Spontaneous chromosomal breakage and high incidence of leukemia in inherited disease

By Traute M. Schroeder and Renate Kurth

Spontaneous chromosomal breakage has been found in six inherited diseases: ataxia telangiectasia, Bloom's syndrome, Fanconi's anemia, glutathione reductase deficiency, Kostmann's agranulocytosis and pernicious anemia. These diseases are not equally well researched and the cytogenetic data are by no means uniform. However, in each disease, the incidence of leukemia is increased. Chromosomal breakage has been investigated primarily in vitro. In order to connect this phenomenon with each syndrome and with the development of malignancy, the relationship between in vitro results and in vivo events must be established. This relationship may be clarified through the use of direct chromosome preparations from the bone marrow and bone marrow smears. A few such investigations have been carried out. Any evaluation of the results must consider the complexity of the bone marrow in general, as well as in each disease and in each individual case. An understanding of in vivo events in turn would allow an evaluation of factors specific to the in vitro condition, and their ability to enhance, provoke or diminish breakage. It is our opinion that contradictory results from in vitro investigations are not yet confirmed. The kind of studies necessary for such confirmation is fairly clear and, until they have been carried out, it is premature to subgroup the various diseases. A complete understanding of these diseases is possible only when the primary metabolic defect is known. Pernicious anemia is well understood although the genetics remain to be clarified. Biochemical investigations of the other five diseases are still at a preliminary stage. However, a comparison of the cytogenetic data, the clinical picture, as well as the course of the diseases may lead to a conceptualization of the unknown metabolic defect. Although the primary causes for each disease differ they manifest themselves at the cellular level in vitro and/or in vivo as spontaneous chromosomal breakage. As a consequence of this phenomenon a change in the genetic material itself may create, by chance, primary yet unknown conditions for malignant growth. This may partially explain the correlation between spontaneous chromosomal breakage and leukemia. Methodical research in this field requires that blood cultures, bone marrow smears and direct chromosome preparations from bone marrow smears and direct chromosome preparations from bone marrow be included in the close following of the diseases. It is also imperative that the designation of types of chromosome aberrations be standardized and published in detail. This, together with continuing biochemical research will finally elucidate the primary defects involved.

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CHROMOSOMAL BREAKAGE AND LEUKEMIA

In the last 5 years, spontaneous chromosomal breakage has been reported in cultured blood cells, fibroblasts and bone marrow cells from patients with different genetic diseases. Such breakage, which has no discernible external cause, is a rare phenomenon and its relevance to the clinical picture of the cases involved has not been clarified.

In Table 1 data from six inherited diseases are summarized. They show a close relationship between chromosomal breakage and the incidence of leukemia or other cancers.

The first disease in which spontaneous chromosomal breakage was detected in vitro and in vivo is Fanconi's anemia. In 1964, Schroeder et al.68 described their cytogenetic findings from two brothers. More than 40 per cent of the analyzed metaphases from peripheral blood cultures demonstrated chromosomal breakage and rearrangements. Today, 36 out of 41 cases of Fanconi's anemia have been published with similar findings. Of about 170 cases known in world literature, four terminated with leukemia, one had skin carcinoma and six members of the families also had leukemia.

Three other hematological diseases, glutathione reductase deficiency anemia, Kostmann's agranulocytosis and pernicious anemia, have been less well researched in this direction. However, the data available also indicate a correlation between chromosomal breakage, leukemia and cancer.

Ataxia telangiectasia and Bloom's syndrome have no hematological symptoms. Ataxia telangiectasia is primarily a neurological disease; however, many patients developed leukemia or lymphoreticular neoplasia. For this reason, ataxia telangiectasia was included in this study, although the cytogenetic findings are as yet ambiguous.

Bloom's syndrome has three cardinal symptoms: telangiectatic erythema, skin sensitivity to sunlight, and stunted growth. The 35 cases which have been reported since 1954 have just recently been reviewed by German,17 who first found, in vitro, the chromosomal abnormalities associated with the disease.16 All cytogenetically investigated patients showed this phenomenon. Again, four patients out of 35 had leukemia or cancer. Spontaneous chromosomal breakage is a very rare finding. Although this control value varies from laboratory to laboratory, the extent of chromosomal damage found in these six diseases is extraordinarily high.

This unusual phenomenon brings to mind two main questions: (1) What is the relationship of chromosomal breakage to the disease? (2) How is chromosomal breakage connected with the development of malignancy?

This paper will discuss these questions and associated problems to give a plausible outline for further investigations.

General Remarks

Aberrations Found in Metaphase Chromosomes (Fig. 1)

The aberrations under discussion refer to structural anomalies found in isolated cells and do not include any consistent numerical or structural change of the karyotype. These single cells sometimes represent more than 50 per cent of the total mitoses evaluated. The aberrations found are roughly classified under the following headings: (A) achromatic regions or "gaps" which refer to
Table 1.—Summary of Data Concerning the Six Inherited Diseases Showing Spontaneous Chromosomal Breakage In Vivo and/or In Vitro and Coincidence with Leukemia and Cancer

<table>
<thead>
<tr>
<th>Disease mode of inheritance</th>
<th>Number of Cases</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ataxia telangiectasia autosomal recessive</td>
<td></td>
<td></td>
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<tr>
<td>Bloom’s syndrome autosomal recessive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fanconi’s anemia autosomal recessive</td>
<td></td>
<td></td>
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<tr>
<td>Glutathione reductase deficiency autosomal dominant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kostmann’s agranulocytosis autosomal recessive</td>
<td></td>
<td></td>
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<tr>
<td>Pernicious anemia hereditary, but pattern undefined</td>
<td></td>
<td></td>
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<thead>
<tr>
<th>Disease mode of inheritance</th>
<th>Reported Investigated for Chromosome Abnormalities</th>
<th>With Chromosomal Breakage In Vitro and Fibroblast Cultures</th>
<th>Without Chromosomal Breakage In Vivo</th>
<th>Without Chromosomal Breakage Direct Bone Marrow Preparations</th>
<th>With Mitotic Disorder in Bone Marrow Smears</th>
<th>With Leukemia *</th>
<th>With Other Cancer</th>
<th>With Cancer in Family</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ataxia telangiectasia autosomal recessive</td>
<td>200/7</td>
<td>4 3</td>
<td>n n</td>
<td>n</td>
<td>2 11 n</td>
<td>3, 23, 27, 48, 55, 75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bloom’s syndrome autosomal recessive</td>
<td>35/26</td>
<td>26 0</td>
<td>1 n</td>
<td>n</td>
<td>3 1 0</td>
<td>4, 11, 16, 17, 18, 19, 24, 30, 32, 33, 37, 56, 62, 65, 74</td>
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<td></td>
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<tr>
<td>Fanconi’s anemia autosomal recessive</td>
<td>170/44</td>
<td>39 5</td>
<td>4 3</td>
<td>6</td>
<td>4 1 7</td>
<td>5, 14, 15, 18, 19, 20, 21, 29, 38, 54, 60, 63, 64, 66, 67, 68, 69, 73, 76, 79, 80, 81</td>
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<td></td>
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<tr>
<td>Glutathione reductase deficiency autosomal dominant</td>
<td>100/8</td>
<td>5 3</td>
<td>n n</td>
<td>8</td>
<td>10 0 0</td>
<td>6, 7, 25, 41, 83, 84, 85</td>
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<tr>
<td>Kostmann’s agranulocytosis autosomal recessive</td>
<td>15/1</td>
<td>n n</td>
<td>1 n</td>
<td>+</td>
<td>+ in other familial agranulocytosis</td>
<td>35, 44, 58</td>
<td></td>
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</tr>
<tr>
<td>Pernicious anemia hereditary, but pattern undefined</td>
<td>?/19</td>
<td>+ +</td>
<td>17 2</td>
<td>typical for pernicious anemia</td>
<td>32 +</td>
<td>2, 8, 26, 34, 36, 43, 51, 58, 59, 66, 70, 71, 86</td>
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n, no specific information; +, present.

* Incidence of leukemia in general population: 6 in 100,000.22
† Pernicious anemia in northern European population: 2.0–4.4 per cent.28
Fig. 1.—Types of chromosomal aberrations. (A) Chromatid gap. (B) Chromatid break with dislocation of the fragment. (C) Isochromatid break with dislocation of the double fragment (arrow, upper right) of a No. 2 chromosome (arrow, lower left), compare with the homologue (arrow, lower right). (D) Acentric double fragment. (E) Chromatid exchange figure, nonhomologous chromosomes. (F) Ring chromosome. (G) Dicentric chromosome.

unstained areas in one or both chromatids; (B) chromatid and (C) chromosome breaks which refer to a complete interruption of one chromatid or both chromatids at the same location, the fragment being dislocated. In desig-
nating gaps or breaks investigators differ because this distinction between "invisible" molecular events is arbitrary. Therefore, any attempt to compile results is inaccurate, but the fact remains that such anomalies are present; (D) fragments refer to isolated acentric chromatid or chromosome segments which are the results of actual breaks; (E) reunion figures or translocation figures refer to the reciprocal exchange of chromatid fragments of two chromosomes. These chromosomes may or may not be homologous. Such an exchange sometimes involves more than two chromosomes; (F, G) abnormal chromosomes refer to distinguishable structural alterations of chromosomes. They are the results of breakage-reunion, exchanges, or other mechanisms; for example a ring chromosome (F). This category includes translocation chromosomes which are specifically the consequences of exchanges, such as a dicentric chromosome (G).

Significance of Chromosomal Aberrations to the Cell (Fig. 2)

In anaphases and telophases the consequences of chromosomal breakage to the individual cell become evident: fragments are not attached to the spindle
and thus often lost; dicentric chromosomes form bridges between the poles (A,B) which eventually must break; a nuclear membrane might form around chromatin masses which have not properly migrated to either pole (C) and then be manifest as a micronucleus in the cytoplasm of the daughter cell (D–G).

All these events generally lead to some imbalance of the genetic material among the daughter cells which results in a shortening of their lifespan. There are important exceptions to this generalization: cells with gross and minor changes of the genetic material may survive.

Cell lines with an abnormal karyotype have been observed in fibroblast cultures of a patient with Bloom’s syndrome and the mother of a patient with Bloom’s syndrome. The authors thought it most likely that these cell lines arose in vitro but did not exclude an in vivo origin. Although there are, as yet, no other reports of abnormal cell lines being maintained in vivo, their existence cannot be ruled out. However, it is probable that cells bearing abnormalities are more likely to be eliminated by the defense mechanisms in vivo than in vitro where many of these factors are lacking. Cells bearing invisible structural anomalies such as point mutations, gene deletions and duplications which result from breakage-reunion may divide unrestrained. As a result, the tissue has an increased spontaneous mutation rate, which together with aberrant cell lines carries the seed of malignancy.

First Problem: What Relationship Exists Between the In Vitro Results and the In Vivo Events? Are the In Vitro or the In Vivo Findings Causally Related to the Disease?

Almost all chromosome studies are based on short term blood cultures and bone marrow cultures; some are based on long term fibroblast cultures.

Direct bone marrow preparations must be examined to determine if the same or similar events take place in vivo. In panmyeloplastic or pancytopenic patients it is often difficult to obtain marrow which is suitable for analysis. Therefore, none of the few studies done have been based on a very large number of cells. The direct bone marrow preparations obtained from seven patients with Fanconi’s anemia showed four with about 10 per cent aberrant metaphases and three with no breakage. Matsaniotis et al. found more than 20 per cent aberrant cells in a baby with Kostmann’s agranulocytosis. Landau et al. reported four out of 50 metaphases with breaks in a patient with Bloom’s syndrome.

The various stages of dividing cells seen in bone marrow smears also represent a direct view of in vivo events. Anomalies, as demonstrated in Fig. 2A–G, are typical findings in Fanconi’s anemia, pernicious anemia, glutathione reductase deficiency anemia and Kostmann’s agranulocytosis. There is no doubt that these abnormalities are related to chromosomal breakage.

German and Crippa also demonstrated mitotic disorder in their fibroblast cultures from Fanconi’s anemia and Bloom’s syndrome which showed a parallel high frequency of chromosome breaks in metaphase preparations. Their results give the ratio of mitotic anomalies to cells with chromosomal breakage as 1:20 in Fanconi’s anemia. When this factor is applied to the number of mitotic
disorders found in the bone marrow smear of patient 21 a 10 per cent occurrence of chromosomal breakage is to be expected, which agrees with the findings of some of the authors mentioned above.

The 10 per cent breakage becomes significant when the complex structure and function of the bone marrow are considered. If it occurs preferentially in the stem cells and not only within the differentiation process, such a loss without compensation would mean disaster after relatively few divisions. However, this does not happen. The bone marrow can cope with the situation by various compensatory mechanisms, the effectiveness of which varies with the primary cause of the diseases and the individual bone marrow constitution. The distinct morphological differences seen in the bone marrow of patients with these diseases indicate a different complex of events for each. The significance of breakage found could be more precisely evaluated if the stages most affected by mitotic disorder were determined. However, the clinical picture of each individual case must always be considered because the amount of breakage in itself can have completely different consequences depending on the disease and the patient. No in vitro model can be used to elucidate this complexity.

Because the incidence of chromosomal breakage in the bone marrow of healthy individuals is unknown, the only available control value is the 0.6 per cent found in nonhematological diseases.

Mitotic disturbances and anomalies of nuclei are rare findings in control groups of patients with pancytopenias or pannmyelopathies of other unknown etiology. No specific studies of bone marrow smears have been reported in ataxia telangiectasia and Bloom’s syndrome.

Examinations carried out with bone marrow from patients with pernicious anemia need special comment. Bottura and Continho, Heath and Krogh-Jenson and Friis-Møller succeeded in demonstrating that chromosomal breakage actually takes place in vivo in untreated pernicious anemia. Together with morphological alterations due to the block in differentiation, mitotic disorder and abnormal nuclei formations are found in parallel bone marrow smears. With vitamin B12 and/or folic acid treatment the bone marrow loses its morphological abnormalities and metaphase preparations no longer show chromosomal breakage. These results illustrate directly the relevance of mitotic disorders in bone marrow smears to chromosome damage seen in direct bone marrow preparations. It also shows chromosomal breakage in vivo causally related to pernicious anemia. Investigations of lymphocytes in vitro from patients with pernicious anemia give ambiguous results, possibly because of the enriched culture medium used for blood cultures.

The positive and negative results in glutathione reductase deficiency anemia are probably not due to culture conditions. The extent of chromosomal breakage in vitro seems to be related to the stage of the disease. Where only the erythropoietic system is involved, mitotic anomalies are found in immature red blood cells in bone marrow smears, but no chromosomal breakage is found in cultured leukocytes. If pancytopenia exists, abnormalities are observed in both bone marrow smears and blood cultures. Hampel and co-workers investigated a mother and her son with quantitatively the same glutathione reductase deficiency. The son, suffering from pannmyelopathy, showed more
breakage in cultured lymphocytes than the clinically normal mother.

From the entire discussion it becomes evident that no general answer to the first question will suffice. In summary: a relationship between in vitro findings and in vivo events has been shown in Fanconi's anemia; in glutathione reductase deficiency this relationship is dependent on the stage of the disease; no in vivo studies have been done in Bloom's syndrome and ataxia telangiectasia; breakage in Kostmann's agranulocytosis has been investigated and shown only in vivo; in pernicious anemia the relationship between in vitro findings and in vivo events seems obscured by culture conditions; however, in vivo breakage has been shown causally related to this disease. Besides pernicious anemia, any hypothesis relating in vivo and in vitro findings to the other hematological diseases must pay special attention to the complexity of the bone marrow.

Chromosomal breakage in vitro reported to be absent in some cases, present in others, in pernicious anemia and glutathione reductase deficiency anemia, has a conceivable explanation. However, this, together with positive and negative results in the other diseases, raises the next question.

Second Problem: Are Both Positive and Negative Results From In Vitro Chromosome Studies Valid?

Assuming that each disease is genetically uniform, cell cultures from all patients can be expected to show chromosomal breakage. However, culture conditions and stages of the disease influence the in vitro results, as indicated in pernicious and glutathione reductase deficiency anemias. All cases reported with negative results have not been investigated thoroughly enough to rule out technical complications or to show true evidence for the nonexistence of breakage. Therefore, it is premature to speak of two different types of a disease simply because positive and negative reports of chromosomal breakage in vitro exist.

In five cases of Fanconi's anemia, investigators claimed no chromosomal breakage in blood cultures. However, they did not include bone marrow studies. It would be important in such cases to know if mitotic anomalies are present or absent in vivo. Also, it is necessary to repeat the blood cultures to see if the negative result is reproducible or if breakage manifests itself in the course of the disease. Although all investigated patients with Bloom's syndrome show positive results, Schoen and Shearn reported that a second and third culture, done some years after the first, demonstrated chromosomal breakage in their patient. With ataxia telangiectasia, glutathione reductase deficiency anemia and Kostmann's agranulocytosis, more studies will have to be done to confirm both positive and negative findings.

All further investigations of these diseases must include repeated blood cultures to look for chromosomal breakage. In publications, negative as well as positive findings must be clearly stated. It is not enough to report that the patient has a normal karyotype. In addition, bone marrow smears and direct bone marrow preparations must parallel the culture studies. However, the consideration of the positive results alone point to concepts which will now be discussed.
Third Problem: What Significance Do Differing Cytogenetic Findings of the Diseases Have and How Are They Relevant to the Clinical Picture?

A comparison of Fanconi's anemia and Bloom's syndrome, the most comprehensively researched of the diseases, offers information which indicates primary differences in causality.

The types of chromosomal aberrations. The cytogenetic data itself points to differences in the mode or mechanism of the damage. In Bloom's syndrome, publications indicate that the majority of the aberrations are of the chromosome type, while in Fanconi's anemia almost all structural changes are chromatid lesions. Furthermore, in Bloom's syndrome the chromatid aberrations lead more frequently to homologous exchange figures than in Fanconi's anemia and in glutathione reductase anemia where almost exclusively nonhomologous exchanges occur. This suggests that more "systematic" breakage occurs in Bloom's syndrome, isolocus breaks in homologous chromosomes, than in Fanconi's anemia.

Chromosomal breakage and the stage of the disease. In one case of Fanconi's anemia, it has been shown that chromosomal breakage in vitro as well as in vivo is present before the manifestation of the anemia. Patient 22, who at the time of that investigation was clinically normal, has now, 5 years later, a fully developed panmyelopathy (Wohlenberg, personal communication). The incidence of in vitro breakage has not changed since 1964 (Schroeder, unpublished data). Three other patients have been reported as showing chromosomal breakage in vitro in the preanemic stage of Fanconi's anemia.

The studies on one patient with Bloom's syndrome indicate that there could be a development or at least an increase in the incidence of breakage in vitro in the course of the disease. The first chromosome analyses at the age of 2 years showed no breakage. In contrast, at the age of 39/12 years and 48/12 years a gradual increase of breakage in vitro was demonstrated; concurrently, no abnormal cells were found in the bone marrow.

It is interesting to speculate that a development of chromosomal breakage in Bloom's syndrome points to an accelerating process which may be initiated by an external factor. In contrast, a continuous presence of breakage with little or no increase in time as found in Fanconi's anemia, is more indicative of a steady process. To date, there is no solid evidence for either of these speculations. However, it is very important that they be substantiated or refuted by future research. Such data would be the first step towards an understanding of the course of these two diseases.

The symptoms of the diseases. In Bloom's syndrome there is, to date, only one case with a therapy-resistant aplastic anemia, excluding preleukemic anemias. No changes in blood cell composition have been observed in patients with Bloom's syndrome whose cells showed chromosomal breakage in culture. In contrast, pancytopenia or pannymelopathy is the cardinal symptom of Fanconi's anemia.

The major question is whether or not chromosomal breakage takes place in vivo in Bloom's syndrome and, if so, how does it affect the bone marrow. In
Fanconi’s anemia it has been shown that there is chromosomal damage in vivo and, consequently, cell destruction in the bone marrow.

To substantiate these effects of differences in causality and to further our understanding of their meaning, it is necessary that blood cultures, bone marrow smears and direct chromosome preparations from bone marrow be included in the close following of the disease. So that the cytogenetic data may be compiled and accurately compared, the methods of evaluation must be standardized. Publications should give detailed information about the various types of aberrations found.

**Fourth Problem: What Is the Cause of Chromosomal Breakage In Vitro and In Vivo?**

In vitro results cannot be numerically applied to the in vivo events. It must be taken into account that: (1) a preexisting break-causing mechanism could be enhanced and/or provoked in culture; (2) an in vivo deficiency of the cells could be corrected by the enriched culture medium; (3) differences in the mechanism of cell injury and in the ability to repair damage may be responsible for the degree of cell destruction in vivo and in vitro.

Specific information concerning all three possibilities is still lacking. To understand the influence of the culture condition on the break-causing mechanism, it is important to compare the first with the second and third division of the cells in vitro. Once more is known about the primary causes of the diseases it will be possible to investigate the effects of various components of the culture medium.

However, that the culture condition does enhance the break-causing mechanism in Fanconi’s anemia has been indicated by Bloom et al. In a direct bone marrow preparation the authors found no aberrations in 32 cells, in contrast to the 72-hour culture, where in four of 22 metaphases breakage was present. Germain et al. also found a high incidence (up to 40 per cent) of aberrant cells in 48-hour bone marrow cultures. This value shows a large increase when compared with the 10 per cent in vivo breakage reported by other authors. Unfortunately, the investigators did not do their own controls.

Despite the ambiguity surrounding the interpretation of chromosomal breakage in vitro, there is no doubt that breakage in vivo and in vitro is a secondary consequence to a primary genetically determined defect. Also, the primary defect is different in each disease.

A comparison of the types of chromosomal breakage in Fanconi’s anemia and Bloom’s syndrome indicates such differences in metabolic defects. In Bloom’s syndrome chromosome breaks seem to be more frequent than in Fanconi’s anemia, where chromatid breaks predominate. This suggests that the damage in Bloom’s syndrome is mainly expressed in G1 and that of Fanconi’s anemia primarily in the G2 phase of the cell cycle. Again, to establish that the breakage in Bloom’s syndrome is truly of the chromosome type, data from the first, second and third in vitro mitoses must be carefully compared.

The hypothesis of Swift and Hirschhorn which attributes the chromosomal breakage in Fanconi’s anemia to increased suscepti-
bility to chromosome breaking agents, especially viruses, does not contradict, but actually presupposes that a primary defect already exists.

To elucidate the causes for chromosomal breakage the future objectives of research are: (1) to find the metabolic defects associated with each disease; (2) to show how these defects are responsible for chromosomal breakage and for specific types of aberrations; (3) to clarify the genetics of the disease; (4) to extend these studies to include related diseases.

A start in the above direction has been made. In pernicious anemia the biochemical defect is known. The addition of vitamin $B_12$ and/or folic acid to the metabolic system can compensate for the defect and consequently the in vivo chromosomal breakage disappears.$^{8,26,36}$ The pattern of inheritance has not yet been defined because the genetic factors involved are still unclear. Ryan et al.$^{61}$ have also demonstrated the metabolic defect in pernicious anemia to be responsible for chromosomal breakage. They investigated patients with psoriasis treated with folic acid antagonists. Aminopterine and amethopterine block the folic acid reductase by noncompetitive inhibition which leads to the same resultant defect in purine and pyrimidine synthesis as in pernicious anemia. The authors observed chromosomal breakage in vitro as well as in vivo. Although no comparable control group is available, it is notable that two out of 171 patients treated with folic acid antagonists developed leukemia.$^{57}$

Glutathione reductase deficiency has been found in blood cells.$^{7,41,85}$ The defect remains to be shown in fibroblasts which could possibly be used in an experimental system designed to relate the metabolic disorder to the chromosomal breakage. In addition, it is necessary to examine individuals who carry the genetic defect but do not have the anemia. Here it would be especially interesting to see if a mitotic disorder already exists in the bone marrow or if it develops right after the dangerous medication has been taken. In this direction Hampel et al.$^{25}$ have shown that chloramphenicol, an anemia-inducing medication in glutathione reductase deficiency$^{6,84}$ does increase the frequency of chromosomal breakage in vitro.

Biochemical analyses of 10 patients with Fanconi's anemia have been carried out. In four of these patients, Löhr et al.$^{42}$ demonstrated a defect in hexokinase, the rate limiting glycolytic enzyme in blood cells. The enzyme was altered in activity and, most significantly, in its biophysical properties. Both results caused a lowering of the cell’s ATP level. The four patients belong to three unrelated families, which justifies the assumption that the hexokinase deficiency is causally related to the anemia. Fibroblasts from surviving patients with hexokinase deficiency in blood cells should be analyzed for the defect. In contrast, the fifth patient showed normal carbohydrate metabolism. Yet in all five patients chromosomal breakage was present in vivo and in vitro.$^{66,67}$

Normal hexokinase activity was reported in three other cases.$^{15,69}$ One case published by Gmyrek et al.$^{21}$ was found to have an increase of ATPase activity, the consequence of which again is a decrease of ATP. Chromosomal aberrations were present in vivo and in vitro. Cells with diminished ATP and normal ATP seem to differ cytogenetically, the former having fewer reunion figures than the latter.$^{67}$ This would agree with the results of Wolff$^{87}$ showing that ATP is necessary for normal functioning of the reunion system.
CHROMOSOMAL BREAKAGE AND LEUKEMIA

Vaccaro et al.\textsuperscript{80} claimed no chromosomal breakage in their male patient. A mild G-6-PD deficiency was found in the boy; the mother was normal. The authors discussed two interpretations of their data: the mild Mediterranean type of G-6-PD deficiency or a mosaicism deriving from an X-chromosomal mutation at an early embryonic stage. However, another possibility is that a G-6-PD deficiency in the red blood cells results from a somatic point mutation in the course of Fanconi’s anemia. Analysis of fibroblasts, including electrophoresis, could shed some light on this interesting problem.

Thus far Bloom’s syndrome has not been investigated biochemically. Perhaps it is worthwhile to give special attention to the case of Schoen and Shearn\textsuperscript{65} in their search for the primary defect. The “development” of chromosomal breakage might point to a cumulative effect of UV light. Since sunlight causes the exacerbation of the skin lesions, it is possibly also correlated to the appearance of chromosomal breakage in cultured cells.

The failure of DNA repair in xeroderma pigmentosum is one explanation for skin sensitivity to UV light. The same experimental approach was used on patients with ataxia telangiectasia with negative results.\textsuperscript{9}

In summary, the cause of chromosomal breakage in vivo and in vitro is the result of a primary, genetically determined defect. Because these primary defects are unknown, the possibility that the cells are influenced by in vitro conditions cannot be ruled out. The biochemical investigations necessary to elucidate the metabolic defects involved, are still at a preliminary stage. Pernicious anemia is well understood, whereas the available biochemical data concerning the other diseases is only derived from single cases. These can give directives to future research but should not be considered final.

Recent studies of different groups of inherited diseases show cross-linkage between single symptoms: chromosomal breakage, anemia, skin disease, a- or dysgammaglobulinemia and leukemia or other malignancies.\textsuperscript{3,33,37,47,48,65,88}

Although presently we do not have a complete concept which would incorporate all these facts meaningfully, the connection between chromosomal breakage and malignancy in these diseases can be discussed further. The discussion will be restricted to the specific situation of genetically defective cells, such as from Fanconi’s anemia or Bloom’s syndrome. The general hypothesis correlating chromosomal breakage with neoplasia through various mechanisms including problems of selective procedures and clonal development is assumed.\textsuperscript{1,12,13,39}

Fifth Problem: How Is Chromosomal Breakage in These Diseases Connected with the Development of Leukemia or Other Malignancies?

The cause of malignancy, that which happens within a single cell, is unknown. There are hypotheses, of course, based on findings obtained from cancer cells themselves, as well as based on experiments with chemical, physical and biological carcinogens.

Cancer cells can have the most peculiar karyotypes: hypodiploid, hyperdiploid, triploid or tetraploid and everything in between, as well as many kinds of structural chromosome abnormalities. Cancer cells can have a normal karyotype without visible changes.\textsuperscript{48} The only constant aberration so far known
is the Philadelphia chromosome, which is found in most cases of chronic
myeloid leukemia.

Hypotheses concerning the induction of cancer in general still pose the
problem: do chromosome aberrations per se lead to cancer or are chromosome
abnormalities found in cancer cells an epiphenomenon? Reports of diploid
cancers suggest that not all carcinogenesis is due to gross chromosome aberrations. Such cancer cells may, however, contain hidden chromosomal rearrange-
ments or bear point mutations, gene deletions or duplications.

Cancer in statu nascendi in vivo cannot be utilized. If virus-induced trans-
formation is relevant to a beginning of malignant growth, then this system is
a means for investigating the early stage of cancer.

Moorhead and Saksela showed that changes of the karyotype do not always
appear before transformation is visible. These results support their hypothesis,
and also those of Levan and Biesele and Nichols, that malignant growth
does not necessarily start with gross chromosome abnormalities but also with
point mutations, gene deletions or duplications.

In the diseases described here, both gross chromosome aberrations as well
as point mutations, gene deletions and/or duplications are possible when
chromosomal breakage and rearrangements take place in vivo.

Chromosomal breakage in vivo, however, is not a prerequisite for the spon-
taneous development of malignancies. Chromosomal breakage in vitro evidently
represents the result of a certain cell property, due to the unknown primary
defect. If it occurs exclusively in culture, factors in vitro must provoke this kind
of damage. However, it is conceivable that in vivo this same defect results in
point mutation, gene deletions and duplications instead of breaks. This is one
way of linking the frequent coincidence of leukemia and other tumors with
the diseases.

Because chromosomal breakage both in vivo and in vitro is related to
metabolic defects in the cells, the tendency to malignancies can also be pro-
voked or enhanced by additional external causes. Stoker and Todaro et al. investigated the viral transformation of cells previously damaged by X rays and
thymidine analogues. They found an increase in the transformation rate, i.e.,
the cells reacted with higher susceptibility to transformation by viruses. This
led to the idea that tissue of patients with Fanconi’s anemia might be more
sensitive to chromosome breaking agents and viruses. Viral transformation
in fibroblasts of patients with Fanconi’s anemia and their relatives was ex-
amined and an increase in the percentage of transformed cells was found when
compared with normal controls. Cells from homozygote patients were more
susceptible to transformation than those of heterozygotes. Schuler et al. treated Fanconi’s anemia blood cultures with an alkylating agent and found an
increased rate of breakage when compared with control experiments.

The extension of the experiments with Fanconi’s anemia and Down’s syn-
drome to tissues from patients with other diseases, as suggested by Miller and
Todaro will show which defects and diseases can be conclusively linked to
an increased susceptibility to viral transformation.

If chromosomal breakage in Fanconi’s anemia is merely caused by a long-
term virus infection then the virus must be isolated and proved to be break-
causing in this disease. This would still not explain why these cells are more sensitive than others to viral infection. Any genetic imbalance could provide a plausible reason for an increased sensitivity to external factors, as well as for an increase in spontaneous malfunction of the cellular system. For example, it is known that not only many congenital defects but also consistent chromosome abnormalities such as mongolism are closely related to a high incidence of leukemia or malignancy.47,48

In xeroderma pigmentosum, which is associated with skin cancer, Cleaver found a failure in the dark repair of DNA lesions induced by UV light. He stated: "... it is not simply that the inability to repair lesions in DNA makes a cell become malignant." However, this mechanism active, at the molecular level, could account for point mutations, deletions and possibly breaks.

It is to be expected that different inherited defects will be found which cause a change in the genetic material itself in single cells and so, by chance, create primary, yet unknown, conditions for malignant growth. On the other hand, there might be genetic factors which are responsible for a "weaker constitution," such as diminished resistance or failure in detoxication. This could make cells more susceptible to any of the carcinogens, including viruses. This can even include normally tolerable pharmaceuticals as seen in glutathione reductase deficiency, where a stress on the defective metabolic system initiates a continued process of spontaneous breakage.

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Analytical Review: Spontaneous Chromosomal Breakage and High Incidence of Leukemia in Inherited Disease

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