The Multifactorial/Threshold Concept — Uses and Misuses [1]

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ABSTRACT The common congenital malformations have familial distributions that cannot be accounted for by simple Mendelian models, but can be explained in terms of a continuous variable, "liability," with a threshold value beyond which individuals will be affected. Both genetic and environmental factors determine liability, making the system multifactorial. Cleft palate is a useful experimental model, illustrating a number of factors that contribute to palate closure, the nature of a developmental threshold, and how genes and teratogens can alter the components of liability to increase the probability of cleft palate. The nature of the genetic component to liability in human malformations is not clear, and various possibilities, ranging from polygenic in the strict sense to a major gene with reduced penetrance are compatible with the data — but the important feature is the threshold. Much of the confusion over the concept results from inconsistent use of terminology. The term "multifactorial" should be used for "determined by a combination of genetic and environmental factors," without reference to the nature of the genetic factor(s). "Polygenic" should be reserved for "a large number of genes, each with a small effect, acting additively." When several genes, with more major effects are involved, "multilocal" can be used. When it is not clear which of these is applicable the term "plurilocal" is suggested, in the sense of "genetic variation more complex than a simple Mendelian difference." Since teratological data often represent threshold characters the concept also has important implications for the interpretation of data on dose-response curves, synergisms, and strain differences in response to teratogens.

The concept of a continuously distributed developmental variable in a population, and a developmental threshold separating the population into those with an abnormality, on the one side, and without it on the other, is generating an increasing amount of controversy. Some hail it as the first (and perhaps only) rational explanation for the familial patterns shown by various relatively common human malformations and other disorders. On the other hand it has recently been referred to as tautological, based on grandiose assumptions, having no experimental support, and providing no testable hypotheses (Melnick and Shields, '76). It seems opportune, therefore, to review the concept, some of the uses to which it can be put, some of its implications, and some apparent paradoxes it can explain.

1. *History*

The concept was probably first introduced by Sewall Wright ('34a,b) on the basis of his study of polydactyly in the guinea pig. He observed that the trait had different frequencies in various sublines of a polydactylous strain and concluded that the dichotomy — Three versus four toes —

Received May 14, '76. Accepted Jul. 1, '76.

[1] Publication No. 445 from the McGill University-Montreal Children's Hospital Research Institute.

[2] Supported by a Medical Research Council Genetics Group Grant.

"cannot correspond to alternate phases of a single factor" (he used "factor" to mean gene) but must be the result of a physiological threshold in a character affected by many factors. Results of crosses between a polydactylous and a normal strain fitted the expectation for an autosomal recessive gene in the F_2 and first backcross generations, but when the backcross offspring were tested the results were no longer compatible with a single-locus model. Further testing led him to conclude that there must be several factors ("more than three"), but not an infinite number. The lesson, that the results of F_2 and first backcrosses may simulate Mendelism, and that proof of determination by a single locus requires testing a segregating generation, is still an important one.

Grüneberg ('52) developed the concept of a threshold character further, coined the term "quasi-continuous variant," described many genetic properties of these variants in the mouse, and pointed out that some common malformations in man also show the "stigmata" of quasi-continuity. One of the first examples that led him to the concept was absence of the third molar tooth in the mouse. He showed, e.g., that the frequency of missing third molar in various strains and crosses was correlated with size of the tooth when it was there, and suggested that a critical mass of the tooth anlagen might be the developmental threshold in this case. The "stigmata" of quasi-continuous variants included: (a) different stable frequencies of the trait in different inbred strains; (b) widely different frequencies of the trait in outcrosses of a strain expressing the trait to different "normal" lines; (c) a correlation between penetrance and expressivity of the underlying genetic system; (d) sensitivity to environmental differences such as diet and maternal physiology; (e) sensitivity to genetic differences, such as sex or major mutant genes.

That the multifactorial/threshold concept could be applied to teratogenetically induced traits was suggested by cortisone-induced cleft palate (Fraser and Fainstat, '51). Movement of the palatine shelves from vertical to horizontal during closure was delayed by cortisone (Walker and Fraser, '57). The differing frequencies of cleft palate induced by the same treatment in different inbred strains and crosses (Kalter, '54), and the fact that the palatine shelves, in untreated embryos, tended to move to the horizontal earlier in those strains and crosses that had the lower frequencies of cleft palate when treated by cortisone (Walker and Fraser, '56; Trasler and Fraser, '58; Trasler, '65), suggested that cleft palate in this case might be a multifactorially determined threshold character. If the shelves were delayed by more than a certain critical amount, a cleft palate would result. The latest stage at which the shelves could come up and still meet would be the threshold between normality and abnormality.

Concurrently, human studies were providing evidence that, for certain common congenital malformations, the frequency of the condition in various categories of relatives of affected probands fitted the expectation for a multifactorial threshold trait in several characteristic ways (Carter, '61, '69, '76). Consider a normally distributed variable termed "liability" to a disorder. Liability is determined by a combination of genetic and environmental factors. The frequency of the disorder will depend on the proportion of the population falling beyond a given threshold of liability. (The reader is invited to draw a frequency distribution, and a threshold near the right hand tail, to help her (him) visualize the following points.) Individuals falling beyond the threshold would, on the average, carry more of the predisposing genes than other members of the population, so the distribution of liability for their relatives would be shifted to the right, and more of this group would fall beyond the threshold and be affected. Assuming a normal distribution, and a given heritability, the expected proportion of affected relatives can be calculated, and certain other predictions made. Thus:

1. In populations with a high and low

frequency, respectively, the absolute risk to the families of affected individuals will be greater in the population with the high frequency, but proportionately the increase will be less. This has been shown for neural-tube defects, for example; in South Wales the population frequency is 0.76 and the risk for relatives is 5.2, a 7-fold increase, whereas in London the corresponding figures are 0.29 and 4.4, a 15-fold increase (Carter, '76).

2. The frequency in the families of affected individuals decreases sharply between first and second degree relatives and much less sharply between second and third degree relatives, rather than linearly (Carter, '69).

Note that both this and the previous feature depend simply on a threshold, and a distribution decreasing toward the tail; without requiring specification of the genetic component, or the form of the distribution.

3. The recurrence rate in first-degree relatives is higher when the defect is more severe in the proband. This is another way of saying that penetrance of the underlying genotype is correlated with expressivity. Thus the recurrence risk is higher if the proband has bilateral cleft lip (± cleft palate) than unilateral (Fraser, '70).

4. The recurrence risk for sibs is higher in families that had had two affected than in those with one affected child — viz., cleft lip (Curtis et al., '61), pyloric stenosis (Carter and Evans, '69), and neural-tube defects (Carter and Roberts, '67).

5. If the malformation affects one sex more than the other the recurrence risk is higher in the relatives of the probands of the less often affected sex. This follows from the assumption that the distribution of liability is shifted to the left, away from the threshold, in the sex less often affected. Therefore affected individuals of that sex must have a relatively greater liability, in order to fall beyond the threshold, and are thus likely to carry more of the predisposing genes than affected individuals of the other sex. The most outstanding example is pyloric stenosis (Carter and Evans, '69), where five times as many males as females are affected, and the risk ranges from 2.5% for the sister of a male proband to 18% for the brother of a female proband. The effect is present also in cleft lip, cleft palate (Fraser, '70), and dislocation of the hip (Wynne-Davies, '70).

Note that all of the features could also be accounted for by a model in which the genetic variation was not polygenic — e.g., a single major gene difference, giving three genotypes, with environmental variation affecting all the genotypes, resulting in a trimodal distribution. However, at least one of the more resistant populations would need to extend beyond the threshold.

In addition, there should be an increased frequency in the offspring of consanguineous parents, since increased homozygosity should increase the variance of the distribution (Newcombe, '63). This effect has not been convincingly demonstrated, but the appropriate data are difficult to obtain (Stevenson et al., '66).

7. Finally the frequency of the defect in first degree relatives is roughly equal to the square root of the frequency in the population (Edwards, '59; Newcombe, '63); i.e., on a log scale the relation is linear, whereas for single-gene models it is curvilinear (Reich et al., '72). However, this is an insensitive criterion for common malformations, since rather large differences in population frequency are needed to produce appreciable changes in recurrence risk for sibs.

A number of common congenital malformations and other diseases have now been shown to fulfill these criteria more or less fully. These include the classical example, congenital hypertrophic pyloric stenosis (Carter, '61), as well as cleft lip and palate (Fraser, '63, '70), pes equinovarus (Wynne-Davies, '65), congenital dislocation of the hip (Wynne-Davies, '70), anencephaly/spina bifida (Carter and Evans, '73), Legg-Perthe disease (Gray et al., '72), and Hirschsprung disease (Passarge, '73).

At this point it is time to consider some questions of terminology and, in particular,

the terms "polygenic" and "multifactorial," since their use by different authors to mean different things has led to considerable confusion. Wright ('34) used neither term originally; later he (Wright, '68) referred to guinea-pig polydactyly as a "multifactorial" trait, but also called genes "factors." Did he thereby mean "multigenic"? At any rate he clearly recognized that the trait was the result of a threshold in a character affected by many "factors," and that these included environmental factors. Grüneberg ('52) referred to multiple genes with additive effects, and avoided the word "polygene" since it was originally introduced to refer to a hypothetical category of genes affecting quantitative characters and intrinsically different from genes affecting discontinuous traits (Mather and Harrison, '49). He suggested that the variation due to genes with minor additive effects on a continuous character probably results from major genes acting primarily on some other character, of which the remote effects are being observed. This would account for their additivity and their interaction with the environment. "As the branches of a tree sway more in the wind than the trunk, so the more remote gene effects become progressively more sensitive to all kinds of influences of the environment" (Grüneberg, '52).

Quantitative geneticists use "polygenic" to refer to characters determined by a large number of loci, each having a relatively small effect, acting additively, and interchangeably — i.e., such that identical phenotypes can be produced by a large number of genotypes (Lerner, '58). Since most of these features cannot be demonstrated in man, for traits where the underlying variable is not defined, the term is often used more loosely, to mean "involving at least several genes, with effects of unspecified magnitude on the character." But to some this is a definition of "multifactorial." Others prefer to reserve "polygenic" (presumably in its loose sense) for the genetic predisposition, and use "multifactorial" to include environmental as well as the genetic factors (Carter, '69). It is small wonder that confusion exists. I would propose that the word "multifactorial" in this context be used in the sense of "determined by a combination of several genetic factors, of unspecified nature, and environmental factors," and that "polygenic" should be reserved for "a large number of genes with small effects, acting additively." The term "multilocal" has been suggested to refer to characters under the control of several genes, but not fulfilling the strict criteria for polygenic variation (Murphy and Chase, '76). One might add the word "plurilocal" when the nature of the genetic variation is not known, which is often the case for threshold disorders in man. At least, those who use the terms should make it clear what they mean by them.

In Carter's ('61) model, proposed to account for the inheritance of pyloric stenosis, the hypothetical distribution was a distribution of genotypes; those beyond the threshold would have the defect if exposed to some environmental "trigger." A more attractive model, because it is more amenable to methods of calculating heritability, considers the continuous variable as "liability" to a given trait. The liability is determined by all the causes, both genetic and environmental, that make an individual more or less likely to develop the trait (Falconer, '65, '67). The model assumes that the genetic component is polygenic (in the strict sense), that the liability can be expressed in units on a scale that renders its distribution normal, and that individuals above a certain threshold of liability are affected. Liability is essentially a statistical concept, and can be measured only in terms of the proportions of affected individuals in various populations. Certain attributes might reasonably be expected to correlate with liability to a disorder (e.g., blood pressure with respect to stroke), but would not be a direct measure of it. For congenital malformations even correlated attributes would be difficult to identifiy. From such a model, or various modifications of it (Edwards, '69; Smith, '70; Reich et al., '72), correlations of

liability between affected individuals and their relatives can be calculated from the frequencies of the defect in various categories of relative. From these the heritability of liability can be estimated.

This conceptual advance was followed by a sudden increase of interest in the genetics of the common malformations and other disorders that had previously been considered not amenable to analysis by standard techniques. It provided a rational explanation for many of the peculiarities that had frustrated previous attempts to make the family patterns fit various modifications of the Mendelian laws. It also made it possible, by extrapolation, to provide estimates of risk for categories of relatives for which there were no empirical data (Bonaiti-Pellie and Smith, '74; Mendell and Elston, '75).

Estimates of heritability for liability to a number of diseases turned out to be quite high. For instance, the heritability for liability to cleft lip and palate is estimated as 70-90%. This was a surprise to those not versed in quantitative genetics, who tended to think that low recurrence risks (5% or less) and low concordance rates in monozygotic twins (less than 50%) implied low heritability. For diseases with a variable age of onset, such as diabetes mellitus, the heritability of liability decreased with age (Simpson, '69) since the longer a person lives the more opportunity there is for environmental factors to influence liability.

It was recognized that, for disorders where there is no measurable quantity related to liability, the assumptions of additivity and normality cannot be tested, and that the model provides no insight into mechanisms. In the moving words of Edwards ('69), "The many factor model, where the factors are so numerous, and individually so feeble (as to) yield the smooth and tractable luxury of the Normal curve, and the single-factor model, in which the influence of one factor is so great that all others can be regarded as trivial, are the extreme interpretations between which reality must lie..... Thus, while various parameters may be estimated on various assumptions, these are in no sense structural parameters relating to mechanism, but rather summarizing indices which convert several numbers to one, or allow a more convenient auditing of the balance-sheet of variability." Nevertheless, "This is the nearest one can get with the human data to the degree of genetic determination of liability" (Falconer, '67).

One cause of scepticism was the realization that the empirical data would also fit other models (Morton, '67; Smith, '71), such as a major gene combined with polygenic and environmental variation (Morton and MacLean, '74), a single locus with two alleles, each with incomplete penetrance (Reich et al., '72), or a heterogeneous mixture of cases determined either by a major locus with incomplete dominance and reduced penetrance or by environmental factors (Chung et al., '74), or various combinations of these (Elston and Stewart, '73; Lange and Elston, '75). This is because the extreme tail of the distribution (which is all one can usually see) are not good indicators of the shape of the main body of the distribution. Reich et al. ('72) presented criteria for distinguishing polygenic from major-gene models if two thresholds can be distinguished (e.g., bilateral vs. unilateral cleft lip, or 1 vs. 2 or more extra digits), but appropriate data are lacking for the common congenital malformations. The importance of the distinction is that the components of polygenic (strict sense) contributions to susceptibility are not likely to be identified in specific terms whereas, if major genes are involved, there is some hope of distinguishing, and perhaps correcting, their effects, or at least identifying high-risk individuals more precisely. However, those who support the multifactorial/threshold model would, no doubt, point out that there is no need (except for the purpose of calculating heritability) to assume that the genetic variation is polygenic in the strict sense, and would not rule out the existence of major genes as long as genetic variation near the tails of the distribution is accepted. The unimodal continuous distribution of

red cell acid phosphatase activity, e.g., represents the summation of five separate but overlapping distributions, representing the phenotypes of only three alleles at one locus and their combinations (Harris, '75). The variation does not have to be polygenic (strict sense) in a multifactorial system. Thus the distinction may be semantic, rather than biological, and the proposed definition of "multifactorial" would include the alternative models mentioned above.

In fact, progress is being made in the identification of specific genes contributing to liability. For example, the predisposition to congenital hip dislocation (Wynne-Davies, '70; Czeizel et al., '75) is determined in part by joint laxity (major locus), acetabular dysplasia (? polygenic), and possibly swaddling practices (environmental). The HLA antigenic type appears to alter susceptibility to several diseases including diabetes (Nerup et al., '74) and some of the rheumatoid diseases (Anonymous, '75). Further progress will probably depend less on the elaboration of better mathematical approaches, useful as they may be (Morton and MacLean, '74), than on identifying the biological attributes of the predisposing factors.

2. *Cleft palate as a multifactorial/threshold model*

Cleft palate is considered briefly here as an example of a congenital malformation that is clearly multifactorial, and where experimental studies have provided some insight into the biological nature of some of the genetic and environmental factors involved (Fraser, '69; Burdi et al., '72).

Closure of the palate appears to involve a force in the palatine shelves that promotes their movement from a vertical position on either side of the tongue to a horizontal position above the tongue, where their edges fuse to form the complete structure. Opposing this force is the intervening tongue. Various factors impinge on the struggle of the shelves to force their way into the space between the tongue and the floor of the skull.

A multifactorial/threshold model for cleft palate is illustrated in figure 1. It postulates that the stage at which the palatine shelves become horizontal and thus able to extend towards the midline, meet, and fuse is continuously distributed. Thus in some embryos the shelves become horizontal relatively early and in others relatively late. Head width continuously increases during the period of closure, so that if shelf movement is delayed by more than a certain critical amount the shelves will become horizontal too late to accomplish fusion, and a cleft palate will result. Thus a discontinuous variable (cleft palate vs. normal palate) is determined by whether a continuous variable (stage at which shelf becomes horizontal) puts the embryo on one side or the other of a developmental threshold (latest stage at which shelves that have reached the horizontal can fuse). Both the distribution of the variable and the threshold can be influenced by both genetic and environmental factors.

The diagram illustrates the position of the distribution (relative to the threshold) as being determined primarily by the interaction of shelf force (promoting shelf movement) and resistance of the tongue (delaying it). The tongue becomes motile and moves forward during closure (Wragg et al., '72), which may aid the shelves in their struggle to move into the space above it. Both shelf force and tongue resistance can be influenced by other factors. The rate of growth of the mandible may influence forward movement of the tongue and alter its resistance. The shelf force presumably depends on the physical structure of the shelf, which may be related to mucopolysaccharide synthesis (Larsson and Bostrom, '66), hydration, etc., and may involve contractile elements in the shelf (Wee et al., '76). It may also vary with the rate of extension of the cranial base (Verrusio, '70; Long et al., '73). The probability of an embryo falling beyond the threshold also varies with the position of the threshold, and this varies with shelf width and shelf length. Both of these can be influ-

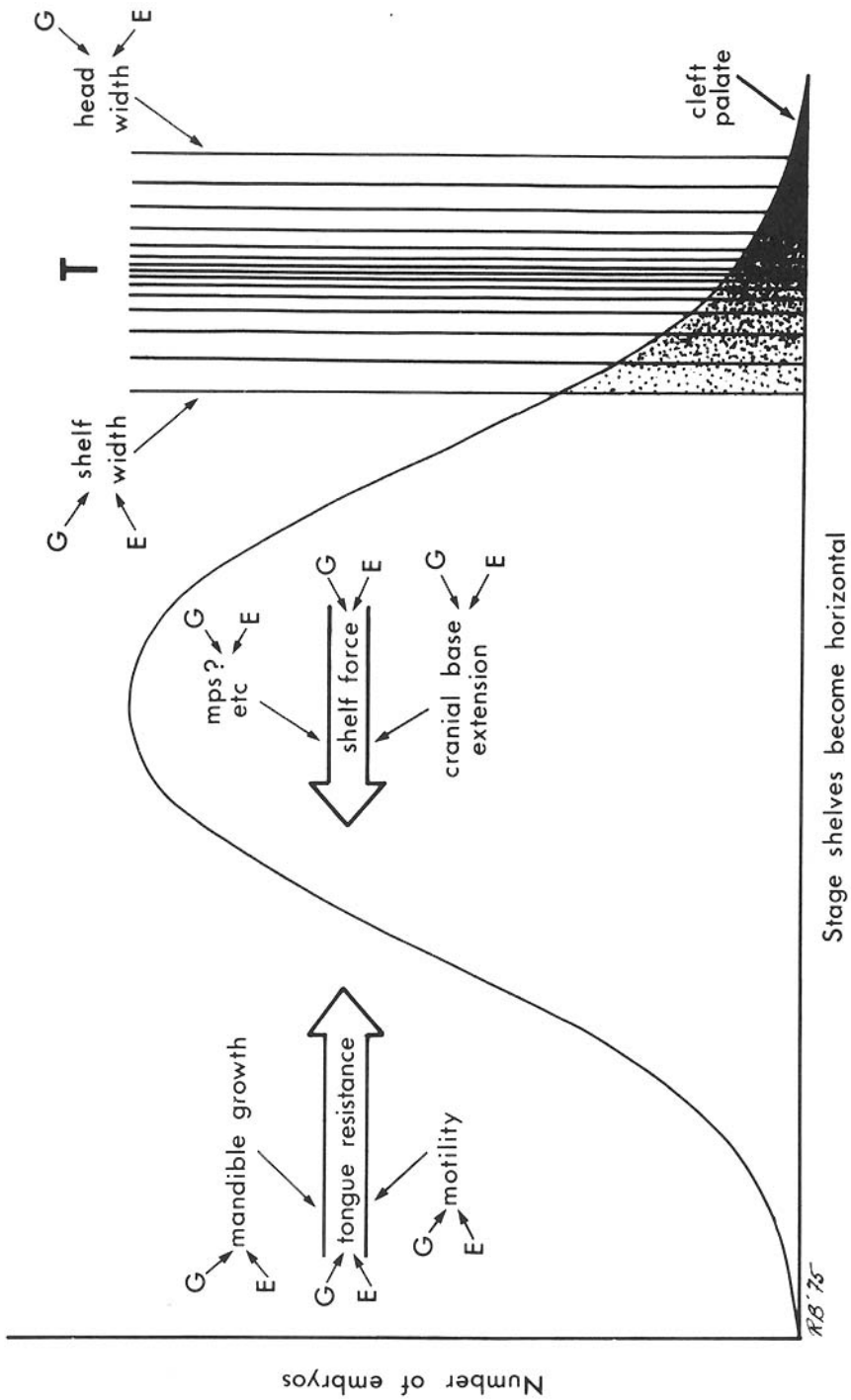

Fig. 1 A diagram illustrating the multifactorial nature of cleft palate. Stage at which shelves become horizontal is represented as influenced by the shelf force and opposing tongue resistance. Position of threshold varies with shelf width and head width. See text for details.

enced by genetic and environmental factors. A relatively wide shelf would allow successful closure even if the shelf moved relatively late (i.e., the threshold is moved to the right, relative to the distribution) and a relatively wide head would require the shelves to become horizontal relatively early to close successfully (the threshold is moved to the left). Thus the position of the threshold is also depicted as a continuous variable. This helps to emphasize that the probability of successful closure is assumed to depend not only on when the shelves become horizontal but on how far apart their medial borders are when they do.

Susceptibility to cleft palate depends on the relation of distribution to threshold. Thus the shelves move to the horizontal later in development in A/J than in C57BL mouse embryos (Walker and Fraser, '56) suggesting that the A/J embryo's genes put it relatively closer to the threshold and make it relatively susceptible to cleft palate induced by any environmental factor that delays shelf movement. Furthermore, the correlation between stage of shelf movement in the untreated embryo and resistance to cortisone treatment (as measured by cleft palate frequency) is reasonably good, for six crosses (Trasler and Fraser, '58; Trasler, '65). Although the evidence that one is causally related to the other is only inferential, it seems unlikely that it is coincidental.

The use of developmental stage as the underlying variable is obviously an oversimplification. The stage at which the shelves move is the reflection of many interacting underlying variables. It may not even be the time of approximation of the shelves that is critical but some other variable, such as the strength of the forces that keep them approximated (e.g., shelf growth vs. head growth) while they are undergoing fusion. Thus there is no simple variable that reflects liability in this model, since liability would be influenced by stage of shelf movement, head width, and their interactions.

Both genes and environmental factors can influence the position of a given embryo relative to the threshold by acting at any of the points depicted or, indeed, at others not depicted. A major increase in tongue resistance accounts for the cleft palate in mice with genetically determined cleft lip; the large median process (prolabium) obstructs forward movement of the tongue (Trasler and Fraser, '63). The mutant *mdg* (Pai, '65) probably causes cleft palate by inhibiting muscle differentiation and innervation in the tongue resulting in an increase in its resistance. Oligohydramnios, environmentally induced by amniocentesis, may also cause cleft palate by increased resistance of the tongue in the constricted embryo (Trasler et al., '56; Poswillo, '66). No genes or teratogens have been positively identified as producing cleft palate by reducing the shelf force, though many have been invoked. The homozygous mutant gene *ur* reduces shelf width (Fitch, '67) and so do vitamin A (Kochhar and Johnson, '65) and X rays (Masuyama, '59; Poswillo, '68). Presumably cleft palate will also occur when relatively small effects — on shelf force, shelf width, cranial-base extension, head width, tongue motility, mandible growth, amniotic-fluid volume, and so on — interact in the direction of delaying shelf movement beyond the critical point, and this would be a truly multifactorial etiology.

3. *Implications for animal and human studies*

In considering the implications of the model one must keep in mind that it represents a very simplified view of reality. It may provide a useful conceptual framework to aid one's thinking about the problem, but must not be accepted glibly.

a. *The contribution of normal developmental patterns of teratogenic susceptibility*

In the example of cortisone-induced cleft palate, the earlier the palate shelves normally closed, the more resistant the embryo was to the teratogen (Trasler and Fraser, '58). This illustrates that *the embryo's teratogenic susceptibility can be influenced*

by its normal developmental patterns, and implies that such a developmental difference would impose a difference in susceptibility to any teratogen that retarded shelf closure. Thus a strain difference in frequency of an induced malformation could occur even though the primary effect of the teratogen was the same in the two strains. This aspect of the threshold model has rapidly achieved widespread oblivion. For instance Larsson ('62) did not find a difference in acid mucopolysaccharide synthesis produced by cortisone treatment in embryos of a susceptible and resistant strain. Contrary to the otherwise excellent review by Greene and Kochhar ('75), this is not evidence against the hypothesis that cortisone causes cleft palate through inhibition of acid mucopolysaccharide synthesis. The strain difference in cleft palate frequency could have resulted simply from a strain difference in palate closure pattern, if this is related to liability. If closure were delayed equally in both strains, but occurred earlier in one strain, there would still be a strain difference in cleft palate frequency.

However, *identifying the basis for a genetic (strain) difference in susceptibility to a teratogen may not tell us anything about the mode of action of the teratogen.* The difference between the A/J and C57BL strains might, for instance, stem from a difference in shelf force, and the teratogen might act by paralyzing the tongue, equally in the two strains. Yet the C57BL strain, being farther away from the threshold, would be more resistant.

b. *Penetrance and expressivity*

If a major mutant gene, rather than cortisone, caused a delay in shelf movement the gene would have full penetrance in the A/J strain and reduced penetrance in the C57BL strain (Fraser, '65). This illustrates one mechanism by which "modifiers" in the "genetic background" of a strain (or individual) can affect the penetrance of a mutant gene.

The model also provides one way of accounting for the fact that penetrance is often positively correlated with expressivity (Grüneberg, '52; Landauer, '55; Sang, '63). That is, in a series of populations carrying a mutant gene, and differing in the relation of distribution to threshold, the closer the population mean was to the threshold the more individuals would fall beyond the threshold (increased penetrance), and the farther out on the scale of liability the distribution would extend (increased expressivity). Related to this is the fact that the recurrence risk for certain human malformations increases with the severity of the malformation in the proband (Carter, '69; Fraser, '70).

c. *Implications relating to effect of a threshold on frequency of a quasi-continuous trait*

(1) If a given malformation is a threshold character, with an underlying continuous distribution of some variable, then the frequency of affected individuals does not vary directly with the distance of the threshold from the mean, but with the area of the distribution curve falling beyond the threshold (Wright, '26). Therefore statistical comparisons of frequencies produced by different doses of teratogen, genotypes, etc., should be based on a probit, or other appropriate transformation, of the data, rather than the means and standard errors of the frequencies (Bliss, '57; Falconer, '60; Finney, '71).

Thus for a threshold character, with the underlying variable normally distributed, a *linear* dose-response curve, where response is measured by how far a given dose shifts the mean of the distribution, would appear to be *sigmoidal,* as measured by frequencies of induced malformations, unless these were converted to probit units.

(2) In practice, the underlying distribution may not be normal, even though it is usually depicted as such in diagrams illustrating the model, and often assumed to be so in the mathematical treatments of the properties and implications of the model. Normality is clearly not the case in some examples. For instance, A/J embryos begin

shelf movement late and close their palates quickly as compared with C57BL embryos; i.e., the distribution of shelf movement (which we postulate is related to liability) is skewed to the left (Walker and Fraser, '56), and for a number of human traits, such as blood pressure, stature, and IQ, the distribution departs appreciably from normality at the extremes (Edwards, '69).

If the distribution is not normal in situations where it can be observed we must be cautious in assuming normality where the distribution cannot be observed (as in most situations involving congenital malformations) which makes the choice of transformation difficult. This (as well as the one discussed next) is a problem in studies attempting to estimate the heritability of malformations from the frequencies in relatives (Falconer, '67; Cavalli-Sforza and Bodmer, '71), or to estimate the number of genes underlying a strain difference in malformation frequency, either spontaneous or induced (Dagg, '65; Davidson et al., '69). A transformation such as probit, if it renders the data linear, provides some reassurance (Biddle, '75), but caution is recommended in appraising results obtained by such statistical manipulations.

(3) In a multifactorial/threshold model an increase in frequency of the malformation can result from either a shift of the distribution to the right (in the conventional diagram) or an increase in variance, which in effect puts more individuals in the tails of the distribution. The latter possibility is often ignored in the interpretation of teratological data. It was pointed out by Newcombe ('63) in relation to the effects of inbreeding in man on multifactorial/threshold traits and can also result from assortative mating. This could be misleading when conclusions are based on the assumption that changes in frequency of the malformation are due entirely to shifts in the mean of the distribution. However Falconer ('67) points out that it will not affect calculations of heritability by his method.

(4) In a multifactorial/threshold model, if two teratogens interact additively — i.e., together they shift the distribution mean by the sum of their separate effects — the result, as measured in terms of frequency of malformations, may appear to be either synergistic (Fraser, '65) or approximately additive, depending on the magnitude of the shifts and the relation of distribution to threshold. Figure 2 illustrates a hypothetical model. The important point is that the effect of the teratogen is reflected by the shift in the distribution, but what is actually measured in a threshold system is the proportion of the population that falls beyond the threshold — i.e., the per cent affected. Thus if we have a control population that is assumed to be normally distributed, with a mean three standard deviations, (SD) from the threshold, only about 1 per thousand will fall beyond the threshold and be affected. If teratogen A shifts the distribution by 1.5 SDs, then (by the properties of the normal curve) 5% of the population will fall beyond the threshold and be affected. Assume that teratogen B has the same effect. Then teratogens A and B together, if they act additively, will shift the distribution by 3 SDs and 50% (not 10%) of the population will be affected. Thus the interaction will appear to be synergistic. If each teratogen moves the distribution by 3 SDs (50% affected), together they would move it 6 SDs (100% affected), so the effect would appear to be additive, as it actually is. Thus it should have been no surprise to Miller ('72) to find that pyridoxine deficiency and cortisone acted synergistically at a low dose of cortisone, and additively at a high dose. Deductions about the synergism of teratogens should be made only if the synergism is still present after a statistical transformation of the data that results in linear dose-response curves.

The apparent synergism of additive effects in a multifactorial/threshold model does not require the assumption, contrary to Runner's ('67) statement, that similar steps in a common enzymatic pathway are involved. To cite the palate-closure model yet again, one teratogen could delay shelf closure by acting on the tongue, and

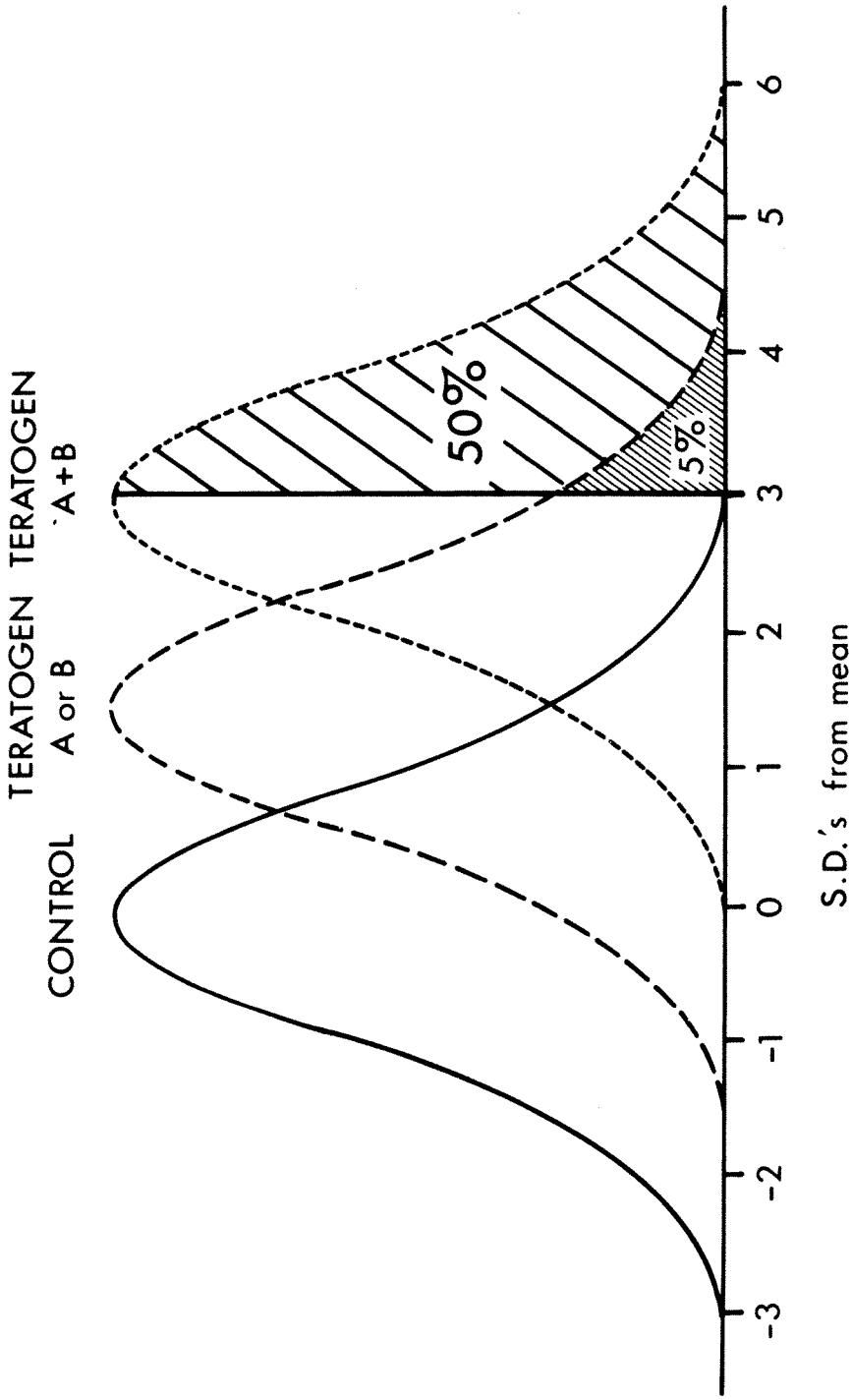

Fig. 2 Hypothetical diagram illustrating two teratogens, each of which shifts the liability by 1 standard deviation, and produces a 5% frequency of a malformation. If, together, they act additively, and shift the liability by 2 standard deviations, the resulting frequency of malformation will be 50%, and the interaction would appear synergistic.

another on the shelf. If they both delayed palate closure by the same amount, relative to the threshold, the effect on cleft palate frequency could be synergistic, as measured by frequency of cleft palate.

Similarly, it has sometimes been assumed that if two recessive mutant genes each produce a similar phenotype when homozygous, and the F_1 between the two homozygotes also show the mutant phenotype, then the two mutant genes must be allelic, but this is not necessarily so. Each mutant could be having an intermediate (additive) effect in combination with its normal allele, which would result in a normal phenotype, but in combination the two mutants, acting at different points of the system, could shift the distribution so that the compound heterozygote fell beyond the threshold and had the mutant phenotype (Harris and Fraser, '68).

4. *Significance for the human problem*

Previous to the popularization of the multifactorial model geneticists either tended to ignore the relatively common "familial" disorders as too messy to be worth analyzing or attempted to force them into some Mendelian mould, often with great ingenuity but poor credibility.

The multifactorial/threshold model had a salutory effect insofar as it provided a rationale that could account for various features of the familial data that had not been accounted for by any other model, and provided a stimulus for further research. On the other hand, it has attracted criticism since it now appears that other models may account just as well for most of the observations. Furthermore some have applied the concept uncritically, giving the impression that it is so all-encompassing as to be entirely nondiscriminating. Others appear to have used multifactorial as a synonym for heterogeneous. It is true that multifactorial systems are heterogeneous, in the sense that two individuals at the same point on the liability scale may be there for quite different reasons. However, the bare fact that a condition appears in some individuals as the result of (say) a mutant gene and others for a nongenetic reason is not sufficient reason to call the condition multifactorial. The term should be reserved for those conditions that demonstrate a reasonable number of the characteristics referred to previously.

Fears have been expressed that acceptance of the multifactorial/threshold model as the etiological basis for a disease may hinder further research by distracting attention from the environmental (and therefore potentially controllable) factors in the system, and because the assumption of high heritability may be taken (falsely) to mean that environmental control is unlikely to be effective, as apparently happened with tuberculosis (Edwards, '69, '70). Yet recognition of the fact that height, e.g., is a multifactorially determined trait does not seem to have impeded research on the genetic, endocrine, nutritional, and other factors contributing to its variation.

One hopes that the multifactorial/threshold concept will not deter, but encourage a better understanding of the distributions and thresholds underlying the common congenital malformations, and of the factors, both genetic and environmental, that modify them.

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