could possibly be used, therefore, for the prenatal investigation of hereditary disorders of human connective tissue.

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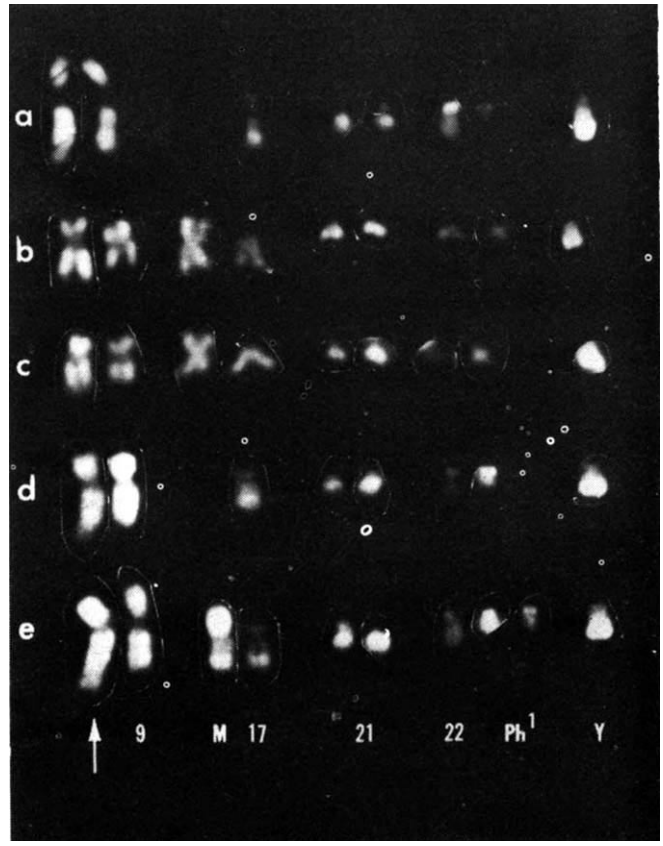

Fig. 1 Partial karyotype of five mitotic cells from cases 1–3 photographed with quinacrine fluorescence. The first pair of chromosomes in each row is the 9, with the 9q+ chromosome identified by the arrow. Note the extra band of dully fluorescing material at the end of each 9q+ chromosome. The next chromosome is the metacentric marker chromosome (M), when one is present, paired with the normal chromosome 17. The pair of chromosomes 21 is followed by the normal chromosome 22 and the Ph¹ chromosome. The Y chromosome is the last one in each row. *a*, Cell obtained from case 1. *b* and *c*, Cells obtained from case 2. The upper arm of the marker has two bands including one near the centromere, which would not be expected in an isochromosome 17. *d*, Cell obtained from case 3 before transformation. Note the relatively bright short arm region of the Ph¹. *e*, Cell obtained from case 3 after transformation. One arm of the marker appears to be homologous to the long arm of 17; the other arm shows relatively even moderate fluorescence. The two Ph¹ chromosomes are arranged with that similar to the initial Ph¹ being placed first.

A New Consistent Chromosomal Abnormality in Chronic Myelogenous Leukaemia identified by Quinacrine Fluorescence and Giemsa Staining

CELLS from nine consecutive patients with chronic myelogenous leukaemia (CML) have been analysed with quinacrine fluorescence and various Giemsa staining techniques. The Philadelphia (Ph¹) chromosome in all nine patients represents a deletion of the long arm of chromosome 22 (22q−)[1,2]. An unsuspected abnormality in all cells from the nine patients has been detected with these new staining techniques. It consists of the addition of dully fluorescing material to the end of the long arm of one chromosome 9 (9q+). In Giemsa-stained preparations, this material appears as an additional faint terminal band in one chromosome 9. The amount of additional material is approximately equal to the amount missing from the Ph¹ (22q−) chromosome, suggesting that there may be a hitherto undetected translocation between the long arm of 22 and the long arm of 9, producing the 9q+ chromosome.

Bone marrow specimens were prepared for chromosomal analysis as previously described[3]. We cultured peripheral blood leukocytes for 24 or 48 h without phytohaemagglutinin to obtain circulating immature leukaemic cells in division, and for 72 h with phytohaemagglutinin to obtain lymphocytes in division. The time in Velban® was reduced to 40 min for bone marrow and 60 min for peripheral leukocytes so that extended chromosomes, which are more useful for quinacrine fluorescence and Giemsa staining, could be obtained.

Some slides were used for the initial analysis and then destained as previously reported[4], while other slides were prepared from material which had been stored for several weeks to 9 months at −20° C in fixative (45% glacial acetic acid, 55% methanol). All slides, either newly prepared or destained, were stained in a solution of quinacrine mustard (approximately 50 μg/ml.) dissolved in McIlvaine's buffer at *p*H 5.5 for 30–60 min, rinsed twice in buffer, *p*H 5.5, mounted in buffer, and examined as previously described[5]. Well-spread metaphases were photographed with a 95X fluoride objective containing an iris diaphragm on Kodak Panatomic X film using exposure times of 8–15 s. After the coverslips had been removed and the slides dehydrated through 70% and 95% alcohol, they were stained with Giemsa (Gurr's Giemsa R66) directly or after treatment either with the ASG technique[6] or with NaOH[7]. Six to fifty cells from each patient were analysed with both quinacrine

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fluorescence and Giemsa stain. Duplicate karyotypes were prepared according to the Chicago[1] and Paris[2] nomenclature from photographs of quinacrine fluorescence and Giemsa-stained cells in metaphase.

The patients had had CML for 2 months to 6 years, and all had responded well to busulphan. Case 2 had received prednisone and 6-mercaptopurine one year before cytogenetic analysis. Cases 2, 4, and 5 were studied only in the acute blast phase, whereas cases 1 and 3 were studied in remission as well as in the acute blast phase of CML. Case 3 showed a change in modal chromosome number from 46 in remission to 50 in the terminal phase of his disease. Cases 6 and 7 were in the early phase, and cases 8 and 9 were in remission at the time of analysis. Cultures of peripheral lymphocytes from five of the patients, three of whom were in blast crisis, have provided no mitotic cells suitable for analysis. No cultures were taken from the remaining patients. Cytogenetic findings are summarized in Table 1.

Analysis of cells from all nine cases confirmed previous reports[8,9] that the Ph[1] chromosome was a deleted 22 (Fig. 1). All cells examined with fluorescence showed that one chromosome 9 had additional dully fluorescing material at the terminal portion of the long arm (9q+) (Figs. 1, 3, and 5). Giemsa-stained cells showed an additional faint terminal band on the same chromosome 9 (Figs. 2 and 4). This change was seen in cells from case 3 obtained before blast crisis (Figs. 1*d* and 2*d*), as well as in samples obtained in the blast phase (Figs. 1*e*, 2*e*, and 3). The additional material is approximately equal to one-half the length of the moderately fluorescing region of the long arm of 9.

The occurrence of an apparently identical chromosomal abnormality in addition to the Ph[1] chromosome in nine patients with CML indicates that the chromosomal pattern in CML is not as simple as it has appeared to be. The source of the dully fluorescing and pale-staining material on the 9q+ chromosome cannot be determined at present. Its appearance is similar to that of the long arm of chromosome 22, which suggests that it may represent a translocation of the portion of chromosome 22 that is missing from the Ph[1] chromosome. In support of this suggestion, the amount of material deleted from 22 is approximately equal to the additional dully fluorescing portion observed on the 9q+ chromosome. Furthermore, other large regions of dull fluorescence, such as the end of the short arm of chromosome 1 or the end of the long arm of chromosomes 12 or 15, which could be the source of this material, appear to be intact.

The mechanism for the production of such a specific chromosomal translocation (if this is the correct explanation for these findings) is not clear. This would constitute the only specific chromosomal translocation in humans that has been

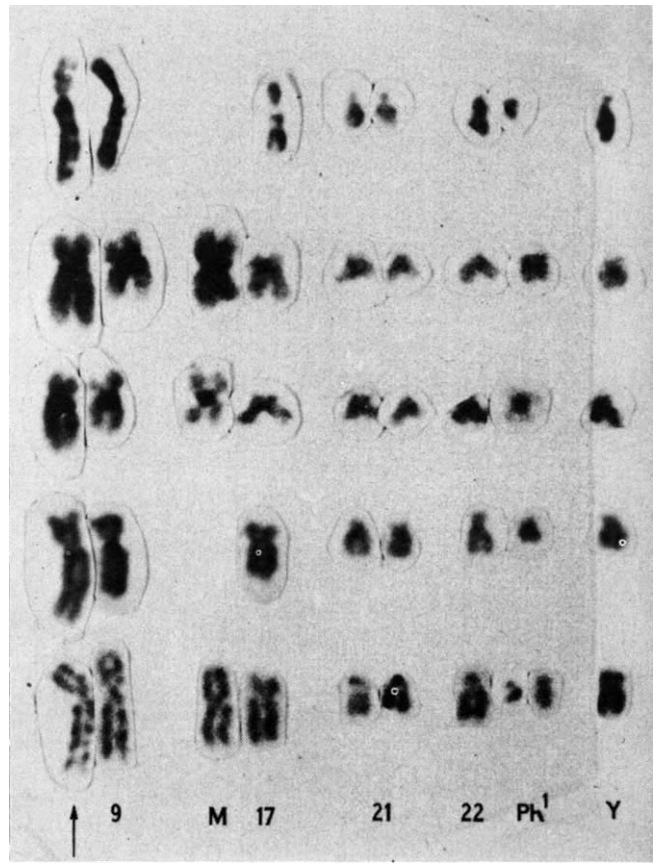

Fig. 2 Partial karyotype of the same five mitotic cells shown in Fig. 1, stained with Giemsa. The arrangement of the chromosomes is as in Fig. 1. Note the additional faint band of material at the end of each 9q+ chromosome (↑). *a*, Slide destained after quinacrine and restained with Giemsa (Gurr's R66) 1: 10 for 10 min. There is a suggestion of banding although the preparation was not specially treated to produce bands. *b*, Slide destained after quinacrine, incubated in 2×SSC for 2.5 h, and stained with Giemsa. Note the fine band near the centromere region in the upper arm of the marker chromosome, which is not present in the lower arm. *c*, Slide destained after initial Giemsa stain and again after fluorescence; treated with 0.07 N NaOH for 1 min and incubated in 2×SSC for 2.5 h. Note the relatively large amount of centromeric heterochromatin in the marker, extending into the upper arm which may not be homologous to 17q. *d*, Slide destained after quinacrine and incubated in 2×SSC for 2.5 h before staining with Giemsa. *e*, Slide destained and incubated in 2×SSC for 2 h and stained with Giemsa. Note the similarity of bands in the lower arm of the marker with those in chromosome 17, and the longer long arm in the second Ph[1] chromosome as compared with the original.

Table 1 Summary of Chromosomal Analysis

Case	Age (yr)	Duration of CML (yr)	Source	Number of cells analysed with quinacrine and Giemsa	Karyotype*[6,7]	Figure number
1§	72	6	Marrow	8	46,XY,9q+,22q−	1*a*, 2*a*
2§	29	3½	Marrow	25	48,XY,9q+,+C,+mar,−17,+?F,22q−	1*b*,*c*, 2*b*,*c*
3§	37	3½	Marrow†	15	46,XY,9q+,22q−	1*d*, 2*d*
			Blood	26	50,XY,9q+,+8,+C,+mar,22q−,+22q−	1*e*, 2*e*
			Marrow	10	50,XY,9q+,+8,+C,+mar,22q−,+22q−	5
4§	71	1⅓	Blood	6	46,XX,9q+,+mar,−17,22q−	3*a*, 4*a*
				3	47,XX,9q+,+C,+mar,−17,22q−	
5§‡	51	2½	Blood	50	48,XY,9q+,+mar,22q−,+22q−	3*b*, 4*b*
6	45	2 mo	Marrow	6	46,XX,9q+,22q−	3*c*, 4*c*
7	25	1	Marrow	12	46,XX,9q+,22q−	3*d*, 4*d*
8	18	3	Marrow	21	46,XX,9q+,22q−	3*e*, 4*e*
9	64	3½	Marrow	11	46,XX,9q+,22q−	3*f*

* (+) additional; (−) absent; (q) long arm; (mar) marker; (22q−) represents the Ph[1] chromosome. †Obtained before blast crisis. ‡ Slides provided by Dr Lorraine F. Meisner, State Laboratory of Hygiene, Madison, Wisconsin. § Patient died in blast crisis.

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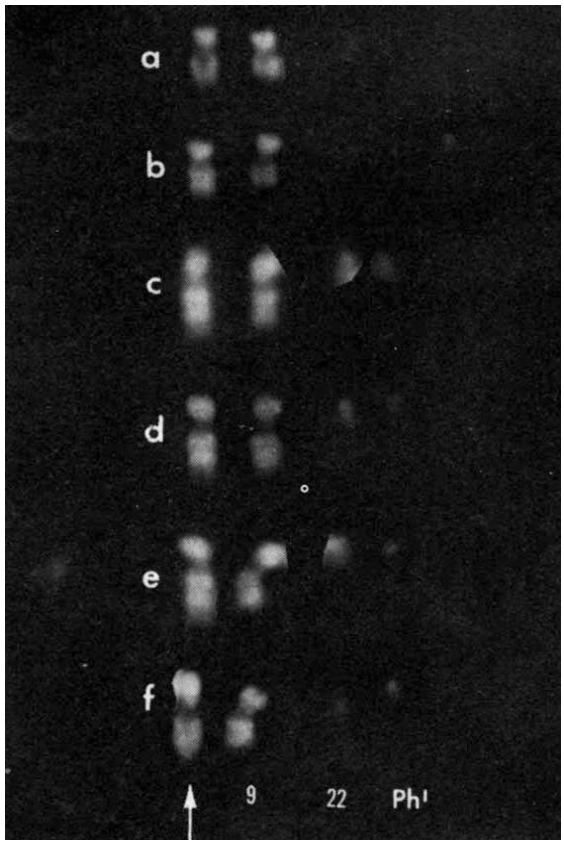

Fig. 3 Partial karyotype of six mitotic cells from cases 4–9 photographed with quinacrine fluorescence. The first pair of chromosomes in each row is the 9, with the 9q+ chromosome identified by the arrow. Note the extra band of dully fluorescing material at the end of each 9q+ chromosome. The second pair of chromosomes is the normal 22 and the Ph¹ chromosome. The cells were obtained from case 4 (*a*), case 5 with two Ph¹ chromosomes (*b*), case 6 (*c*), case 7 (*d*), case 8 (*e*), and case 9 (*f*).

identified. Previous attempts to determine whether the Ph¹ chromosome represented a loss of chromosomal material rather than a translocation floundered because it was not possible to identify precisely all the human chromosomes[10]. The 9q+ chromosome was observed in case 3 before the acute blast phase, as well as in cases 6–9 who were in remission. This suggests that the 9q+ chromosome may be present in the initial development of the somatic mutation, and that the translocation is not solely a result of progressive changes in the karyotype which frequently occur in later stages of blast crises.

Patients with CML having cells with the Ph¹ chromosome fare better clinically than patients who do not have this abnormality[11,12]. If it is assumed that the 9q+ represents a translocation from 22, then leukaemic cells with or without the Ph¹ chromosome appear to have approximately the same amount of chromosomal material. In this case, differences in the clinical course cannot be accounted for by a gross loss of chromosomal material. The assumption that the additional material on chromosome 9 comes from 22 can be tested by examination of the chromosomes from patients with CML whose cells are Ph¹ negative.

For reasons that are not understood, chromosomes from leukaemic cells are often "fuzzy" with poorly defined centromere regions. This makes accurate karyotyping of cells from leukaemic patients difficult and sometimes impossible. Nevertheless, in the present study using quinacrine fluorescence and Giemsa stains, additional findings have emerged. For example, case 3 in the terminal phase showed two Ph¹ chromosomes, but these were not identical. One consistently had a brightly fluorescing short arm region similar to that of the Ph¹ chromosome found in the analysis before blast formation. The other Ph¹ chromosome showed dull fluorescence in the short arm region and had more material in the long arm. Case 5 also had two Ph¹ chromosomes, but they were identical in size and fluorescence pattern.

Two other findings are of interest: (1) The extra C group chromosomes in two patients in blast crisis (cases 2 and 3) most closely resemble chromosome 8 in size, centromere index, and general fluorescence pattern. (2) At least one arm of the metacentric marker chromosome found in cases 2, 3, and 4 appeared to be homologous to the long arm of 17 (17q), and the marker in case 4 may be an isochromosome for the long arm of 17 (17qi). Lobb *et al.*[13] observed a metacentric marker chromosome in three patients who had CML and, with the use of the ASG technique, identified the marker as an isochromosome for the long arm of 17. The apparent frequency of metacentric marker chromosomes occurring at the time of acute blast crisis and involving the long arm of chromosome 17 remains an enigma.

As has been stated earlier[7], a combination of quinacrine fluorescence and various Giemsa staining techniques provides much more information than any one technique used separately. Application of these new techniques to the study of other types of tumours has also been fruitful. Analysis of cells from a patient with acute myelogenous leukaemia not only permitted identification of the chromosomes involved in a translocation, but also pointed to the sites of chromosomal breakage[14]. An extra terminal bright band on chromosome 14 has been observed recently by Manolov and Manolova[15] in biopsies and cell cultures from Burkitt's lymphomas in ten out of twelve

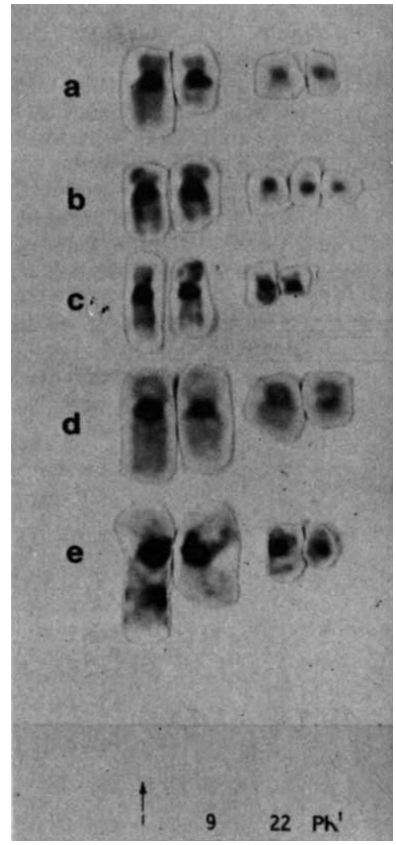

Fig. 4 Partial karyotype of five of the six mitotic cells shown in Fig. 3, stained with Giemsa. All slides were treated with 0.07 N NaOH for 2 min, incubated in 2×SSC at 60° C overnight, and stained with Gurr's Giemsa R66 1: 20 for 30 min. Note the additional faint band of material at the end of each 9q+ chromosome, and the 2 Ph¹ chromosomes in case 5 (*b*). Giemsa preparations for case 9 were unsuccessful.

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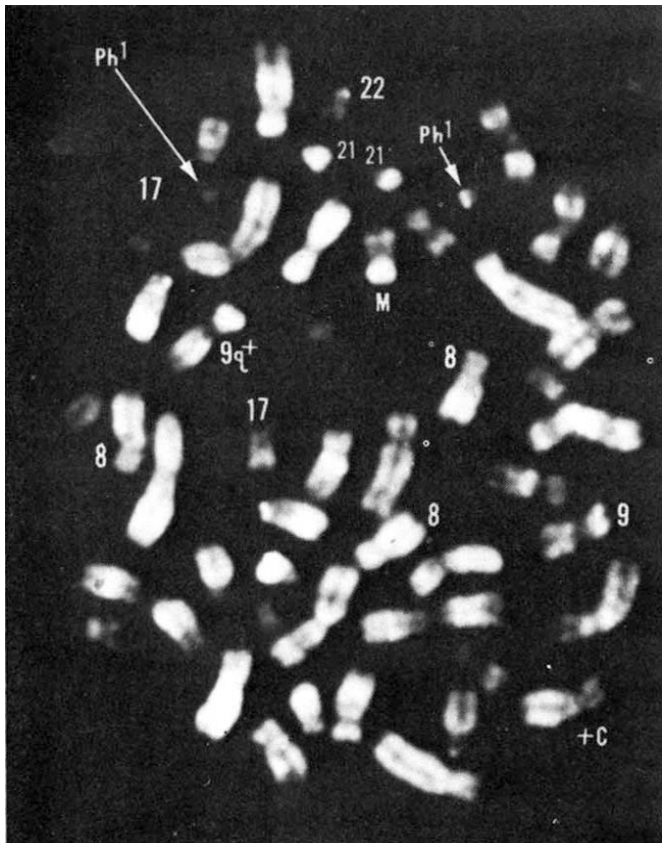

Fig. 5 Intact metaphase from case 3 stained with quinacrine mustard. This cell contains 50 chromosomes, including two extra C chromosomes, the metacentric marker, and two Ph¹ chromosomes. The 9 and 9q+ chromosomes are identified. One of the two extra C chromosomes is identical to an 8, and therefore three chromosome 8s are identified; the relative similarity in size and fluorescence pattern between the other C chromosome (labelled +C) and chromosome 8 can be observed. The arm of the metacentric marker (M) away from the label is 17q. The normal 17s are labelled, as are the normal 21s and the 22. The original Ph¹ is identified by the short arrow, and the second Ph¹ by the long arrow. In each case, the arrow points to the short arm of the chromosome. The brighter short arm and shorter long arm of the original Ph¹ chromosome as compared with the second Ph¹ are clearly demonstrated. (The difference in brightness between the two Ph¹ chromosomes seen here was noted consistently in all mitotic cells, whereas the dullness of the peripheral chromosome 17 was an artefact of this cell.)

patients. The loss of chromosome 22 in meningiomas has been confirmed with fluorescence techniques[16].

Whereas it appeared earlier from the bizarre chromosomal abnormalities identified in leukaemic cells that almost any kind of genetic imbalance might be viable, the new techniques may well demonstrate that excesses or deficiencies of only certain chromosomes can be tolerated. Careful analysis of cells from patients with leukaemia may identify certain chromosomal regions that are frequently aneuploid in leukaemic cells. This information could contribute to an understanding of the role of chromosomal changes in malignancy.

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Experimental Model for von Willebrand's Disease

IT has been reported[1] that Ristocetin induces aggregation of platelets in normal platelet-rich plasma (PRP) but not of platelets from patients with von Willebrand's disease. Results presented here demonstrate that the aggregation of platelets induced by Ristocetin involves a factor present in normal and haemophilia A plasma and absent in Willebrand plasma. Lack of such a factor would then explain the inability of platelets to aggregate in response to Ristocetin in von Willebrand's disease. In eight patients fulfilling the criteria of von Willebrand's disease (Table 1), platelet aggregation induced by Ristocetin was markedly deficient when compared to healthy normal plasmas or did not occur at all. The abnormal responses of platelets from von Willebrand's patients could be corrected by the addition of small volumes of normal platelet-poor plasma (PPP) (Fig. 1); similar correction was achieved by Haemophilia A PPP but not by PPP from patients with von Willebrand's disease.

Table 1 Biological Data in Eight Patients with von Willebrand's Disease

Patients	Platelet retention %	Factor VIII activity %	Factor VIII-like antigen %	Initial velocity of aggregation to Ristocetin % 2 mg ml^{-1}	% 1 mg ml^{-1}
1	15	37	38	4.5	0
2	6	4	<5	0	0
3	8	10	<5	0	0
4	0	3.8	<5	0	0
5	27	10	<5	0	0
6	12.5	51.5	8	30*	3
7	0	20	<5	26.5*	0
8	0	25	<5	51*	9*
Controls	80–100	50–200	50–200	70–118	24–67

* Lag phase of 15 s.

Platelet retention to glass beads was measured by the method of Bowie *et al.*[2] and Factor VIII antigen was assayed on plasma by a modification[3] of the Laurell technique[4]. Platelet aggregation was studied in the MK III aggregometer[5] by the turbidimetric method[6] using citrated PRP. The initial velocity of aggregation was obtained by drawing a tangent to the steepest part of the aggregation curve on a potentiometric chart recorder and expressed as a percentage of the maximum transmitted light (PPP) after 30 s.

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