Abnormalities Associated with a Chromosome Region in the Mouse

Variant *t*-alleles, widespread in natural populations, may control differentiation of ectodermal and neural structures.

I. Transmission and Population Genetics of the t-Region

Most newly occurring mutations have something abnormal about them and might thus become objects of interest to the teratologist. In the work discussed here, the first mutation to be discovered was followed by a succession of related genetic changes all having effects on the spinal column and associated structures. The genetical analysis of these revealed a system of balanced lethal factors which is still unique in mammals, and the embryological study of these lethal factors showed that those controlling certain related processes in early development were properties of a limited area of one chromosome. At the same time, it was discovered that these same genetic factors were responsible not only for abnormal development but also for striking abnormalities in the mechanism by which such genes are transmitted to offspring. This abnormality turned out to constitute a new kind of evolutionary force by which lethal genes may not only be maintained in natural populations but actually enabled to spread. All of these peculiarities led to an intensive study of this region. The results of this to date, while far from complete, serve as a demonstration of the interrelations and interdependence of the genetical system, that is, the transmission mechanism, the mechanisms which control development, and those which control gene frequency, and thus evolutionary processes. Since these are parameters which are encountered or implied in many biological problems, and especially in teratological ones, we shall try to outline the study of these three aspects sufficiently to show the connections among them.

It will be my part to tell you enough about the genetical transmission mechanism in this region to serve as a background for the exploration of the manner of origin of the abnormalities in the embryo which will be discussed in the accompanying paper by D. Bennett (*1*). I shall extend my account to include something about the population genetics and evolutionary dynamics of this group of genes.

The history of the occurrence and the study of the first of these abnormalities has been reviewed by Grüneberg (*2*) and the genetic analysis of later cases has been reviewed by Dunn, Bennett, and Beasley (*3*). I shall therefore not attempt a detailed account or documentation of work which has been carried out by a number of workers in several different laboratories. The points of chief interest are that from a mutant mouse with a short tail, first found by Dobrovolskaia-Zavadskaia at the Institut Curie in 1927 and called by her Brachyury, there descended a stock of mice which, when inbred and outcrossed in various ways, produced offspring with a variety of abnormalities in addition to the short tail. These included spina bifida, paraplegia, and a variety of localized abnormalities of the vertebrae. From outcrosses of short-tailed (Brachyuric) to normal animals arose lines of entirely tailless animals; in some of these, spina bifida in the sacral region was common as well as a uro-rectal-caudal syndrome consisting of imperforate anus combined with a common urogenital sinus and, occasionally, other abnormalities of the gut. Crosses among tailless lines produce some normal-tailed offspring with marked abnormalities of the head region: microcephaly, microphthalmia, anophthalmia, and occasional otocephalics.

The genetical background against which these abnormalities occurred appeared to be remarkably simple. A dominant mutation T when heterozygous produced a short tail; when homozygous it acted as a lethal factor at the time of placentation. This allele when combined with any one of a series of recessive alleles, known as *t*-alleles, produced taillessness. Some of these *t*-alleles are lethal and also suppress recombination in the region of T so that they may be maintained in balanced lethal lines without selection. The genotypes and phenotypes are as follows:

Genotype	*Phenotype*
$+/+$	Normal tail
$T/+$	Short tail
T/T	Abnormal lethal embryo
$+/t^1$	Normal
T/t^1	Tailless
t^1/t^1	Abnormal lethal embryo

where (t^1 is any lethal *t*-allele)

The ability to maintain lethal mutants in balanced lines is of especial importance since it permits control of the production of specified abnormalities as homozygous embryos for study. The system operates as follows:

	T/t^1	x	T/t^1
F_1 genotypes	T/T	T/t^1	t^1/t^1
	lethal	viable tailless	lethal

Each of the several *t*-lethal alleles has its own syndrome of abnormalities and a characteristic period when death is most likely to occur.

The system also permits detection of changes in the effects of *t*-alleles, since occasionally, and usually as a result of an exceptional kind of crossover, an established *t*-allele gives rise to a different one. This occurs with a low frequency (about 2 per thousand offspring) in all balanced lethal lines (*2*). In cases in which a marker gene near the locus of T is present, it appears that most *t*-lethal alleles suppress regular recombination between T and the marker, and that the new *t*-allele arises by a process of exceptional recombination since it acquires the marker probably by crossing over. From the excep-

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tion, a new balanced tailless line with the marker can be established, as shown in Fig. 1.

Likewise, intercrosses between balanced lethal lines of different origins can be made to reveal whether the lethal alleles they carry are the same or different, different ones being defined as those which in combination give a normal phenotype as follows: t^n/t^n, lethal; t^x/t^x, lethal; t^n/t^x, viable with normal tail.

Thus the system above is one that detects genes which have their locations near each other and which take part in the determination of early developmental processes underlying induction and differentiation. We have observed some 75 occurrences of such genes, many arising as exceptions in laboratory balanced lines, others detected in different wild populations. Additional occurrences have been recorded in other laboratories and in wild populations in Britain, Japan, and Australia. Each of these separate occurrences does not necessarily involve a different allele in this region. We know that some of them are duplicates of alleles previously observed. The total variety is nevertheless quite large and includes at least a half dozen different lethal alleles and at least as many alleles which are viable when homozygous. All of them have one effect in common, to interact with the original mutant T to cause the dedifferentiation and resorption of the embryonic tail and thus produce taillessness. But they may differ in other properties.

One of the most unusual and interesting of these properties is the effect which all lethal alleles have on the male transmission ratio. Although all are transmitted in normal proportions by female heterozygotes, male heterozygotes transmit a lethal t-allele usually in high (90 to 99 percent) or moderately high (70 to 89 percent) ratios. One lethal allele is transmitted in a low ratio. Viable alleles may be transmitted in high ratios when found in wild populations or in normal or low (20 to 40 percent) ratios when they are derived from exceptions.

In the case of certain high-ratio alleles first analyzed by Braden (*4*), the effect appears to be due to a direct influence of the t-allele on the behavior of sperm carrying it after the sperm has entered the female reproductive tract. The variety of effects of factors in this region on this and other properties is shown in Fig. 2.

Genes with some of these effects occur at other loci in the mouse. For example, several years ago we tested some 16 different mutations with effects on tail and vertebral column resembling in some degree those associated with Brachyury. Two of them (kinked tail and fused tail) were found to be in the same linkage group with Brachy and about eight units away. The others were distributed more or less at random through the other linkage groups. Only those which behaved as alleles of T showed the other peculiarities listed in Fig. 2—namely, the type of lethal effect, the specific interaction with T, and effects on transmission ratio, male fertility, and recombination. These effects are obviously related to one chromosome region whereas abnormalities in tail development result from mutation at many other different loci. There is no longer any doubt that the first mutation T occurred in a region of the ninth chromosome which has a peculiar and possibly unique structure and that the other t-alleles were discovered and continue to be discovered because (i) the prior occurrence of T permits them to be easily detected; (ii) the structure of the region is related to its function in development and in evolution.

There is no evidence that T is other than a point mutation. Most t-lethal alleles on the other hand are certainly sectional changes in the vicinity of T

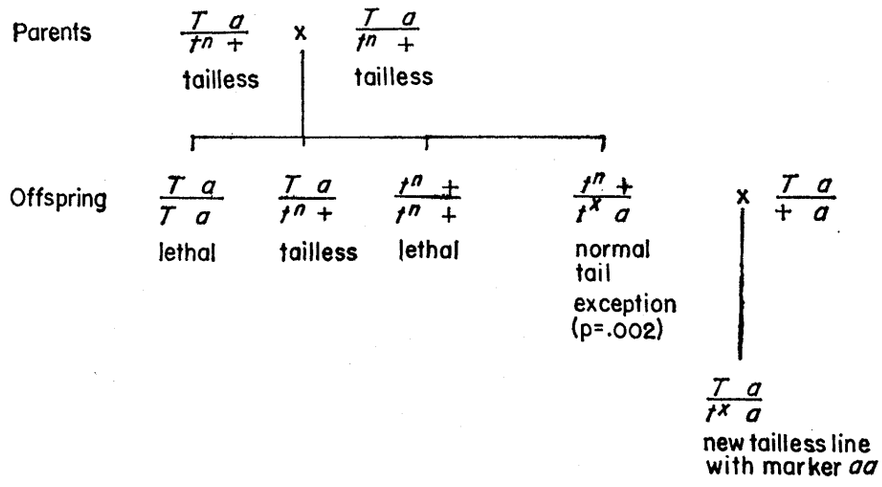

Fig. 1. The occurrence and manner of testing of exceptions from balanced lethal lines (T/t^n) from which new tailless lines (T/t^x) are derived. Gene t^n is lethal and suppresses recombination in the region T — a; a is a recessive marker about ten crossover units from T.

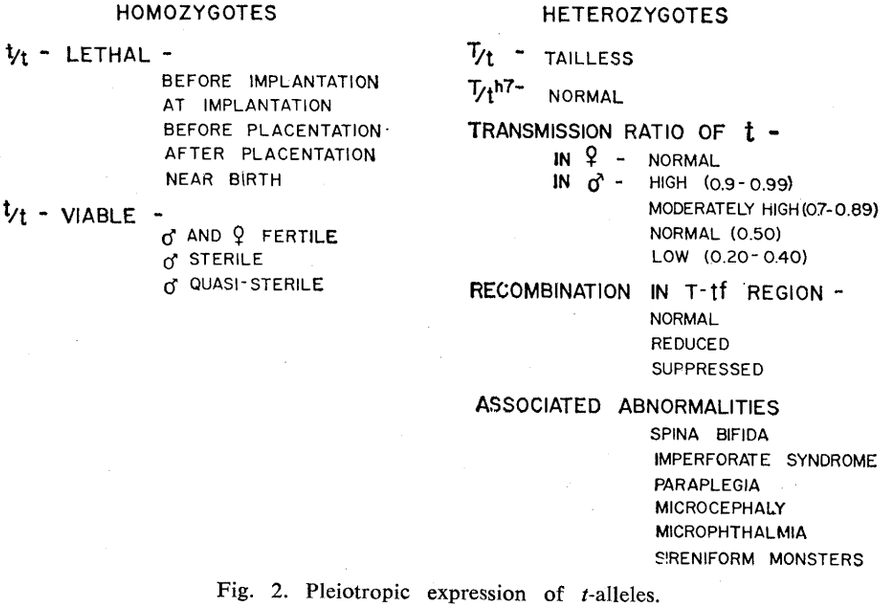

Fig. 2. Pleiotropic expression of t-alleles.

Table 1. Influences on changes in gene frequency of a *t*-allele in a given population (evolutionary forces). Where the evidence at present is in favor of the change indicated, it is followed by +; where it is against the change, −; where it is neutral or no evidence exists, 0; the symbols in parenthesis (+) mean that the evidence is indirect, in this case from computed analogue populations.

Tending to increase frequency of *t*		Tending to decrease frequency of *t*	
Mutation pressure $+\rightarrow t$	−	Mutation pressure $t\rightarrow +$	−
Selection favoring $+/t$ (heterozygote advantage)	+	Selection against $+/t$ (dominance of *t*)	−
		Selection against *tt* (lethality; sterility)	+
Drift = loss of + allele in small breeding groups	−	Loss of *t* in small groups including inbreeding effect	+
		Fixation of *tt* in sterile males with extinction of small population	(+)
Selective immigration of $+/t$ (or *tt*)	0	Selective emigration of $+/t$ (or *tt*)	0
Gametic selection of *t* i.e. > 0.5 *t* gametes	++	Gametic selection favoring normal allele	$(+^{v})$

since they suppress ordinary recombination for a distance of at least ten crossover units. One lethal and most viable *t*-alleles permit recombination and there is no evidence connecting these with chromosomal abberations. Cytogenetic evidence on the structure of this chromosome region is at present indecisive.

Facts suggesting hypotheses about the structure of the locus have come from breeding experiments (*3*, *5*). The exceptions which arise by exceptional recombination in balanced lethal lines contain derived alleles which have usually lost the lethal effect, the high male transmission ratio, and the crossover suppressing effect; that is, they are viable alleles transmitted in normal or low ratios. But there are a few cases in which the allele derived by recombination is lethal, is transmitted in high ratio, and suppresses recombination, so that more than one kind of exceptional recombinant is found. The number of kinds is limited, however, since eight lethal alleles derived from exceptions fall into three groups of duplicates. Two other groups of lethal alleles were found in populations. The members of each group resemble each other in lethal effect, transmission ratio, and effect on recombination but alleles in different groups differ in these respects and complement each other. Altogether 31 lethal *t*-alleles have been studied genetically and embryologically and found to fall into five complementation groups. In addition there are several different kinds of viable *t*-alleles. This suggests that there are a number of regions in and near this locus separable by recombination. Probably a considerable stretch of chromosome is involved since the crossover-suppression characteristic of some of the alleles extends for at least ten recombination units.

This juxtaposition of genes affecting related processes in development is discussed in the accompanying article (*1*). I should like now to consider the relation of this region to evolutionary processes.

Evolutionary Processes and Observations on t-Alleles

The list of effects associated with *t*-alleles (Fig. 2) contain several which should be decisive in determining the fate of such alleles in natural or wild populations. Those alleles which kill embryos homozygous for them would be eliminated, and other things being equal, should be found in natural populations only at the low rate expected for new mutations (actually at a frequency which is the square root of the mutation frequency). The same fate should overtake (but more slowly) *t*-alleles which form homozygotes which are viable but male-sterile.

But in fact nearly all wild populations of this species contains animals which are heterozygous for a lethal or a male-sterile *t*-allele; and many laboratory populations of apparently normal mice also harbor such lethals. Altogether we have studied 26 *t*-lethal alleles found in 22 different wild populations and four different laboratory stocks, and four viable male-sterile *t*-alleles each derived from a different wild population. Other *t*-alleles have been found in wild populations in Japan and Australia. In only one of the North American wild populations which was adequately sampled did we fail to find a *t*-allele. Moreover, heterozygotes carrying a *t*-allele, usually a lethal one, have been found in high frequencies—around 50 percent—in the wild populations. This means that a specific lethal gene may attain a gene frequency of 25 percent. Thus most wild populations are polymorphic for *t*-alleles and incidentally also for variant genes with visible effects on coat colors and patterns.

This is not astonishing since natural populations of several *Drosophila* species and of some other animals and plants have been shown to consist of a variety of gene and chromosome arrangements. In some "normal" populations of *Drosophila* a third or more of the chromosomes contain a lethal gene. We have come to look upon such variety as the normal thing, getting some of our impressions from looking at our own species. It is now pretty well established that each human being is likely to carry at least one lethal gene, probably more.

Why this should be so has been one of the most discussed questions of population genetics and indeed of evolutionary biology. No single explanation can be given but a prevalent view has been that this kind of population structure is due to natural selection, heterozygotes being at a selective advantage. This view seemed to its proponents to be dramatically strengthened by the evidence that under certain conditions persons heterozygous for an allele for sickle-cell anemia or for Mediterranean anemia which has a lethal or sub-lethal effect were more fit in the reproductive sense of leaving more progeny than their normal siblings, mainly owing to better resistance to falciparum malaria. On the other hand many of the carriers of lethal genes in *Drosophila* are less fit; and a hematologist would probably insist that the reduced efficiency of oxygen transport in sickle-cell trait or thalassemia minor would impose some selective disadvantage on such heterozygotes at times of crisis or stress.

At any rate, ideas about the persistence of disadvantageous genes in populations were strongly colored by selectionist ways of thinking and rightly so. But when the prevalence of lethal genes in wild-mouse populations was discovered an explanation of quite a different kind presented itself almost automatically. This made itself evident when it was found that males heterozygous for any *t*-allele from a wild population always transmitted it to over 90 percent of their offspring instead of the 50 percent characteristic of female heterozygotes and of heterozygotes for most other genes. This excess transmission of lethal alleles through sperm had the same effect as a mutation rate of 40 percent, and was a much more

powerful way of increasing the population frequency of *t*-alleles than any heterozygote advantage that had previously been found. It could be computed that this form of gametic selection would more than compensate for the loss of genes through the death of homozygotes so that the frequency of such lethal alleles should increase in populations in which the usual conditions for Hardy-Weinberg equilibrium were approximated. Theoretical predictions however called for a higher frequency of such alleles in natural populations than had actually been found, so it could be inferred that other forces opposed the spread of the lethal alleles.

Thinking about any evolutionary problem generally involves the drawing up of a tentative kind of balance sheet of opposing forces, since the state of a population at any time represents an equilibrium, and evolutionary change is essentially a shift in the equilibrium.

In the case of the *t*-alleles the possibilities are shown in Table 1. Without describing the evidence in detail I shall merely indicate what seems to us the preponderance of probabilities for each item in this balance sheet.

The chief evolutionary forces in natural populations are clearly gametic selection (male transmission ratio advantage) tending to increase *t*; and the effect of both selection and drift against homozygotes (lethal or sterile) tending to decrease *t*. These can be to some extent played off against each other, that is to say, those *t*-alleles will increase in the populations which are favored by a sufficiently high transmission ratio. These are of course the alleles regularly found in both natural and laboratory populations. We know from observation of the origin of new alleles in the laboratory that most of them have no transmission ratio advantage and hence would disappear within a few generations. Only the high ratio alleles persist and spread in the species.

Other forces oppose the spread of such alleles. Selection against homozygotes (that is, no survival) is ineffective against very high transmission ratios; selection against heterozygous carriers has been looked for but not found. Although the experimental tests are difficult and not decisive, they favor selective advantage of heterozygotes.

The influences most effective in limiting the spread of *t*-alleles are probably loss by drift in small breeding groups and the curious fate to be suffered by those which produce male sterility. In small groups the only males may by chance be sterile homozygotes, a distribution which may extinguish not only the allele but the whole group (*6*).

Opposing forces such as these will always make the fate of *t*-alleles a precarious one, and persistence in the species must thus depend on infection of new populations by migrant heterozygotes. Males which transmit such alleles to nearly all progeny are of course very effective as spreaders of such genes. The role of mutation in originating new alleles is unknown. We have never detected such an occurrence in some 25 years of breeding observations. Change of one allelic form to another by recombination as described here probably occurs in natural populations but does not of course increase the total gene frequency. Curiously enough the origin of a viable from a lethal allele by this method is unlikely to extend the life of the polymorphism in the population since the male sterility of viable alleles makes their existence more precarious than that of the lethal alleles. In agreement with this view is our experience that most of the alleles found in both wild and laboratory populations have been lethals.

It is interesting that the deleterious effects on early development of genetic changes in this region are more than compensated by another effect which has been called an "abnormal" transmission ratio, although this is obviously a misnomer for a condition which is a regular constituent of natural populations. The transmission ratio effect appears to be locked into the regional change by crossover suppression so the whole complex is inherited as a block. The loss of embryos which ocurs when two heterozygotes mate (approaching 50 percent when male transmission ratio of *t* approaches 100 percent) may thus be a minor effect and might even have a useful function under certain conditions. The persistence of this block of genetic material, even in association with a lethal effect, suggests that it makes some positive contribution to the life of this ubiquitous species.

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References and Notes

1. D. Bennett, *Science*, this issue.
2. H. Gruneberg, *The Genetics of the Mouse* (Nijhoff, The Hague, ed. 2, 1952).
3. L. C. Dunn, D. Bennett, A. R. Beasley, *Genetics* **47**, 285 (1962).
4. A. W. H. Braden, *Nature* **181**, 786 (1958).
5. M. F. Lyon and R. J. S. Phillips, *Heredity* **13**, 23 (1959).
6. R. C. Lewontin, *Am. Naturalist* **96**, 65 (1962).

7. Supported in part by contract with AEC.

II. Embryological Effects of Lethal Alleles in the t-Region

It is evident that there are two basic components in any teratological study: one requires a knowledge of the actual nature and biochemical action of the teratological agent, the other a knowledge of its effect on the developmental processes of the embryo.

The experimental teratologists—those who study the effects on developing embryos of drugs or other chemical agents —have often been fortunate in knowing a great deal about the pharmacological action or cellular metabolism of the agents studied. Perhaps for this very reason, however, they have tended to study only the effects of such agents as seen at the end of development, and to neglect the detailed study of the early pathways of embryonic differentiation and development which lead to the final abnormalities. This undoubtedly means that many useful insights into the metabolic control of embryonic differentiation have been passed over.

If the study of mammalian developmental genetics is considered as an exercise in teratology, it will be evident at once that one of the two essential components mentioned far outweighs the other. Developmental genetics has contributed heavily to our knowledge of the casual relationships in the epigenetics of mammals; in fact until relatively recently almost all the knowledge we had about the "experimental embryology" of mammals came from observations in which the mutant gene was used essentially as a tool to produce developmental abnormalities that could then be studied by the methods of classical embryology. But although the analysis of the primary or basic action of the genes whose mutants were used thus as tools was always one of the stated aims of such experiments, it must be admitted that small progress has been made in the analysis of gene action in mutants affecting morphological processes in mammals.

The *T*-locus in the mouse appears to provide uniquely favorable material for studying both of these major components of developmental genetics, namely, the gene and the embryological effect. As the preceding paper (*1*) has indicated, the locus appears to stretch over some length of chromosome, all of which length can therefore be presumed to participate in functions concerned with early organizational processes in the embryo.

At this locus there have already been

identified six different complementation groups whose alleles are lethal in the homozygous conditions. Each one seems to affect a very basic process of differentiation, most often taking part in the differentiation or maintenance of ectodermal or nervous structures. Each lethal allele is at least partially complementary with lethal alleles of all other complementation groups. Since these alleles are all members of the same chromosomal locus, it is probably not unreasonable to assume that they are all related to each other in genetic structure, and that consequently the processes which they control are also in some way related to one another. Therefore, studying the actions and interactions of this relatively large number of mutant factors may give us an advantage in interpreting developmental disturbances and in relating them back to the function of the chromosomal segment.

The genetics of the locus and methods of breeding analysis have been set forth (*1*). A summary of the identified complementation groups, and the heterozygous and homozygous effects of their alleles are shown in Table 1.

The first mutant found and studied at this locus (*2*), and the one which serves to detect all recessive alleles, is *T*. This mutant is the only one of the lethal series which behaves as an ordinary point mutation. Its embryological effects are directed quite clearly at the structure and function of the components of the primitive streak and notochord (*3*).

In embryos heterozygous for *T* (either *T*/+ or *T*/*t*) the tail develops normally, as far as can be seen externally, until about 11 days of gestation. Interestingly, it is just at this time that the tail of the normal embryo begins very rapid growth. However, at this time in the mutant an externally visible constriction develops around the tail. In *T*/+ embryos, the constriction is usually about halfway to the end of the tail; in *T*/*t* embryos it is at the base of the tail. Distal to the constriction, all the tissues, except sometimes skin, atrophy and are resorbed.

Histologically, abnormalities can be detected earlier. By the 8th or 9th day of gestation, cross sections of the embryo reveal abnormalities in lumbar and sacral as well as caudal regions. These abnormalities involve both notochord and neural tube. The notochord, particularly, is irregular; it is sometimes small and disorganized, sometimes larger than normal, often shows diverticula, and frequently has a lumen. The neural tube shows abnormalities of shape and size which appear to depend on abnormalities of the notochord in its vicinity, since neural abnormalities tend not to occur alone. Nevertheless, prior to the time when the tail constriction occurs, both the notochord and the neural tube are present throughout the length of the tail, so the atrophy of the distal portion cannot be ascribed to the initial absence of either one of these structures.

The homozygous effects of *T* are comparable to those seen in heterozygous condition but are much more severe. The final phenotype of *T*/*T* is an embryo lacking not just tail struc-

Table 1. Morphological effects of alleles at *T*-locus.

ALLELIC GROUP	HETEROZYGOTE		HOMOZYGOTE	
	PHENOTYPE	FIRST EFFECTS	FINAL PHENOTYPE	PROCESS AFFECTED
T	SHORT TAIL	ABNORMALITIES OF NOTOCHORD	POSTERIOR HALF ABSENT NO UMBILICAL SYSTEM LETHAL: 11 DAYS	GROWTH AND MAINTENANCE OF NOTOCHORD AND ALLANTOIS
t^{12}	$^{+}/t$: NORMAL TAIL T/t: TAILLESS (SOME SPINA BIFIDA)	ENHANCES T EFFECT ON NOTOCHORD	MORULA LETHAL: 4 DAYS	BLASTOCYST FORMATION, NUCLEOLAR DIFFERENTIATION
t^{0}	SAME	SAME	UNDIVIDED EGG CYLINDER LETHAL: 6-7 DAYS	DIVISION OF INNER CELL MASS INTO EMBRYONIC AND EXTRA-EMBRYONIC ECTODERM
t^{w5}	SAME	SAME	"EXTRA EMBRYONIC ORGANISM" LETHAL: 7-10 DAYS	GROWTH AND MAINTENANCE OF EMBRYONIC ECTODERM
t^{w18}	SAME	SAME	NEURAL TUBE DUPLICATIONS LETHAL: 8-10 DAYS	GROWTH OF PRIMITIVE STREAK (OVERGROWTH)
t^{w1}	SAME	SAME	MICROCEPHALIC, ABNORMAL NEURAL TUBE LETHAL: 9 DAYS - BIRTH	GROWTH AND MAINTENANCE OF NEURAL TUBE AND BRAIN
t^{h7}	$^{+}/t^{h7}$: NORMAL TAIL T/t^{h7}: NORMAL TAIL or SHORT TAIL	SUPPRESSES T EFFECT ON NOTOCHORD	SAME AS t^{0}	SAME AS t^{0}
t^{viable}	$^{+}/t^{v}$: NORMAL TAIL T/t^{v}: TAILLESS	ENHANCES T EFFECT ON NOTOCHORD	NORMAL (♂ MAY BE STERILE)	NONE

tures, but the whole posterior body behind the anterior limb buds.

Again, T/T embryos go through the early stages of embryonic development without showing any obvious abnormalities. The notochord is formed initially and is present throughout the length of the embryo. The first obvious sign of abnormality appears at about 8 days gestation, the stage when the first somites are forming. At this time large fluid-filled blebs of unknown origin or meaning appear under the skin of the dorsal surface of the embryo. At about the same time (in embryos studied histologically) the notochord can be seen to be grossly abnormal; it is uneven in size, tends to become incorporated in the neighboring structures of gut and neural tube, and often can be detected only as clumps of notochord-like cells. By 9 or 10 days of gestation, T/T embryos are markedly abnormal. Their body ends just posterior to the anterior limb buds, the somites are degenerating, and the neural tube is severely kinked and folded. Histologically, no trace of notochordal cells can be identified. These embryos possess no, or only scanty, allantoic connections to the placenta; it is presumably the failure of this system which leads to their death at about 10½ days of gestation, the time when the umbilical circulation begins to function in normal embryos.

In summary then, all genotypes containing T produce some degree of incorporation of the notochord into both gut and neural tube. This seems to implicate the notochord (or even the primitive streak from which it is derived) as being primarily affected by T; the primary effects seem to be on the cellular construction of the notochord and on its surface properties (for example, "stickiness"). It is perhaps as a result of these effects that the capacity of the notochord for continued longitudinal growth and extension is reduced. It is most likely that this failure of notochordal extension leads to the kinking of the neural tube and to the failure of the posterior body that is seen in T/T embryos.

The study of the development of abnormalities in embryos carrying T thus indicates almost unequivocally that T affects the chordamesoderm, the primary inductive system of the embryo.

Additional evidence that the mutation T has its primary effect on the mesodermal system of the embryo comes from experiments in tissue culture (*4*). It had previously been elegantly demonstrated by Grobstein and Holtzer (*5*) that somites from 12 to 24 somite mouse embryos would form cartilage in tissue culture only under the influence of an inducer, the most effective one being ventral spinal cord from embryos of the same stage.

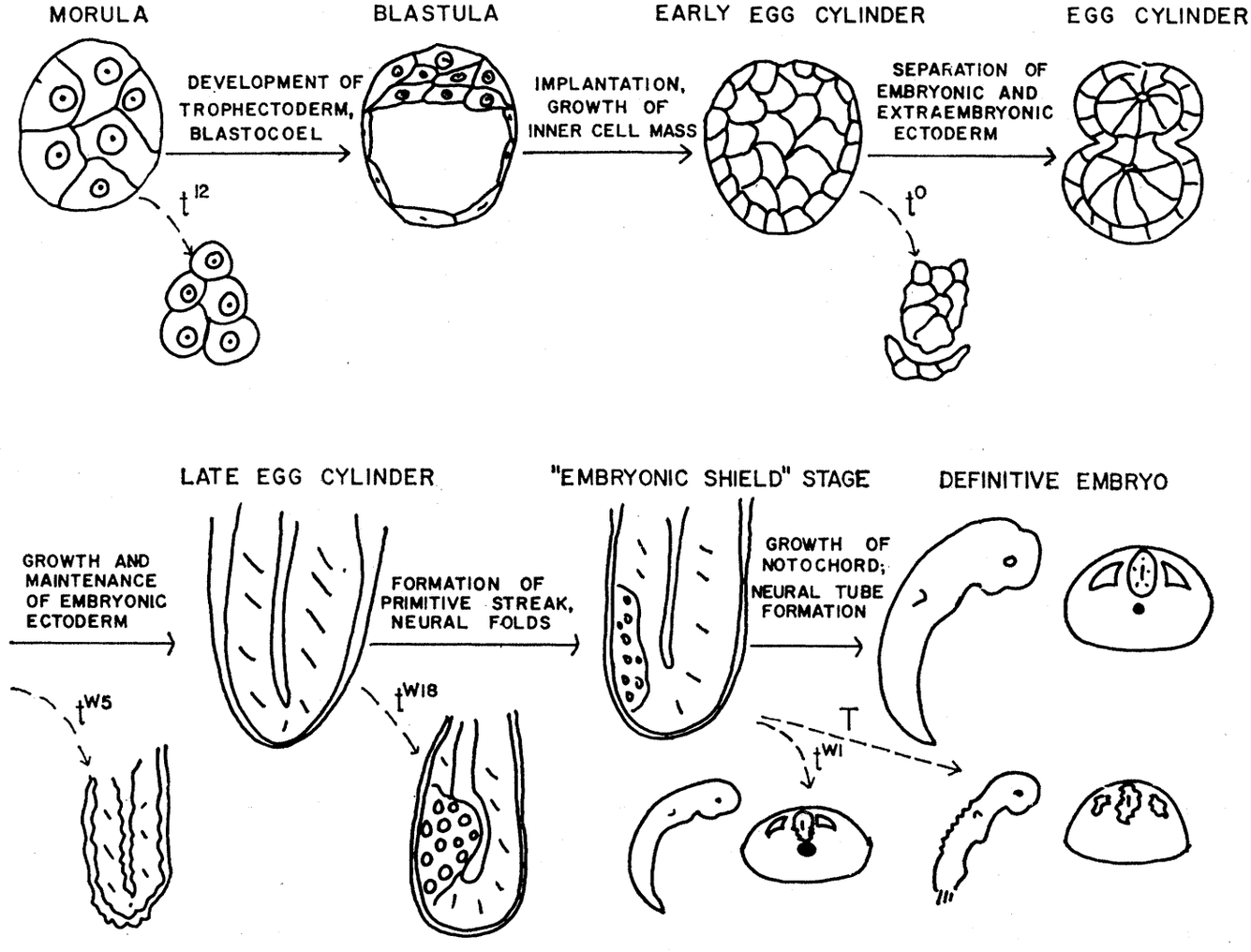

Fig. 1. Differentiation of ectodermal and nervous structures of mouse embyro showing the points at which the recessive *t*-alleles interfere with normal development.

The use of tissues from slightly younger T/T embryos in similar tissue-culture experiments indicated that T/T somites were totally unable to respond with the production of cartilage to the presence of *normal* ventral spinal cord used as inducer. Ventral spinal cord from T/T embryos, however, was quite capable of inducing cartilage formation in somites from normal mouse embryos. Such evidence strengthens the supposition that T has its effects primarily on the notochord–mesoderm system.

Lethal Effects of Recessive t-Alleles

The recessive alleles at this locus seem, with one exception, however, to have their effects primarily in tissues of ectodermal derivation. If one examines the development of a mouse egg into a fully formed embryo which is beginning to undergo organogenesis, the course of this early development can be seen to be marked by certain transitional points, periods at which major and rapid changes take place. In the early embryo, as will be seen from the schematic representation in Fig. 1, many of these changes are concerned with the development or differentiation of ectodermal and nervous structures. Also represented in Fig. 1 are the points at which the different recessive t-alleles interfere with normal development. It can be seen that t-alleles have been recognized which seem to interfere with almost every one of these transitional events.

The abnormalities produced by the various t-alleles will be discussed separately below in more detail, in chronological order of their developmental effects.

The allele with the earliest effects is in fact the earliest acting lethal allele known in mammals (*6*). Embryos homozygous for t^{12} develop apparently normally through the morula stage, but degenerate at that stage without undergoing any transition into blastocysts. Since blastocoel formation involves the differentiation of the trophectoderm, that function might be ascribed to t^{12}. Several concomitant abnormalities have been described for t^{12}/t^{12} embryos. Normal embryos, in undergoing the transition from morula to blastocyst, show pronounced changes in nucleolar shape; the nucleoli in morulae are generally rather round and smooth; in blastocysts they are much more elongate and rough. The nucleoli of t^{12} morulae, however, never become elongate; instead, as the embryos approach death they seem to become even more uniform and smooth. Together with the nucleolar change, normal embryos show a sharp increase in the cytoplasmic concentration of RNA as they pass from morula to blastocyst stages. The t^{12}/t^{12} morulae do not show any such increase. Unfortunately, however, both of these factors come into evidence only as the normal embryos are also changing morphologically into blastocysts; therefore they cannot be used to differentiate t^{12}/t^{12} embryos from normal when all are in the morula stage. The abnormalities in nucleolar shape and RNA concentration can be interpreted as meaning that t^{12} interferes with RNA synthesis. It is not at all certain, however, whether defective RNA synthesis is the cause of defective differentiation in t^{12}/t^{12} embryos, or whether it is merely a symptom of approaching death, and thus a secondary effect of the t^{12} allele.

The next lethal allele to consider is t^0. This was the first t-allele to be detected (*7*) and described (*8*). Embryos homozygous for t^0 become implanted in the uterus and appear to grow normally until the stage when in normal embryos the extraembryonic ectoderm and the embryonic ectoderm become differentiated from the inner cell mass of the former blastocyst. In t^0/t^0 embryos the inner-cell mass grows little or not at all, becomes pycnotic, and the differentiation of the two types of ectoderm does not take place. Concomitantly, abnormalities of the endoderm appear; the cells become vacuolated and the endoderm layer in many cases lifts off and forms a loose cap over the undifferentiated ectoderm. The abnormal embryos become progressively more pycnotic, deteriorate, and are resorbed within a day or two after the first abnormalities appear. Thus the primary effect of t^0 appears to be on the initial differentiation process leading to the formation of the definitive ectoderm.

Another allele which appears to interfere more or less directly with ectodermal differentiation and maintenance is t^{w5} (*9*). The t^{w5}/t^{w5} homozygotes pass successfully and normally through the stage of separation of embryonic and extraembryonic ectoderm, and through the beginning of fusion and growth of these components to form the elongate egg cylinder. Once this has been achieved, however, the embryonic portion of the egg cylinder ectoderm becomes pycnotic and appears to be incapable of maintaining itself or undergoing further growth. This seems to be a very specific effect, with the extraembryonic portion frequently being quite unaffected. The embryonic ectoderm becomes progressively more pycnotic and disappears by a process of resorption. The eventual result in such cases is the formation of what we have called an "extraembryonic organism," a remnant of embryo which survives for 2 or 3 days without any actual embryonic structures at all, but with all extraembryonic membranes—amnion, chorion, and allantois—essentially normal and intact. In some rare cases, a small remnant of embryonic ectoderm apparently persists, and we find a small, often spherical, "micro-embryo," which nevertheless contains neural tissue, a notochord-like structure, and mesoderm, perched on top of the normal-sized embryonic membranes.

The next t-allele to be considered is one which seems to show more similarity to T than to the other recessive lethals. t^{w18} is the only lethal t-allele which does not suppress recombination, which has a low or normal transmission ratio, and which does not clearly affect primarily ectodermal structures. This lethal seems to affect the growth of the primitive streak, leading to the apparent over-production of primitive streak cells (*10*). The first effects are obvious at the late egg cylinder stage. At the time when the primitive streak just begins to form it is more massive than normal and bulges into the ectoderm which overlies it and into the proamniotic cavity. The bulging primitive streak does not seem to have any effect on the overlying ectoderm other than a mechanical one, but the bulge frequently forces the neural folds into a W-shaped structure, which often becomes an essentially double neural tube, especially in the posterior. Thus t^{w18}, by its production of primitive streak overgrowth, leads to an appearance of partial twinning, without, however, any process leading to a basic duplication of either the organizer or the responding ectoderm.

The latest acting and in some ways the most interesting t-allele is t^{w1} (*11*). The t^{w1} alleles appear to have an effect which is directed specifically at the nervous system, and in fact specifically at the ventral portion of the neural tube and brain. Homozygotes for this allele have a very extended lethal period, some beginning to die as early as the 9th day of gestation, and others

surviving until birth. Those which survive so long have abnormally small heads, hydrocephalus, structurally abnormal brains and spinal cord, extreme edema, but normal tails!

The first signs of abnormality which can be recognized in young t^{w1}/t^{w1} embryos appear at about 8 or 9 days of gestation. At this time pycnosis begins to appear in the *ventral* neural tube; the process of pycnosis begins most often in the cervical region and spreads both anteriorly and posteriorly. The distribution of cell death remains, however, strictly confined to the ventral regions of the neural tube and leads in many cases to the virtual death of the whole ventral portion. In some embryos, however, viable cells apparently remain, and in embryos which survive, a process of repair ensues which restores the integrity of the neural tube, although its architecture is not normal. Thus any one region in such embryos undergoes successively pycnosis, extreme degeneration, and repair. The brain in such embryos also undergoes degeneration of the ventral portion, and consequently is smaller and less well differentiated than normal. The skull is also retarded in differentiation, and in fact is smaller, in relative proportion, than the brain; thus these embryos are at the same time microcephalic and hydrocephalic. The relative disproportion of the skull might be taken as evidence for some inductive action of the ventral portions of the brain on the formation of skull cartilage.

In recapitulation, it can be said that the recessive alleles at the *T*-locus all appear to have effects on early and basic processes of axial organization, especially those which are concerned with increasingly complex differentiation of the ectoderm. As yet, no clear evidence is available on the matter of how the effects of alleles are related to one another, and whether they represent qualitative or quantitative differences. The fact that many of them, at least, must have some qualities in common is indicated by the abnormalities frequently found in animals of compound (t^x/t^n) genotype (*12*). As was shown in the preceding paper, complementation (meaning the production of at least some viable normal-tailed compounds of genotype t^x/t^n) exists between members of all these different groups. However, such complementation is not always complete or perfect, and animals of various compound genotypes are often poorly viable and morphologically abnormal. The interesting thing is that all such combinations of *t*-alleles seem to produce essentially the same types of abnormality. These are almost always abnormalities showing varying degrees of otocephalic characteristics, such as microcephaly, micrognathia, or microphthalmia.

The existence of such abnormal compounds gives additional evidence for the idea that the *t*-alleles control some common process which is involved with the differentiation of ectodermal and neural structures.

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References and Notes

1. L. C. Dunn, *Science*, this issue.
2. N. Dobrovolskaia-Zavadaskaia, *Compt. Rend. Soc. Biol.* **97**, 114 (1927).
3. P. Chesley, *J. Exptl. Zool.* **70**, 429 (1935); H. Grüneberg, *J. Embryol. Exptl. Morphol.* **6**, 424 (1958).
4. D. Bennett, *Nature* **181**, 1286 (1958).
5. C. Grobstein and H. Holtzer, *J. Exptl. Zool.* **128**, 333 (1955).
6. L. J. Smith, *ibid.* **132**, 51 (1956).
7. N. Dobrovolskaia-Zavadskaia and N. Kobozieff, *Compt. Rend. Soc. Biol.* **110**, 782 (1932).
8. S. Glueksohn-Shoenheimer, *Genetics* **25**, 391 (1940).
9. D. Bennett and L. C. Dunn, *J. Morphol.* **103**, 135 (1958); L. C. Dunn and D. Bennett, *Genetics* **45**, 1531 (1960).
10. D. Bennett and L. C. Dunn, *J. Exptl. Zool.* **143**, 219 (1960).
11. D. Bennett, L. C. Dunn, S. Badenhausen, *Genetics* **44**, 795 (1959); D. Bennett, S. Badenhausen, L. C. Dunn, *J. Morphol.* **105**, 105 (1959).
12. S. Silagi, *Develop. Biol.* **5**, 35 (1962).

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SCIENCE AND PUBLIC AFFAIRS

The Political Good Fortune of Medical Research

Two strategically placed legislators regularly assure congressional generosity for the NIH budget.

Milton Viorst

Congress' generosity in the field of medical research stands in sharp contrast to its response to other domestic welfare needs. Every year, for example, little is done to meet the problems of unemployment, air pollution, urban congestion, and education. Though the existence of these problems is generally acknowledged, Congress continues to argue about how to resolve them but reaches no consensus. Since the war, however, medical research has been a congressional favorite, almost as sacrosanct as national defense. While the proponents of other good causes plead vainly for dollars, medical researchers have had no such problem.

The figures record the story:

In 1940 Congress voted $3 million for health-related research. By 1957 the figure had reached $186 million. Last year, it exceeded $916 million and will, in this year's budget, come close to $1 billion.

In 1957 private sources provided more funds than government for medical research. Last year, though private contributions had almost tripled, the government provided nearly twice as much money as private sources.

In other terms, $1 out of every $4000 of federal expenditures went to medical research in 1940; last year the proportion was almost $1 out of every $100.

The explanation for this phenomenon lies, in large measure, in the universality of disease and the remarkable advances made in medicine since the war.

Congressmen and senators who live in spacious suburbs and send their children to excellent schools may be badly equipped to recognize the wel-

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