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Cytogenetic studies on peripheral blood cultures of neonates treated in an intensive unit

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The possible causes of an increased rate of structural chromosomal aberrations and sister chromatid exchange in peripheral blood cultures of mechanically ventilated newborns in an intensive care unit were investigated.

No cytogenetic abnormalities were found in low-birth-weight babies affected by hypoxia and acidosis during their first week of life. The rate of chromosome breakage and sister chromatid exchange was increased in blood cultures of neonates continuously ventilated with 70—80 vol% oxygen for a long period of time. The incidence and degree of chromosomal damage, although showing wide individual variations, was related to the duration of oxygen treatment.

In addition to high oxygen tension, other environmental factors of intensive care therapy like antibiotic and chemotherapeutic agents may be responsible for the mutagenic effect. The results indicate once again the importance of continuous pO_2-monitoring of ventilated newborns.

The incidence of structural chromosomal aberrations in peripheral blood cultures of term babies is 0.8—3.2% during the first fifteen days of life [4, 7, 18]. Some authors [3, 15] demonstrated a 4—10-fold increase of chromosome breakage in blood cultures of low-birth-weight neonates, while others [16] found no chromosomal abnormalities in newborns weighing less than 1000 g.

Hatcher and Hook [10] produced evidence that chromosomal breakage develops postnatally in both preterm and term newborns; this points to environmental mutagenic factors.

During the first days of life the homoeostasis of newborns is menaced by hypothermia, hypoxia, hypoglycaemia, infection and resulting acidosis. The question emerges whether the therapy directed against these conditions was responsible for damage to the chromosomes.

Patients and Methods

1. Fourteen preterm babies with a gestational age less than 36 weeks, admitted to the neonatal intensive care unit in hypothermia, hypoxia and severe acidosis were included into the study. The number of chromosome breaks per cell in peripheral blood cultures was determined during the first eight days of life (Table I).

2. The rate of chromosome breakage and sister chromatid exchange (SCE) was determined in blood cultures of 10 newborns on 1—4 weeks uninterrupted intermittent positive pressure breathing (IPPB) or positive expiratory end pressure (PEEP) after termination of ventilatory treatment.

TABLE I

Cytogenetic finding encountered in prematures affected by hypoxia and acidosis

Patient No.	Birth weight g	Age, days	Diagnosis	Blood pH	pO_2 mmHg	Blood glucose m/dl	Body temperature °C	No. of evaluated metaphases	No. of structural aberrations
1.	1250	3	Centralis lesion	7.07	—	90	35.8	30	
2.	1400	4	Perinatal infection	6.8	—	140	36	50	
3.	1600	5	IRDS I—II	7.2	—	45	36.4	30	1
4.	1000	8	Perinatal infection	7.0	—	90	36.7	30	
5.	1200	2	Prematurity	7.2	—	175	36.4	30	
6.	1830	7	Oesophageal atresia	7.1	32	150	36.4	30	1
7.	1440	4	Oesophageal atresia	7.0	28	200	36	40	1
8.	1730	1	Asphyxia in utero	7.24	36	100	35.5	30	
9.	1080	2	IRDS, intracerebral haemorrhage	7.19	39	130	36.4	30	
10.	2100	2	Sepsis, jaundice	7.09	14	250	35.4	30	2
11.	1200	1	IRDS III—IV	7.15	16	90	35.5	30	
12.	660	3	Cerebral haemorrhage	7.32	53	130	36	30	1
13.	1550	4	Hyperbilirubinaemia	7.3	44	—	36.5	30	
14.	950	8	Pneumonia	7.14	42	90	36.2	30	
								450	6

The gas mixture used for therapy contained more than 60 vol% O_2. pO_2 values were either monitored transcutaneously or measured in arterial blood samples by the Astrup method. Cytogenetic studies were performed simultaneously in O_2-treated neonates and newborns of comparable age admitted to the same unit but needing no oxygen therapy.

In addition to oxygen treatment, the patients received parenteral glucose, electrolyte and amino acid solutions, vitamins and antibiotics. The control group was treated with these substances in a similar way, as demonstrated in Table II.

Newborns treated with phototherapy, or with caffeine because of apnoeic spells were excluded from the study, as these factors are supposed to possess a chromosome damaging action [8, 21].

Chromosome studies were carried out on cultured peripheral blood by the method of Sumner et al (24); the breakage points were evaluated by light-microscopy after traditional staining. The SCE phenomenon was studied by the method of Perry and Wolff [19], as modified by Raposa [20].

RESULTS

1. The number of breaks per metaphase was 0.013 immediately after admission in prematures with acidosis and hypoxia (normal value for this laboratory: 0.010 ± 0.013) as shown in Table I.

2. Most pO_2 values of the patients treated with continuous mechanical ventilation were higher than 80 mm Hg. After termination of ventilatory treatment they exhibited a mean rate of aberration of 0.16 as contrasted to the value of 0.05/metaphase, found in newborns cared for in the same unit but not treated with oxygen ($p < 0.05$). The mean rate of SCE amounted to 7.1 in the oxygen treated group and to 5.7 ($p > 0.40$) in the control group (Table III). The highest breakage rates were encountered in the cul-

TABLE II

Clinical data of newborns receiving long-term oxygen therapy

Serial number of patient	Diagnosis	Drugs administered	Type of oxygen treatment	Duration in days
1.	Pneumonia, sepsis, central lesion	Tobramycin, meticillin, oxacillin, bromohexine	IPPB—CPAP	26
2.	Acute fetal distress, pneumonia	Tobramycin, azlocillin, polymyxin furosemide, dopamine, dobutamine, phenobarbital, human lyophil Ig	IPPB	11
3.	IRDS, bronchopulmonary dysplasia	Tobramycin, penicillin, oxacillin, bromohexine, furosemide, dopamine dobutamine, tolazoline	PEEP—CPAP IPPB, nasal gavage	4 30
Control	Ileal atresia	Tobramycin, penicillin, cephamandol, ampicillin, oxacillin, azlocillin, dexpanthenol, human lyophil Ig	—	—
4.	Connatal tuberculosis	Tobramycin, penicillin, azlocillin, cephamandol, furosemide, dexpanthenol, human lyophil Ig	PEEP—CPAP PEEP—IPPB	18 28
5.	In utero infection	Tobramycin, penicillin	PEEP—CPAP	7
Control	Hydronephrosis	Tobramycin, ampicillin,	—	—
6.	E.coli meningitis	Tobramycin, oxacillin, azlocillin, cephamandol, chloramphenicol, human lyophil Ig	IPPB-incub. O_2	12
7.	Intrapartum asphyxia, infection	Tobramycin, ampicillin, diazepam, phenobarbital	PEEP—CPAP	7
8.	Intrauterine growth retardation, bronchopulmonary dysplasia	Tobramycin, oxacillin, human lyophil Ig, heparin	IPPB—CPAP	16
9.	Perinatal infection	Tobramycin, oxacillin, dopamine, dobutamine	CPAP-incub. O_2	13
10.	IRDS III—IV, cerebral haemorrhage	Penicillin, ampicillin, cephamandol furosemide, phenobarbital, diazepam, dopamine, dobutamine	CPAP-PEEP	18
Control	Neonatal pyelonephritis	Tobramycin, penicillin	—	—

tures of newborns treated with 90—100 vol% oxygen for a considerable time. The two cytogenetic parameters showed mostly no parallelism and there was considerable individual scatter within the results obtained by either method. Only three patients had a SCE rate exceeding the mean, i.e. these patients exhibited excessively high values. In one patient the increased breakage rate was not accompanied by SCE induction (Table III). It appeared that the duration of oxygen therapy was of importance. In an illustrative case, a baby needing continuous positive airways pressure (CPAP) resp. PEEP because of intrauterine infection and serial

TABLE III

Cytogenetic findings of newborns receiving prolonged oxygen treatment

Patient No.	Oxygen concentration of inhalation mixture, vol%	Duration of oxygen therapy, day	Number of metaphases studied	Aberration per cell	Type of chromosome aberration		SCE per cell
					chromatid	chromosome	
1	100—70	26	50	0.28	12	2	9.7
2	100—90	11	50	0.14	4	3	not investigated
3	100—70	14	50	0.24	11	1	8.3
Control			50	0.10	5		5.7
4a	100—90	18	50	0.06	2	1	5.5
4b	100—95	28	50	0.18	8	1	6.3
5	60—50	7	50	0.06	2	1	not investigated
Control			100	0.02	1	1	5.7
6	50—40	12	50	0.12	5	1	5.2
7	60—40	7	50	0.02	1		6.0
8	70—60	16	50	0.24	9	3	5.0
9	65—30	13	50	0.12	5	1	not investigated
10	100—60	18	50	0.20	7	3	7.7
Control			50	0.06	3		5.0
Total/mean			550	0.16	66	17	7.1
Control values			200	0.05	9	1	5.7

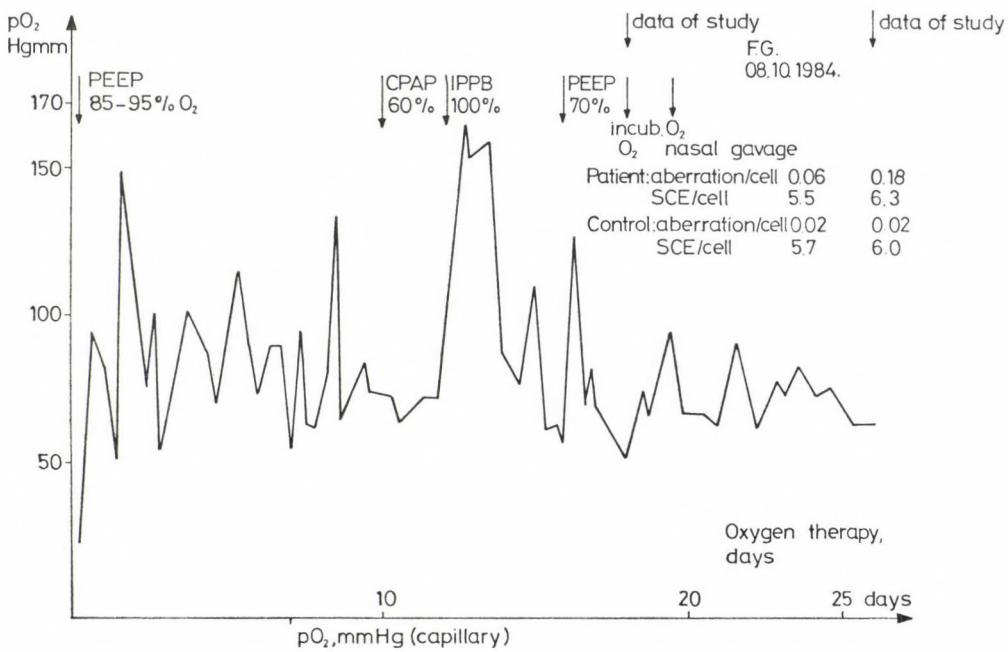

FIG. 1

apnoeic spells over 18 days had 0.06 aberrations and 5.5 SCE per cell; 40 days later, after a period of uninterrupted PEEP-IPPB treatment, these values increased to 0.18 and 6.3 respectively (Figure 1).

Discussion

Environmental mutagenic effects must play a role in the development of chromosomal aberrations observed in newborns since there are no structural aberrations or SCE in the cord blood of neonates born of healthy mothers [10]. There is an elevated rate of chromosomal breakage in newborns under intensive care or those exhibiting signs of intrauterine growth retardation [10].

Oxygen seems to play an outstanding part among the environmental factors leading to mutagenesis [25]. Within certain limits, the degree of free oxygen pressure may increase the concentration of peroxy radicals and thereby exert an indirect mutagenic effect. It is known from culturing human lymphocytes that free oxygen radicals produced in the medium by light or enzymes cause chromosomal breakage or induce SCE [5].

The genotoxic effect of high oxygen pressure was shown in bacterial tests [14] while others [23] observed cell death, micronuclear positivity, chromosomal gaps and breaks in Chinese hamster fibroblast cultures. The aberration rates observed on exposure to oxygen showed a dose and duration dependent character. Increased oxygen tension causes elevation in the rate of SCE in Allium cepa Linné cell cultures [9]. In other words, molecular oxygen may cause genome changes in both procaryote and eucaryote organisms.

Several defense mechanisms against free radicals have been developed by all kinds of live matter (nuclear membrane, reducing enzymes, catalases, peroxidases, etc.). It was assumed [6] that all aerobic organisms possess an enzymic defense system protecting the cells from oxygen induced damage. A similar role has been ascribed to plasma uric acid, an antioxidant compound capable of protecting the erythrocytes from lipid peroxidation [1].

In phytohaemagglutinin stimulated blood cell cultures of patients with Fanconi anaemia, elevation of the O_2 concentration provokes a commensurate increase in the rate of chromosomal breakage. This oxygen dependent chromosomal instability shows interindividual fluctuations which are further enhanced by the addition of mitomycin C [13]. In Fanconi anaemia a faultive defense mechanism has been assumed to be at work [12].

Clinical cytogenetic studies cannot help in deciding whether the increased rate of breakage and SCE, observed in lymphocytes of newborns ventilated with 60—100 vol% oxygen should be ascribed to the indirect damaging effect of oxygen itself or to a failure of the assumed enzymic defense mechanism.

The rate and degree of chromosomal damage is appreciably influenced by

the duration of oxygen therapy. Importance of the time factor is corroborated by data collected from several perinatal intensive care centres [17], who showed no cytogenetic aberrations in blood cultures of neonates exposed to ventilation therapy not exceeding 4—5 days.

In evaluating the individual variability, the possible role of mutagenic factors other than oxygen also emerges. When selecting the control group, patients comparable in respect to antibiotic and chemotherapeutic treatment were chosen since these drugs may cause cytogenetic aberrations [2, 11, 22]. The present data do not allow an evaluation of a possible relationship between acquired chromosomal aberrations and specific antibiotic regimens. However, in a patient of the control group, affected by ileal atresia, who had had to be treated with numerous antibiotics in addition to cephamandol, a markedly increased breakage rate (0.10 aberration per cell) was observed.

It may be speculated that pathological chromosomal phenomena have a multiple cause; beside a high oxygen pressure, antibiotics and other hospital pollutants (detergents, chemicals used for disinfection or cleaning, etc.) may be suspected. It appears that exact registration of the oxygen content of the gas used for ventilation and continuous monitoring of the patients' pO_2 values are indispensable in proper oxygen dosage and in attem pts to reduce the cumulative effect of deleterious factors present in intensive care units.

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