Part I

Introduction

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The problem of aneuploidy

The human impact of aneuploidy

This book is about a major human problem, one which affects human existence from conception to death. Although sheer numbers cannot tell the whole story, the impact of aneuploidy, in quantitative terms, can be assessed in a variety of ways (Table 1.1) (see also discussions of Golbus, 1981, and of Bond and Chandley, 1983). An exact figure for the frequency of aneuploidy at conception is not available, but it has been estimated to range from as low as 8–10% (Alberman and Creasy, 1977; Kajii, Ohama, and Mikamo, 1978) to as high as 50% (Boué, Boué, and Lazar, 1975; Schlesselman, 1979). The most germane data presently available are those of Martin (quoted in Bond and Chandley, 1983), based on the cytogenetic status of human spermatozoa used for the in vitro fertilization of hamster ova (Rudak, Jacobs, and Yanagimachi, 1978; Martin *et al.*, 1982). In these studies, 8.6% of the human spermatozoa had chromosome anomalies, 5.6% of which were frank aneuploidy. If it is assumed that the frequency of such abnormalities in human oocytes is of a similar or perhaps even greater degree, then the incidence of aneuploidy at conception might be as high as 15–30%. That such a figure is not unreasonable is suggested by the observation that 33% of conceptions prospectively identified by detection of human chorionic gonadotropin in the urine do not survive beyond implantation (Miller *et al.*, 1980).

It is likely that a specific estimate for the frequency of chromosomal abnormality will eventually come from work on human in vitro fertilization, but reliable data are not yet available (Edwards, 1983). However, whatever the figure is, it seems clear that a significant number of aneuploid embryos die in the periimplantation period, including virtually all embryos with autosomal monosomy (Epstein and Travis, 1979), so that by three to five weeks of gestation (after ovulation), 9.3% of therapeutically aborted fetuses are chromosomally abnormal (Yamamoto and Watanabe, 1979). This figure decreases dramatically during pregnancy as more and more such abnormal fetuses are spontaneously aborted or miscarried. Chromosome abnormality thus constitutes the principal known cause of first- and second-trimester abortions, reaching proportions as high as 50–60% between 8 and 20 weeks of gestation (after the last menstrual period) (Warburton *et al.*, 1980). Of the aborted fe-

Table 1.1. *Frequency of chromosome abnormalities at various stages of development*

Stage	Nature of data	Derivation of data	Frequency	References
At conception	Chromosome abnormalities in spermatozoa[b]	Observation	8.6%[c]	Martin, in Bond and Chandley (1983, p. 6)
During gestation[a]				
3–4 wks	Chromosome abnormalities in induced abortions	Observation	9.3	Yamamoto and Watanabe (1979)
3–10 wks		Observation	5.0[d]	Yamamoto *et al.* (1981)
5 wks	Living fetuses with chromosomal abnormalities	Estimation	4.7	Hook (1981)
8 wks			3.8	
12 wks			2.1	
16 wks			0.8	
28 wks			0.33	
Birth	Chromosomally abnormal newborns	Observation	0.37[e]	Higurashi *et al.* (1979)
			0.31[f]	Bond and Chandley (1983)
			0.27[g]	Hook (1981)
Childhood and adult life	Institutionalized individuals with moderate to severe mental retardation	Observation	10.9[h]	Jacobs *et al.* (1978)
			15,3	Sutherland *et al.* (1976)
			32.6	Gripenberg *et al.* (1980)

[a]Timed from estimated time of conception.
[b]As judged from in vitro fertilization of hamster ova by human spermatozoa.
[c]For all chromosome aberrations; for aneuploidy only, 5.2%.
[d]Adjusted for maternal age.
[e]All chromosome abnormalities.
[f]Aneuploidy only; mosaics excluded.
[g]Clinically significant aneuploidy.
[h]These figures represent the fraction of mentally retarded individuals who have chromosome abnormalities.

tuses with chromosome abnormalities, half have autosomal trisomy, nearly a quarter are polyploid, and a fifth are 45,X (data summarized in Bond and Chandley, 1983).

By 28 weeks of gestation, about 0.33% of fetuses are chromosomally abnormal, and this proportion is about the same at birth. Of the newborns with chromosome abnormalities, nearly three-fifths have sex chromosome anomalies and the remainder have autosomal aneuploidy, overwhelmingly trisomy 21 (data summarized in Bond and Chandley, 1983). The prevalence of cytogenetically abnormal individuals during childhood and the adult years has not been established. Although sex chromosome aneuploidy does not have a high mortality rate associated with it, autosomal trisomies other than trisomy 21 do, and trisomy 21 itself has a mortality of about 50% from birth to age 30 to 40 (Thase, 1982). (The last figure is difficult to estimate because of the decreasing mortality rate, especially in the early years, of individuals with trisomy 21 as medical care and social attention improve.) Therefore, the incidence of all forms of aneuploidy can be roughly estimated as being about 0.25% at age 40, and of autosomal aneuploidy about 0.06%. Although these proportions may seem low, their impact assumes much greater significance when considered in terms of the total number of individuals in the population.

Another way to evaluate the impact of aneuploidy on the human population is to consider its contribution to the causation of mental retardation. Although the estimate arrived at is, of course, influenced by the choice of individuals to be studied, it is clear that a significant proportion of individuals with moderate to severe mental retardation do have chromosome abnormalities (Table 1.1). And, regardless of one's opinions about the nature of the association, it is also clear that there is an increased frequency of sex chromosome aneuploidy (XYY, XXY) among individuals hospitalized or imprisoned for certain forms of behavioral deviance (Hamerton, 1976).

Finally, we may consider the economic burden of aneuploidy. In this regard, trisomy 21 has the greatest impact because of both its frequency and compatibility with long survival. On the average, the expected length of life of a newborn with Down syndrome is close to 36 years (Jones, 1979), resulting in a loss of life calculated to be 53.6 years per 1000 livebirths. In addition, considerable costs are incurred during the period that the affected individuals are alive. A recent analysis placed these costs at approximately $196,000 (in 1980 U. S. dollars), of which $27,000 was for medical costs, $55,000 for education, and $114,000 for residential care (Sadovnick and Baird, 1981). If we add to these figures the costs for long-term medical attention to individuals with some of the sex chromosome abnormalities and the costs for shorter-term management of infants with the more severe autosomal abnormalities, the heavy costs to both individuals and society can be appreciated.

This brief survey indicates that, however it is estimated, the impact of aneuploidy on the affairs of mankind is significant. It probably constitutes a

major cause of early postconceptual pregnancy loss and is certainly an important cause of spontaneous abortion. It is a major contributor to infant and childhood morbidity, including both mental retardation and physical abnormalities, and also to morbidity in the adult population. For virtually all forms of aneuploidy, the social, financial, and psychological impact of the condition on the families of affected individuals, on the individual themselves (if they are aware of their condition), and on society at large is profound.

While the discussion so far has been concerned only with constitutional aneuploidy – chromosome imbalance present at birth – it must also be pointed out that acquired aneuploidy is a prominent feature of malignancy, appearing sooner or later in the evolution of virtually every tumor (Sandberg, 1980). Therefore, if we broaden our consideration of the impact of aneuploidy to include malignancy also, it is clear that chromosome imbalance does indeed affect humans from the point of conception to the time of death.

Causation

The "problem" of aneuploidy can be divided into two essentially unrelated parts: causation and effects. The first, that of causation, is concerned with why and how aneuploidy occurs and has received most of the attention in nonclinical discussions of aneuploidy [see, for example, the monograph by Bond and Chandley (1983) entitled simply *Aneuploidy*, and the monograph by de la Cruz and Gerald (1981)]. That this should be the case is understandable, since the premise that prevention is preferable to cure, especially when no cure is in sight, would dictate considerable attention to trying to understand how aneuploidy arises and how it might be prevented. Nevertheless, the prevention of the birth of aneuploid individuals by means other than universal prenatal diagnosis and selective abortion is still only a dream. Therefore, if any practical justification is required for attention to the second part of the aneuploidy problem, that of effects, it is that for the foreseeable future we shall have to be concerned with aneuploid individuals and their problems. To be able to ameliorate their situation in more than a symptomatic manner will require an understanding of the mechanisms by which their difficulties arise. Only by a knowledge of such mechanisms will it be possible to develop strategies that are based on more than empirical and often groundless assumptions.

Approach to understanding the mechanisms of the deleterious effects of aneuploidy

This book will be concerned with issues of mechanism: how does aneuploidy interfere with normal development and function? My intent is not in cataloging the phenotypic abnormalities resulting from chromosome imbalance but,

rather, in understanding why and how a particular state of chromosome imbalance produces a specific set of abnormalities. A trivial answer to this question is that the genomes, which we regard as balanced, of humans and other species have evolved so as to optimize the probability of normal development. Therefore, it is quite understandable that any disturbances of its genetic balance should adversely affect the functions which a genome controls. I would not argue against the validity of such a notion, but would only point out that it is more a truism than a statement of cause and effect. Further, while it might say something about *why* imbalance is deleterious, it still does not address the *how*, and this is the matter of principal concern here.

My approach to the analysis of the effects of aneuploidy has a quite straightforward conceptual basis, which is that it will ultimately be possible to deduce the phenotype from the genotype. While not denying a role for environmental and stochastic factors in the final determination of the phenotype, my fundamental premise is that it should be possible to reduce the complex phenotypic effects of an aneuploid state to separable elements which can be attributed to the imbalance of a specific genetic locus or sets of loci. Therefore, the connection between imbalance of a particular chromosome or chromosome segment and the consequent developmental and functional abnormalities will ultimately be explicable in terms of the conventional principles of gene expression, embryology and development, cell biology, and metabolism. Of course, there are many principles which still have not been worked out and can be appreciated only in the most general terms. Nevertheless, it will be these principles and not some group of mysterious effects of chromosome imbalance that will finally provide the sought-after explanations.

This approach to understanding the effects of aneuploidy, which is essentially a reductionist one, is heavily influenced by work on the genetic determination of the three-dimensional (tertiary) structure of protein molecules in which I participated over twenty years ago (Epstein, Goldberger, and Anfinsen, 1963). The essence of the latter, for which Anfinsen received the Nobel prize, was the demonstration that the folding and final three-dimensional structure of a protein molecule is determined solely by the sequence of amino acids which constitutes its primary structure, taking into account, of course, the molecular environment (the milieu) in which the folding occurs. While the precise mechanisms of folding and the rules for translating sequence into three-dimensional structure (and thereby permitting predictions to be made about the effects of amino acid substitutions on structure) are still being worked out, the general validity of the overall principle, which was severely attacked when first enunciated, is firmly accepted today. If we substitute "genetic locus" for "amino acid" and "phenotype" for "tertiary structure," the basis of my conceptual approach to the problem of understanding the effects of aneuploidy becomes clear. However, lest it be argued that this view of the mechanisms of the effects of aneuploidy suffers from the same disabilities as

the reductionist or biological determinist approach to intelligence and behavior so roundly excoriated by Lewontin, Rose, and Kamin (1984), I would only echo these authors' own sentiments: "We are in no doubt that, were the processes of development sufficiently well understood, and given a sufficient amount of detailed information about the genotype of an organism, we could predict the phenotype in any given environment." This is just what I am striving to achieve with regard to understanding the consequences of chromosome imbalance.

Earlier in this chapter, I suggested a practical reason for being interested in the mechanistic basis of the effects of aneuploidy. I would now like to suggest two more reasons. The first is that it is an interesting problem in its own right, one which is concerned with a prevalent biological phenomenon. How can one look at a person with a chromosome abnormality syndrome and not wonder how the genetic imbalance produces the phenotype associated with it? The second reason is that the study of aneuploidy may ultimately tell us much about the processes of normal development and function. Just as the study of the inborn errors of metabolism has provided crucial insight into the normal aspects of intermediary metabolism, the study of genetic abnormalities that affect development will provide similar insights into the normal processes of organogenesis and morphogenesis (Epstein, 1978). Furthermore, any mechanisms proposed for processes based on the quantitative aspects of gene expression will become testable in the light of the quantitative perturbations introduced by the aneuploid state. This will occur in much the same way that quantitative differences introduced by heterozygosity for mutant alleles test metabolic relationships and that both heterozygosity and homozygosity for qualitatively aberrant loci test the relationships between the loci and their proposed mechanisms of action (Epstein, 1977). Aneuploidy thus becomes one more class of experiments of nature with which to probe normal development and function.

Organization of the book

The remainder of this book is divided into five parts. In deciding on the organization of the initial part of this volume, I had two choices. The first was to start with a discussion of the critical issues and then to proceed to an analysis of the clinical data relevant to these issues. The other was to do it the other way round, and for the reasons advanced at the beginning of the next chapter, this is what I elected to do. Therefore, this book will begin (in Part II) with an exploration of several aspects of human aneuploid phenotypes, the aim being to determine what general principles about the phenotypic effects of aneuploidy might be inferred from the clinical evidence. This analysis will be restricted to autosomal aneuploidy in order to avoid the complications which might be introduced by the role of X-chromosome inactivation in the

determination of the phenotype. The latter will be considered later in the book.

Following the examination of clinical phenotypes, I shall use both this information and other relevant material as the basis for a broad theoretical consideration of the mechanisms which may be involved in generation of the phenotypic effects of aneuploidy (Part III). In addition to the human clinical data, material will be drawn from reports of work involving a variety of different biological approaches carried out on humans and several other organisms ranging from bacteria to mice. The next part (Part IV) of this volume will be concerned with experimental approaches to studying the effects of aneuploidy. It will explore the in vitro and in vivo systems and methods that are now being used and may in the future be developed to investigate the mechanisms whereby aneuploidy produces its effects. Particular attention will be devoted to the development of model systems for studying human aneuploidy.

The next to last part (Part V) will consist of detailed discussions of three situations which might be considered as prototypic problems in aneuploidy. The first, trisomy 21 (Down syndrome), is of course the most frequent of the human autosomal aneuploidies compatible with viability and among the most important causes of moderate to severe mental retardation. The second is X-chromosome monosomy (Turner syndrome, gonadal dysgenesis), the best-studied example of X-chromosome aneuploidy. It has been included to provide a basis for discussing certain aspects of the control of X-chromosome expression which are relevant to an understanding of the effects of X-chromosome aneuploidy. The third situation is not an aneuploid phenotype or syndrome per se but is the intriguing association of aneuploidy and malignancy. The prevalence and specificity (or at least nonrandomness) of this relationship suggest that aneuploidy plays an important role in the establishment or maintenance of the malignant state, and that once again an understanding of the mechanisms by which it produces its effects may be relevant to the prevention or control of these effects.

This volume will conclude (Part VI) with a recapitulation and synthesis of the principal facts and inferences derived from the analyses of the clinical and experimental data and from consideration of the theoretical implications of aneuploidy.

A few definitions are in order at this point. The term "aneuploidy" is used to refer to any state of chromosome imbalance other than polyploidy, irrespective of whether changes in chromosome number are involved. "Trisomy" and "monosomy" are used to refer, respectively, to the addition and loss of a chromosome which, in turn, changes the total number of chromosomes. The addition or loss of a chromosome segment is termed, respectively, a "duplication" or "deletion," although it is sometimes difficult to avoid using the terms "partial trisomy" or "partial monosomy." Finally, in referring to the

number of copies of a locus present when there is a trisomy or duplication, the term "triplex" is used. With a monosomy or deletion, the term is "uniplex."

To assist in following the cytogenetic descriptions, the standard human and mouse karyotypes and human cytogenetic nomenclature are presented in the Appendix. A Glossary of clinical terms is also included to facilitate the understanding of clinical syndromes by readers unfamiliar with medical terminology.

Part II

Clinical observations