

# Extensive cytological damage caused by measles in African children

ULLA GRIPENBERG\* and C. FORBES

*International Agency for Research on Cancer, Nairobi Regional Centre and Kenyatta National Hospital, Nairobi, Kenya*

**Summary.** The occurrence of mitotic and chromosomal aberrations was examined in cultured lymphocytes from 25 African children suffering from acute measles at an early stage (0–5 days after onset of rash).

In eight cases the mitotic response to phytohaemagglutinin was impaired. In two cases extensive changes were observed, involving the formation of giant polykaryotic syncytia with chromosome pulverization. It is suggested that the development of giant cells *in vivo* and the damage to epithelial membranes characteristic of severe measles in the tropics are to be correlated with these changes.

A varying incidence of minor chromosome aberrations, mostly small gaps, was found in 15 cases of measles as well as in controls. The specific relationship of this type of aberration to the virus is questionable.

Mitotic activity was induced in two replicate lymphocyte cultures in the absence of phytohaemagglutinin.

The possible oncogenic potential of the measles virus is discussed in connection with the fusion-pulverization phenomenon.

In tropical countries measles is known to be a severe disease (Morley *et al*, 1967). Mortality is high, and the age of onset is early. In East Africa, for instance, the case fatality is reported to be 5.7%, in West Africa 12.3%; in Uganda, Kenya, and Malawi the median age is found to be 18.5 months (Morley, 1969a, b). These figures deviate very considerably from corresponding observations from the Western countries (World Health Organization, 1969). At the present time measles is unique among infectious diseases in its range of severity.

The clinical picture shows characteristic traits. In the severe form, the rash darkens to a deep red or violet colour, and is followed by desquamation, the skin separating in plaques more than 1 cm across. Equivalent changes occur on other epithelial surfaces as well, inducing lesions of the conjunctival sac, the mouth, the upper respiratory passages, and the gastro-intestinal tract. Fatalities are mostly caused by bronchopneumonia, diarrhoea with intes-

tinal bleeding, and encephalitis. Blindness, and malnutrition due to inability to suck are further severe complications.

The reasons for the severity of measles in the tropics are not known. In acute measles a high frequency of chromosome breakage has been observed (Nichols *et al*, 1962), but contrary findings have also been reported (Tanzer *et al*, 1963; Harnden, 1964). The cytological changes possibly occurring in the severe form of measles in the tropics were studied. Lymphocyte cultures from 25 African children were established in the acute stage of the disease. Large syncytia were found in the cultures, including numerous cells with prematurely condensed chromatin, representing all stages of pulverization. The clinical consequences of these remarkable cytopathological changes are discussed.

## Material and Methods

The measles patients selected for chromosome studies were all seen as outpatients at the measles clinic at Kenyatta National Hospital, Nairobi, during the period February–May 1972. The children were all black

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\* Present address: Department of Genetics, University of Helsinki, Helsinki, Finland.

TABLE I

THE OCCURRENCE OF ABERRATIONS IN LYMPHOCYTE CULTURES FROM MEASLES PATIENTS AND CONTROLS

Patient						Cell and Chromosome Changes					
No.	Initials	Sex	Age (mth)	Day of rash	Mitosis without PHA	Syncytia (%)	No. of Mitoses Studied	No. of Cells With:			Total Aberrations (%)
								Breaks	Gaps	Multiple Changes	
<i>Measles patients</i>											
1	K.K.	M	7	2			50	1	5	2	16
2	S.K.	M	15	2			41	0	2	2	10
3	B.J.	M	12	2			43	2	0	1	7
4	M.W.	M	18	2	-		33	0	1	2	9
5	M.W.	F	18	2			38	0	1	2	8
6	F.D.	M	24	5			50	0	2	1	6
7	N.J.	F	12	2	-		28	3	2	0	18
8	N.J.	M	14	1			123	3	17	15	28
9	S.O.	M	18	1		55					
10	B.W.	F	34	5			38	0	2	0	5
11	K.M.	M	8	1			50	0	2	1	6
12	M.D.	F	48	1		6					
13	Y.N.	F	6	4			45	1	2	2	11
14	S.K.	M	12	1	+		30	0	3	0	10
15	H.W.	F	10	1	-		51	2	2	2	12
16	C.M.	M	18	3			62	1	4	0	8
17	A.J.	F	10	1	+		44	1	3	1	9
Mean							48.4	1.9%	6.6%	4.2%	12.8%
<i>Controls</i>											
18	W.	M	1	—			34	1	1	0	6
19	A.	M	1	—			50	0	6	0	12
20	M.A.	F	10	—			50	2	2	0	8
21	B.N.	M	1	—			50	0	5	4	18
Mean							46	1.6%	7.6%	2.1%	11.0%

Africans belonging to the population living in and around Nairobi.

Table I gives details of age, sex, and day of rash when the blood samples were drawn.

The patients were between 5 months and 4 years old, mean age 16.7 months. Most of them were in a very early stage of the disease when bled. No special attention was paid here to the severity of the symptoms in the individual cases, since many of the complications only become apparent at later stages of the disease.

Five to 10 ml. of blood was used for starting two or three replicate cultures from each sample. The blood of these small children was drawn from the femoral vein.

Phytohaemagglutinin (Wellcome) was added to two of the cultures. When possible, a third culture was started without addition of the mitogen. The cells were grown in Parker TC 199. No foreign serum was added. Cells were harvested on the second or third day after 1 hour's pretreatment with Colcemid (Ciba). As hypotonic treatment Hank's solution diluted 1:4 with distilled water was given. Slides were prepared by air drying and Giemsa staining.

In screening, attention was first paid to the overall mitogenic response. In slides with a fair number of mitoses 50 or more good metaphases were usually selected for detailed examination. The aberrations were classified as (1) chromosome and chromatid breaks and fragments, (2) single gaps, and (3) multiple aberrations per cell. Typical secondary constructions were excluded from the counts. Specimens from four controls referred for karyotype diagnosis were handled in exactly the same way by culture, harvesting and slide-making.

When bled, these children were free from any sign of infection.

## Results

In the lymphocytes from the measles patients the mitotic response to phytohaemagglutinin stimulation varied considerably.

Lymphocyte cultures from eight of the 25 patients showed few if any mitoses. These mitotically inhibited cases had to be excluded from the series.

The cultures from two patients contained highly abnormal cells. Giant polykaryotic syncytia involving one or sometimes several groups of normal-looking metaphase chromosomes were observed. Some of the nuclei in the syncytia showed prematurely condensed chromatin, also called pulverized chromosomes. This condition is produced by fusion of nuclei at different stages of the cell cycle (Fig. 1). The frequency of polykaryotic syncytia in these cultures is expressed as the percentage of syncytia among all cells containing metaphases. In one of the cases (no. 9) the frequency of fused cells containing pulverized chromatin was remarkably high, 48 and 62% in the two replicate cultures. In the other case (no. 12) the frequency was lower, 5 and 9%. In these two samples cell and chromosome damage is expressed only as the incidence of the syncytia in the cultures.



FIG. 1. A syncytium with prematurely condensed chromatin from a measles patient.

With respect to the occurrence of chromosome breaks they are not regarded as comparable with the other samples.

Cells from cultures of 15 measles patients were subjected to detailed examination of the chromosomes at metaphase.

Altogether 726 cells (mean 48.4 cells per case) were screened for the occurrence of aberrations. The frequency of metaphases showing aberrations varied between 5 and 28%, mean 12.8 (control 11.0). As seen from Table I, the commonest type of aberration was a single gap in one or both chromatids of a chromosome (mean 6.6; 7.6 in the controls). In 4.2% of the cells the metaphases had two or more aberrations per cell (control 2.1); mostly two different gaps or one gap and one break. Only 1.9% of the cells had true breaks (control 1.6). No significant differences occurred with regard to the incidence of breaks and gaps in the metaphases from measles cases and from controls. A slightly

larger number of cells with multiple aberrations was found in the measles group.

Some of the cultures from measles patients contained abundant cell debris. Additional cell damage, such as chromatin condensation and cell necrosis was observed sporadically. No micronuclei were found.

In two different replicate cultures (nos. 14 and 17) some mitotic activity occurred without addition of any mitogen. The number of good metaphases was too low, however, for evaluation of the frequency of chromosome aberrations.

A certain clustering of changes was found in cultures established on the first day after the appearance of the rash (Fig. 2). Syncytia with chromatin pulverization were seen only in cultures from this stage. The highest frequency of breakage was seen in a culture from the first day. Mitotic stimulation without addition of any mitogen was observed only at this early stage.

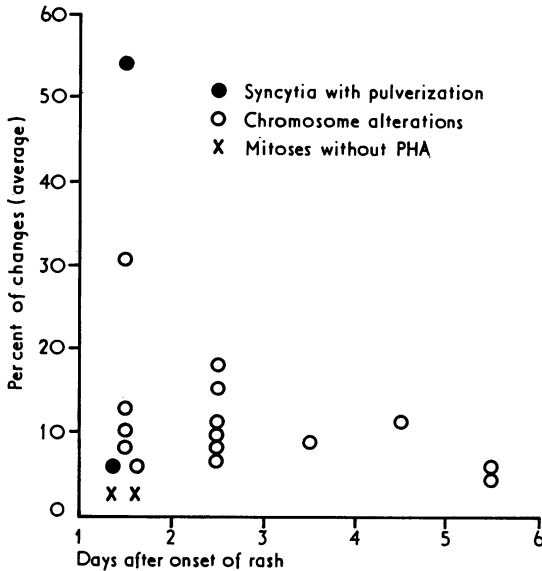


Fig. 2. Incidence of cell and chromosome changes in cultures established 1-5 days after onset of rash.

### Discussion

The cellular damage induced by the virus will be considered under three separate headings.

**Inhibited Lymphoblast Transformation.** As shown in the results, no blast cell transformation or mitotic activity occurred in the cultures from eight of the measles patients. This may have been due to a number of factors affecting the cultural conditions. During the same period, however, there were no failures in eight comparison cultures established from children suspected of having abnormal karyotypes. Hence, it seems probable that some factor related to the virus infection was responsible for the failure.

Viral inhibition of phytohaemagglutinin-induced lymphoblast transformation has been described by Olson, South, and Good (1967) and Montgomery *et al* (1967). In measles, signs of depression of mitotic activity have been reported by Nichols *et al* (1964). On the other hand, two of the present cultures showed mitotic stimulation even without addition of mitogen.

Whether different cell populations react in opposite ways or whether a sequence of processes leads via growth inhibition and possible cell destruction to repopulation by cells capable of transformation is not known. The stimulation and inhibition of blast cell transformation may have resulted from competing influences.

**Cell Fusion and Chromosome Pulverization.** Chromosome pulverization is closely related to cell fusion, a phenomenon known to be induced by measles and other myxoviruses (Stenman and Saksela, 1969). Chromosome pulverization has been studied extensively *in vitro* (Heneen *et al*, 1970; Johnson and Rao, 1970; Stenman, 1971). It is due to premature condensation of chromatin during interphases before the cell is ready to undergo mitosis. A prerequisite for the premature condensation of chromatin is the presence of at least one metaphase nucleus in the syncytium. The result of the condensation mechanism seems to be synchronization of the G<sub>1</sub>, S, and G<sub>2</sub> nuclei within the syncytium. So far, chromosome pulverization has been described mainly as a phenomenon induced experimentally *in vitro*. Harnden (1964) has published pictures indicating the unequivocal occurrence of pulverization in cultures from subjects vaccinated against yellow fever. Partial pulverization has been described in a ring chromosome (Gripenberg, 1967). In this case delayed spiralization of the structurally abnormal chromosome seems to have caused the pulverization. As a consequence, micronuclei were formed (Leisti *et al*, 1968) and the abnormal chromosome was lost from part of the cells.

Pulverization is usually induced very rapidly. Exposure to the virus for as little as 20 minutes has been found to be sufficient (Aula and Saksela, 1966). In the present study the virus affected the cells from the start of the culture and probably throughout the incubation time, *viz*, 48 or 72 hours.

Poste (1970), however, has shown that the rapid fusion of the cells is essentially a laboratory artefact, demonstrable *in vitro* only after infection at extremely high virus multiplicities. Late polykaryocytosis is a cell fusion beginning a number of hours or, more commonly, days after infection at moderate or low virus input multiplicities. He further stresses that cell fusion coincides with the most intensive phase of virus multiplication.

The pulverization phenomenon is here demonstrated to occur in phytohaemagglutinin-stimulated cultures, from patients in the acute stage of measles. This *in-vitro* phenomenon is thought to correspond to events occurring *in vivo*. Giant cells, pathognomonic for measles, are a regular finding in nasal secretions from the patients. Very large multinucleate cells also occur in a measles induced lesion called giant cell pneumonia (Cheatham, 1959). Cell fusions, by interfering with mitotically active cells, probably destroy a portion of the dividing cells. Leucopenia, a frequent feature of measles infection, may well be the result of disturbed cell divi-

sion. That formation of syncytia is usually followed by cell degeneration and death is known from studies *in vitro* (Heneen *et al*, 1970). Cell fusions probably contribute to the epithelial damage that develops in the bronchus, conjunctiva, and other mucous membranes. Symptoms such as sore mouth, conjunctivitis, laryngitis, and bronchopneumonia can be understood as clinical consequences of the cytopathological changes described here.

Similarly, diarrhoea may originate from necrotic changes in mitotically active centres in the intestinal wall. Syncytia may cause focal lesions with haemorrhages.

Although probably not the only factors responsible for the clinical picture of severe measles, cell fusion and chromosome pulverization seem likely to play a striking role. Whether the fusion-pulverization phenomenon is caused chiefly by variants of the virus, by a poor immune response of the patients or by still other factors remains to be clarified.

It has been shown that in some cases multipolar polykaryocytes are able to undergo complete cell divisions *in vitro* (Heneen *et al*, 1970). As a result cells with a multiplicity of structural and genetical constitutions will appear. The progeny derived from syncytia are characterized by the diversity of the cell population. A wide spectrum of chromosome numbers can be expected. This condition in connection with loss of regulatory mechanics may have far-reaching consequences regarding oncogenesis.

**Chromosome Breakage.** Viruses have repeatedly been observed to cause chromosome breakage. Their effect on the chromosomes seems to be very inconsistent (Gripenberg, 1965), and the incidence of breaks varies from series to series. Although this effect has been studied in different systems *in vitro*, the underlying mechanism is not yet known. Recently, Henry, Cohen, and Karzon (1971) have shown that cell debris alone can induce increased breakage, possibly by liberating lysosomal enzymes. The severity of the cell damage may be expected to vary in accordance with the amount of cell debris.

In the present series the frequency of aberrations in the measles cases as a whole was rather similar to that of the controls. A considerable proportion of cells was affected in both groups. A higher frequency of breaks was found in a single case of measles.

Compared with the fusion-pulverization effect, the incidence of single breaks seem to be a less specific type of aberration.

## Conclusion

Of the three classes of virus-induced cell and chromosome changes, the fusion-pulverization mechanism is thought to have the widest bearing cytologically as well as clinically. Mitotic inhibition is still too poorly understood for a valid assessment of its significance. Chromosome breakage probably depends on other factors besides the virus infection; a new approach to this question may be necessary to clarify the role of the virus.

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