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Cytogenetic Studies in a Case of Benzene Leukaemia*

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THE **CHRONIC** exposure to inhalation of benzene can produce toxic effects on the bone marrow, which result in hyporegenerative anaemias or pancytopenias. In some cases the haemopathy can evolve into a leukaemia, mostly of the myeloblastic type [1–3].

One such case is presented, which was followed up by cytogenetic studies from the phase of hyporegenerative anaemia to the leukaemic phase.

Case *report.* Z.G. was a 38-year-old female, when first seen at the Clinica del Lavoro in December 1965. Nothing important in the family history. One normal pregnancy at the age of 26. The patient had been working from 1942 to 1964 as a finisher of electric cables. The cables were covered by a rubber muff, and were cleaned with solvents containing benzene; the solvent was poured on cotton-wool and this was rubbed on the external surface of the cable muff. From 1960 to 1964 the patient complained of weakness, headache, vertigo, irregular fever. In September 1964 a severe anaemia was discovered (Hb 6·4 g/100 ml, RBC 2,100,000/mm³). She had two hospital admissions and improved under treatment with transfusions.

From September to November 1965 she was admitted to a university hospital. The blood findings were: Hb 4·5 g/100 ml, RBC 1,900,000/mm³, TVBC 4,800/mm³, with normal differential count, platelets 90,000/mm³. No bone marrow could be obtained despite several attempts. She was discharged with the diagnosis of benzene-induced hypoplastic anaemia. The case was reported to the Insurance Institute against Occupational Diseases and sent to the Clinica del Lavoro.

On first admission (11/30/65–3/12/66) no important findings were observed by physical examination. The laboratory findings were: Hb 6·4 g/100 ml, RBC 2,000,000/mm³, WBC 3200/mm³ with neutrophils 70%, eosinophil 1%, lymphocytes 27%, monocytes 2%; reticulocytes 1%; platelets 120,000/mm³, free protoporphyrin nine µg 41/100 ml RBC; plasma iron µg 149/100 ml; UIBC µg 110/100 ml plasma, MCV µ³ 108; total serum bilirubin mg 0·7/100 ml; normal bleeding and coagulation times and thromboelastogram. At sternal puncture (2/21/66), the bone marrow was very poor with rare normoblasts and some cells of the myeloid series, mostly myeloblasts and neutrophil promyelocytes with abundant azurophilic granules.

When seen in the out-patient clinic on 4/19/66, the spleen was palpable for the first time; TYBC 3000/mm³ with neutrophils 74%, lymphocytes 16%, monocytes 2%, metamyelocytes 4%, myelocytes 3%, myeloblasts 1%; normoblasts 2%.

On second admission at the Clinica del Lavoro (5/25/66–7/30/66), the laboratory findings showed **an** accentuation of the anaemia, an increase of immature cells in the peripheral blood (10% of 3000 WBC/mm³), low platelet count (48,000/mm³). The bone marrow at sternal puncture on 6/13/66, **was** rather scanty;

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Table 1.

Date	Hb (g/100 ml)	WBC /mm³	Myelo-blasts %	LAP	Sample	Distribution of chromosome counts ≤43	44–45	46	47	48–51	~tetra-ploid	Total counts
12/20/65	9	5000	0		PBlc	6	17	124	3			150
2/21/66	8·9	2600	0		BM	3	3	10	6			37
4/19/66	7.5	3500	1	10	PBlc	2	5	24	1			32
6/ 1/66	5	3000	10	168	PB	2	1	3*	11	3		20
6/13/66					BM	2		1*	11	5		19
7/28/66	12	3000	7	101	PB				2		1	3
9/ 5/66	8	10,000	16	108	PB		4	2*	11		1	18
11/12/66	5	10,000	17	90	PB	1		2*	26	1	2	32
3/ 7/67	4·3	10,000	18	80	PB		2	1*	25			28

LAP: Leucocyte alkaline phosphatase.
BM: Bone marrow, direct preparation.
* Pseudodiploid cells.
PBlc: peripheral blood, lymphocyte culture.
PB: peripheral blood, direct preparation.

the differential count showed an increase of cells of tlie myeloid series, some of which with atypical features; the rare cells of the erythroid series were mostly of basophilic type.

During further observation as an out-patient, a progressive enlargement of liver and spleen was noted. The anaemia was always severe, the WBC counts varied between 6000 and 24,000/mm³ with 15–18% myeloblasts.

During the third admission at the Clinica del Lavoro (11/7/66–11/25/66) and the clinical check-ups in the next months, the patient complained of fever and showed **a** progressive spleen edargement. The Hb and RBC values decreased progressively, despite the treatment with transfusions; the WBC counts were around 10,000/mm³ with 18–20% myeloblasts.

From 3/3/67 to 3/30/67 the patient was admitted for the fourth time at the Clinica del Lavoro in a very poor condition. She presented mycosis of the oral cavity, hyperpyrexia and bloody sputum. The chest X-ray showed numerous infiltrates, which were interpreted as leukaemic infiltrates. The patient wished to be discharged despite her severe condition and died at home on April 7, 1967. No autopsy was performed.

During the whole course of her illness, the patient was treated only with transfusions, corticosteroids, antibiotics and vitamins.

Material and methods. From Dcccmbcr 1965 to March 1367 several cytogenetic studies were performed, at first on peripheral blood lymphocyte cultures, then on direct preparations of bonc marrow and peripheral blood (Table 1). The lymphocytes from peripheral blood were cultured *in vitro* for 72 hr in the presence of phytohaemoagglutinin according to a slight modification of the method of Moorhead *et al.* [4]. The direct preparations of bone marrow and of peripheral blood with immature cells were performed according to a modification of the method of Punturieri [j]; the Colcemid treatment lasted 45 min for the bone marrow and 1½ hr for the peripheral blood. Direct chromosome counts were performed. Most metaphases from direct preparations of bone marrow and peripheral blood were also karyotyped.

The leucocyte alkaline phosphatase was determined according to the histochemical score of Kaplow [6].

Chromosome studies. The results of chromosome counts are summarized in Table 1, together with some blood data at tlie time of study. The first study was performed on pcripheral blood lymphocytes. The preparations were very rich in transformed lymphocytes and mitoses, and the counts were performed on 150 elements. Of these cells, 4 showed chromatid aberrations {gaps), 5 showed unstable chromosome aberrations (acentric fragments, dicentric chromosomes) with a total of **8** caiculated breaks. One cell with 46 chromosomes showed an abnormal karyotype, doe to translocation of part of the long arm of one C and one G chromosomc on a chromosome of the D group; the deleted chromosome of the G group was similar to the Ph¹ chromosome. Three cells had 47 chromosomes ; the extra chromosome was a large submetacentric not classifiable in one cell, an acrocentric of intermediate size between those of the D and those of the G group in another cell; in one more cell the extra chromosome was a submetacentric approximately of the size of pairs 17–18 and one chromosome of the G group had deleted long arms and was similar to Ph¹.

In February 1966, when the patient was

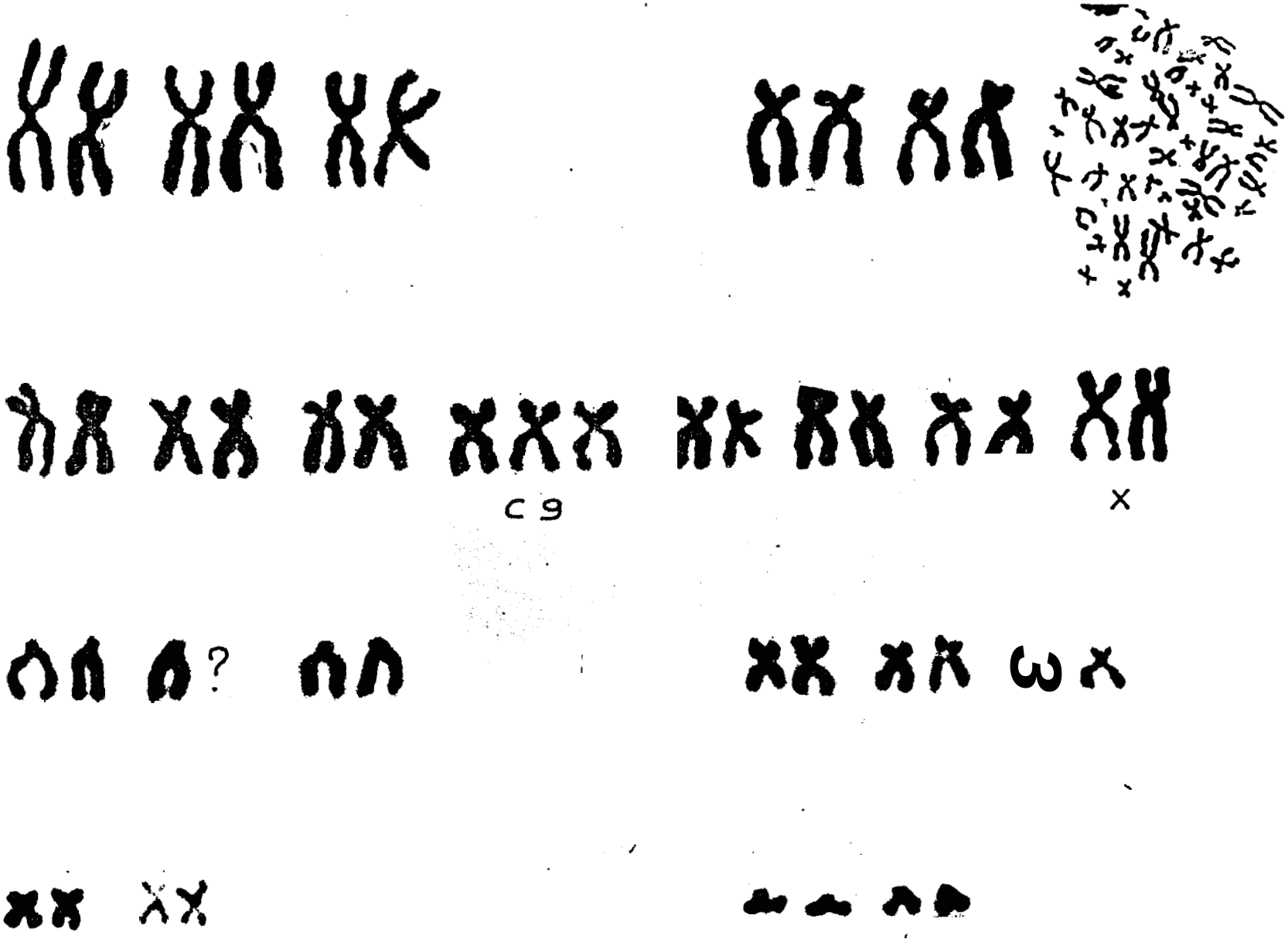

Fig. 1. Pseudodiploid cell with one extra chromosome in group C and one missing chromosome in group D. The extra chromosome has an evident secondary constriction and is indistinguishable from those of pair 9.

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Fig. 2. Cell with 94 chromosomes. Six chromosomes of group C have an evident secondary constriction.

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anaemic and leucopenic, still with no immature cells in tlie peripheral blood, one cell with 46 chromosomes presented an abnormally long acrocentric chromosome in a direct preparation of bone marrow. Six out of 22 cells had 47 chromosomes. In 4 analyzed cells with 47 chromosomes, the extra chromosome was similar to one of the C group in one cell, to those of pair 16 in one cell, to those of the F group in one cell and to those of the G group in one more cell.

In April 1966, when for the first time immature cells appeared in the peripheral blood, no mitoses were seen in direct preparations of pcripheral blood. In lymphocytes cultured for 72 hr with PHA, 2 out of **32** cells (one with 47 chromosomes) showed unstable chromosome aberrations, and 2 metaphases with 46 chromosomes had an abnormal karyotype due to deletions and translocations. The leucocyte alkaline phosphatase (LAP) score was low.

In June 1966 the patient had still low blood counts with 10% myeloblasts in the peripheral blood. The chromosome counts on direct preparations of peripheral blood and bone marrow showed a modal number of 47 in 22 out of 39 metaphases. Most cells with 47 chromosomes had an extra chromosome in the C group, which could not be classified further. Three cells with 48 chromosomes had an extra chromosome in the C group and one in the G group; another cell had an extra C and an extra D chromosome. One cell with 49 chromosomes had an extra chromosome in the C, D, E groups, and one had 2 extra C and one extra G. Another cell with 49 chromosomes had 4 extra chromosomes in tlie C group and one missing of pair 2. One cell with 51 chromosomes had one missing chromosome in the C group, one extra chromosome in the D group, 3 extra chromosomes in the G group and 2 "minute". The cells with 46 chromosomes were pseudodiploid with an extra C and one missing chromosome in another group, mostly G.

In further chromosome studies, the same pattern of group C trisomy was constant in cells with 47 chromosomes, escept for 2 cells with an extra G. Cells with **46** chromosomes were pseudodiploid due to the presence of an extra C and one missing chromosome of another group. Of interest was the appearance after July 1966 of occasional near-tetraploid cells; this fact coincided with the appearance in peripheral blood smears of some binucleatcd cells of the myeloid series, both immature and mature. In occasional cells the estra chromosome could be classified **as** C 9, due to the presence of 3 chromosomes of the same length with evident secondary constriction in group C (Fig. 1). This was particularly clear in a metaphase with 94 chromosomes, in which **6** chromosomes of the C group showed the secondary constriction (Fig. **2).**

DISCUSSION

In the case described here, a leukaemia occurred after a long-standing hyporegenerative anaemia from chronic exposure to benzene for occupational reasons. On the first and third chromosome studies, the patient's lymphocytes cultured *in vitro* with PHA showed a high rate of chromosome abnormalities. Chromosome changes in people with benzene haemopathy have been described before [7, 8]. Also subjects who have recovered from benzene haemopathy [9] and subjects exposed to benzene without signs of toxicity [9, 10] show a high rate of chromosome aberrations both of the unstable and of the stable type in their lymphocytes. It seems therefore that chromosome changes in lymphocytes may be an indicator of exposure to the toxic agent, provided the exposure to radiation and to cytotoxic drugs may be ruled out.

Of interest is the fact that in the present case manifold chromosome changes were present in the bone marrow before the onset of leukaemia. The cells with 47 chromosomes showed different karyotypes. Cells with abnormal karyotypes had been observed also in people with past benzene haemopathy, apparently recovered both from the clinical and the haematological point of view [9].

After the onset of leukaemia, the leukaemic cells both in the bone marrow and in the peripheral blood showed a stable abnormal karyotype with a C group trisomy. The extra chromosome could sometimes be identified as belonging to pair 9. The problem of the relationship of group C trisomy and leukaemia or leukaemoid reactions is an actual one. Several reports have been published of group C trisomy in acute myeloblastic leukaemia, blastic crisis of chronic myelogenous leukaemia and myeloproliferative disorders with leukaemoid reaction [11, 12] (see review of the literature in [13]). As suggested by Sandberg [13], the development of **a** clone with C 9 trisomy might really play a role in the onset of leukaemia. The findings in our case seem to support this hypothesis.

The occasional cells with 46 and 48 chromosomes with one missing or one extra chromosome in other groups (mostly D and G) and C trisomy, might be part of clones resulting from

abnormal chromosome distribution during mitosis in the leukaemic cells.

The development of the leukaemic phase was accompanied in our case by the finding of a high leukocyte alkaline phosphatase level. This fact does not seem to be related to the C 9 trisomy, since also cases with C trisomy and low granulocyte alkaline phosphatase have been described [12]. The fact that in our case a high score of leucocyte alkaline phosphatase was first found at a time of an infectious complication might be of interest.

As for the mechanism of induction of leukaemia by benzene, one can suggest the hypothesis that multiple chromosome aberrations might be produced by the toxic agent on bone marrow cells, resulting in cells with abnormal chromosome complement. If the abnormal cells are able to undergo mitotic division, different clones may develop, one of which might eventually become predominant due to selective advantage and become the leukaemic clone. This seems to have happened in the case described. It is possible that a benign course of benzene haemopathy might occur, when there is no formation of potentially leukaemic clones. On the other side, the toxic action of benzene on the lymphocyte population might play a role in the development of leukaemia, by affecting the potentiality of the immune system.

RESUME

Un cas d'anémie due au benzène, évoluant uers la leucémie aigue myéloblastique, après une longue période de latence, a été contrôlé par de multiples analyses cytogénétiques. Au moment où le patient présentait une anémie sévère, une moelle aplastique, et une absence totale de leucocytes immatures dans le sang périphérique, de nombreuses aberrations chromosomiques "stables" et "instables" furent observées dans les lymphocytes des cultures de sang. Quelques mois plus tard, toujours en l'absence de formes immatures dans le sang, de nombseuses cellules à 47 chromosomes furent mises en évidence par l'examen direct de la moelle osseuse. Les cellules hyperdiploïdes appartenaient à des clones différents, le chromosomes supplémentaire ressemblant soit à un chromosome du groupe C, soit à un chromosome des groupes F ou G. Au moment où des myéloblastes apparaissaient dans le sang, toutes les cellules à 47 chromosomes, tant par l'examen direct de la moelle que par la culture du sang, montraient le même caryotype, avec un chromosome supplémentaire du groupe C. Quelques cellules avaient plus de 47 chromosomes. Les auteurs émettent l'hypothèse que les cellules auec différentes aberrations chromosomiques induites par le benzine peuvent être à l'origine de clones anormaux. L'un de ceux-ci pourrait devenir leucémique et supplanter les autres par un processus de sélection.

SUMMARY

A case of benzene anaemia evolving into acute myeloblastic leukaemia after a long latent period was followed up by multiple cytogenetic studies. At a time when the patient showed severe anaemia, aplastic bone marrow, no immature leucocytes in the peripheral blood a high rate of "unstable" and "stable" chromosome aberrations was found in cultured peripheral blood lymphocytes. A few months later, still in the absence of immature leucocytes in the peripheral blood, numerous cells with 47 chromosomes were detected in a direct preparation of bone marrow. The hyperdiploid cells belonged to different clones, the extra chromosome being similar at times to one of the C groups, at times to one of the F or G groups. When myeloblasts appeared in the peripheral blood, all cells with 47 chromosomes in direct preparations of bone marrow and peripheral blood showed the same karyotype with an extra chromosome in the C group; occasional cells with more than 47 chromosomes were also observed. The hypothesis is suggested that cells with diffesent chromosome alterations induced by benzene may give origin to abnormal clones. One of these might become the leukaemic clone by selective advantage.

ZUSAMMENFASSUNG

Ein Fall von Benzolanämie, der nach einer langen Latenzperiode in eine akute Myeloblastenleukämie umschlug, wurde mit Hilfe zahlreicher zytogenetischer Untersuchungen verfolgt. Zu einer Zeit, als der Patient eine schwere Anämie, ein aplastisches Knochenmark und keine unreifen Leukozyten im peripheren Blut hatte, wurde eine hohe Rate von stabilen und unstabilen Chromosomenaberrationen in Kulturen con peripheren Lymphozyten

gefunden. Einige Monate spater, immer noch beim Fehlen unreifer Leukozyten im peripheren Blut, konnfen in einem direkten Knochenmarkspräparat zahlreiche Zellen mit 47 Chromosomen gefunden werden. Die hyperdiploiden Zellen gehorten uerschiedenen Klonen an. Die Extrachromosomen waren einmal denen der C-Gruppe, wieder andere Male denen der F- oder G-Gruppe ähnlich. Als Myeloblasten im peripheren Blut auftauchten, zeigfen alle Zellen mit 47 Chromosomen, die in direkten Praparaten von Knochenmark und peripheren Blut gefunden wurden, den gleichen Kerntyp mit einem Extrachromosom in der C-Gruppe. Gelegentlich wurden auch Zellen mit mehr als 47 Chromosomen gefunden. Es wird die Hypothese vorgeschlagen, daß Zellen mit verschiedenen Chromosornenveranderungen, die durch Benzol ausgelöst sind, zum Ursprung abnormer Klonen werden können. Einer von diesen könnte der leukamische Klonus werden aufgrund von Selektionsvorteilen.

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