The effects of severe mixed environmental pollution on human chromosomes

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SUMMARY Cytogenetic studies were conducted on healthy young mothers, shortly after child birth, in two residential areas each with an approximate population of 20 000, situated about 25 km from Athens, Greece. One of the areas, Elefsis, is subject to severe mixed industrial pollution, and the other, Koropi, is relatively free of pollution. Chromosomal aberrations were investigated in 16 women from each area in 72 hour lymphocyte cultures treated with gentian violet to enhance any chromosomal instability induced by the pollution. The women were of a comparable socioeconomic level, aged between 20 and 31 years, and with no history of factors associated with mutagenesis. Venous blood samples were taken from the two groups and processed concurrently. The slides were coded and examined independently by two observers, who were unaware of the source of the samples. A total of 100 cells was examined on each sample. The two observers obtained highly comparable results. Women from Elefsis had an average of 0.42 anomalies per cell and those from Koropi had 0.39. The absence of a statistically significant difference between the two groups clearly shows that the severe mixed environmental pollution of Elefsis has no significant visible effect on human chromosomes in most residents. However, two Elefsis women had abnormal results and could be at risk. Their presence is not sufficient to raise significantly their group's average, but the induction by pollution of an increased rate of chromosomal anomalies in only a few people at risk could account for the known association between urban residence and cancer mortality.

The problem of population monitoring for health effects of environmental pollutants is complex especially in the case of mixed, low level chemical exposures. Chemical mutagenesis and chromosomal changes have been reported in human population groups with occupational exposure to specific chemicals, such as lead,^{1 2} vinyl chloride,^{3 4} benzene,⁵ pesticides,⁶ nickel,⁷ and styrene.⁸ There has also been limited investigation of the mutagenic effects on non-occupational exposure to individual chemicals.⁹ Mixed exposure studies have been conducted mainly in populations residing in the vicinity of old chemical dumps¹⁰ and have been criticised for methodological faults. Excellent guidelines are available¹¹⁻¹³ to avoid the pitfalls frequently encountered in the design and execution of such cytogenetic studies.

The gentian violet (GV) method of investigation¹⁴⁻¹⁷ provides an excellent short term cyto-

genetic test for constitutional or induced chromosomal instability of various types. The addition of GV to lymphocyte cultures for a few hours before harvest greatly enhances chromosomal instability and raises the baseline level of anomalies, so that very small or even undetectable differences in conventional cultures become striking. Its action precludes the use of other toxic agents such as BrdU. An additional advantage of the method is that the raised baseline makes the otherwise tedious analysis of thousands of normal cells more interesting to the observer. The results are thus more reliable, especially when two observers work blindly and obtain comparable results.

Areas investigated

The polluted area in this study is centred in and around the town of Elefsis in the Thriasian Plain in Greece, bordering the south coast of Attica, about 25 km west of Athens. It has oil refineries, distilleries, steel mills, cement works, shipyards, and

a large number of small industrial plants producing plastics, paints, and a wide variety of other products. The liquid waste drains into the sea, which is heavily polluted, and the various industrial processes discharge into the atmosphere fumes and particulate matter visible to the naked eye and unpleasant in smell. Climatic and geographical conditions result in frequent episodes of thermal inversion, and in the summer months the hot, dry, windy weather increases the area of dispersion of particulate matter and its inhalation by the residents.

Measurements of particulate matter in the air samples from the residential area of Elefsis¹⁸ frequently exceed the limits recommended by the World Health Organization. Between 1979 and 1981, a survey of certain stable pollutants in the surface soil and vegetation of the Thriasian Plain showed markedly increased concentrations (two to 50 times the levels accepted as normal) of cadmium, lead, nickel, chromium, and sulphur over wide areas.¹⁹ In the immediate vicinity of Elefsis, high levels of calcium carbonate and fluoride were found, corresponding to wind dispersion from the cement works.²⁰ It is probable that other pollutants, less amenable to measurement and possibly more toxic, are also widely dispersed throughout the residential area.

The Thriasian Plain has a total population of 40 000 to 50 000, of whom about 20 000 are permanent residents of the borough of Elefsis.

In 1981, the Athens Institute of Child Health started to conduct studies on the effects of the environmental pollution on the health of children in Elefsis. One of these studies was on the outcome of pregnancy, which is considered an appropriate indicator of environmental hazards.²¹ This study included the total pregnant population of Elefsis and, for comparison, of two smaller communities of a similar population, located in a relatively unpolluted rural area about 25 km east of Athens (Koropi). This study provided the opportunity for selection of two well matched populations of young women for chromosomal study, one from a heavily polluted area and one from a relatively pollution free area.

Material and methods

The populations studied consisted of 26 healthy women from Elefsis and 23 from the control area (Koropi). In each area, pregnant women were identified who were between 20 and 35 years old, non-drinkers,²² non-smokers,⁸ and known not to be heterozygotes for β thalassaemia.²³ Women were excluded who had a history of malignancy in a first degree relative living outside the study area.

The women from both areas gave birth in the same three Athens maternity hospitals where procedure is comparable. Between two and four weeks after the women had given birth, further selection was made, excluding those women who in the previous month had taken medication other than that standard for childbirth, or had suffered a viral illness,²⁴ or in the previous three months had undergone radiography.²⁵ Consecutive women from each area, over the same time period, who fulfilled these conditions and agreed to participate, were included in the study.

From these women, venous blood samples were withdrawn and transported in heparinised syringes at a stable temperature. The samples were coded and the examiners were unaware of the origin of each sample.

CYTOGENETIC PROCEDURES

Upon receipt of the blood sample, conventional three day lymphocyte cultures were established in Ham's F10 supplemented with 18% fetal calf serum. GV (Merck) was added for the last three hours of incubation at a critical final concentration of 5 μ g/ml from an aqueous stock solution of 5 mg/ml renewed every three weeks. (GV from other firms is known to require other concentrations or timing or both for the same results.) Colcemid (Gibco) 0.1 μ g/ml medium was added during the last 90 to 120 minutes. This was followed by a 15 minute hypotonic shock in 0.075 mol/l KCl and fixation in three changes of 3:1 ethanol-acetic acid for a total of one hour. Slides were air dried, stained with Giemsa, later coded by one person, and examined blind by two others. Scanning for apparently complete metaphases was done at low power and analysis at higher power under the microscope, provided that there was a total of 45 or 46 chromosomes. A few cells too difficult to analyse under the microscope were photographed and later analysed from prints. One hundred cells per subject (50 by each observer) were scored for breaks, gaps, deletions, chromosome rearrangements and chromatid exchanges, minutes, acentrics, monosomies, and trisomies. A square root transformation was applied to the data before the statistical analysis.

Results

Out of the 49 cultures established, 32 yielded the desired number of metaphases (16 from each group). The discarded cultures were randomly distributed among the two groups, both in number and in time, and their frequency did not significantly differ from that of parallel experiments where GV was not used. The two observers obtained highly

comparable results, with one consistently scoring more anomalies than the other, and their overall correlation coefficient of 0.64 significantly differed from zero ($p < 0.01$). Accordingly, their results were pooled and the means are shown in tables 1 and 2.

The overall range of anomalies per cell was 0 to 18 in Elefsis women and 0 to 14 in Koropi women. Those from Elefsis had an average of 0.42 anomalies per cell (that is, 0.65 ± 0.210 on the transformed data) while those from Koropi had 0.39 (0.63 ± 0.163 when transformed). Both variances ($F_{(15,15)} = 1.63$, two tailed test, $0.2 < p < 0.5$) and mean ($t_{(30)} = 0.390$, one tailed test, $0.25 < p < 0.45$) appeared to belong to the same population, without significant differences between the two groups.

(The same conclusion is reached when each observer's results are analysed separately, when aberrations

implying two breakpoints (cte) are scored as two lesions instead of one anomaly, and when anomalies other than breaks and gaps are analysed separately.) The overall average for all women was 0.41 anomalies per metaphase, with a Gaussian distribution after a square root transformation. Woman No 46 from Elefsis had the only abnormal average, 1.34 anomalies per cell, more than 2 SD from the overall population mean. She gave a second blood sample seven months later after the data were decoded. Her new level of 0.94 anomalies per cell fell within normal limits, but was still the highest of all. Woman No 44, also from Elefsis, was the only one in whom more than three cells were found to have five or more anomalies. She had five such cells with five, six, seven, eight, and 14 sporadic anomalies respectively.

TABLE 1 Characteristics of women included in study and cytogenetic results: Elefsis.

Subject serial No	Age	Years of residence	Work outside home	Work during pregnancy	Husband's work	Father's work	cte/cell	Total anomalies per cell
1	21	21	Secretary	No	Mechanic	Driver	0.02	0.56
4	29	21	Shopkeeper	Yes	Postman	Farmer	0	0.25
5	20	20	—	No	Welder	Waiter	0	0.19
9	28	5	Plastics worker	No	Welder	Labourer	0	0.17
16	21	21	—	No	Clerk	Café proprietor	0.02	0.27
23	27	5	Labourer, paper factory	No	Labourer, chemical plant	—	0	0.16
27	20	20	—	No	Driver	Sailor	0.04	0.53
28	26	26	Weaver	No	Labourer, distillery	Fisherman	0.03	0.21
31	27	27	—	No	Plumber	Labourer, cement works	0.05	0.35
36	20	20	Clerk	No	Shopkeeper	Bargeman	0.02	0.44
39	25	25	Munitions worker	No	Electrician	Operator, cement works	0.03	0.77
40	31	3	Hairdresser	No	Shopkeeper	Painter	0.02	0.45
42	25	25	—	No	Doctor	Farmer	0.03	0.30
43	30	6	Plastics worker	No	Driver	Builder	0.10	0.69
44	21	12	Toy factory worker	No	Driver	Shipyard worker	0.02	0.76
46	20	20	—	No	Foreman, cement works	Taxi driver	0.05	1.34
Mean	24.4	17.25						0.42

TABLE 2 Characteristics of women included in study and cytogenetic results: Koropi (controls).

Subject serial No	Age	Years of residence	Work outside home	Work during pregnancy	Husband's work	Father's work	cte/cell	Total anomalies per cell
3	22	22	—	No	Builder	Farmer	0.01	0.20
7	31	31	Clerk	Yes	Farmer	Gun repairer	0.01	0.34
8	26	26	Accountant	Yes	Teacher	Farmer	0.02	0.15
13	20	20	Salesgirl	No	Machine operator	Builder	0	0.14
17	21	3	Weaver	No	Welder	Farmer	0	0.34
18	28	28	—	No	Driver	Drill operator	0	0.66
21	21	3	Cannery worker	Yes	Furniture worker	Café proprietor	0	0.39
29	28	4	Furniture worker	No	Painter	Farmer	0	0.21
30	29	29	Waitress	No	Cafeteria owner	Metal worker	0.02	0.38
35	26	26	Clerk	No	Tile worker	Farmer	0.005	0.44
37	25	25	Clerk	Yes	Clerk	Driver	0	0.30
38	26	26	Teacher	Yes	Bank clerk	Farmer	0.06	0.67
41	25	25	—	No	Printer	Teacher	0.04	0.50
45	23	23	Seamstress	No	Builder	Farmer	0.05	0.49
48	28	28	Teacher	No	Clerk	Driver	0.06	0.94
49	31	3	Nurse	Yes	Farmer	Farmer	0	0.50
Mean	25.6	20.1						0.39

Discussion

The greatest possible attention was given to the experimental design in order to obtain simultaneous matched controls, to minimise possible biological and technical confounding factors, and to assure absolutely blind examination and thereby avoid unpleasant episodes of social and political character.¹³

The results clearly show that the severe mixed environmental pollution of Elefsis has no significant visible effect on human chromosomes in most residents. The findings of an abnormally high average of anomalies in woman No 46 and of an excess of very abnormal cells in woman No 44 may constitute the chance discovery of two subjects at risk. Their presence is not statistically sufficient to alter significantly their group's average, but it is consistent with the view that a polluted environment is more harmful to them than to the others. It has been suggested that a substantial part of the known association between urban residence and cancer mortality²⁶ can indeed be accounted for by the presence of heterozygotes for cancer predisposing genes, who are more susceptible to pollution effects than the rest of the population.^{27 28}

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