DISTURBANCES of the mitotic process have for many years been known to occur in leukemia, especially in the acute forms, and the existence of abnormal mitoses and polyploid cell forms has furnished one of the arguments in favor of the neoplastic nature of the disease. No accurate examination of the human chromosome complement was, however, possible until the development, some 7 years ago, of new technics. The subsequent establishment by Tjio and Levan, and by Ford and his colleagues of the normal diploid number at 46—and not 48, as had been taught for decades—constituted as impressive a volte-face as has been seen in biology and led in turn to a detailed examination of the chromosomes in the leukemias. Some hundreds of cases of leukemia have by now been studied by cytogenetic methods, and there is a consensus of opinion on the more important findings, although not necessarily on their interpretations.

The technics used in this work consisted at first of short-term cultures of bone marrow and of blood. Immature cells such as the myelocytes in chronic granulocytic leukemia will readily divide as soon as they are transferred to an artificial medium, and following culture and treatment with colchicine and hypotonic solutions good preparations are obtained in which the individual chromosomes can be identified. In cultured normal blood no mitoses occur because mature granulocytes do not divide and neither do lymphocytes under ordinary circumstances. The addition, however, of a phytohemagglutinin (PHA) extracted from Canadian red beans has the surprising effect of causing small “mature” lymphocytes in culture to become transformed to much larger, immature, mitotically active cells, an effect for which there is as yet no clear explanation, although it has been lately suggested to have an immunologic basis.

When leukemic blood is cultured with the addition of PHA, any mitoses seen may be derived either from leukemic cells or from normal lymphocytes present in the blood. Abnormal mitoses occurring in quantity in such cultures may reasonably be interpreted as belonging to leukemic cell lines. Not infrequently, however, there are only normal mitoses. This may be so either because all of them are derived from normal lymphocytes, the leukemic cells—as in many acute leukemias—having failed to divide; or alternatively some apparently normal mitoses may have come from leukemic cells. Although divisions of leukemic cells may be obtained in 1- or 2-day cultures without PHA, the interpretation of the findings in blood cultures is often difficult; the same is true of marrow cultures which are likewise apt to contain mixtures of normal and leukemic cells, each line capable of mitotic

From the B.E.C.C. Cytogenetics Unit, Christchurch Hospital, Christchurch, New Zealand. Submitted July 30, 1963; accepted for publication Sept. 17, 1963.
division. Moreover all cultures have the disadvantage that mitotic abnormalities, if found, may be artifacts induced by the artificial growing conditions. This disadvantage can be overcome by examining the mitoses in uncultured preparations of marrow, a method technically more difficult than culture but which, because of its relative freedom from artifacts, has lately commended itself to increasing numbers of workers in the field.

The principal agreed results of cytogenetic studies in leukemia are as follows:

In the acute forms there is a very high incidence of chromosome abnormalities consisting of alterations in number (aneuploidy, polyploidy) as well as structure. These are probably produced by mitotic abnormalities such as non-disjunction and spindle failure, and by chromosome deletion, translocation and other types of change. In some series7-9 chromosome abnormalities are found in nearly all cases, in others10-13 there is a lower incidence; differences are probably accounted for mainly by matters of technic. The most striking feature is that the abnormalities are extremely variable, being apparently, with few exceptions, unique in each case. However, once established they may be constantly present throughout the course of the disease. It has recently been suggested14 that the various types of acute leukemia may differ to some extent in the degree of aneuploidy.

In chronic granulocytic leukemia there is an entirely different state of affairs. Here an almost constant abnormality is found.15,16 It consists in the loss—either by deletion or translocation—of a segment of one small acrocentric chromosome, probably No. 21 in the Denver classification, the abnormal chromosome being termed Ph1 after Philadelphia, the city in which it was discovered. The Ph1 is present in well over 70 per cent of all cases of chronic granulocytic leukemia17-19 and has not yet been found in seemingly normal people or in any abnormal condition other than CGL. It may therefore be provisionally termed specific for this condition. Occasionally Ph1 cells have been found in the marrow before the development of full clinical symptoms of the leukemia.20 An abnormal chromosome resembling Ph1, but believed to be a Y chromosome, has been reported recently21 in two male mongol infants, and in their fathers, all of whom were without signs of leukemia.

In chronic lymphocytic leukemia no abnormalities have been found in the great majority of cases, but two siblings with the disease, as well as several unaffected members of the same family, have been shown to have an inherited abnormal small acrocentric chromosome from which the short arms are missing. This chromosome is also probably No. 21, and has been called Ch1.22

These are some of the chief findings. What is their meaning? The relation of chromosome abnormalities to the onset of leukemia and other malignancies has been considered recently by Ford23 and Hauschka.24 The possibilities are essentially three: The chromosome changes may, in Ford’s23 words, be primary and causal, secondary but contributory, or ir-
relevant. Taking the last possibility as a beginning, “irrelevant” would mean changes arising more or less accidentally in the course of the leukemia, epiphenomena of no consequence either in the origination of the disease or in its further development. This appears an unlikely explanation for the findings in the acute leukemias in which distinct cell lines with characteristic abnormalities are almost constantly present at the clinical onset and persist, often unchanged, throughout its course; in chronic granulocytic leukemia, with its constant and uniform Ph1 chromosome, the suggestion clearly cannot apply.

Should changes in the chromosome apparatus be then regarded either as causative or associated with the causative event in leukemogenesis? Mutations have of course been thought for many years to initiate malignant processes, and though this term, as commonly used, refers to submicroscopic “point” changes, there is no reason why the grosser chromosome aberrations should not be included in it. Ionizing radiations, for instance, are known to lead at times to leukemia, and radiation also produces chromosome damage. It is therefore only a short step to postulating that chromosome aberrations are involved in the causation of leukemia when it follows exposure to radiation. It may therefore be significant that chronic granulocytic leukemia is one of the forms known to occur after radiation exposure, and that its characteristic Ph1 abnormality is probably the result of simple chromosome breakage such as is readily caused by radiation, although this does not mean that all cases of CGL in which radiation is suspected as an etiologic factor will necessarily show the Ph1 chromosome. Chromosome damage has been shown to persist for months or years after radiation in the lymphocytes of the blood, and it has been suggested that this may also be the case in the leukopoietic cells of the marrow. However, the persistence of chromosome aberrations in lymphocytes may result from a mechanism peculiar to these cells and inapplicable to other tissues.

The relation between chromosome abnormalities and leukemia is most obvious when the former are congenital in origin and therefore clearly antedate the disease. This is so in at least two conditions which appear to predispose to leukemia. One is mongolism: this, in most instances, is characterized by trisomy of chromosome No. 21, and mongols have a leukemia incidence nearly 20 times higher than the general population. The other is the presence of the Ch1 chromosome, an inherited abnormality, which, at least in one family, appears to predispose to chronic lymphocytic leukemia.

Since the No. 21 chromosome is abnormal in both chronic granulocytic (Ph1) and lymphocytic (Ch1) leukemia and is reduplicated in mongolism, which might also be called a preleukemic condition, it has been suggested that this chromosome may be the locus of a gene or genes regulating leukopoiesis. Leukemia would then result from mutations or other changes at these loci. Though this may be so, there could also be a wider explanation for the observed associations: it might be that the changes in No. 21 are only one example of chromosomal imbalance, and that imbalance however
produced might predispose to leukemia. In favor of such a concept are the associations observed in several cases between sex chromosome abnormalities and leukemia\textsuperscript{29,31} and between leukemia and major congenital defects other than mongolism in the same patient.\textsuperscript{32} Further, the occurrence in the same sibship of mongolism and leukemia,\textsuperscript{33,34} sex chromosome abnormalities and leukemia\textsuperscript{35,37} and multiple miscarriages and leukemia\textsuperscript{32} may point to an unstable condition of the parental chromosomes causing non-disjunction in germinal cells and predisposing to leukemia in the offspring. Such instability could be expected also to lead to leukemia in the parent; thus the mother of a typical trisomic mongol was recently found by us to have Ph\textsuperscript{1}-positive chronic granulocytic leukemia.

It may well be that the mechanism of the genetic change in many leukemias is a disturbance, either inherited or acquired, in an unknown locus regulating normal cell division in general, that this leads to chromosomal imbalance, and that such changes may in turn encourage alterations in other chromosomes, thus leading eventually to the astonishing pleomorphism of the abnormalities in acute leukemia, as well as in some experimental animal leukemias.\textsuperscript{38} It is perhaps rather naive to ask if these chromosome changes merely further the development of leukemia rather than cause it. The classical two-stage concept of carcinogenesis, a stage of initiation and one of promotion, may be an oversimplification. Burch\textsuperscript{39} has adduced cogent reasons for postulating as many as four mutations as necessary for the development of leukemia of which one may be inherited and three acquired. Such visible chromosome alterations as trisomy 21 and Ch\textsuperscript{1} may correspond to the first of these, and acquired abnormalities, as in acute leukemia, may be next in such a series; nor should it be forgotten that submicroscopic point mutations may be as important as grossly visible changes in leukemogenesis, but impossible to demonstrate directly. In this connection the position of the Ph\textsuperscript{1} is of interest. This is certainly an acquired chromosome abnormality; it occurs in hematopoietic but in no other somatic cells, and probably only in the granulocytic series (although on the strength of indirect evidence, it has lately been surmised that it may also be present in erythroid cells and perhaps in megakaryocytes\textsuperscript{19,40}). Its very high incidence and apparent specificity in chronic granulocytic leukemia has given cause to the suggestion that the abnormality may be causally related to this type of leukemia. It may be preferable to regard it as only one essential step in the production of a specific type of leukemic proliferation, a step which is neither causative by itself nor, in most cases, final; for evidence is accumulating\textsuperscript{18,29,41} that other chromosome changes may be superadded when the acute “blastic” transformation occurs in this type of leukemia. In terms of Burch’s thesis, the Ph\textsuperscript{1} may be only the penultimate of the mutations necessary for the full development of the disease.

Whatever the precise relationship between the newly discovered chromosome aberrations and the onset of leukemia, there is no question that these form excellent markers for many types of study. Thus it is possible, by
tracing in the blood and marrow the rise and fall of the Ph¹ in chronic granulocytic leukemia or the characteristic abnormalities in acute leukemias, to gain a clear picture of the means by which remissions are produced in the course of therapy;¹⁸,¹⁹,⁴² by cross-transfusion experiments the survival of homologous leukemic cells can be followed in leukopenic recipients;⁴ and new insights may well be obtained into the genetic control of certain chemical factors such as neutrophil alkaline phosphatase, or some blood group substances, by comparing their concentrations in normal cells and in those carrying abnormalities like the Ph¹ or trisomy 21. The study of the leukemias by cytogenetic technics holds promise of contributing materially to the better understanding of the nature of leukemia, as well as its successful treatment.

REFERENCES

18. Fitzgerald, P. H., Adams, A., and Gunz, F. W.: Chronic granulocytic...


400


Frederick W. Gunz, M.D., Ph.D., Haematologist, North Canterbury Hospital Board, Christchurch Hospital, Christchurch, New Zealand

P. H. Fitzgerald, M.Sc., Associate Director, Cytogenetics Unit, Christchurch Hospital, Christchurch, New Zealand
Editorial: Chromosomes and Leukemia

FREDERICK W. GUNZ and P. H. FITZGERALD