A REVIEW OF THE INVESTIGATIONS CONCERNING THE ETIOLOGY OF MEASLES

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INTRODUCTION

The etiology of measles and, indeed of all the exanthemata of man, constitutes one of the perplexing problems in medicine. At one time, the somewhat discouraging impression prevailed that these infections are probably caused by a type of parasite wholly unlike any of the pathogenic micro-organisms with which we are familiar; on the other hand, an opinion, decidedly too hopeful, has sometimes been expressed that the discovery of the etiologic agent of one of the acute exanthemata would lead to the clearing up of the entire group. A little reflection shows that this latter view is rather sweeping. The underlying pathological processes of the various exanthematous lesions are often dissimilar and micro-organisms as diverse as (1) *Treponema pallidum*, (2) *Rikettsia prowazeki*, and (3) *B. typhosus*, are each capable of producing a well marked skin rash.

In the investigation of infectious diseases of unknown origin, the clinical features frequently suggest the general group of parasites in which the etiologic agent would most likely be found. In the study of measles, it is important to note its mode of transmission, the portal of entry of the virus, the self-limited course of the infection and the resulting immunity. Occasionally, investigators have described protozoan-like bodies as the causative agent of the disease, apparently disregarding the consideration that typical protozoa are not transmitted by droplet infection and usually produce a more or less chronic type of disease which is not followed by a sterilizing immunity. It would be extremely surprising if the etiologic agent of measles should prove to be a protozoan parasite.

In reviewing the experimental work concerning measles, three general subjects will be considered, namely (1) the histologic pathology of the specific lesions, (2) the artificial inoculation of the disease in man and lower animals, and (3) cultural studies on artificial media. These procedures represent the underlying principles upon which the study of an unknown virus is usually undertaken. Various investigators have studied these features rather extensively in measles with results that are sometimes difficult of interpretation, but which possess considerable interest. The divergent observations described in the literature will often necessitate the introduction of considerable detail in this review in order to bring these conflicting data into harmony as far as possible.

HISTOLOGICAL INVESTIGATIONS

The chief interest in the microscopic examination of the lesions of measles in the skin and mucous membrane lies in the search for the specific etiologic agent. Since the portal of entry and the primary lesions of measles occur in the mucous membrane of the respiratory tract, it seems reasonable to assume that the Koplik spots are produced by the specific organism of the disease *per se*. The clinical features indicate that the exanthem is caused by the presence of a specific microörganism and not solely by a toxin. The generalized rashes caused by agents in solution, such as drugs and sera, make their appearance irregularly over the surface of the body. Areas that are widely separated may become involved simultaneously. In measles there is a definite and rather gradual progress of the rash from the uppermost parts of the body downward over the trunk, arms, and lower extremities.

Careful microscopic examinations for parasitic organisms has revealed very little, either in the Koplik spots of the mucous membrane or in the skin lesions. In the endothelial cells of the capillaries running through the skin lesions and also in Koplik spots, Mallory and Medlar (1) found coccoid bodies of variable size occurring, in the cytoplasm, either singly or in pairs. These bodies stained positively by Gram's method. Their exact nature could not be determined. The authors considered it unlikely that these bodies could

be the remains of phagocyted and digested leucocytes. Neither did it seem probable that they were retrograde changes since these cells tended to proliferate rather than degenerate. These bodies in some respects were similar to enlarged centrosomes but the authors considered that they were probably cocci in various stages of dissolution. They were found in the digestive vacuoles in the cytoplasm and they did not occur in the blood vessels in unaffected tissues of the body. Nothing resembling these bodies could be found in control examinations of a variety of other skin lesions.

A thorough examination of fresh and stained blood smears for parasites was made by Mallory and Medlar in 60 instances but with negative results. In 15 cases, examinations were made every four hours beginning twenty-four to forty-eight hours before the eruption started and continuing twenty-four to thirty-six hours after its appearance. This failure to recognize microörganisms does not by any means preclude their presence in the skin lesions. One need only recall the difficulty not infrequently encountered in demonstrating microscopically in tissues such organisms as *B. tuberculosis* or *T. pallidum.*

The histological pathology of the lesions of measles becomes of direct interest in view of its possible bearing upon the diagnosis of experimental measles in lower animals.

The older writers considered that the roseolae of measles, developed principally around the sebaceous glands, the sweat glands, and the hair follicles. It is definitely shown by Mallory and Medlar that the lesions commence around the capillaries and venules in the skin, starting at a given point and spreading along these vessels. Incidentally the glands and hair follicles become secondarily involved. Their findings may be stated very briefly as follows:

> The essential cellular reaction of the skin lesions in measles may be summed up as consisting of a proliferation of vascular endothelial cells, of an emigration of endothelial leucocytes and of a rapid proliferation of them in the tissues surrounding the vessels. The endothelial cells become swollen and finely granular and show occasional mitoses. The endothelial leucocytes are young and active, showing numerous mitoses. They are often larger than normal and they phagocyte and digest the occasional polymorphonuclear leucocytes which wander into the infiltrated area.

Epithelial structures, adjacent to the lesions become infiltrated with endothelial leucocytes. In all early lesions, collections of exudate were noted in the skin beneath the cornified layer, containing serum, fibrin and endothelial leucocytes. No evidence was found of any necrotic lesions such as the pyogenic cocci produce. Very little diapedesis of red cells was observed.

The authors considered that these findings signify that the micro-organisms of measles is phagocyted by the endothelial cells of the capillaries and venules of the skin and mucous membrane, producing an inflammatory reaction in the immediate neighborhood.

It is significant that, in an excised typhoid rose spot, the authors report "a cellular reaction like that of measles, but rather more abundant."

Ewing (2) in 1909, in describing the lesions of measles, noted the mitotic figures in the endothelium and the extensive infiltration of the lesions of the mucous membrane and of the skin with large mononuclear leucocytes. No single type of cellular change was found constantly. Indeed, some peculiar changes observed in a case of hemorrhagic measles were duplicated almost exactly in a case of *pityriasis rosea*. Ewing considered that, from the histological picture alone exclusive of clinical considerations, one might think that the diagnosis of measles included more than one disease. He surmised that measles is caused by an actively multiplying microörganism, of the class of bacteria, producing an active toxin having a special affinity for superficial epithelial cells.

Field (3) in 1905, studied some of the supposedly protozoan micro-organisms which had been described in measles. He concluded that these bodies were not protozoa, but that they were probably degenerating and cytolyzed epithelial cells and leucocytes, which within certain limits are specific for measles and scarlet fever.

The significant and at the same time unfortunate feature of the preceding observations lies in the fact that no pathognomonic cellular reaction in measles has as yet been clearly established. Therefore in studying sections of doubtful rashes such as may occur in experimental animals, one may determine whether the lesions are consistent with measles but an absolute diagnosis can not be established solely from the histological picture.

ARTIFICIAL TRANSMISSION

Inoculation of man. The early experiments on the artificial infection of man with measles were unfortunately conducted without adequate precautions for avoiding the possibility of accidental infection. Hektoen (4) has compiled a thorough review of this early work.

In the experimental study of measles, it becomes of the utmost value to the investigator to know whether the virus is present in the blood stream. From the pathology of the disease, it is self-evident that the causative organism is not invasive to the extent of setting up lesions of the viscera. The decidedly mechanical nature of the progress of the exanthem over the body at once raises doubt about the presence of the virus in the circulating blood.

In 1905, Hektoen (5) conducted the first modern work under carefully controlled conditions upon the experimental inoculation of measles in man. Two volunteers, injected with blood of measles patients, developed symptoms of measles after an incubation period of ten to twelve days. The details of this experiment are very important. The first subject was inoculated with a specimen of blood taken from a patient about six hours after the first appearance of the rash. In order to detect any secondary invaders, the blood (3 cc.) was incubated in ascitic broth (50 cc.). After twenty-four hours there was no gross or microscopic evidence of any microorganisms and the first volunteer was injected subcutaneously with 4 cc. of this blood, diluted in broth. The quantity of patient's serum injected was approximately 0.1 cc. No local symptoms appeared at the site of injection. Twelve days later, the temperature commenced to rise, reaching 104°F. on the fourteenth day. During the morning of the fourteenth day, a red papular eruption appeared on the forehead and spread over the greater part of the body in about five hours. During convalescence a branny desquamation appeared. There were no catarrhal symptoms and no definite evidence of malaise.

The blood for the inoculation of the second subject was taken from a patient thirty hours after the appearance of the eruption. After incubation in ascitic broth for twenty-four hours, several cubic centimeters were injected subcutaneously as in the preceding case without producing any local reaction. The temperature began to

rise ten days later, reaching 103°F. on the twelfth day. On the following day, a rash appeared on the face spreading to the chest, back and abdomen. No mention is made of any subsequent desquamation. Mild respiratory symptoms, a little conjunctivitis, and slight malaise were present in this patient.

The incubation period observed in these two cases of experimental measles corresponds to that of the spontaneous disease. The exanthem is described as typical of measles. Moreover, the rash appeared first on the face as in spontaneous cases, a point which is of interest in view of the altered portal of entry of the virus.

There are several respects in which the symptoms differed from the ordinary course of the disease. The description in the first case of the rapid progress of the rash over the body within a few hours is quite unlike the usual slow progression as seen under natural conditions. Apparently no desquamation occurred in the second case. The absence of conjunctivitis and of respiratory signs does not, as suggested by Ustvedt (6) militate against the diagnosis of an inoculated form of measles. Neither case showed a pre-eruptive rise in temperature, a feature, however, which is not constant in spontaneous infections. It is unfortunate that no information is supplied in these cases concerning the leucocyte count, the occurrence of Koplik spots and the behavior of the lymphatic glands.

Hiraishi and Okamoto (7) in attempting active immunization against measles, inoculated 44 children with blood from early cases. They conclude that the minimum infective dose lies between 0.001 and 0.002 cc. and that 0.0001 cc. of blood is harmless. The work was carried out during an epidemic. It is by no means clear that adequate precautions were taken to protect these children from accidental infection. No description is given of the experimental disease.

During the winter of 1918 to 1919, the writer (8) inoculated a series of volunteers with blood from early cases of measles in an effort to confirm Hektoen's results. In working with such a common infectious disease, considerable difficulty was experienced in obtaining susceptible adults. Eight volunteers were eventually accepted who, as far as could be determined from correspondence with their families, had never been exposed to measles. These men

were injected in various ways with blood but no symptoms developed in any instance.

The description of these injections may be summarized as follows: For the first inoculation, blood was taken from a patient twelve hours after the eruption appeared. The serum was separated by centrifugalization and diluted with nine parts of isotonic salt solution. One individual was given 5 cc. of the diluted serum subcutaneously.

For the next series of inoculations, a specimen of blood was taken from a case of measles twelve hours after the rash appeared. A portion of this specimen (4 cc.) was incubated in ascitic broth (50 cc.) according to Hektoen's technique and another part was defibrinated. The latter was injected at once subcutaneously in 2 cc. quantities into each of 2 men. The portion in ascitic broth was incubated for one day and 10 cc. quantities were injected subcutaneously into 2 individuals.

Since no symptoms followed the preceding inoculations, some more intensive injections were carried out. Blood was taken in citrate from 2 cases of measles in the pre-eruptive stage, six hours before the rash appeared in 1 patient and thirty hours before its appearance in the other. These citrated specimens were mixed and the equivalent of 3 cc. of blood was injected into each of 2 individuals, part of the injection being given subcutaneously and part intramuscularly. Twenty-four hours later each of the 2 volunteers received a second injection from these 2 patients in the same manner. One of the measles cases was now in the eruptive stage and in the other the rash appeared six hours later. One of these two volunteers gave an unusually clear history of susceptibility to measles. He was the sixth of 8 children and had always lived on an isolated farm in West Virginia. According to the statement of the mother and eldest sister, measles had never occurred in the household. But several members of the family had left home and eventually had contracted measles. Of the older brothers and sisters, 4 out of 5 developed the disease away from home. Of the two younger children, one, a brother, enlisted in the army and developed measles at Camp Shelby, Miss.

Neither of these 2 individuals receiving intensive injections from patients in the pre-eruptive and eruptive stages developed any

symptoms. After an interval of three weeks, they were exposed to an early case of measles and also inoculated on the mucous membrane with secretions from this case in the pre-eruptive stage, four days before the rash developed. The volunteers remained free from symptoms. This result, therefore, suggests that they were immune to measles at the time this final test was made. It is not possible to determine definitely whether their immunity may have been due to some previous unremembered or undiagnosed attack of the disease, or whether it resulted from the injections of measles blood which they received. Certainly the evidence of their susceptibility at the beginning of these injections is more concrete than the generalization that few adults have escaped an attack of the disease in childhood.

Finally, an injection was made in 1 volunteer with whole blood taken from a patient six to twelve hours after the rash appeared. Immediately after withdrawal, without the use of citrate, 0.5 cc. was given subcutaneously and 1.5 cc. intravenously. He remained free from symptoms.

These 8 successive failures indicate that measles cannot be transmitted by the injection of patient's blood as readily as would be expected from the results of the 2 cases reported by Hektoen. Moreover, a thorough analysis fails to suggest any simple or definite explanation of these divergent results. Except in 2 cases, the technique which I followed differed from that of Hektoen, the blood from the measles patient being injected directly without preliminary incubation. At the time these experiments were conducted, it was thought that the direct injection of a moderate amount of blood would be more likely to infect than the use of a minimal quantity after twenty-four hours incubation. Hektoen used approximately 0.1 cc. of patient's serum. However, it is theoretically possible that multiplication of the virus of measles may have occurred during the incubation. If such development did take place, then the preliminary incubation would surely enhance the possibility of reproducing the disease.

One must consider the possibility of producing a fever and rash by toxic constituents contained in the media which was injected. The writer has carried out injections of ascitic broth incubated with normal blood in a series of 20 individuals. Only minor reactions

developed and they could not in any way be confused with the symptoms of measles.

The evidence presented by Hektoen indicates that the fever and the accompanying rash, developing after a period of two weeks constituted true infections with the virus of the disease. Careful precautions were taken to guard against accidental infection during the period of experimentation. Although the resulting symptoms did not conform fully with the naturally acquired disease, it is not to be expected that the injection of a virus under highly artificial conditions would reproduce, in detail, the usual symptoms of the natural infection. The absence of a pre-eruptive rise in temperature, the rapid spread of the rash over the body, the lack in 1 case of inflammation of the mucous membranes, and the very moderate degree of malaise might readily be accounted for by the artificial mode of inoculation.

It is perhaps natural to feel that the blood of a measles patient taken early in the disease would either consistently fail to infect or else regularly reproduce the disease upon injection in a susceptible individual. Such an assumption, however, is not justifiable as a general conclusion. Indeed, the blood of an active case of pneumonia or of typhoid fever, during the stage of bacteriemia, might give very inconstant results upon injection into susceptible individuals. The failure in my own work to produce measles in volunteers by the injection of the blood of patients cannot, in my opinion, be explained merely on the supposition that the apparently susceptible volunteers were in reality immune on account of some previous attack of this disease. It is entirely possible that the blood of measles patients, even though the virus be present, would not consistently infect susceptible men. Hektoen's successful results are very important in demonstrating that the virus is present in the blood and that infections can be produced in man by the subcutaneous route even though the normal portal of entry is by way of the mucous membranes. It would be extremely important to know whether the likelihood of successful infection is increased by the preliminary incubation of the patient's blood in ascitic broth as practised by Hektoen. Unfortunately, the results of my experiments throw no light on this question.

Inoculation of monkeys. Experiments upon the transmission of measles to lower animals have been carried out extensively with monkeys, principally those of the genus *macacus*, the injections having been made with blood and mucous secretions of measles patients. Following Hektoen's work with volunteers, Anderson and Goldberger (9) reported the successful inoculation of monkeys in a manner analogous to the production of typhus fever in lower animals. Subinoculation through a series of monkeys produced mild symptoms which these authors interpreted as a reaction to the virus of measles. Confirmation of this work has been reported by several observers though the results of the individual investigators vary rather markedly. One would hardly expect that the typical clinical features of measles could be reproduced in monkeys with sufficient clearness to permit a diagnosis from the symptoms alone. It would be sufficient to produce a perfectly definite reaction which, by the exclusion of other factors, may be proved to be caused by the virus.

There are in all six signs or symptoms which have been reported in monkeys; namely, (1) fever, (2) rash, (3) Koplik spots and other forms of enanthem, (4) leucopenia, (5) conjunctivitis and rhinitis, and (6) evidence of malaise.

Anderson and Goldberger employed three species of monkeys, namely, *M. rhesus*, *M. cynomolgus*, and *M. sinicus*, using in all, more than 100 animals. Apparently these three species were equally satisfactory, though the symptoms were very mild and many individual animals failed to react. The authors summarize the results of the inoculation of blood of early cases as follows: ". . . . at least 50 per cent of the animals react in a characteristic manner. After a variable incubation period of not less than five days there is a more or less marked rise in temperature with or without catarrhal symptoms referable to the respiratory passages, such as sneezing and cough, and with or without an exanthem."

In the subinoculation of the virus in monkeys, the maximum incubation period was twenty-one days. Such irregularity complicates the interpretation of the data and increases considerably the difficulties of practical work. Unfortunately many details of the work are not available at present. In the majority of instances, the temperatures of the inoculated animal are not stated, since the com-

plete report of the work has not yet appeared. The character of the exanthem was extremely variable. Sometimes only an erythematous blush was noted. Frequently the rash was copper-colored from the beginning. Occasionally discrete pink macules and papules were observed which disappeared on pressure and were followed by a branny desquamation. These rashes occurred at very irregular intervals after inoculation; they developed most commonly on the face and chest but appeared sometimes on the thighs and abdomen. Rhinitis, coryza, and malaise were sometimes noted but these were not striking symptoms. No observations are recorded concerning leucocyte counts or examinations for Koplik spots.

Several strains were subinoculated from monkey to monkey. One in particular was passed rapidly through a series of 6 monkeys in forty-four days, but no evidence was noted of any alteration in its virulence. Experiments were also conducted to determine the infectivity of the blood for monkeys after infiltration, and after exposure to unfavorable conditions. Four specimens of blood were passed through a Berkefeld filter. Negative results were obtained with the first three; with the fourth specimen, 1 of 2 animals developed an exanthem twenty-one days after inoculation. Subinoculation of blood from this animal produced a slight febrile reaction in 1 of 2 monkeys. The authors conclude that the virus of measles is capable of passing through a Berkefeld filter.

Additional experiments were made concerning the effect of drying, heating, freezing, and of age upon the virus. They draw the following conclusions: "The virus in measles blood may resist desiccation for twenty-five and one-half hours, lose its infectivity after fifteen minutes at 55°C., resist freezing for twenty-five hours, and possibly retain some infectivity after twenty-four hours at 15°C."

Anderson and Goldberger also inoculated monkeys with mucous secretions of measles patients. Two monkeys inoculated on the mucous membrane with material taken twenty-five hours after the rash appeared, developed no symptoms. Subsequent work was carried out by subcutaneous injection of secretions. The contaminating bacteria produced a prompt rise in temperature and a local abscess. The latter was usually incised and some drop in temperature usually occurred. In some animals the temperature subsequently

rose again with or without the development of a rash. There were 5 experiments in which secretions were taken not later than twenty-six hours after the first appearance of the exanthem. In 4 instances the results were negative or doubtful. Secretions were obtained from 1 patient at the beginning of the rash and again twenty-four hours later. Successful inoculation of monkeys was reported with both specimens.

Hektoen and Eggers (10) supplied data more especially concerning the leucocyte counts in monkeys inoculated with measles blood. They report a more or less definite initial leucocytosis followed by a leucopenia of variable degree involving principally the neutrophils and resulting in a relative increase in the lymphocytes. In control animals injected with normal blood they noted either no change or else a slight transitory leucopenia. Two monkeys received measles blood obtained during the first twenty-four hours of the rash. One of these, on the twelfth and thirteenth days after inoculation showed signs of malaise, but there was no rash and no respiratory complications. The other developed evidence of malaise on the twelfth day; a faint masculo-papular rash appeared about the eyes and forehead on the fifteenth day, and a similar rash developed in both groins on the following day. These rashes disappeared after one to two days without any distinct desquamation. No Koplik spots were present. Subinoculation of monkeys was performed with blood taken late in the incubation period and no definite symptoms resulted.

The authors conclude that their results, when combined with those of Anderson and Goldberger, indicate that the *M. rhesus* is susceptible to a mild kind of measles.

Lucas and Prizer (11) desribed the occurrence of Koplik spots in monkeys. Two animals (*M. rhesus*) were injected with blood from a pre-eruptive case of measles. They report a leucopenia and the development of Koplik spots ten days after injection. On subinoculation into 2 other monkeys, spots, which were interpreted as Koplik spots, appeared in one, after ten days. The duration of these spots is not stated. The 2 animals injected with measles blood from man showed a transient erythema but no rash. No febrile reactions developed. The interpretation of these results is difficult because of an intercurrent infection of unknown etiology which killed several

control monkeys and also some of the inoculated ones about two weeks after their injection with measles blood.

Nicolle and Conseil (12) in 1911, reported confirmation of the work of Anderson and Goldberger. One monkey (*M. sinicus*) was injected with blood taken from a case of measles twenty-four hours before the rash appeared. The animal developed no symptoms except very slight malaise and a rather transient rise in temperature, most noticeable on the eleventh and twelfth days of the incubation period. Blood taken on the eleventh day was injected into a very young monkey (*M. sinicus*) but the animal remained entirely normal. The authors conclude that they have confirmed the work of Anderson and Goldberger.

In 1920, Nicolle and Conseil reported very briefly the results of some experiments conducted in 1913, concerning the transfer of measles from a child to monkeys (*M. sinicus*), re-inoculated successfully into a child, and again in monkeys. No symptoms other than a febrile reaction were observed in the monkeys; the temperatures are given for only a short portion of the incubation period. It is, therefore, inadvisable to draw any conclusions without knowing the normal temperature for these animals. As regards the child injected with blood from a monkey, there is no description of the symptoms, such as the respiratory involvement, Koplik spots, leucopenia, or glandular enlargement. There is no description of the rash, nor any reference to subsequent desquamation. It is certainly very important to know whether the course of the disease resembled the spontaneous infections, or whether some of the modifications occurred which were noted by Hektoen. This information is particularly desirable since there is no description of the precautions which were taken to avoid contact infection with measles.

Tunnicliff (13) inoculated one animal (*M. rhesus*) with blood from a measles patient taken at the end of the first twenty-four hours of the rash. There was no definite febrile reaction. The temperature at the time of inoculation was 104°F. It rose from 102.6°F. on the seventh day to 103.5°F. on the eighth day and then fell slightly. Tunnicliff considered that this rise may have been caused by the virus of measles. A protracted leucopenia developed, the count remaining relatively low, for fifteen days, a period which is much

longer than other observers have recorded in monkeys inoculated with measles; it is also much in excess of the duration of the leucopenia occurring in human cases. There was neither rash nor Koplik spots, nor other indication of measles.

Jurgelunas (14) endeavored to produce measles in monkeys by inoculation of blood, of mucous secretions, and by exposure of animals in a measles ward. He concludes that his results were negative.

One monkey (*Pavian*) was injected with defibrinated blood from a patient showing Koplik spots at that time; the rash appeared on the following day. Ten days after injection, the animal developed small rose colored spots over the abdomen. There was no rise in temperature. Death occurred on the following day. The autopsy failed to reveal the cause of death. The liver and spleen were enlarged. Cultures from the blood and various organs showed no growth. Jurgelunas considers that the rash did not conform to the exanthem of measles and that measles was not the cause of death in this animal. He injected one other monkey (*M. cynomolgus*) with the blood from an active case of measles, the specimen being taken during the first day of the rash. No symptoms developed. A third monkey injected with blood showed no symptoms, but it should be noted that the specimen was not taken till the second day of the rash.

Two monkeys were exposed to natural infection in a measles ward, being five days among acute cases and two days with convalescent patients. Neither developed any symptoms of measles; one, however, died of an acute streptococcus peritonitis two weeks after the last exposure in the ward.

Several experiments were conducted with mucous secretions, all of which were negative. One animal (*M. cynomolgus*) was injected subcutaneously with specimens taken on the day preceding the appearance of the rash. In another (*M. cynomolgus*), the mucous membranes of the mouth were rubbed with secretions from a patient showing Koplik spots but no exanthem. Another monkey (*M. rhesus*) was inoculated in the same way with specimens taken during the first day of the eruption. Lastly, the secretions from another case taken during the first day of the rash were rubbed into the scarified mucous membrane of the mouth of a *M. rhesus*.

Jurgelunas made no comments concerning leucocyte counts and Koplik spots, relying apparently on the temperature and an exanthem for indications of an infection.

Blake and Trask (15) have reported the successful infection of monkeys (*M. rhesus*). Ten monkeys were inoculated with the mucous secretions of early cases and 8 are regarded as having developed symptoms of measles. The authors confirm the occurrence of a rash, the febrile reaction and the malaise noted by Anderson and Goldberger, the leucopenia first noted by Hektoen and Eggers and occasionally found Koplik spots as reported by Lucas and Prizer. Many of their animals developed more or less conjunctivitis but none showed any rhinitis nor bronchitis. The filterability of the virus of measles was also confirmed. In 2 instances, mucous secretions of patients were passed through a Berkefeld N filter. The filtrate upon injection into monkeys, produced an exanthem and an enanthem but no fever developed.

The evidence of leucopenia as recorded in the charts is not particularly constant nor striking. However, the authors state that they do not regard the temperature and leucocyte counts as evidence of successful inoculation, but merely as additional data.

The characteristic enanthem in the monkey as noted by Blake and Trask consisted usually of a bright erythematous discrete or granular rash occurring most commonly on the labial mucous membrane and the gums. In one instance whitish lesions occurred resembling the Koplik spots of human cases. Histologically the cellular reaction of the enanthem and exanthem occurring in monkeys conformed to the description of the human lesions as given by Mallory and Medlar. Apparently no examinations were made for the Gram-positive coccoid bodies found by Mallory and Medlar in measles. These histological studies would be considerably strengthened in case the picture of these lesions proved to differ sharply from that of the spontaneous maculopapular rashes which often occur in monkeys.

The authors stress emphatically the very close resemblance of experimental measles in monkeys as compared with the human disease. The two differ significantly, in their opinion, only in the inconstant febrile reaction and the absence of rhinitis and bronchitis. To this I would add the usual absence of typical Koplik spots in monkeys and the inconstancy of a definite leucopenia.

The usual immunity tests were carried out, employing 6 monkeys which had shown a reaction to the virus of measles and 2 control monkeys. The 2 controls developed symptoms but the 6 which had previously reacted remained negative. The data concerning the temperature and leucocyte counts are not given.

Subinoculations from monkey to monkey were carried out, using either blood or the ground skin and mucous membrane of inoculated monkeys. The authors consider that the early transfers gave successful infections but that after repeated passage (8 to 12 transfers) a strain eventually dies out. In the inoculations made directly from patients and also in the subpassages, no febrile reactions developed except in those animals injected with contaminated material. In the course of the subinoculations, whitish areas resembling Koplik spots were noted in the enanthems which developed in 2 of 12 or more animals.

Four monkeys were injected intravenously with blood and all of these developed conjunctivitis. This result in a rather refractory species stand out in more or less contrast to the observation of Hektoen. It will be recalled that 1 of 2 volunteers, injected subcutaneously, escaped any signs of involvement of the mucous membranes and in the other only a mild conjunctivitis and some cough developed.

In the beginning of their work Blake and Trask applied the procedure of intratracheal injection for the inoculation of the virus in monkeys but they appear to have obtained satisfactory results with equal ease by rubbing infective material on the mucous membranes or by the injection of blood. Their experiments, however, were not designed to test the relative value of the various methods of inoculation.

Kawamura (16) took blood from a measles patient sixty hours before the appearance of the eruption and injected rather less than 1 cc. of blood into each of 3 monkeys (*M. fuscatus*). After an incubation period of eight or nine days, a fever, leucopenia, rash, conjunctivitis and rhinitis developed. Koplik spots were noted in 1 animal. Two successful subpassages were obtained by the injection of blood. Histologically, the rash in monkeys appears to have resembled both the cellular reaction seen in measles and also that of Japanese flood river fever.

In the course of their work on the inoculation of rabbits with measles, Nevin and Bittman (17) had occasion to inject 2 monkeys. One of these monkeys was injected intratracheally with mucous washings from an early case of measles; the other received blood of 2 patients taken early in the eruptive stage. The animals developed more or less leucopenia, an exanthem and an enanthem but no fever. In some later work these authors inoculated a third monkey with patient's blood under similar conditions and obtained a similar result.

In 1918 and 1919, Wentworth and the writer (18) carried out some experiments upon the inoculation of monkeys with measles. In a preliminary experiment 3 animals (*M. rhesus*) were used for blood injections. The first (A) was given 10 cc. of blood from a patient eighteen hours after the rash appeared. In transmitting typhus fever to monkeys, Ricketts and Wilder (19) recommend dilution of the blood. Accordingly this quantity of 10 cc. was diluted with 40 cc. of isotonic salt solution, defibrinated, and injected intraperitoneally. The animal remained well and there was no evidence of any rash or Koplik spots. The temperature and leucocyte count did not fluctuate beyond the normal limits.

A second animal (B) was injected with blood from a patient within six to twelve hours after the onset of the rash; 10 cc. were diluted with an equal volume of isotonic salt solution, defibrinated, and injected intraperitoneally. This animal was kept under observation for ten days before injection. During the early part of this period, a marked erythema with a few macules was present over the face and eyebrows. This rash practically disappeared during the first week of the incubation period, and then increased very slightly ten days after inoculation. Two months after the last injection it was more marked than at the beginning of the experiments. Otherwise, the findings in this animal were negative.

Very frequently, an animal which fails to respond to an injection with blood from a case of typhus fever may subsequently react typically to a similar injection. Accordingly these 2 animals (A and B) and a third young adult monkey (C) were given rather intensive injections of blood from measles patients. They were injected on three successive days with blood taken from 3 cases of measles

in the early stage of the exanthem. On the first day, blood was obtained from a patient four to five hours after the rash appeared; on the second and on the third day, from patients in each of whom the rash had started about twelve hours previously. The blood for these injections was either defibrinated or collected in sodium citrate.

There was no evidence of any reaction in these 3 monkeys. On the eleventh day after the first of the three injections, 3.5 cc. of blood was withdrawn from monkey C and injected subcutaneously in a susceptible volunteer. There was no change in his temperature or leucocyte count and no symptoms developed.

The leucocyte counts and the temperatures of these monkeys are given in charts I, II, and III. As an additional control, the room-temperature is also included since the body temperature of monkeys is sometimes influenced by this factor. These charts represent very clearly the disappointing type of reaction that may commonly be expected in monkeys even when inoculated under favorable conditions.

In a continuation of this work (20) some rather interesting results were obtained from an experiment in which portions of the same specimen of measles blood were injected simultaneously in 2 volunteers and in 2 normal monkeys (*M. rhesus*). As already described neither of the 2 men developed any symptoms; 1 of the 2 animals showed a suggestive reaction. In the interpretation of this result it must be recalled that 1 of these 2 volunteers gave exceptionally clear evidence of never having been exposed to measles. Blood was obtained from 2 patients for these injections, specimens being taken on 2 successive days. For the sake of convenience, the description of these cases will be repeated here. On the day of the first injection both patients were in the pre-eruptive stage. Pooled specimens of blood taken in citrate solution were injected at once. Each of the volunteers received the equivalent of 3 cc. of blood, the first portion being injected subcutaneously and the remainder intramuscularly. Each of the two monkeys received the equivalent of 2 cc. of blood, part of which was injected subcutaneously and the remainder intraperitoneally. One of the 2 measles patients developed a rash six hours after withdrawing the first specimen of blood. On the next day the patients were seen again; one was still in the pre-eruptive

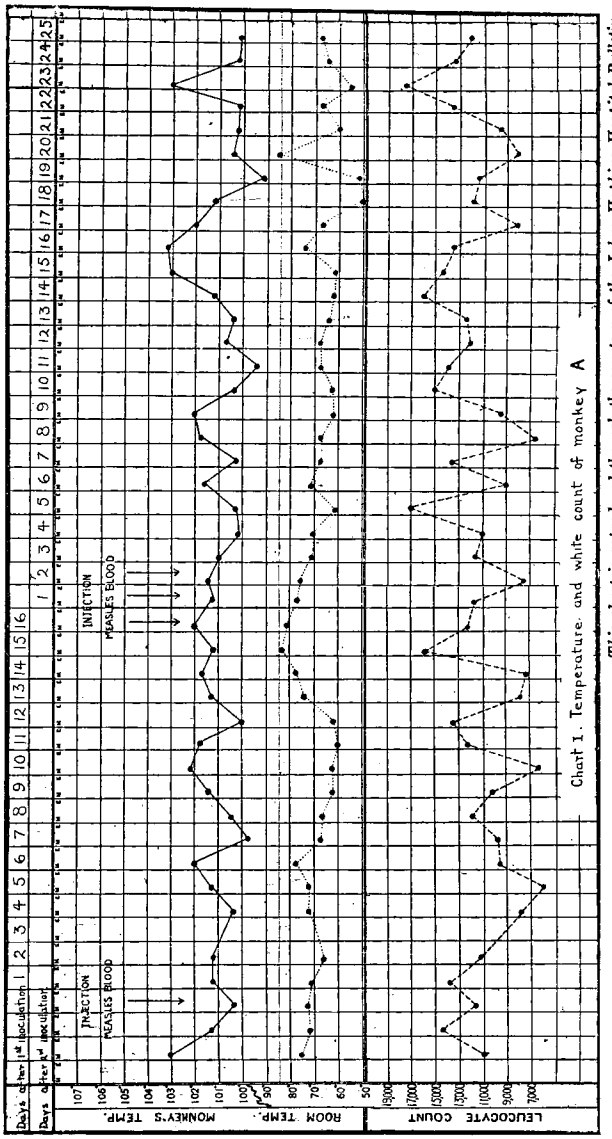

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CHART I

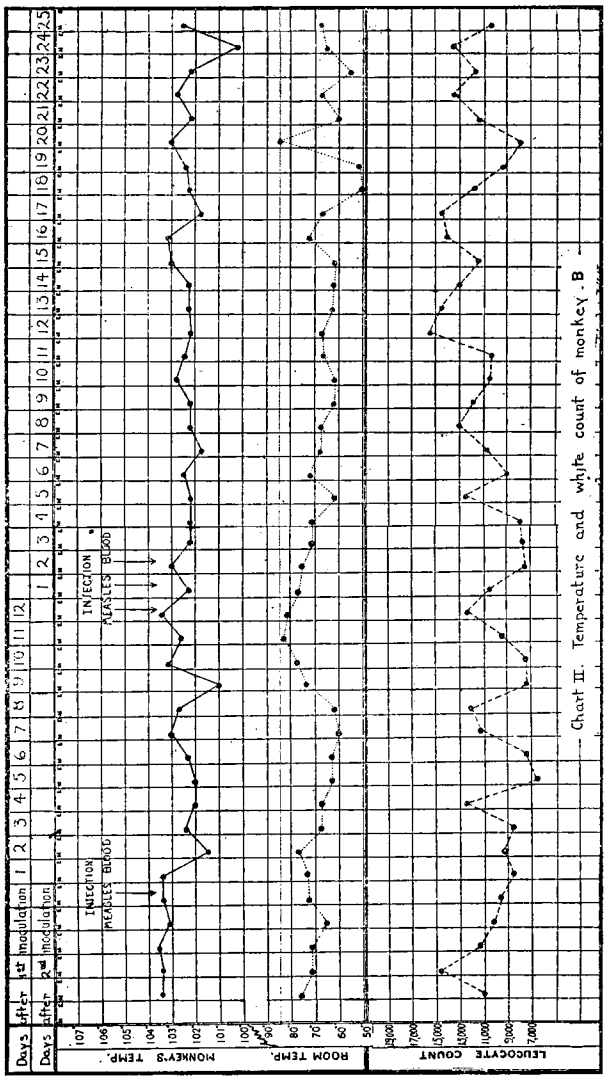

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CHART II

stage but the rash appeared about six hours later. Blood was taken from both patients, the specimens were pooled and all of the injections were repeated as on the preceding day, employing the same quantities. The 2 monkeys varied somewhat in their reaction. One (D) showed a low leucocyte count on the ninth and again on the eleventh and twelfth days after injection. There was no febrile reaction, no respiratory nor constitutional symptoms, and no exanthem nor Koplik spots. The other animal (E) developed a leucopenia

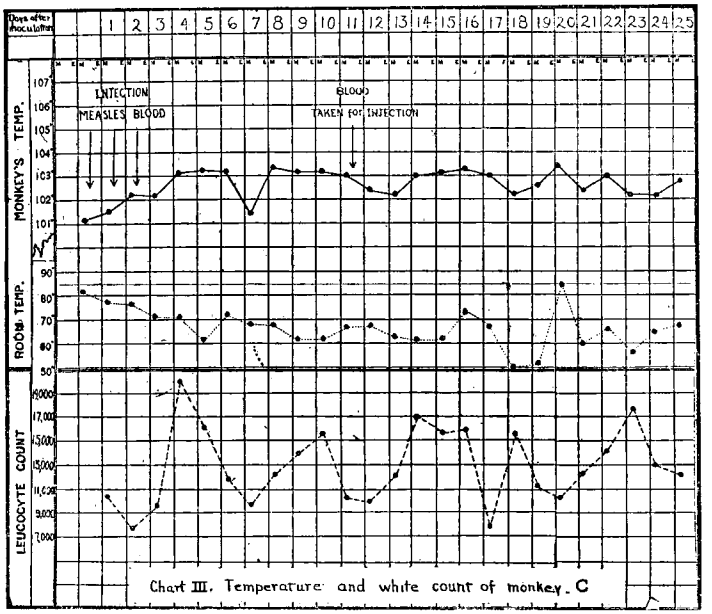

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CHART III

beginning on the sixth day after his first injection, and persisting for three consecutive days. On the twelfth day, a faint rash developed over the face, neck, and uppermost part of the chest. This was principally a diffuse erythematous blush but there appeared around the eyes and nose discrete red macules 1 to 2 mm. in diameter from which the color could be readily expressed. On the next day, the rash faded almost completely leaving behind only slightly pigmented areas. These disappeared on the following day, and they were not followed by desquamation. On the first day of the rash a moderate

degree of malaise was noted. These symptoms were not accompanied by any febrile disturbance. There was no rhinitis and no Koplik spots were found at any time. On the fifteenth day after injection a well marked pneumonia developed.

On the seventh day of the incubation period, when the leucopenia appeared, 3 cc. of blood were withdrawn and injected subcutaneously

TABLE 1

DAYS AFTER FIRST INOCULATION	RHESUS D			RHESUS E			ADDITIONAL OBSERVATIONS ON RHESUS E
	Temperature		White count per cubic millimeter	Temperature		White count per cubic millimeter	
	a.m.	p.m.		a.m.	p.m.		
	°F.	°F.		°F.	°F.		
1		101.2	18,900		101.8	13,900	
2	99.0	102.0	11,100	100.4	102.2	10,700	
3	99.4	101.8	10,100	101.8	101.0	18,500	
4	101.0	102.4	11,800	102.0	102.0	10,000	
5	101.2	102.0	12,500	100.6	102.0	11,800	
6	100.8	102.2	14,900	102.2	102.8	5,500	
7	101.6	101.8	8,300	102.4	102.0	5,200	Bled for inoculation of volunteer
8	100.8	101.2	9,500	101.2	101.8	6,400	
9	100.8	102.4	4,700	101.6	102.0	7,900	
10	101.6		7,400	101.2		9,900	
11	101.4	101.8	4,400 4,900	102.8	103.6	11,110	
12	101.2	102.6	5,700	101.0	101.4	12,600	Slight rash
13	101.6	102.9	7,800	101.0	102.0	8,200	Slight rash
14	100.9	102.2	9,200	100.0	101.0	6,400	
15	101.4	102.0	8,900	100.0	100.0	6,000	Early signs of pneumonia
16	100.6	101.0	8,300	99.4	99.8	6,600	Definite pneumonia
17	100.6	102.2	17,300	98.8	100.6	18,400	Critically ill
18	102.0	102.2	13,900	101.0		9,400	Critically ill
20	101.2		14,500	100.8		44,000	Marked improvement by crisis

and intramuscularly into a volunteer. No local nor general symptoms developed.

The record of the temperatures and white counts for the two monkeys are given in Table 1.

The rash and leucopenia developing in this second monkey, unaccompanied by rhinitis, fever or Koplik spots are difficult of interpre-

tation. The absence of any symptoms following the corresponding injection of measles blood in man constitutes strong evidence against ascribing the reaction in the monkey to the virus of measles. This is especially true in view of the direct concrete evidence of susceptibility in one of these volunteers. On the other hand, the reader may on purely general grounds feel skeptical about the susceptibility to measles of any adult. It must also be remembered that each of the monkeys received some measles blood intraperitoneally. In view of the ultimate results, the experiment is faulty in this respect; for it is theoretically possible that a refractory animal might be overwhelmed by an intraperitoneal injection although a susceptible host escaped infection after subcutaneous and intramuscular injection.

It is noteworthy that the first of these 2 monkeys remained free from any characterisitc reaction notwithstanding the intensive injection of extremely favorable material. This result might be taken as an illustration of Anderson and Goldberger's view that many individual animals are altogether refractory.

My own experience with the inoculation of monkeys with mucous secretions has given only negative results. I have endeavored to infect 2 monkeys (*M. syrictus*) by inoculation with secretions taken four days and one day before the patient's rash developed. Swabs moistened with the conjunctival secretions of the patient were rubbed over the conjunctivae and nasal and pharyngeal mucous membrane of the monkeys. Similarly swabs from the nasal and pharyngeal mucous membrane of the patient were thoroughly rubbed over the corresponding mucous membranes of the animals. Neither monkey developed any fever or leucopenia. There was no rash nor Koplik spots, no inflammation of the mucous membranes, and no malaise. Two additional monkeys (*M. rhesus*) were inoculated with secretions from a measles case taken two hours after the first appearance of the rash. The inoculations were made in the same manner as for the syrictus monkeys. In addition, scarified areas of the mucous membrane of the monkey's mouths were rubbed with swabs from the conjunctival and nasal mucous membrane of the patient.

For the sake of convenience, table 2 has been prepared showing the general results obtained by various investigators upon injecting monkeys with the blood of measles patients. This outline covers

only those experiments which were designed to determine whether the monkey is susceptible to measles. It does not include the records of those injections in which the patient's blood was subjected to various procedures such as filtration or aging, for the purpose of studying the properties of the virus. Reports based upon the injection of a single animal are also omitted.

The results concerning the inoculation of the mucous secretions as obtained by various investigators are given in the table 3. The negative experiments conducted with late cases are not included.

TABLE 2

Inoculation of monkeys with blood of early cases of measles

EXPERIMENTAL RESULTS	ANDERSON AND GOLDBERGER	HEKTOEN AND EGGERS	LUCAS PRIZER	NICOLLE CONSEIL	JURGELUNAS	KAWAMURA	NEVIN BITTMAN	SELLARDS AND WENTWORTH
Incubation period, days	5–11	12	10	11	----	8–9	4	----
Fever	+	+	0	+	0	+	0	0
Leucopenia	----	+	+	----	----	?	+	+
Exanthem	+	+	?	0	0	+	+	?
Enanthem	----	0	+	----	----	----	+	0
Conjuctival or respiratory signs	+	0	?	----	----	+	+	0
Malaise	+	+	----	?	----	+	+	?
Subinoculation in monkeys	+	?	?	+	----	+	----	----
Re-inoculation in man	----	----	----	+	----	----	----	0
Number of animals inoculated	7+	2	2	6	2	3	3	5
Number showing symptoms	4+	2	2	5	1?	3	3	1

0, none; ----, no observations; +, present; ?, irregular or doubtful.

Localized lesions. In the attempts to reproduce measles in animals, practically all of the attention has been directed toward obtaining a systemic infection. In this connection, a consideration of smallpox is instructive. The virus of smallpox certainly gains access to the circulating blood at some periods of the infection. Yet the experimental transfer of the disease by the injection of blood has not been conclusively demonstrated. However, subinoculation from skin lesions in man to the skin of lower animals, readily produces a local lesion but a generalized infection typical of the spontaneous disease has not been obtained. In the course of some unpublished work, Bigelow and the writer carried out an analogous procedure in measles.

Early skin lesions and Koplik spots were excised from patients and implanted in the skin and mucous membrane of monkeys. Several of the results were entirely negative but some were very suggestive. In one instance in particular, an implant of skin lesion into the skin was followed after two weeks by the development of bright pink papules in an area approximately 5 cm. in diameter surrounding the implanted tissue. These papules faded gradually in the course of three days and were followed by pigmentation and desquamation.

TABLE 3

Inoculation of monkeys with mucous secretions of patients

EXPERIMENTAL RESULTS	ANDERSON AND GOLDBERGER		JURGELUNAS		SELLARDS	BLAKE AND TRASK
	Swabbing mucous membrane	Subcutaneous injection	Swabbing or scarifying mucous membrane	Subcutaneous injection	Swabbing or scarifying mucous membrane	Swabbing mucous membrane or intratracheal injection
Incubation period, days	----	8 and 9	----	----	----	6–10
Fever	0	+	0	0	0	+
Leucopenia	----	----	----	----	0	+
Exanthem	0	+	0	?	0	+
Enanthem	----	----	----	----	0	+
Conjunctival or respiratory signs	0	+	0	0	0	+
Malaise	0	+	0	----	0	+
Subinoculation in monkeys	----	+	----	----	----	+
Number of animals inoculated	2	6	3	1	4	10
Number of negative or doubtful reactions	2	2	3	1	4	2

0, none; ----, no observations; +, present; ?, irregular or doubtful.

Normal human skin implanted in control monkeys was gradually absorbed without producing any eruption.

In seeking for a method of active immunization against measles, Blake and Trask (21) report the development of a localized lesion in monkeys by the intramuscular injection of an attenuated virus. It is well to recall that Hektoen, injecting the virus of measles subcutaneously in man, observed no trace of any local reaction.

Discussion of the reaction in monkeys. There is certainly, at present, no exact proof of the susceptibility of monkeys to measles. The work of Nicolle and Conseil suggests that the virus of measles is

conserved in monkeys and may produce a mild febrile reaction. It will be recalled that these observers noted the development of measles in a child which they inoculated with blood from a monkey showing a mild reaction some days after being injected with virus. This observation cannot be accepted as final without knowing the precautions which were taken agaist accidental infection.

In contrast to experimental typhus fever, and spotted fever, no criteria have as yet been established by which experimental measles can be recognized definitely and unmistakably. Spotted fever in the guinea pig runs a fatal course with a characteristic pathology. A guinea pig inoculated with typhus fever develops only a moderate febrile reaction and no symptoms appear which would suggest the human disease. However, microscopic lesions occur with great regularity in the brain; the histologic picture of these corresponds to those found in human cases and furnishes an accurate method of recognizing the experimental disease. The rashes sometimes occurring in monkeys inoculated with measles agree fairly well in the histologic picture with the skin lesions of human cases. This histologic picture, however, is not pathognomonic of the disease.

Several species of the genus *macacus* have been utilized more or less extensively, namely, *rhesus*, *cynomolgus*, *sinicus*, and *fuscatus*. Thorough comparative studies are not available but there is no indication that the characteristics of the reaction to the virus is dependent on the type of monkey employed.

The mass of evidence though conflicting in many respects, suggests on the whole that occasional individual monkeys (genus *macacus*) may show mild reactions of variable character when injected with the virus of measles. Are these signs and symptoms sufficiently frequent and definite to constitute a reliable method for the experimental study of measles? If, for example, only 1 animal in 4 or 5 gives a definite dependable reaction, then the method soon becomes unwieldy and impractical for any extensive studies.

Observations in monkeys may be controlled in a measure by the "immunity test." But this test loses much of its significance since, even after intensive inoculation with ideal material, a considerable proportion of animals remain essentially free from symptoms.

The acceptability of these delicate reactions occurring in monkeys as a reliable method for the study of measles resolves itself ultimately into a question of the standards which the individual investigator considers essential. To me, they are not satisfactory. Personally, I am not willing to accept as established the various characteristics of the virus of measles as worked out in this way. Thus the important conclusion that the virus is filterable rests primarily upon more or less vague results obtained in three monkeys. I prefer to consider the filterability of the virus as an entirely open question.

Inoculation of rabbits and guinea-pigs. In the past two years, a few attempts have been made to simplify the study of measles by the substitution of rabbits or guinea-pigs for monkeys in experimental work. Nevin and Bittman took blood from 6 cases of measles, two to four days "after the onset of the disease." Six rabbits were inoculated intravenously and all gave evidence of a reaction. There was no characteristic fever nor leucopenia. The animals were shaved before inoculation. The redness caused by shaving became more intense in those receiving blood and subsequently desquamation occurred. In the control series, the redness after shaving faded without desquamation. Subinoculations of blood were made from rabbit to rabbit and 9 of 11 animals reacted. One strain, after five passages in rabbits, was inoculated into a monkey, *M. rhesus*. A somewhat suggestive leucopenia developed on the third day; the following day two spots somewhat resembling Koplik spots appeared on the labial mucous membrane; then a maculo-papular rash appeared on the face and later a red granular rash on the mucosa of the lips. The exanthem was followed by a marked desquamation. Subsequently, this animal showed no reaction to an intratracheal injection of 10 cc. of mucous washings from a patient with measles. The authors consider that this monkey developed typical measles as a result of the injection of blood from the inoculated rabbits and was, therefore, immune to the injection of secretions from a patient with measles. To me it seems equally possible that the rash developing in the monkey after an inoculation of rabbit's blood was not necessarily produced by the virus of measles; also the failure to react to a test injection of mucous secretions may have been nothing more than the corresponding failures which have been noted from time to time in normal monkeys.

In some later work, Nevin and Bittman passed a strain of measles virus through three rabbits and then through a series of three monkeys in order to eliminate as far as possible any question of rashes due to a foreign protein. Leucopenia, Koplik spots, an enanthem and an exanthem were noted in all of the monkeys after injection with blood from inoculated rabbits. Some of the rabbits, in addition to an erythema, developed a generalized maculo-papular rash followed by pigmentation and extensive desquamation. Koplik spots and enanthems were also noted. The authors conclude that the virus of measles "survives passage in rabbits."

Simultaneous with the studies of Nevin and Bittman on the blood of measles cases, Grund (22) working in the same laboratory collected mucous secretions from these same patients and injected rabbits intratracheally. Of 23 animals a rather large number proved refractory. No definite febrile reaction or leucopenia occurred. In 1 or 2, a maculopapular eruption developed and in 10 or 11 an erythema occurred. Sub-passages in rabbits gave somewhat more encouraging results. Immunity tests on convalescent animals proved rather "contradictory." Grund concludes that no one individual animal gives a typical picture of measles but that the series, taken as a whole, encourages the belief that rabbits are susceptible to the virus of measles.

Duval and D'Aunoy (23) conclude that rabbits are susceptible to measles developing a specific reaction which they regard as analogous in all essential features to the human disease. They consider "only temperatures of 102°F. or over as pyrexia" and regard white counts under 9000 cells as evidence of leucopenia. After the intravenous injection of patient's blood in rabbits, they noted the development of coryza, conjunctival injection, and enanthem similar to Koplik spots and in 40 per cent of the animals an exanthem appeared. A number of rabbits developed an acute hemorrhagic nephritis.

After several subpassages in rabbits, a very remarkable phenomenon was noted by Duval and D'Aunoy. They report a striking increase in virulence and conclude that a number of animals died undoubtedly from the direct effect of the virus of measles and not on account of intercurrent infection. This finding would require extensive confirmation and elaborate control in order to eliminate the possibility of epizootic disease.

The susceptibility of guinea-pigs to measles was studied also by Duval and D'Aunoy. They conclude that the guinea-pig reacts specifically to the virus of measles showing a definite and constant rise of temperature with a coincident fall in the total number of leucocytes after an incubation period of nine to twelve days.

Several large series of experiments were conducted but unfortunately some of the essentials of these data do not appear in the report. Those portions of the data concerning the temperature and leucocyte count which the authors present are not sufficiently complete to permit a logical conclusion. The situation in brief is as follows: The temperatures and leucocyte counts of 30 normal guinea-pigs were taken for thirty-one days and the daily average result of this series is recorded. In a similar manner, 15 guinea-pigs were injected with normal human blood and the daily average temperature and leucocyte count is recorded. Finally, the blood from 7 cases of measles was injected into guinea-pigs. In each experiment of this series, 6 animals were used, 4 for blood from measles patients and 2 for controls. Thus 28 pigs received measles blood and 20 of these showed evidence of reaction. However, only two charts are given of temperature and leucocyte counts and it is entirely impossible to determine whether these charts represent the data of a single animal or the composite data of more than one. Since some of these animals were sacrificed, the curve is not a composite of the entire group. Obviously the chart of a single experiment or of an entirely unknown number of animals cannot be compared with the composite chart of 30 control animals, studied one or two months previously. The 14 control animals inoculated simultaneously with those receiving measles blood showed no reaction but no data are given. It would appear that any temperature above 102° was regarded as abnormal. In passage experiments from guinea-pig to guinea-pig, the virus increased in virulence even to the extent of killing "a number" of the animals. Acute hemorrhagic nephritis is reported as a constant finding but unfortunately the number of animals examined is not indicated.

Tunnicliff and Moody (24) injected 9 rabbits intratracheally with the virus of measles using presumably mucous secretions. Good rashes were observed in 8 of these animals but no other definite

symptoms developed. None of 15 control rabbits showed any rashes similar to those produced by the virus of measles. Two guinea-pigs were inoculated intratracheally with the virus of measles, the results suggesting a rise in temperature and in one instance a leucopenia.

Kawamura inoculated monkeys with the blood of measles patients and subinoculated from the monkeys into guinea-pigs and rabbits with entirely negative results.

Nicolle and Conseil inoculated rabbits and guinea-pigs and conclude that these animals are not susceptible to measles.

In conclusion, it would seem clear that the symptoms in rabbits appear even less definite than those described in the monkey and the evidence that the virus survives in rabbits rests, in a large measure, on the re-inoculation from rabbits to monkeys. Acceptance of the susceptibility of rabbits and guinea-pigs to measles, or even the survival of the virus in these animals, is not warranted on the evidence which has been submitted.

BACTERIOLOGICAL INVESTIGATIONS

Cultural work on measles has been restricted, for the most part, to the aerobic bacteria. The types of media and the methods which have been employed have not differed strikingly from the standard routine bacteriological procedures. The materials usually selected for examination have been the secretions of the conjunctival and upper respiratory mucous membrane, the circulating blood and the lesions of the skin. From these sources, many representatives of the common types of bacteria have been isolated, none of which occur constantly or exclusively in measles. The organisms which have been described are not very remarkable. The earlier observations have been summarized by Hektoen (25).

B. influenzae. Of the various bacilli observed in measles, *B. influenzae* of Pfeiffer has attracted the most attention. It was first isolated from uncomplicated cases of measles during the active stage of the disease by Giarre and Picchi (26) in 1900. Subsequent observers have found that this bacillus appears with considerable frequency in measles. It occurs rather abundantly in the mucous membrane of the conjunctivae and upper respiratory tract, and in the lungs in cases complicated by pneumonia, but it has not been found in the blood nor in the skin lesions.

Several weeks after the subsidence of the first wave of the pandemic of influenza in 1918, the occurrence of *B. influenzae* in cases of measles was investigated at Camp Devens, Massachusetts, by Lieutenant Sturm and the writer (27). As this was one of the camps in which the Pfeiffer bacillus was prevalent during the epidemic of influenza, it is obvious that this organism may have been rather widely distributed at this time. We recovered an organism indistinguishable from *B. influenzae* from the sputum in 80 per cent of a series of 31 consecutive cases of measles. Moreover in three-fourths of these patients, the bacillus disappeared with the subsidence of the acute symptoms. In a group of 7 control individuals, some of whom had had influenza a few weeks previously, repeated examinations failed to show the presence of the Pfeiffer bacillus. The strains obtained from measles cases were compared carefully with similar strains isolated at autopsy from the lungs of patients dying from complications of influenza. No significant differences were found either in morphology, staining properties, cultural characteristics, immunity reactions, nor in the behavior of these strains to freezing, drying, and the action of bile and sodium hydroxide. Two monkeys were inoculated with strains obtained from measles patients. One remained well but the other, after six days, developed pronounced malaise with cyanosis and a fall in the leucocyte count but without a rise in temperature. Two human volunteers who, as far as could be determined, had had neither measles nor influenza, were inoculated by rubbing strains from the cases of measles on the mucous membrane of the conjunctivae, the nose, mouth, and throat. No symptoms developed and no change occurred in the temperature or white count. Moreover the inoculated organisms could not be recovered in subsequent cultures. Under natural conditions the Pfeiffer bacillus frequently establishes itself upon a mucous membrane. Therefore, the failure to produce symptoms would have had much more significance if successful colonization had been obtained. Mallory and Medlar found *B. influenzae* frequently in measles cases in 1916 and only rarely in 1917.

Various interpretations have been suggested concerning the occurrence of the influenza bacillus in measles. Several of the early observers considered that the presence of this organism justified the diagnosis of a complicating influenza. This conclusion is not war-

ranted since, as a rule, the cases of measles harboring the bacillus do not show clinical evidence of influenza. A few observers, notably Giarre and Picchi, consider the possibility that a Pfeiffer-like organism may be the etiological agent in measles. In order to give favorable consideration to this view it is of vital importance to establish differences between the strains of the Pfeiffer bacillus occurring in measles and in influenza. Such differences have not been forthcoming. In their absence, the weight of evidence indicates strongly that the Pfeiffer bacillus is merely a secondary invader which multiplies more readily during the period of inflammation produced by the virus of measles. Indeed, the acceptance of the Pfeiffer bacillus as the specific cause of influenza is somewhat compromised by the frequency with which this organism appears in uncomplicated cases of measles.

Cocci. Many observers have described micrococci, very commonly in the mucous secretions, and much less often in the blood stream. The most recent observations are those of Tunnicliff (28) who isolated, by anaerobic methods, a micrococcus from the blood during the preeruptive and eruptive stages of the disease. In the latter stage, a very considerable variety of other organisms was also obtained. The micrococcus isolated from the blood, developed aerobically on subinoculation, producing green pigment on blood agar plates. A similar coccus was also found in the secretions from the mucous membrane. In a subsequent study, Tunnicliff confirmed the occurrence of this micrococcus in the mucous secretions but no report was made in regard to blood cultures.

In a recent paper Tunnicliff and Moody (24) describe the effect on animals of this micrococcus. Upon intratracheal injection in rhesus monkeys, a leucopenia developed but no rise in temperature occurred. Occasional small red papules appeared in the skin, the histological picture of these being consistent with the diagnosis of measles.

The cultures of this green producing coccus were also found to be pathogenic for rabbits, dogs, mice, rats and guinea-pigs. In the guinea-pig, a rise in temperature accompanied by a fall in the leucocyte count was noted, but no rash developed. In rabbits (22 in all) no definite fever nor leucopenia occurred but 42 per cent developed spots which were interpreted as Koplik spots and in 87 per cent an

exanthem was observed. Rats and young dogs injected with this micrococcus became ill but recovered. It is noteworthy that these animals are ordinarily regarded as being entirely refractory to measles.

Tunnicliff and Moody cautiously, and it seems to me wisely, refrain from concluding definitely that this green producing coccus is the etiologic agent of measles. In this connection it is interesting to recall that other micrococci are capable of producing rashes, notably the meningococcus and probably some of the streptococci as recently reported by Dick and Dick (29).

Wentworth and the writer, as well as Mallory and Medlar, were unable to demonstrate any micrococci in cultures from the blood of measles patients.

Diphtheroids. The diphtheroid bacilli represent one additional group of organisms which has been found more or less frequently in measles. Bacteria of this type appear to be almost omnipresent as a part of the normal flora of the body tissues. Observations of some interest were made by Ciaccio (30). In the autopsy of 8 cases of measles an organism, which was apparently a small diphtheroid, was found rather widely distributed in the lymph glands and in various organs, but never in the skin lesions. Mallory and Medlar in the cultural examination of the nasal and nasopharyngeal secretions noted the presence of diphtheroid bacilli almost constantly.

Bigelow and the writer (31) have frequently obtained diphtheroids, often in pure culture, from the conjunctivae of measles patients. The original cultures grew feebly and slowly, but eventually subcultures on ordinary egg or Loeffler's medium gave abundant growth. Subsequently we found repeatedly that a small Gram-staining pleomorphic bacillus developed in blood cultures from measles patients. This organism was obtained in 25 of 31 cases. In control blood cultures in 24 instances, growth occurred in 5 cases. The organisms obtained from the controls resembled those from the measles cases in their morphology and staining reactions but differed in their fermentation tests rather markedly from the majority of those obtained from the measles cultures.

Three rhesus monkeys were inoculated with cultures from the measles cases. In 2, the symptoms were vague but in the third a suggestive cluster of macules and papules developed. The histological picture conformed to the description of the human lesions.

Neither the micrococcus described by Tunnicliff nor the bacillus cultivated by Bigelow and myself have been confirmed by independent observers. The simplest explanation for both of these microörganisms is that, during the extensive inflammation of the mucous membrane, some of the normal flora are swept into the blood stream. Such an explanation is, of course, not an easy one to demonstrate experimentally. However, all workers in problems of etiology must bear in mind the now numerous examples in other infectious diseases of the cultivation, apparently from the blood stream, of bacteria which are surely without direct etiologic relationship to the disease.

RÉSUMÉ

Of the acute exanthemata, measles is the most important cause of infant mortality. The disease does not *per se* produce fatal results but only through its complications; one attack confers marked immunity. There is, therefore, an excellent theoretical basis for the development of a method of active immunization, this being obviously the most desirable procedure for bringing the disease under control. Practically, the experimental problem of developing such a method of inoculation has proven to be very difficult of approach. The difficulty might be solved, of course, either by obtaining a suitable source of supply of the virus with the development of a process for its attenuation or more ideally by the isolation and cultivation of the causative microörganism.

The clinical features of measles indicate that one might reasonably expect to find the virus of measles in the circulating blood of a patient and that the injection of such blood into a susceptible individual might reproduce the disease more or less regularly and perhaps in a somewhat modified form. The symptoms of the disease, the mode of infection and the resulting immunity suggest that the causative agent is not a typical protozoan; it may not unlikely prove to be a member of the general group or bacteria or the bacteria-like microörganisms, either visible or ultramicroscopic in size.

Histology. Careful histologic examinations of the skin lesions and the Koplik spots have not revealed any definite microörganisms. Nevertheless, the causative organism is probably present in these

lesions, perhaps in very scanty numbers. Neither has any cellular reaction been described which is diagnostic of the disease, the principal characteristic being some proliferation in the tissues around the vessels, of the endothelial leucocytes, the latter often showing mitoses. There is no evidence of primary necrosis or acute exudation of polymorphonuclear leucocytes such as the ordinary micrococci produce.

Bacteriology. Cultures of the inflamed mucous membranes have shown for the most part only the flora commonly occurring in the upper respiratory tract such as the cocci, the diphtheroids and frequently the influenza bacillus. A number of microörganisms have been found from time to time in cultures of the blood; two are worthy of mention; namely, the micrococcus obtained by Tunnicliff and a Gram-positive pleomorphic bacillus reported by Bigelow and the writer. Each of these organisms when inoculated in monkeys produced maculopapular lesions, the histology of which was consistent with that of human measles. In my opinion, this finding is not sufficiently distinctive to justify one in placing confidence in either of these organisms as the etiologic agent.

Transmission to man. It has already been emphasized that the existence of the virus of measles in the circulating blood of a patient does not necessarily presuppose that the injection of such blood in a susceptible person would produce an infection. The most valuable and the one most definite experimental contribution to the study of measles was made by Hektoen when he produced measles artificially in 2 volunteers by the inoculation of blood from a patient. He demonstrated at the same time that the virus will survive in ascitic broth at 37°C. for at least twenty-four hours. The clinical symptoms in these volunteers differed in minor respects from the usually constant picture of the natural infection. Information is lacking concerning certain features such as Koplik spots and the leucocyte counts. Indeed it is not yet established in how far "measles inoculata" might vary from the spontaneous disease.

My own work on the inoculation of volunteers with blood of measles patients has given only negative results, indicating that the injection of a patient's blood will not regularly and constantly reproduce the disease in individuals who are apparently susceptible.

Susceptibility of monkeys. Experimentally, one of the most important factors in the study of measles is the question of the susceptibility of monkeys. Attempts have been made in two directions to establish proof of the susceptibility of monkeys to measles. Nicolle injected blood from a measles patient into a monkey and noted a mild febrile reaction. A child inoculated with blood from this animal developed measles. Unfortunately the precautions which were taken to prevent accidental infection are not described.

Blake and Trask found that the histologic picture of the skin rashes occurring in monkeys inoculated with measles corresponded to the histology of the lesions of human cases. This histologic picture is not pathognomonic. We have, therefore, no convincing proof of the susceptibility of monkeys.

Although my own attempts to infect monkeys have been disappointing, nevertheless it seems to me that the weight of evidence in the literature favors the conclusion that occasionally individual animals develop mild reactions when inoculated with the virus of measles. However I am not willing to place dependence on this method for studying the disease. Practically all observers agree that the symptoms are rather vague, many individual monkeys being entirely refractory. Variation occurs in this respect to a much greater degree for example than in the case of the experimental production of typhus fever. Moreover, experienced investigators report altogether conflicting results in the study of measles regarding such cardinal factors as the development of a skin rash and the occurrence of a febrile reaction. There is also marked variation concerning details such as the incubation period, the presence of Koplik spots, of leucopenia, rhinitis and malaise. Anyone contemplating the study of measles in monkeys will find that very naturally no uniform technique has as yet been evolved. In the choice of material for inoculation, equally good results have been reported by the use of either blood or mucous secretions. Three modes of procedure have been employed for the inoculation of mucous secretions; namely, (1) swabbing the mucous membranes with or without preliminary scarification, (2) subcutaneous injection, and (3) intratracheal injection. No comparison of these methods has been attempted but theoretically the intratracheal injection when followed by regurgitation with coughing

and sneezing would give opportunity for a thorough inoculation of the mucous membranes.

Whatever the mode of inoculation, the chief difficulty arises in the interpretation of the reactions. Of the various findings reported in the monkey, there are three features of cardinal imporance; namely, (1) fever, (2) leucopenia, and (3) rash, either of the skin or mucous membranes. These symptoms supposedly characteristic of experimental measles, are too mild to determine convincingly the etiologic relationship of suspected microörganisms isolated from patients.

This may seem to be an unhopeful view. On the contrary, it is merely suggested that attention should be directed toward a further study of the reactions in animals. It seems to me important to establish first of all an exact method of study rather than to increase the mass of data that has been founded on more or less doubtful methods.

Of the cardinal problems yet to be solved in measles we may mention: (1) the demonstration of the causative microörganism, (2) its cultivation, and (3) the infection of lower animals in such a manner as to provide a reliable and practical method for the recognition of the virus. By contrast with measles, let us consider a disease such as spotted fever, in which the causative organism is readily demonstrated microscopically in tissues and which produces in guinea-pigs a fatal infection with characteristic lesions. In any attempts at cultivating this organism, suspected cultures can be tested readily and conclusively. However, in measles, in working on any one of the three features just mentioned, it is necessary to contend with two unknown factors.

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