Isolation of Measles Virus from the Washed Leucocytic Fraction of Blood.* (28465)

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(Introduced by J. F. Enders)
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The presence of virus in the various blood fractions in viremia has received little attention. A few investigators have found, however, that some viruses can be recovered with greater ease from the leucocytic fraction than from the other blood elements(1-9). The study reported herein extends these previous observations and demonstrated that measles virus can be easily and consistently recovered in primary cultures of human amnion cells from the washed leucocytic fraction. This technique may thus prove suitable for the isolation of other viruses from the blood.

Methods/materials. Seventeen children (3-6 years) and 2 adults (19 and 28 years) were hospitalized with the clinical diagnosis of measles in the measles ward at the Claude Bernard Hospital for Infectious Diseases. Heparinized blood was obtained from patients 2-48 hours after admission to the hospital, and with one exception (Table I, patient No. 2,) was delivered to the laboratory for testing within 6 hours. Dextran§ (final concentration 1%) was added to the blood which was kept for about 30 minutes at 4°C to accelerate the sedimentation of most erythrocytes.

The plasma supernate was centrifuged at 1500 rpm, and the sediment was washed twice with Hanks' salt solution. This cellular sediment referred to as the "leucocytic fraction" contained in addition to leucocytes some platelets and erythrocytes. After resuspension in Eagle's salt solution to provide a leucocyte concentration of 3-6 × 10^6 cells/ml, 0.05 ml was added to each of 5 cultures of human amnion cells. In all cases an equal number of cultures were maintained uninoculated and others were inoculated with this fraction obtained from "normal" blood donors. The inoculum was allowed to settle on the amnion layer and left undisturbed for several days thereafter. In 3 instances, patients Nos. 8-10, whole blood was also tested and in one instance (patient No. 10) plasma as well.

Primary cultures of human amnion cells were grown as previously described(10) and

TABLE I. Isolation of Measles Virus.

Patient No.	Day of rash	Virus isolation (human amnion) Leucocytic fraction	Virus isolation (human amnion) Whole blood	HI antibody Acute plasma†	HI antibody Convalescent plasma
1	-2	+	—	<10‡	1280
2*	1	+	—	80	160
3	1	+	—	40	1280
4	1	+	—	80	1280
5	1	+	—	320	2560
6	1	+	—	10	320
7	1	+	—	—	1280
8	1	+	0	20	640
9	1	+	0	40	—
10	1	+	0	80	—
11	1	0	—	40	640
12	2	+	—	<10	>1280
13	2	0	—	80	1280
14	2	0	—	80	640
15	2	0	—	320	—
16	2	0	—	160	—
17	2	0	—	640	1280
18	2?	0	—	40	640
19	3	0	—	160	1280

* Blood was kept 24 hr at 4°C prior to testing.
† At time of viral isolation.
‡ Reciprocal of the titer.
+, isolation of virus; 0, no viral isolation; —, not tested.

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§ Dextran grade H, M.W. 186,000, Pharmachem Corp., Bethlehem, Pa.

maintained vertically in Kahn tubes in 5% casein hydrolysate medium.

Presence of measles virus was determined by the appearance of the characteristic syncytial CPE(10) in cultures inoculated with the leucocytic fraction of blood from patients with measles, and the absence of CPE in cultures inoculated with similar material from normal blood donors. All strains of virus induced a similar CPE, and all strains were passed successfully to fresh cultures of human amnion cells or to primary cultures of cynocephalus (baboon) kidney cells grown at 40.5°C(11). The CPE of 4 representative isolates was suppressed by measles convalescent plasma and not by acute phase plasma free of antibody(12). Hemagglutination inhibition tests of patients' plasma were performed as previously described, employing 4 units of antigen (Edmonston strain adapted to monkey kidney cells)(13).

Results. The clinical diagnosis of measles was confirmed either by viral isolation, a 4-fold or greater HI antibody response, or both, in 16 of the 19 patients in this study (Table I). Measles virus was isolated from the washed leucocytic fraction of blood taken during the first 24 hours of the exanthem from 10 of 11 patients and from this fraction taken during the second 24 hours of the exanthem from one of 8 patients. In 3 instances in which whole blood was also tested (Nos. 8, 9, 10) and in one case (No. 10) in which plasma was tested as well as the leucocytic fractions, measles virus was recovered *only* from the latter.

Since the culture tubes were maintained vertically, the blood cells accumulated at the bottom of the tube forming a central button. CPE, characterized by syncytia, was often first observed directly beneath the button, though subsequently typical cytopathic changes also developed at the periphery of the cell sheet. Syncytia were first noted between the 4th and the 12th day, with one exception (patient No. 9, 17th day).

Despite this mass of "foreign cells" present for several days, no toxic changes in the amnion cells were ever observed. In fact, cells in cultures exposed to either viral infected leucocytic fractions or "normal" leucocytic fractions often appeared "healthier" than uninoculated control cultures. A similar beneficial effect due to the introduction of a second cell type had been previously observed when WS cells (a human amnion cell line) were grown on primary cultures of human amnion cells(14).

Discussion. Using the techniques described, agents exhibiting the characteristic cytopathogenicity of measles virus were isolated from 10 of 11 patients in the first 24 hours of rash. These patients all exhibited clinical manifestations consistent with measles, and 8 of the 11 patients subsequently demonstrated an increase in measles HI antibody. The identity of 4 of these agents with measles was confirmed in neutralization tests. In only 1 of 8 cases of the disease in which blood was obtained after the first day of the rash was a comparable agent recovered. Virus was not recovered when specimens of whole blood or plasma from selected patients were employed as inocula. No virus was demonstrated in leucocytic fractions from the blood of 19 healthy donors. These results therefore indicate that measles virus can be isolated with high consistency from the washed leucocytic fraction during the first 24 hours of the exanthem.

These findings are consistent with those of Papp(7), who transmitted the disease to 3 human volunteers by inoculating them with the washed leucocytic fraction obtained from patients with measles and with those of Peebles(15,16,17) who recovered the virus in cultures of human kidney cells from the washed leucocytic fractions of patients with measles. In relation to our observations the results of Berg and Rosenthal(15) are also relevant, for these workers demonstrated the multiplication of measles virus in suspensions of human leucocytes.

Though this virus has been isolated on a number of occasions from whole blood during the first 24 hours of the exanthem(18,19), several investigators have reported difficulty in consistently recovering the agent at this favorable time. Bech and von Magnus(20) allowed the blood to clot and then utilized erythrocytes resuspended in the serum as inoculum. Presumably this procedure elim-

inated most of the leucocytes (and platelets) from the inoculum. The negative results of Halonen and his coworkers(21) may have been in part due to storage of whole blood at −60°C, since freezing and subsequent thawing disrupts cells thus releasing virus which would be neutralized if antibody were present. In this study it was demonstrated that even during the first 24 hours of exanthem HI antibody was detectable in most cases. In this regard it is appropriate to recall that Douglas and Smith(5) and Sabin (6) recovered vaccinia virus from the washed leucocytic fraction of blood of vaccinia immune rabbits but not from whole blood, which contained antibody. It would thus seem likely that the highest efficiency in the isolation of measles virus and perhaps other viruses from the blood may be achieved by testing the intact and washed leucocytic fractions.

Primary cultures of monkey and human kidney cells have been extensively employed in the isolation of measles virus. Despite their sensitivity to "wild strains" of virus, monkey kidney cells often harbor latent infectious agents which render identification of measles virus difficult, and human kidney cells are not readily available. It should therefore be emphasized that in our experiments measles virus was recovered in primary cultures of human amnion cells. Though this virus can be easily adapted to these cells(10), they have hitherto proved less sensitive to infection by virus present in the circulating blood (22). Several possible explanations for the difference between our results and those of other investigators may be presented: a) the dose of virus to which amnion cells are exposed may be greatly increased as a result of multiplication in previously infected cells after their addition to the culture, b) the conditions in our experiments were different (*i.e.*, the cultures were held vertically, thus giving a greater depth of fluid with resulting lower oxygen tension) from the usual slanted monolayer cultures, c) infected cells in contact with susceptible elements may possibly induce syncytial formation more effectively than cell free virus(23). This possibility is supported by the observation that when infected culture media from the original cultures was transferred to fresh human amnion cultures CPE developed more slowly and was less extensive.

Lastly it should be emphasized that some platelets and erythrocytes were also present in the "leucocytic fraction." From our results, therefore, it is not possible to define the blood element(s) associated with measles virus. It seems likely, however, that leucocytes represent at least one viral containing component, since it has been shown that these cells *in vitro* support multiplication of measles virus(15).

Summary. Peripheral blood was obtained from 19 patients with measles. Virus was isolated (employing primary cultures of human amnion cells) from the washed leucocytic fraction of blood of 10 of 11 patients in the first 24 hours of the exanthem and of 1 of 8 patients in the second 24 hours of the exanthem. It is suggested that this procedure may prove effective in the isolation of other viruses from the blood.

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Improved Mental Functioning with Premarin Therapy in Atherosclerosis. (28466)

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Intellectual decline in the elderly is often attributed to atherosclerosis in the cerebral vasculature. Experimental work with animals and studies of patients with coronary artery disease have both suggested that estrogens may be helpful in retarding or even reversing atherosclerosis(1). It has also been reported that survival rate increased with administration of conjugated equine estrogens (Premarin) to men who had recovered from an acute myocardial infarction(2,3). Kountz(4) concluded that administration of estrogens to postmenopausal women reduced atherosclerosis in uterine vessels and also improved the general constitutional condition and some aspects of the mental state. Rivin and Dimitroff(5) reported less severe atherosclerosis of cerebral arteries in patients with carcinoma of the prostate who were treated with estrogens than in those who were not. Their findings were not confirmed, however, by Meissner and Moehring(6). To the extent that estrogens may promote restored cerebral physiological processes in patients with atherosclerosis, improvement in mental functioning might also be anticipated.

This report concerns the effect of Premarin on certain aspects of cognitive funtioning, the hypothesis being that mental impairment decreases in patients with atherosclerosis treated with Premarin. The hypothesis was tested with the use of the Rorschach ink blot technique, which has frequently proved to be sensitive to impaired mental functioning accompanying organic changes in the brain(7).

Methods. One hundred and one patients were included in the analysis, 51 who had had cerebral thrombosis and 50 who had had myocardial infarction. They were participating in a long-term, controlled study of the effects of Premarin therapy in cardiovascular and cerebrovascular disease, in progress at the Los Angeles County Hospital. Prior to assignment to treatment all patients had been carefully evaluated to ensure accuracy of diagnosis. They were free of concomitant illness threatening survival, and were free of clinical need for hormone therapy other than insulin or thyroxin. In general they came from low socioeconomic backgrounds and had had limited education. Patients were randomly allocated by a statistical control office to either Premarin or placebo treatment, and were then seen in special outpatient research clinics at approximately 6-week intervals.

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