead of the whole blood, and injected five instead of three cubic centimeters. In monkey No. 113, on the contrary, five cubic centimeters of unwashed corpuscles mixed with serum was the material used to carry the infection. Moreover, the blood with which Hektoen and Eggers inoculated their monkey No. 5



(Chart IV.) was drawn thirty hours after the appearance of the skin eruption, and that with which they inoculated their monkey No. 10 (Chart V.) six hours after the appearance of the eruption, while the blood with which our first two monkeys were inoculated was taken from a case of severe onset and obtained six hours before the appearance of the



skin eruption. Monkey No. 115 was inoculated with blood drawn from our monkey No. 112 on the day preceding the appearance of Koplik spots. It may well be, therefore, owing to the larger volume of material used for inoculation

and the earlier stage of the disease at which our blood was drawn, that our blood pictures represent a more severe reaction. This assumption is rendered more plausible by the fact that in monkey No. 10, which Hektoen and Eggers inoculated with blood drawn at a stage twenty-four hours earlier than was the case with their monkey No. 5, the early mononuclear leucocytosis was less marked and the leucopenia developed earlier and was more severe, so that a lower count was obtained in monkey No. 10 after six days than in monkey No. 5 after ten days.

That Hektoen and Eggers err in supposing that the leucopenia involves principally the neutrophils is shown, we believe, by a careful analysis of the five charts. To be sure the neutrophils suffer more severely in Hektoen and Eggers' monkey No. 5 (Chart IV.), as is the case in our monkey No. 115 (Chart III.), but even here the leucopenia seems to involve all the white corpuscles with the possible exception of the eosinophiles and mast cells, concerning the variations of which no conclusions can be drawn from our work. In our monkeys Nos. 112 and 113 (Charts I. and II.) the lymphocytes and large mononuclear leucocytes seem if anything relatively fewer than the neutrophils, while in Hektoen and Eggers' monkey No. 10 (Chart V.) the curve is approximately midway between the two extremes.

No red corpuscle counts were made but the smears showed evidence of but trifling anemia and this only late in the course of the disease. Once in a while a normoblast was noted but these were always rare.

The later blood pictures, which correspond with the eruptive and post-eruptive stages of the disease, are not included in our discussion since it seems probable that in all instances this part of the curve was complicated in our monkeys by an intercurrent infection the exact nature of which is unknown. After the death of our first two monkeys a number of the uninoculated monkeys became ill and several died. Only one of these had a leucocyte count under twenty thousand and in no instance were Koplik spots present. Autopsy failed to reveal the cause of death.

CONCLUSIONS.

We conclude from these experiments:

1st. That measles can be experimentally reproduced in Macacus rhesus and that so reproduced it is a disease of definite incubation period.

2d. That besides the fever, conjunctivitis, rhinitis, and skin eruptions described by Anderson and Goldberger it is characterized by Koplik spots (to which we believe we are the first to call attention in the monkey) and by a typical blood picture at least during the pre-eruptive stage.

3d. That the virus of measles is present in the blood serum at some time exceeding twenty-four hours before the appearance of the Koplik spots and persists until more than thirty-six hours after the appearance of the skin eruption.

4th. That during the pre-eruptive stage of the disease there is a leucopenia involving the polymorphonuclear neutrophils, the lymphocytes and the large mononuclear leucocytes. This leucopenia develops in from five to ten days after inoculation and may be preceded by a transient lymphocytic and large mononuclear leucocytosis, which is probably lacking or only poorly developed in the severe form of the reaction but is strongly developed in less severe cases.

Monkey No. 112:

Monkey No. 112, injected with human serum as described above, showed an immediate and rapid fall in the number of leucocytes which reached their minimum at the end of the sixth day. Both polymorphonuclear neutrophils and mononuclear leucocytes (including lymphocytes) participated about equally in this reaction. On the sixth day the monkey seemed listless, chilly and respiration was hurried, but on the day following all evidences of illness had disappeared. From the sixth to the ninth day there was a slight relative increase in the number of lymphocytes and large mononuclear leucocytes. From the ninth day there was more fluctuation in the number of polymorphonuclear neutrophils than of lymphocytes and mononuclear leucocytes, the neutrophils being relatively numerous. At the end of nine days the monkey again seemed ill and ten cubic centimeters of blood were drawn under ether from the saphenous vein and used to inject monkey No. 115. On the next day Koplik spots were observed, but otherwise the animal seemed again normal. On the

fourteenth day the monkey became suddenly very ill and died before next morning. Autopsy by Dr. Boretti failed to find any cause to explain the death.

Monkey No. 113:

Monkey No. 113, injected with corpuscles and serum from the human measles blood as described, showed an initial polymorphonuclear leucocytosis followed by a leucopenia which affected polymorphonuclear neutrophils and mononuclear leucocytes (including lymphocytes) to about the same extent. The neutrophils and the total number of leucocytes both reached their minimum at the end of the eighth day, but the lymphocytes and mononuclear leucocytes continued to fall until the end of the tenth day when Koplik spots were first observed. On the twelfth day ten cubic centimeters of blood were drawn under ether from the saphenous vein and used to inject monkey No. 116. On the following day our monkey seemed sick, was listless and chilly, had a marked conjunctivitis and erythema of the face and forehead. He was much the worse on the following day and died before the next morning. Autopsy by Dr. Boretti did not find any cause to explain the death.

Monkey No. 115:

Monkey No. 115, injected with serum from monkey No. 112 as already described, showed an immediate fall in the number of leucocytes, especially the neutrophils, followed by a transient leucocytosis in which the lymphocytes and mononuclear leucocytes predominated. The total of leucocytes, the polymorphonuclear neutrophils and the number of lymphocytes and large mononuclear leucocytes all reached their minimum towards the end of the sixth day. Between the eighth and tenth days there was a slight increase in the number of lymphocytes and mononuclear leucocytes accompanied by a slight fall in the number of neutrophils. On the evening of the tenth day Koplik spots were observed in considerable numbers and persisted until the end of the eighteenth day. The blood picture after the tenth day was not significant. The neutrophil leucocytosis occurring on the seventeenth day was probably due to slight sepsis of one ear. Death occurred on the twenty-eighth day. Autopsy failed to reveal the cause of death.

Monkey No. 116:

Monkey No. 116, injected with serum from monkey No. 113, died on the eighth day. The blood picture was in no way significant. There was an initial transient leucocytosis followed by a gradual leucopenia, the decline beginning with the third day and the lymphocytes and mononuclear leucocytes falling more rapidly than the polymorphonuclear neutrophils. The lowest leucocyte count obtained was 11,000 three hours before death. There is no conclusive evidence that this monkey contracted measles.

Cause of death is unknown.

[We wish to thank Dr. E. H. Bradford for permission to work in his ward and to express our deep indebtedness to Dr. M. J. English for examining one of our monkeys and verifying the appearance of Koplik spots.

To Dr. A. F. Boretti we are indebted for complete autopsies on two monkeys.]

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