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Measles Virus: A Summary of Experiments Concerned with Isolation, Properties, and Behavior

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Presented here is a clarifying summarization of the many studies upon which any forthcoming advances in measles prophylaxis will be based.

Study of the etiologic agent of measles began with Home at the end of the 18th century and has been continued intermittently until the present. Among the more significant contributions to this subject in modern times was the demonstration by Goldberger and Anderson [1] in 1911 that macaque monkeys are susceptible to infection. This observation was later confirmed and extended by Blake and Trask,[2] among others. During the course of these earlier studies evidence for the filterable nature of the agent was obtained.

Using the monkey to test for the presence of virus, Plotz [3] in the 1930's reported its successful cultivation in cultures of chick embryonic tissues. In 1939–1940 Rake and Shaffer [4, 5] described the serial propagation of the agent in chick embryos and in 1941 with Jones [6] confirmed Plotz's observations. In the same year Shaffer and his associates [7] also noted that during the course

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of passages in the chick embryo the virulence of the virus for man was reduced and they attempted to determine whether this attenuated virus might be employed as a vaccine. The results were inconclusive and since that time we have seen no account of further trials with such materials, although it is our understanding that similar experiments are now in progress within the Soviet Union.[7a] Other workers in the past,[8] including one of us, were not able to induce measles in the monkey with regularity and also failed to obtain convincing evidence that the virus multiplied in living chick embryos or in chick embryonic tissues cultivated in vitro.

Such experimental discrepancies as well as the failure to make more rapid progress toward the precise definition of the nature and properties of the measles agent were largely attributable to the lack of a convenient and inexpensive technic whereby viral multiplication could be unequivocally determined. Following the demonstration of the growth and cytopathogenic effect of poliomyelitis virus in cultures of extraneural human tissues it seemed likely that application of the same method might prove effective in the case of measles virus.

Isolation of Measles Virus in Tissue Culture

Accordingly, Enders and Peebles [9] in 1954 undertook experiments in which cultures of human postnatal tissues in roller tube cultures were exposed to whole blood and throat washings obtained from a patient with measles during the first 24 hours of the exanthem. After an interval of from four to 10 days abnormal changes were observed of a character that we shall presently describe and which were shown to be induced by a virus. Comparable materials from seven other typical cases of measles subsequently tested in cultures of human renal or monkey (rhesus) renal cells have yielded agents exhibiting the same cytopathogenic properties. In three of these cases virus was demonstrated in both blood and throat washings; in two the blood was positive and washings were negative. In each of the two remaining cases in which only blood or throat washings was tested virus was also found. In addition, an agent was recovered in tissue culture from the lung of a patient dying during the acute stage of measles that was indistinguishable from the others. It is to be emphasized that these nine viruses were isolated from cases occurring in different geographic areas at two different times, i.e., in the spring of 1954 and 1955. The association with measles of viruses conforming in their characteristics with those we have isolated has been subsequently reported by Cohen and his co-workers [10] and by Ruckle.[11]

Cytopathogenic Properties of the Virus

Upon isolation and during serial passages in cultures of human renal cells our nine strains of virus have induced the formation of syncytia or multinu-

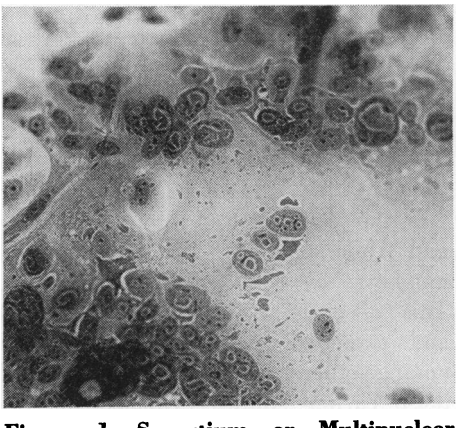

Figure 1—Syncytium or Multinuclear Giant Cell in Culture of Postnatal Human Renal Cells; 16 Days After Addition of 23rd Renal Cell Passage of Edmonston Strain of Measles Virus. H and E Stain x 430

clear giant cells within the sheet of normal epithelial elements such as those shown in Figure 1. Gradually all or nearly all the cells become involved in the process and eventually are destroyed. Several days after the syncytia are apparent, eosinophilic inclusion bodies appear in most of the nuclei and irregular masses of eosinophilic material accumulate in the cytoplasm. Examination of many preparations has convinced us that both these abnormalities are the result of viral multiplication.

When virus that has been propagated in renal cells is introduced into cultures of certain other cells essentially the same cytopathogenic effects are observed at least during a limited number of passages. In Figure 2 are shown the changes that occurred 16 days after inoculation into human amnion of the so-called Edmonston strain of virus. The agent had been previously passed 23 times in cultures of human renal cells. Following the addition of this strain to cultures of the KB line of human carcinoma cells (developed by Eagle) Dekking and McCarthy [12] in our laboratory observed the formation of multinuclear giant cells. Black and his co-workers [13] have described similar changes in cultures of the Hep-2 line of human carcinoma cells. McCarthy [14] and Rustigian [15] independently have noted the same effect in cultures of HeLa carcinoma cells. In unpublished experiments we have demonstrated the growth of virus in the Detroit 6 line of cells that Berman and Stulberg derived from human bone marrow, but we have not noted the formation of syncytia, although in stained preparations intranuclear inclusion bodies were conspicuous in individual cells. We have also seen cells of abnormal appearance in cultures consisting predominantly of human embryonic fibroblasts that were accompanied by multiplication of the virus within the system. So far we have observed no cytopathogenic effects in cultures of bovine amnion cells, chick amnion, or chorioallantoic tissues, or in chick embryonic tissues to which the virus was added.

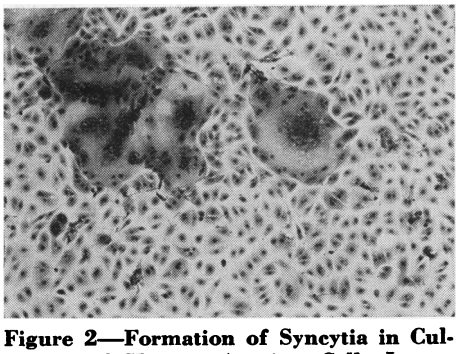

Figure 2—Formation of Syncytia in Culture of Human Amnion Cells Inoculated 16 Days Earlier with Edmonston Strain, from 24th Human Renal Cell Passage. Note Normal Appearance of Surrounding Epithelial Cells. H and E x 160

Lately we have found that after prolonged cultivation, in vitro, the cytopathogenic properties of the virus may become altered. Thus the Edmonston strain after 23 passages in human renal cells has been continuously propagated in human amnion cells. During the 14th amnion cell passage, in addition to the presence of multinuclear giant cells, increasing numbers of refractile, fusiform, or stellate cells were noted that somewhat resembled fibroblasts. Their appearance in the 19th passage is shown in Figure 3 along with typical giant cells. In successive passages these forms tended to predominate or, indeed, in certain cultures came to represent the only manifestation of viral multiplication. In stained preparations intranuclear inclusions were often found in these affected cells. This newly recognized cytopathogenic effect is of much interest from the general biological standpoint: it also led us to attempt again the cultivation of the virus in chick embryos. Later we shall summarize the results of these experiments.

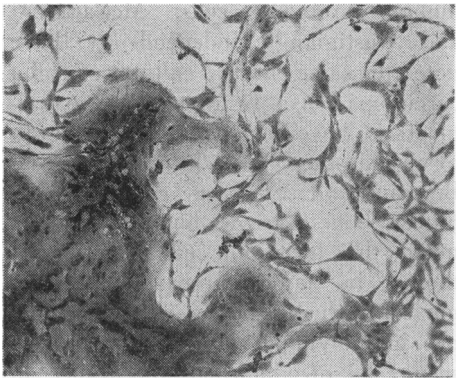

Figure 3—Cytopathogenic Effects in Human Amnion Cells of Edmonston Strain After 23 Passages in Human Renal Cells and 19 Passages in Human Amnion Cells. Note Numerous Fusiform Cells Together with Syncytia. H and E x 150

Serological Reactions

Tests for Virus-Neutralizing Antibodies—Tests of the capacity of acute and convalescent phase sera from 12 measles patients to prevent the cytopathogenic effects of the virus have been carried out using 100 ID_{50} of virus and dilutions of serum increasing by a factor of two. The results have clearly shown that antibodies develop during the course of the disease that prevent the occurrence of both types of cytopathic change in cultures of human renal and amnion cells.[16, 19] Thus in the acute phase sera of six of these 12 patients no neutralizing effect was found in the lowest dilutions of serum tested, i.e., 1:2–1:8. The mean of the convalescent phase serum titers was 1:260 with a range from 1:160 to 1:512. The antibody emerges soon after the exanthem is declared and may attain a moderate or high level within the following seven to 10 days or even earlier as appeared to be the case in the remaining six cases that were studied. Investigation of the persistence of the neutralizing antibody over prolonged periods has not been undertaken, but it can be stated that in a few cases the high concentrations developed early in convalescence are maintained for at least two to four months.

Tests for Complement-Fixing Antibodies—More extensive data on the formation of antibody during the disease have been obtained by means of the complement-fixation test, since this procedure can be carried out with greater convenience and rapidity. As antigen we have used the fluid removed from cultures of human renal or amnion cells two or three weeks after infection with the virus. By means of the drop technic of Fulton and Dumbell 25 pairs of acute and convalescent-phase serum from patients with measles have been tested.[16, 17] In all cases development of antibody or an increase in its initial concentration was demonstrated. The mean increase for these 25 cases was at least 30-fold. In seven instances the first specimen of serum was either taken several days after the onset when an elevated titer was recorded or the end point of the convalescent-phase specimen was not obtained. Excluding these, the mean acute phase serum titer of the remaining 18 cases was less than 1:4 (range: $<$1:2 to 1:8). In contrast the mean convalescent phase serum titer was 1:160 with a range from 1:32 to 1:512. These results, similar to those of the neutralization tests, not only afford strong evidence for the etiologic role of the virus in measles, but also suggest that the complement-fixation test should provide a practical diagnostic procedure.

Further evidence for the relationship of the virus to measles was obtained by comparing the results of complement-fixation tests on the sera of persons with either a positive or negative history of the disease.[17] In this study most of the subjects were children under the age of 10 years. The results are summarized in Table 1. It is clear that in a group of 54 persons giving a positive history the proportion with titers exceeding 1:8 is about six times greater than among

Table 1—Correlation of History of Measles with Results of Complement-Fixation Tests

History Positive			History Negative		
CF Titer *	No.	Per cent	CF Titer	No.	Per cent
<4	8	15	<4	12	48
4–8	6	10	4–8	10	40
>8	40	75	>8	3	12

* Reciprocal of initial dilution of serum giving fixation in presence of two units each of antigen and complement.

the group of 25 with negative history. Although the numbers in the groups are not equal, this difference is clearly significant. The relatively large proportion of sera giving positive tests in dilutions four to eight from the group with negative history suggests that in this range nonspecific reactions may often occur.

Pathogenicity of Virus for Monkeys

The results of experiments in susceptible cynomologus monkeys have indicated that two of the strains of virus tested after cultivation, in vitro, are capable of producing a mild disease comparable in most respects to measles in man.[16, 18] The criteria for determining the susceptibility of monkeys will be subsequently discussed. When animals were inoculated by both the intravenous and intranasal routes with virus of the first, second, and 23rd tissue culture passages several phenomena were noted. Viremia was established beginning on the fourth or fifth day and continuing thereafter for two to five days. An exanthem appeared soon after the beginning of the viremic phase which extended over the thorax and abdomen and was especially prominent in the axillary and inguinal regions. Slight to moderate leucopemia developed that was maximal on the ninth day. Shortly after the period of exanthem, i.e., about two weeks after inoculation of the virus, specific complement-fixing antibodies appeared in the blood and soon attained maximal concentrations. These high levels were maintained for several weeks when they usually began to diminish. Antibodies, however, were detectable in significant amounts at least eight months after inoculation of the virus. Not all these phenomena were apparent in other animals that were inoculated at the same time with portions of the same preparation of virus. In one monkey no exanthem was seen, although viremia and antibody response were comparable to those of animals exhibiting a rash; another responded only by the development of a slight leucopemia and the production of antibody. It is evident, then, that susceptible monkeys may vary considerably in their reaction to infection with this agent.

We have emphasized the fact that only susceptible animals may be expected to react in these various ways because early in our work [17] we found that a majority of normal rhesus and cynomologus monkeys that had been held for some time in three laboratories in this country possessed complement-fixing antibodies for the measles agent. In these tests the sera of 16 rhesus and eight cynomologus monkeys were examined. Antibody was found in 14 of the rhesus sera and in all the cynomologus sera. Neutralizing antibody was also detected in the few specimens that were tested. Ruckle [11] has lately reported similar findings and in addition has isolated an agent from monkey kidney tissue that so far is indistinguishable from human measles virus. The problem, however, of the origin of the agent responsible for the presence of these antibodies in apparently normal monkeys has not yet been solved. We are inclined to believe, for reasons that have been discussed elsewhere at length,[18] that it is probably identical with human measles virus and is derived either di-

rectly or indirectly from cases of the disease in man. The most cogent evidence in favor of this view lies in the fact that no complement-fixing antibodies capable of reacting with the Edmonston strain of virus were found in the sera of 31 cynomologus monkeys bled soon after their capture in the forests of Malaya and the Philippines. However this may be, it is obvious that the presence of antibodies reacting with measles virus in laboratory monkeys probably accounts for the discrepant results obtained by past workers in attempts to reproduce the disease in these animals.

Multiplication of the Virus in Chick Embryos

We referred at the beginning to the uncertainty that has continued respecting the capacity of measles virus to multiply in the developing chick embryo. Accordingly, after the means of isolating the agent and assaying its infectivity in tissue culture had been established, we proceeded to determine whether certain of our nine strains could be propagated in this host. Five of these strains maintained in renal cells since their isolation have been tested. The various routes of inoculation included the amniotic and yolk sacs and the chorioallantoic membrane. An incubation period of from four to six days was adopted following the procedure of Rake and Shaffer.[5] In our experiments two or three egg passages were made in the case of each strain and tests for the presence of virus in embryonic materials were carried out in cultures of human renal cells. In no instance was evidence of viral multiplication obtained.

Subsequently, when the variation in cytopathogenic properties of the Edmonston strain occurred, the possibility arose that this change might be accompanied by an alteration in the capacity of the virus to multiply in the chick embryo. A series of egg passages was therefore initiated. The inoculum consisted of virus from the 28th passage in human amnion cell and was introduced into the amniotic sac of the chick embryo. This route was selected with the thought that virus adapted to growth in human amnion cells might more readily proliferate in the analogous cells of the developing chick. After inoculation the embryos were incubated for nine days at 35° C—a longer period than previously allowed. The inoculum used as routine in subsequent egg passages consisted of amniotic fluid. In collateral passage lines suspensions of amniotic and chorioallantoic membranes mixed with the corresponding fluid were also employed. In each passage after the first the concentration of virus in various embryonic constituents was determined by titration in cultures of human amnion cells. The agent has now been maintained throughout seven serial passages. It continues to induce the formation of fusiform and stellate cells when transferred to cultures of human amnion cells (see Figures 4 and 5). In each of the last four passages multiplication was demonstrated by the titrational data. For example, the mean number of 50 per cent tissue culture infecting doses intro-

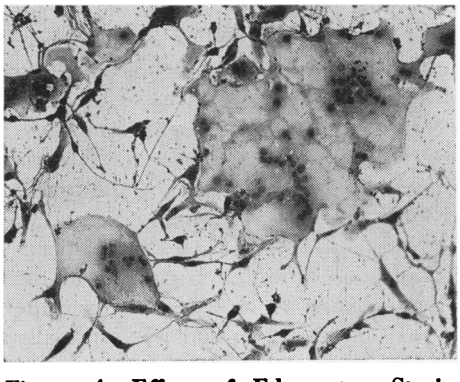

Figure 4—Effect of Edmonston Strain from Third Chick Embryo Passage in Human Amnion Cells, 14 Days After Inoculation of Virus. H and E x 180. Note Fusiform Cells and Syncytium

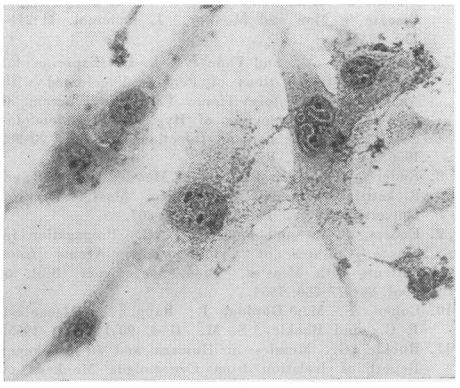

Figure 5—Cells from Same Culture Shown in Figure 4 x 750. Note Inclusion Bodies in Fusiform Cells

duced in the amniotic fluid employed as inoculum was 16, whereas on the average about 2,000 TC ID_{50} per 0.1 ml were found in the amniotic membrane at the time of harvest. The identity of the agent recovered from the fourth egg passage was confirmed in neutralization tests using human and monkey acute and convalescent phase sera as well as in complement-fixation tests in which the antigen consisted of fluid from infected human amnion cell cultures inoculated with the egg-passaged virus. A complete account of these experiments is in preparation.[19]

When it was found that multiplication of this strain occurred in the developing chick it obviously became of interest to determine whether it could be cultivated in cultures of chick embryonic tissues. Experiments of this sort are now in progress and the results so far suggest that multiplication does occur under these conditions.

Discussion

In conclusion, we shall offer brief comment on the results of the investigations that have here been summarized. We believe that they have provided tools whereby the virus can be readily isolated, its infectivity accurately determined, and its pathogenic, immunogenic, and other biologic properties analyzed. Using these tools progress already has been made toward the elucidation of certain long-standing problems, such as the cause of variation in the natural resistance of monkeys and the question of the susceptibility of the chick embryo. Among others that remain to be explored is the nature of the relationship of the virus to postmeasles encephalitis, determination of the true incidence of inapparent infection and of second attacks of measles, the duration of immunity as indicated by the persistence of antibody in the blood, and the determination of whether dermal hypersensitivity to the virus develops after infection.

From the more immediate, practical standpoint it is evident that methods of directly assaying the antibody content of different preparations of gamma globulin or other materials used in the production of passive immunity are already at hand.[16, 17] It also seems that groundwork has been established for future studies directed toward the development of vaccines whether composed of attenuated or inactive virus. We are encouraged to believe that progress toward this objective was made when we succeeded in propagating the agent in chick embryos. In our opinion virus grown under these conditions or in cultures of chick tissue would represent the most suitable material for the preparation of vaccine. There is a potential risk in employing cultures of primate cells for the production of vaccines composed of attenuated virus, since the presence of other agents possibly latent in primate tissues cannot be definitely excluded by any known method. For the preparation of inactive virus vaccine chick embryonic tissue would also appear to be advantageous. Not only would it afford relative assurance of the absence of extraneous viruses in the starting material, but also greatly reduce the cost of manufacture.

Much investigation, however, will be necessary before such considerations become of major importance, since first it must be determined whether multiplication of the virus in chick tissue will continue indefinitely, whether sufficient viral antigen is produced under these circumstances to be immunogenic when inactivated, or whether attenuation of pathogenicity for monkey and man may occur as a result of passage in chick tissue. All these questions future investigation must answer.

REFERENCES

1. Goldberger, J., and Anderson, J. F. An Experimental Demonstration of the Presence of the Virus of Measles in the Mixed Buccal and Nasal Secretions. J.A.M.A. 57:476–478, 1911.
2. Blake, F. G., and Trask, J. D. Studies on Measles. II Symptomatology and Pathology in Monkeys Experimentally Infected. J. Exper. Med. 33:413–422, 1921.
3. Plotz, H. Culture "in vitro" du virus de la rougëole. Bull. Acad. de méd., Paris 119:598–601, 1938.
4. Rake, G., and Shaffer, M. F. Propagation of the Agent of Measles in Fertile Hen's Egg. Nature 144:672–673, 1939.
5. Rake, G., and Shaffer, M. F. Studies on Measles I. The Use of the Chorio-Allantois of the Developing Chick Embryo. J. Immunol. 38:177–200, 1940.
6. Rake, G.; Shaffer, M. F.; and Jones, H. P. Studies on Measles. III The Use of Tissue Culture in Propagation of Measles Virus. J. Infect. Dis. 69: 65–69, 1941.
7. Shaffer, M. F.; Rake, G.; Stokes, J., Jr.; and O'Neil, G. C. Studies on Measles. II Experimental Disease in Man and Monkey. J. Immunol. 41:241–257, 1941.

7a. Zhdanov, V. M., and Fadeyeva, L. L. Experimental Data and Observations of Persons Vaccinated with Antimeasles Allantois—Tissue Vaccine. Program of XIII All-Union Congress of Hygienists, Epidemiologists, Microbiologists, and Infectionists. June 20–28, 1956. Leningrad, Russia.

8. Enders, J. F. The Etiology of Measles in Virus and Rickettsial Diseases. Cambridge, Mass.: Harvard University Press. 1940, pp. 237–267.
9. Enders, J. F., and Peebles, T. C. Propagation in Tissue Cultures of Cytopathogenic Agents from Patients with Measles. Proc. Soc. Exper. Biol. & Med. 86:277–286, 1954.
10. Cohen, S. M.; Gordon, I.; Rapp, F.; Macaulay, J. C.; and Buckley, S. M. Ibid. 90:118–122, 1955.
11. Ruckle, G. Measles in Humans and in Monkeys: Report of Isolation from Cynomolgus Monkeys of an Agent Immunologically Related to Human Measles Virus. Federation Proc. 15:610, 1956.
12. Dekking, F., and McCarthy, K. Propagation of Measles Virus in Human Carcinoma Cells. Proc. Soc. Exper. Biol. & Med. 93:1–2, 1956.
13. Black, F. L.; Reissig, M.; and Melnick, J. L. Propagation of Measles Virus in a Strain of Human Epidermoid Cancer Cells (Hep-2). Ibid. 93:107–108, 1956.
14. McCarthy, K. Unpublished data.
15. Rustigian, R. Personal communication to the authors.
16. Enders, J. F. Observations on Certain Viruses Causing Exanthematous Disease in Man. Am. J. M. Sc. 231:622–637, 1956.
17. ————. Recent Observations on the Behavior in Tissue Culture of Certain Viruses Pathogenic for Man. Fourth R. E. Dyer Lecture (1954). Public Health Service Publication No. 467. Washington, D. C.: Gov. Ptg. Office.
18. Peebles, T. C.; McCarthy, K.; Enders, J. F.; and Holloway, A. J. Immunol. (In press.)
19. Milovanović, M.; Enders, J. F.; and Mitus, A. To be published.)

Mental Health Week Begins April 28

Mental Health Week has been scheduled for the week of April 28. As in the past the National Association for Mental Health is directing this nation-wide observance in cosponsorship with the National Institute of Mental Health, Public Health Service. The week of concentrated attention to mental health problems is designed to get year-round and concerted public support for the care, treatment, and understanding that the mentally ill require in order to come back.

The 1957 slogan is "The Mentally Ill Can Come Back—Help Them—Give" (of understanding, service, and funds). The Association has prepared a kit of materials that is available for publications such as state health bulletins as well as for newspapers and magazines. 10 Columbus Circle, New York 19.