# Malignant Neoplasms of Genetic Origin in *Drosophila melanogaster*

Some developmental genes that control differentiation can also cause malignant neoplasms when mutated.

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*Summary.* Malignant neoplasms that develop in 12 recessive-lethal, larval mutants of *Drosophila melanogaster* are discussed. These mutations affect the adult optic neuroblasts and ganglion-mother cells in the larval brain, the imaginal discs, and the hematopoietic organs. The malignant neoplasms exhibit fast, autonomous growth, loss of the capacity for differentiation, increased mobility and invasiveness, lethality in situ and after transplantation, and histological, fine structural, and karyotypic abnormalities. Intermediate neoplasms are also found. These combine both benign and malignant qualities. They grow in a noninvasive, compact fashion, typical of benign tumors, yet they also exhibit malignant qualities such as fast, autonomous, and lethal growth, loss of differentiation capacity, changes in cellular morphology, and lethal growth after transplantation into wild-type hosts. Thus *Drosophila* and vertebrate neoplasms show striking similarities.

Cancer is defined as a malignant neoplasm, which implies a new, lethal pattern of growth (*1*). Malignant neoplastic development closely relates to fundamental biological questions, such as determination, differentiation (*2, 3*), and the maintenance of the differentiated state (*3, 4*). It is generally accepted that these processes are under the dual control of genetic and epigenetic factors (*5*). The relative roles of genetic as opposed to epigenetic factors in the etiology of vertebrate cancers are unresolved (*6*). In most vertebrate tumor models, more than one mutant gene seems to be involved, which renders it difficult to prove which particular gene in a gene complex is primarily responsible for malignant neoplastic transformation (*7*). Recent genetic and developmental studies with the fruit fly *Drosophila melanogaster* have demonstrated that mutant genes, causing malignant neoplastic transformation of specific tissues and cells, are distributed over the entire genome. Moreover, the mutations involve developmental genes which control crucial events during determination and differentiation of the tissue or cell type.

My attention was first drawn to the existence of such genetic aberrations in *Drosophila* by the occurrence of a spontaneous, recessive-lethal mutation in one of our wild-type stocks. The new mutation was the fourth allele at the locus of the already well-known mutant lethal (2) giant larvae [*l(2)gl*], discovered in 1930 by Bridges and studied by Hadorn (*8*). Developmental studies of the larval neoplastic tissues showed that the primordia for the adult integument, the imaginal discs, represented lethal, transplantable, and noninvasive neoplasms, and that the presumptive adult optic centers of the larval brain developed into a malignant neuroblastoma (*9–12*).

After these initial studies, it was important to decide whether the *l(2)gl* locus represented a unique case of a mutated gene causing malignancy, or whether the *Drosophila* genome contained other similar loci (*13*). Therefore, I began to isolate tumor mutants and to characterize their lethal neoplasms. Before discussing the abnormal development caused by the malignant neoplastic growth in the different mutants, I shall first consider the normal development of *D. melanogaster* and the criteria used in characterizing *Drosophila* neoplasms.

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## Normal Development of *Drosophila*

The fruit fly is a holometabolous insect, developing through an embryonic stage, three larval stages, a pupal stage, and an adult stage. The generation time is 11 to 12 days at 25°C.

In the embryo two types of anlagen (precursors for specific organs) are determined, the larval and the adult. The larval anlagen differentiate during the course of embryonic development into the respective larval organs, while the precursors for adult organs, such as the integument, the adult optic centers, the gonads and others, consisting of 10 to 40 cells, remain in an undifferentiated state (*14*). After the hatching of the larva from the egg, the larval tissues grow by cell enlargement (without cell division), becoming progressively polyploid or polytene. The undifferentiated adult precursors, on the other hand, divide rapidly and become, for instance, the imaginal discs which during metamorphosis and adult development give rise to the adult integument. Similarly, nests of optic nerve precursor cells (optic neuroblasts) in the larval brain give rise to the adult optic centers. In the gonads, precursor germ cells divide and differentiate during adult development into eggs and sperms. Furthermore, distinct cell nests within larval organs form the respective adult organs, such as the salivary glands or the gut.

The capacity for neoplastic transformation depends primarily on the ability of the cells to divide. Postmitotic cells, which are found in many organs of both the larva and adult, are presumably not capable of malignant transformation. Most larval and adult cells grow by endomitosis and cell enlargement and, thus, are considered incapable of neoplastic transformation. The only cells in the larva capable of malignant neoplastic growth are the imaginal disc cells; the adult optic neuroblasts and ganglion-mother cells in the larval brain; the blood cells; the cells in the gonads; and other smaller cell nests within different organs. Finally, in the adult, only the blood cells and the gonads can be expected to become neoplastic.

## Criteria for Malignant and Benign Neoplasms

Vertebrate neoplasms are classified into two main groups: malignant and benign (*1*). Malignant neoplasms are characterized by rapid autonomous growth; invasiveness into adjacent healthy tissue; metastasis to distant sites of the body; lethality to the host; loss of struc-


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ture and function; lethal autonomous growth after transplantation; and lack of contact inhibition in culture in vitro. Benign neoplasms, in contrast, show slow autonomous nonlethal growth; are noninvasive and nonmetastasizing; do not grow after transplantation into a new wild-type host; and show in their structure and function high similarities with the tissue of their origin. In addition there are numerous intermediate neoplasms which exhibit characteristics of both classes (*1*).

The neoplasms of *Drosophila* can also be classified as malignant and benign (*13*). Furthermore, *Drosophila* exhibits neoplasms with intermediate characteristics. Those neoplasms which grow in a compact, noninvasive manner often possess qualities of malignant neoplasms, such as fast autonomous growth, loss of the capacity for differentiation, and lethality in situ and after transplantation. Truly benign neoplasms in *Drosophila* are the ovarian tumors developing in some female sterile mutants (*13, 15*), but these will not be discussed herein.

Vertebrate tumor classification divides malignant neoplasms into sarcomas and carcinomas. This classification is intentionally avoided in *Drosophila*, since homologies between insect and vertebrate organs and tissues are by no means established. Instead, the different *Drosophila* neoplasms are simply designated by the name of the affected organ, tissue, or cell type, for example, lethal, benign, and transplantable imaginal disc neoplasm or malignant blood cell neoplasm.

## Isolation and Characterization of Tumor Mutants

I mentioned earlier the tissues and cells of the larva which grow by cell division and, thus, should be capable of malignant neoplastic transformation, for example, the imaginal discs, the adult optic neuroblasts and ganglion-mother cells in the larval brain, the blood cells, and the gonads. Mutations which promote excessive and uncontrolled growth in the above tissues and cells and, thus, destroy the larva, are candidates for tumor mutants. Since all cells of the body carry the mutant gene, but it is expressed only in some cells, we can assume that the mutant gene has tissue-specific activity during development and in some way controls the proliferation rate and differentiation of a particular cell-type. Thus, a tumor mutant in *Drosophila* is simply a special type of developmental mutant.

In searching for recessive-lethal tumor mutations affecting the larva, we used standard mutagenesis and genetic isolation procedures (*13*). Among 2500 tested chromosomes, each of which was derived from a single male gamete that was exposed to a mutagen, approximately 30 percent recessive-lethal mutations were found.

Individual mutant lines were checked for the presence of malignant neoplasms in the larvae by means of a procedure involving four steps: (i) examination of the external anatomy and appearance of the larvae; (ii) dissection of the larvae and search for enlarged organs; (iii) application of a series of transplantation tests to determine the degree of the neoplastic transformation of the tissues in question; and (iv) histological and fine-structure investigations of the tissues in situ and after transplantation into wild-type hosts. Of the investigated recessive-lethal mutants 0.3 percent showed malignant or intermediate neoplastic growth (*13*). All mutations described below are lethal at the end of larval life, and the mutants are maintained in stocks in which the mutated chromosomes are complexed with so-called "balancer" chromosomes (*16*). These chromosomes prevent crossing-over between the homologous chromosomes and thus preserve the original genetic content of the mutant chromosomes. In these stocks the mutant genes are propagated by the heterozygous animals, while the homozygous individuals develop the neoplastic growth. Table 1 shows the known tumor mutants located on three of the four *Drosophila* chromosomes. The table also shows the various larval tissues in which tumorous growth has been found. Mutants with neoplastic larval gonads have not yet been detected.

## Malignant Neuroblastoma

A short description of the wild-type central nervous system in the embryo and larva may facilitate an understanding of the aberrant development of the mutant brain (*17, 18*). The *Drosophila* nervous system differs from the typical insect nervous system in that the single ganglia are not segmentally arranged but form a fused central nervous system, consisting of two brain hemispheres and a compound ganglion (Fig. 1a, *A*). The larval nervous system differentiates in the embryo and consists of a cellular cortex surrounding the centrally located neuropile. In the lateroposterior region of each brain hemisphere one finds 13 to 20 large neuroblasts which represent the primordia for the adult optic centers (Fig. 1c). These neuroblasts begin to divide after the larva hatches from the egg and continue to do so until the end of larval life. During the first portion of larval life the large optic neuroblasts divide equally forming additional neuroblasts (*19*). At the end of the second larval instar the neuroblasts separate into an inner and an outer formation center (*18*). Throughout the third instar the optic neuroblasts divide unequally giving rise to large neuroblasts and smaller ganglion-mother cells. The large neuroblasts remain in the formation centers while the smaller ganglion-mother cells enter the space between them forming the optic glomeruli (Fig. 1b). Here the ganglion-mother cells divide an unknown number of times before differentiating into preganglion cells and finally into optic neurons (*19*).

There are six nonallelic mutants that develop malignant neuroblastomas in the adult optic centers (Table 1). Since all of these mutants show similar neuroblastomas, I will describe the tumor in the mutant lethal (2) giant larvae[4] [*l(2)gl*$^4$] in which the first neuroblastoma was discovered (*9–12*) [the superscript 4 designates the fourth allele at the *l(2)gl* locus].

The *l(2)gl*$^4$ brain exhibits no anatomical or histological aberrations in the embryo or during the first two larval instars. However, during the third larval instar the size, shape, and histology of the brain becomes more and more distorted. Histological and fine-structure studies showed that the optic neuroblasts of the inner and outer formation centers and the ganglion-mother cells fail to differentiate into adult optic neurons, as witnessed by the absence of the three optic glomeruli. Instead, the optic neuroblasts and ganglion-mother cells proliferate extensively causing an up to twofold enlargement of the brain hemispheres. Moreover, histological preparations show that optic neuroblasts and ganglion-mother cells invade the region of the larval neurons and the neuropile and destroy the original topography of the brain (Fig. 1d).

Thus, the mutant adult optic neuroblasts and ganglion-mother cells in situ undergo a novel type of growth which exhibits some of the typical features of malignant neoplasms, such as loss of differentiative capacity, an excessive proliferation, and invasiveness. The death of the mutant animals results most probably from the neuroblastoma and the tumorous imaginal discs.

A crucial test of malignancy in vertebrates is the unlimited malignant growth after transplantation into a new wild-type host. The absence of transplantation immunity in *Drosophila* makes this test relatively easy. Pieces of mature

Table 1. Recessive-lethal mutations causing malignant and intermediate neoplasms in the larva of *Drosophila*. In accordance with conventional *Drosophila* nomenclature lethal mutations are shown with the number of the affected chromosome in parentheses followed by a brief name or number. The number behind the designation of the mutant indicates the location of the gene on the chromosome and the last number in parentheses is the reference; −, gene not located.

| Designation of neoplasm | Chromosome 1 | Chromosome 2 | Chromosome 3 |
|---|---|---|---|
| Malignant neuroblastoma of the adult optic neuroblasts and ganglion-mother cells | Lethal (1)2, −; (*52*); lethal (1)2269, −; (*52*) | Sixteen alleles of lethal (2) giant larvae, deficiency (2) net lgl, 2–00; (*13*); lethal (2) giant disc, −; (*54*); lethal (2)1542, −; (*52*) | Lethal (3) giant larvae, −; (*29*) |
| Intermediate imaginal disc neoplasm with compact mode of growth | Lethal (1)2, −; (*52*); lethal (1) 2269, −; (*52*); lethal (1) disc large-1, 1–36; (*53*); lethal (1) benign wing imaginal disc neoplasm, 1–34; (*29*) | Thirteen alleles of lethal (2) giant larvae, deficiency (2) net lgl, 2–00; (*13*); lethal (2) 1542, −; (*52*) | Lethal (3) giant larvae, −; (*29*) |
| Intermediate imaginal disc neoplasm with invasive mode of growth | Lethal (1) disc large-1, 1–36; (*53*); lethal (1) benign wing imaginal disc neoplasm, 1–34; (*29*); lethal (1) disc large-2, 1–24.9; (*52*) | | |
| Malignant blood cell neoplasm | Lethal (1) malignant blood neoplasm, 1–39; (*28*) | Lethal (2) malignant blood neoplasm, −; (*29*) | Lethal (3) malignant blood neoplasm, −; (*30*) |

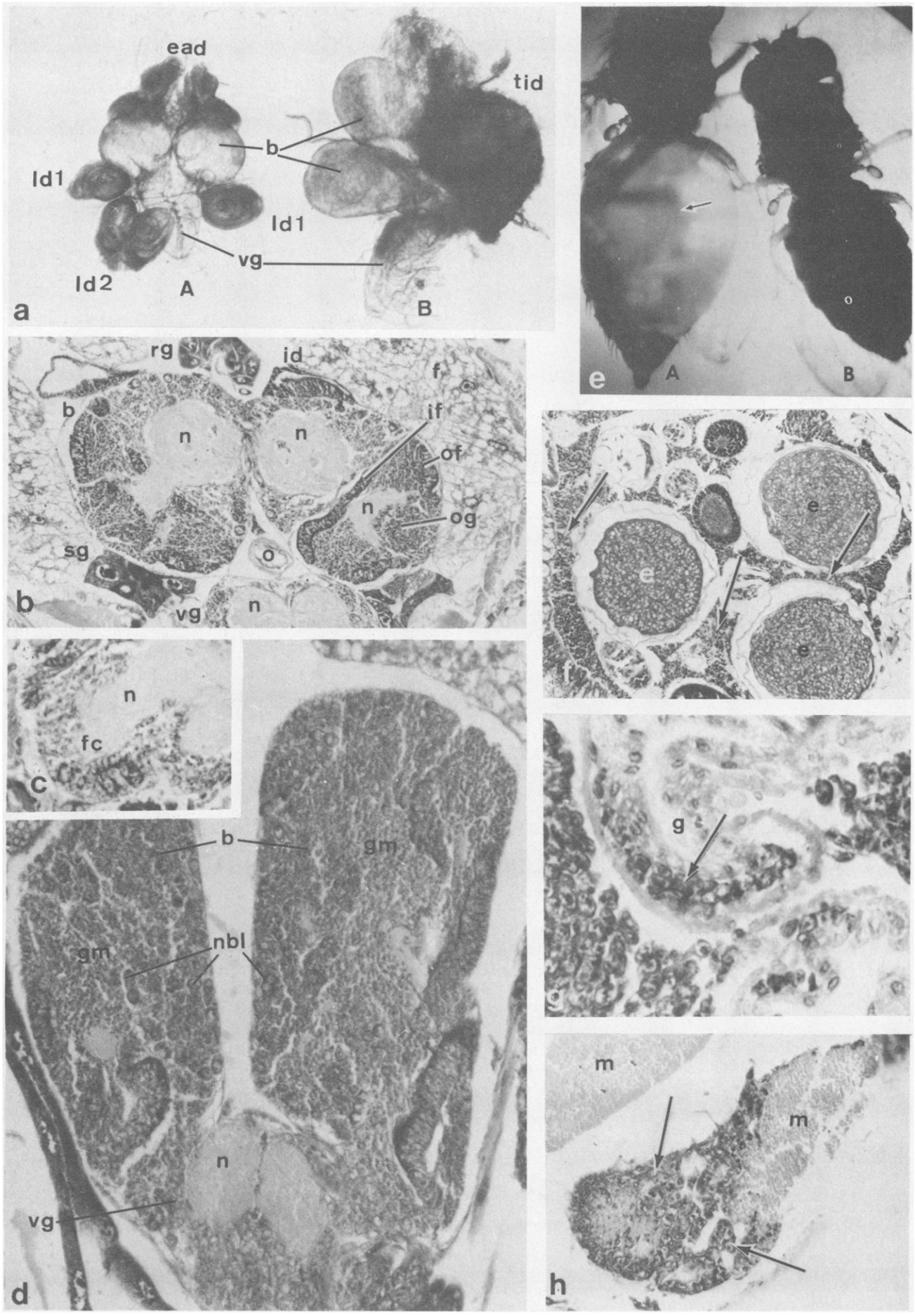
wild-type larval brain implanted into the abdominal cavity of female adult flies neither grow nor kill their hosts and cannot be detected in the adult host upon dissection. In contrast, small pieces of mutant brain implanted in the same way grow rapidly and reach a size up to 300 times the size of the original implant. Tumor-bearing hosts become bloated (bloating syndrome; Fig. 1e) and die in 7 to 14 days. Histological preparations of such hosts show the malignant cells throughout the body cavity, in the abdomen, the thorax, and the head capsule, and invading the ovaries, the gut, and the thoracic muscles (Fig. 1, f, g, h). Except for sporadic attachment points, most cells do not form contacts with their neighbors (Fig. 2a). In the wild-type and *l(2)gl*$^4$ brains the neuroblasts and ganglion-mother cells are not in direct contact with each other but are enveloped by thin glial cell processes (Fig. 2b). Comparative histological and fine structure observations in situ as well as after transplantation reveal three cell types in the malignant growth: large neuroblasts, small ganglion-mother cells, and preganglion cells (Fig. 2a). Glial cells and other cell types found in the brain in situ could not be detected in the histological preparation of the wild-type hosts.

The neoplastic development of the neuroblasts in the mutant presumptive adult optic centers is programmed already in the embryo. The *l(2)gl*$^4$ embryonic brains implanted into adult flies give rise to a malignant growth similar to the one derived from a small piece of a mature mutant brain (see above). In contrast, when wild-type embryonic brains are implanted into the abdomens of adult flies, they grow only to a limited extent, reach the size of a second-instar larval brain, and never kill the host.

While they are in situ, no karyotypic aberrations occur in the neuroblastomas; however, karyotypic abnormalities increase with the period of culture within a host after transplantation. Approximately 30 percent of the cells from the seventh transfer generation (3 months in culture in vivo) were aneuploid. In electron micrographs large numbers of viruslike particles can be observed primarily within the nuclei of the malignant cells (*20*). Figure 3d (inset) shows viruslike particles within the nucleus of an *l(2)gl*$^4$ imaginal disc cell which are identical with the ones found in the *l(2)gl*$^4$ neuroblasts. Nothing is known concerning their role in mutation, neoplastic transformation or other peculiar non-Mendelian genetic phenomena (*21*).

Similar malignant neuroblastomas develop in 15 additional *l(2)gl* alleles, in a *net l(2)gl* deficiency (*deficiency (2) net lgl*) and five further nonallelic mutants (Table 1). The mutants are located on chromosomes 1, 2, and 3. The nature of the brain tumors in the different mutants was established by using the same histological and developmental methods as applied in the initial studies with *l(2)gl*$^4$ (*9–12*). The studies on the 15 *l(2)gl* alleles, the *net l(2)gl* deficiency (*22*), and on each individual nonallelic mutant (*13*) revealed that the optic neuroblasts and ganglion-mother cells behave, in situ as well as in the transplantation tests, in a manner similar to the *l(2)gl*$^4$ neuroblasts and ganglion-mother cells. Thus, a considerable number of genes seem to control the differentiation of the adult optic neuroblasts into adult optic neurons. The six nonallelic mutations represent such developmental genes which prevent dif-

Fig. 1. Wild-type and *l(2)gl*$^4$ brain-ventral-ganglion complexes in situ and after transplantation into female flies. (a) Whole mount of the brain-ventral-ganglion complex of a mature wild-type (*A*) and *l(2)gl*$^4$ (*B*) larva. Note the enlarged mutant brain hemispheres (*b*) and the clumped imaginal discs (*tid*); *ead*, eye-antennal imaginal disc; *Id1*, first leg imaginal disc; *Id2*, second leg imaginal disc; *tid*, tumorous imaginal discs; *vg*, ventral ganglion (×80). (b) Median section through the brain-ventral-ganglion complex of a mature wild-type larva. Note the inner and outer formation centers (*if, of*) and the differentiating outer optic glomerulus (*og*). The remaining structure represents the larval portion of the brain; *b*, brain; *f*, fat body; *id*, imaginal disc; *if*, inner formation center; *n*, neuropile; *o*, oesophagus; *of*, outer formation center; *og*, optic glomerulus; *rg*, ring gland; *sg*, salivary gland; *vg*, ventral ganglion (×200). (c) Median section through the left brain hemisphere (the right brain hemisphere is partially deleted) of a 0- to 5-hour-old wild-type larva showing the young optic formation center (*fc*) which consists of 15 to 20 large optic neuroblasts. The neuropile (*n*) is surrounded by larval neurons; *b*, brain; *fc*, formation center; *n*, neuropile (×350). (d) Median section through the brain-ventral-ganglion complex of a mature *l(2)gl*$^4$ larva. The brain hemispheres (*b*) are much enlarged when compared to the wild-type brain hemispheres shown in (b). Large optic neuroblasts (*nbl*) and small ganglion-mother cells (*gm*) have proliferated excessively, have failed to differentiate the presumptive adult optic centers, and have destroyed by invasion the larval brain structures; *b*, brain; *gm*, ganglion-mother cell; *n*, neuropile; *nbl*, neuroblast; *vg*, ventral ganglion (×235). (e) Female fly showing the "bloating syndrome" caused by the transplantable malignant neuroblastoma (*A*) compared with an uninjected fly (*B*) [from Gateff and Schneiderman (*10*)] (×20). (f) Invasion of the transplanted mutant neuroblasts (arrow) into the ovary of a wild-type female fly; *e*, egg chamber (×125). (g) Invasion of transplanted mutant neuroblasts (arrow) into the gut epithelium (*g*) of the adult host (×280). (h) Invasion of transplanted mutant neuroblasts (arrows) into the thoracic flight muscles (*m*) of the wild-type fly (×230). All histological sections were stained with hematoxylin and eosin. For further explanations see text.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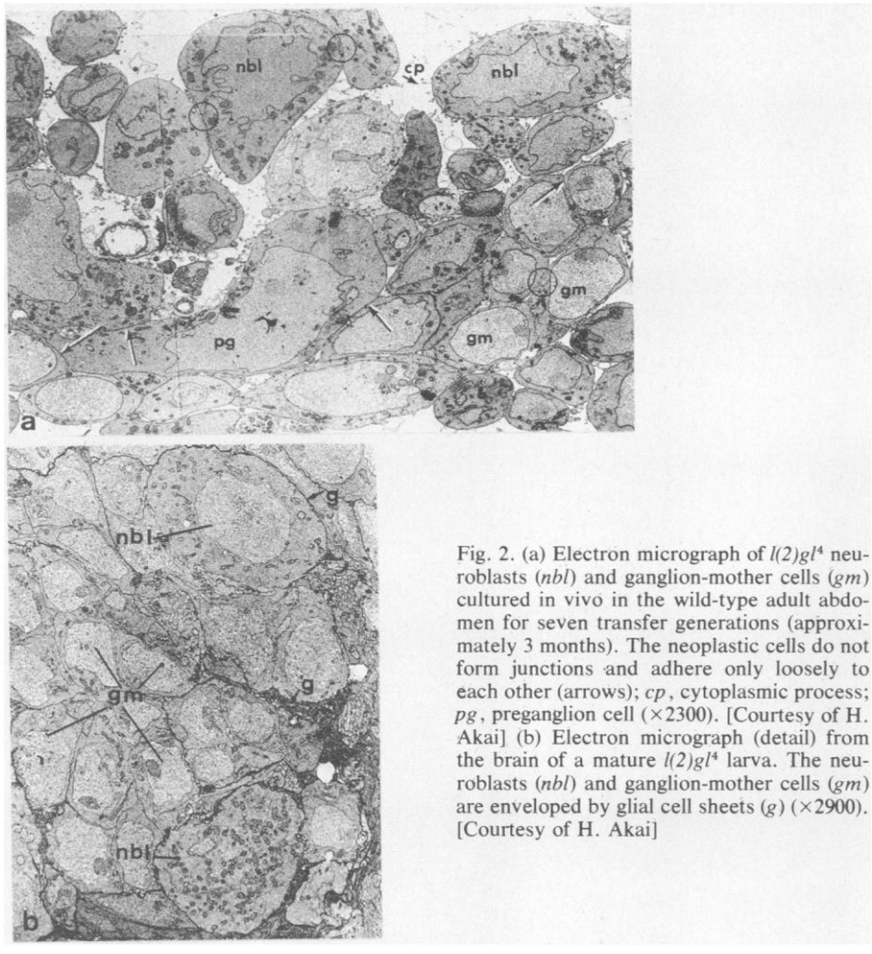



Fig. 2. (a) Electron micrograph of *l(2)gl*$^4$ neuroblasts (*nbl*) and ganglion-mother cells (*gm*) cultured in vivo in the wild-type adult abdomen for seven transfer generations (approximately 3 months). The neoplastic cells do not form junctions and adhere only loosely to each other (arrows); *cp*, cytoplasmic process; *pg*, preganglion cell (×2300). [Courtesy of H. Akai] (b) Electron micrograph (detail) from the brain of a mature *l(2)gl*$^4$ larva. The neuroblasts (*nbl*) and ganglion-mother cells (*gm*) are enveloped by glial cell sheets (*g*) (×2900). [Courtesy of H. Akai]

ferentiation by causing an uncontrolled, autonomous, invasive, lethal, and transplantable growth of the adult optic neuroblasts and ganglion-mother cells.

## Hormones and Malignant Neoplasms

From indirect evidence it is assumed that *Drosophila* development is controlled by hormones (*23*). At the end of larval life the moulting hormone ecdysone initiates pupation, metamorphosis, and adult development. Under the influence of ecdysone, wild-type adult primordia stop growing and differentiate into specific adult structures. Neoplastic cells of *Drosophila* respond generally in one of two ways to hormonal stimuli at metamorphosis: either they stop dividing but fail to differentiate, or they do not respond to ecdysone at all and continue to divide throughout adult development (*13*). Mutant neuroblasts can respond in both ways to hormones. Pieces of mutant brain implanted into third-instar larvae, where they become exposed to ecdysone, stop growing and show no lethal behavior. By the same test mutant neuroblasts that have been cultured for more than five transfer generations in the abdomens of female flies continue to divide throughout adult development, irrespective of their exposure to ecdysone, and kill the host. This indicates that some neoplastic cells, even though they are incapable of differentiation, retain parts of their behavioral repertoire, whereas other neoplastic cells have become unresponsive to hormonal signals.

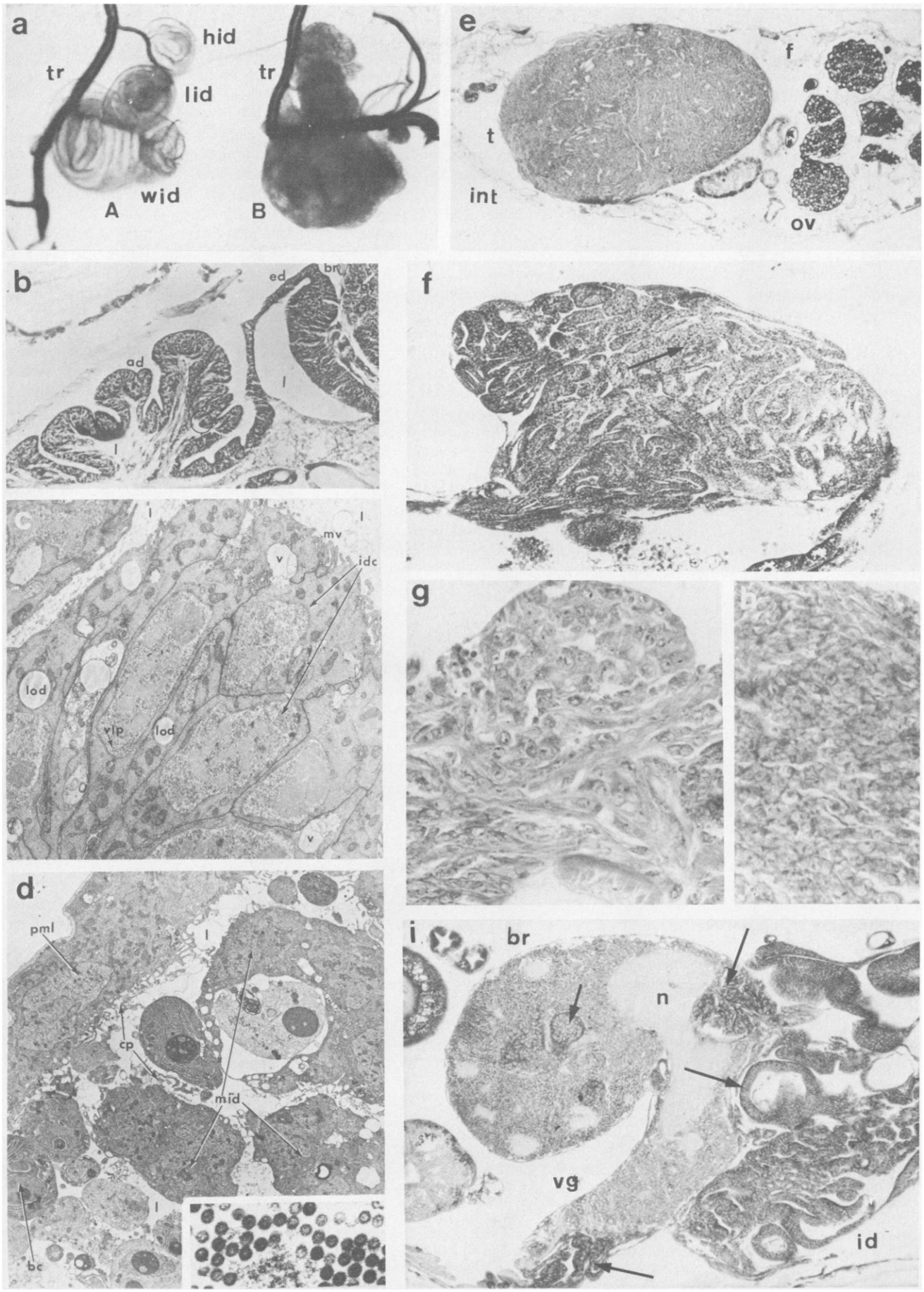
## Imaginal Disc Neoplasms

In the wild-type larva, imaginal discs represent the primordia for the adult integumental structures (*14*). These discs, which give rise to the head capsule, eyes, antennae, and first and second pairs of legs, are closely associated with the central nervous system (Fig. 1a, *A*). Spacially separated from the central nervous system and in close association with the main tracheal trunks one finds the third leg, wing, and haltere imaginal discs (Fig. 3a, *A*). In the caudal portion of the larva is the genital imaginal disc which gives rise to the somatic structures of the genital apparatus.

The wild-type imaginal discs are sacklike structures consisting of 20,000 to 50,000 cells (Fig. 3a, *A*, and Fig. 3b). The cells are arranged in monolayers (Fig. 3b) held together by complex junctions along the lateral surface and by a continuous basement membrane along the basal cell surface (*24*). The apical surfaces, facing the lumen, exhibit numerous microvilli (Fig. 3c) which secrete the chitinous integument during metamorphosis.

Table 1 shows eight recessive-lethal mutants which develop imaginal disc neoplasms. If one takes into account the behavior of the neoplastic imaginal discs in situ, two types of mutants can be distinguished. (i) Mutants in which all imaginal discs represent intermediate neoplasms, exhibiting a compact, noninvasive mode of growth as well as a number of malignant characteristics, such as lethality in situ and fatal autonomous growth after transplantation into a wild-type adult host, loss of the capacity for differentiation, and morphological and histological aberrations (see Table 1). (ii) Mutants in which the imaginal discs associated with the central nervous system grow invasively while the remaining discs are noninvasive (see Table 1).

Fig. 3. Morphology and histology of wild-type and neoplastic mutant imaginal discs. (a) Whole mount of wild-type (*A*) and *l(1)d. lg-2 (B)* haltere (*hid*), third leg (*lid*), and wing (*wid*) imaginal disc connected to the trachea (*tr*). Note the clumped appearance of the mutant imaginal discs (×40). (b) Frontal section through mature wild-type eye-antennal imaginal disc. Note the characteristic folding of the monolayered epithelium; *ad*, antennal imaginal disc; *br*, brain; *ed*, eye imaginal disc; *l*, lumen (× 100). (c) Electron micrography of portion of wild-type imaginal disc epithelium; *idc*, imaginal disc cell; *l*, lumen; *lod*, low opacity droplet; *mv*, microvilli; *v*, vacuole; *vlp*, viruslike particles (×6300). [Courtesy of H. Akai] (d) Electron micrograph of portion of *l(2)gl*$^4$ imaginal disc epithelium; *bc*, blood cell; *cp*, cytoplasmic processes; *l*, lumen; *mid*, mutant imaginal disc cell; *pml*, peripodial membrane-like cell (×3700). [Courtesy of H. Akai] (d inset) A group of viruslike particles from the nucleus of an *l(2)gl*$^4$ imaginal disc cell (×60,000). [Courtesy of H. Akai] (e) Cross section through adult abdomen showing a large noninvasive neoplasm derived from an *l(2)gl*$^4$ imaginal disc; *f*, fat body; *int*, integument; *ov*, ovary; *t*, tumor (×50) [from Gateff and Schneiderman (*10*)]. (f) *l(1)bwn* wing imaginal disc exhibiting clumped (arrow) and aberrantly folded, monolayered morphology (×100). (g and h) Details of *l(1)d.lg-2* imaginal discs lacking monolayer arrangement of the cells (×350). (i) *l(1)bwn* imaginal disc (*id*) in situ invading the brain (*br*) and ventral ganglion (*vg*) (arrows); *n*, neuropile (×80). All histological sections stained with hematoxylin and eosin. For further explanation see text.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Of the eight neoplastic imaginal disc mutants, five exhibit imaginal discs which grow exclusively in a compact, noninvasive fashion (Table 1 and Fig. 3a, *B*). In the remaining three mutants, the imaginal discs associated with the central nervous system show an invasive pattern of growth. They invade the neighboring imaginal discs and the adjacent central nervous system which becomes partially destroyed (Fig. 3i). The wing, third leg, and haltere imaginal discs, in contrast, grow in a noninvasive compact way (Table 1 and Fig. 3f). The mutant imaginal discs were studied by anatomical dissections, in histological and fine-structural preparations, and their developmental capacities were tested by the transplantation method into wild-type larvae and female flies (*13*). Upon anatomical dissection mutant imaginal discs show little resemblance to wild-type discs. They exhibit abnormal shapes, are often enlarged, and sometimes fused (Fig. 3a, *B*). Mutant imaginal discs invading the central nervous system are intimately connected with it (Fig. 3i) and the two structures cannot be separated from each other without the destruction of imaginal disc and nervous tissue.

In histological and fine-structural preparations the mutant imaginal discs show loss of the orderly monolayered cellular arrangement typical of wild-type imaginal discs (Fig. 3, d, f to i) and the cells cluster together forming structures with aberrant shapes and sizes. Electron micrographs show the mutant imaginal disc cells often completely free from contacts to other cells and with numerous cytoplasmic processes and highly lobulated nuclei (Fig. 3d) with numerous viruslike particles (Fig. 3d, inset).

Morphological variations exist, however, among imaginal discs from different mutant stocks. The imaginal discs of the majority of mutant stocks exhibit, besides large portions with cells in clustered arrangement, also small regions with cells in monolayered arrangement [lethal (2) giant larvae [*l(2)gl*], lethal (3) giant larvae [*l(3)gl*] and lethal (1) benign wing imaginal disc neoplasms [*l(1)bwn*] for example] (Fig. 3, f and i). The lethal (1) disc large-2 [*l(1)d.lg.-2*] imaginal discs, in contrast, consist exclusively of cells in clustered arrangement (Fig. 3, g and h). Morphological aberrations in the imaginal disc epithelium are usually an indication of changes in its differentiation capacities (*25*). Wild-type imaginal discs differentiate into normal cuticular patterns upon implantation into third-instar wild-type larvae. When any of the neoplastic mutant imaginal discs shown in Table 1 are implanted into third-instar wild-type larvae, the only normal response to the hormonal stimulus is cessation of cell division. Differentiation of cuticular integumental structures does not take place, which indicates that the neoplastic imaginal disc cells behave autonomously in the wild-type larval environment.

The transplantation test into the abdomens of wild-type female flies represents another crucial proof for malignancy and demonstrates the autonomy of the neoplastic pattern of growth. This test again was applied to all mutant imaginal discs. Pieces of invading and noninvading neoplastic imaginal discs grow rapidly into compact ball-like structures in the adult abdomens, depleting the host of its fat body and ovaries (Fig. 3e) and killing it. The time required for the neoplastic imaginal disc implants to kill their hosts varies with the different mutant imaginal discs from 7 to 14 days, and apparently reflects differing growth rates among mutant imaginal discs. For instance, lethal (1) disc large-1 and -2 imaginal disc implants kill their hosts 1 to 2 days sooner than lethal (2) giant larvae[4] imaginal disc implants. Furthermore, even among the 13 lethal (2) giant larvae alleles variations in the growth rates and thus the time required to kill the host were detected (*22*). Tissue sublines from all neoplastic imaginal discs can be propagated for many transfer generations in the adult female abdomen. They also show increased amounts of viruslike particles (Fig. 3d, inset). In contrast, pieces of normal imaginal discs grow in the wild-type abdomens at a moderate rate, eventually ceasing cell division and causing no harm to the host.

The transplantation test was used also to determine the approximate time of the gene action during development of the lethal (2) giant larvae[4] [*l (2) gl*$^4$] allele. Imaginal discs from different embryonic and larval developmental stages were implanted into the abdomens of wild-type female flies. Beginning with the 10-hour embryo, the mutant implants grew in a tumorous fashion exhibiting the characteristics of the already described intermediate imaginal disc neoplasms found in mature mutant larvae (*10, 12*). Thus, the time of action of the *l(2)gl*$^4$ allele falls sometime during the first portion of embryonic life and seems to coincide with the time of gene activity in the *l(2)gl*$^4$ neuroblasts.

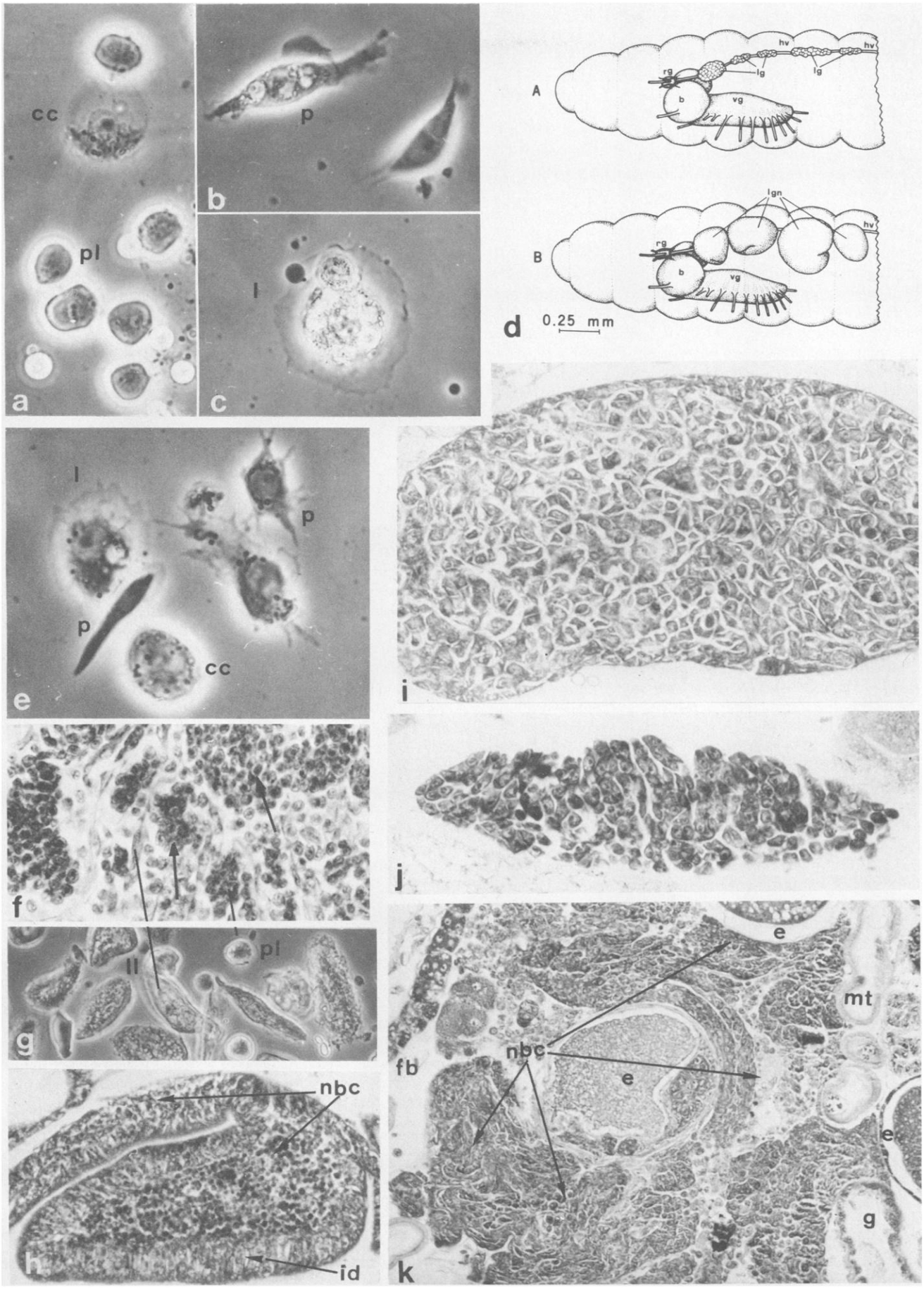
Thus, neoplastic imaginal discs in situ as well as after transplantation possess qualities of both malignant and benign neoplasms: they engage in fast autonomous and lethal growth in situ as well as after transplantation, have lost their capacity to differentiate, and show abnormal morphology and histology. Mutant imaginal discs differ in their pattern of growth in situ; some are noninvasive while others possess an invasive mode of growth.

The capacity for malignant neoplastic transformation is not only confined to *D. melanogaster* mutants. Srdic *et al.* (*26*) have studied a new mutant in *Drosophila hydei*, named lethal (3) giant larvae. This mutant develops a malignant neuroblastoma in the presumptive adult optic centers in the larval brain and lethal, benign, and transplantable imaginal disc neoplasms which are similar to those of lethal (2) giant larvae[4].

## Reversal of the Neoplastic State

The developmental capacity of wild-type and mutant imaginal disc cells can be studied further by using somatic recombination in imaginal disc cells heterozygous for a wild-type (+) chromosome (+++ *c*; *c* = centromere) and a chromosome containing two markers, *m1* and *m2*, that occur on either side of the tumor gene (*m1 tg m2 c*). For the first (X) chromosome the marker alleles most commonly used are yellow body color

Fig. 4. Blood cells and hematopoietic organs from mature wild-type and mutant larvae. Phase contrast micrographs of (a) wild-type plasmatocytes (*pl*) and crystal cell (*cc*); (b) wild-type podocytes (*p*); and (c) wild-type lamellocyte (*l*) (×760). (d) Schematic representation of wild-type (*A*) and *l(1)mbn* (*B*) hematopoietic organs (*lg, lgn*) located along the dorsal heart vessel (*hv*); *b*, brain; *rg*, ring gland; *vg*, ventral ganglion. (e) *l(1)mbn* podocytes (*p*), lamellocytes (*l*), and crystal cell (*cc*) (×760). (f) Nests of hematopoiesis (arrows) in the hemolymph of *l(2)mbn* (×170). (g) Phase contrast micrograph of *l(2)mbn* lamellocyte-like cells (*ll*) and plasmatocyte-like cells (*pll*) (×800). (h) *l(1)mbn* blood cells (*nbc*) invading an imaginal disc (*id*). The imaginal disc epithelium is partially destroyed by the mutant blood cells (arrows) (×120). (i) Longitudinal section of a hematopoietic lobe derived from a mature *l(1)mbn* larva. Compare with (j) and note the high degree of differentiation of its cells (×390). (j) Longitudinal sections of a lobe from the hematopoietic organ of a mature wild-type larva (×370). (k) Detail from cross section through wild-type adult abdomen bearing *l(1)mbn* malignant blood cell neoplasm consisting of numerous neoplastic blood cells (*nbc*) (arrows). Note the invasion into an egg chamber (*e*); *fb*, fat body; *g*, gut; *mt*, Malpighian tubule (×100). All histological sections stained with hematoxylin and eosin. For details see text.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(*y*) and forked bristles (*f*). A crossing-over event between the centromere and the forked allele in a heterozygous imaginal disc cell with the genotype *y tg f c*/ +++ *c* produces two homozygous cells, one homozygous for the tumor gene, *tg*, and the markers yellow and forked (*y tg f c*/*y tg f c*), and the other for the corresponding wild-type alleles (+++ *c*/ +++ *c*). The progeny of the two cells will give rise to clones. The clone produced by the cell containing the wild-type alleles, however, will not be detectable, since this chromosome contains no marker alleles. The clone formed by the homozygous tumor cell, on the other hand, can be recognized by the *y* and *f* markers. The presence of *y f* clones on the integument indicates nonautonomy and thus a capacity of the homozygous tumor cell for reversal to the differentiating state. The absence of *y f* clones on the integument, on the other hand, shows an incapacity for reversal to the differentiating condition and autonomy of the neoplastic state.

Three imaginal disc tumor mutants were tested for survival in clones: lethal (2) giant larvae[4] [*l(2)gl*[4]] (*27*), lethal (1) disc large-1 [*l(1)d. lg-1*] (*13*) and lethal (1) benign wing imaginal disc neoplasm [*l(1)bwn*] (*13*). Two basic types of neoplastic tumor mutants could be distinguished. The imaginal disc cells of lethal (1) disc large-1 mutants did not produce clones and, thus, showed autonomy as they did in the transplantation test (*13*).

The imaginal disc cells in the remaining two mutants, lethal (2) giant larvae[4] and lethal (1) benign imaginal disc neoplasm, which during transplantation behaved autonomously, survived in clones and, thus, behaved nonautonomously and reverted to the differentiating state (*13, 27*). The difference between the developmental capacities of the mutant imaginal disc cells in the transplantation and somatic recombination tests shows that some neoplastic imaginal disc cells are irreversibly transformed, while others are reversible and capable of differentiation when they develop in close contact with wild-type cells. Cline (*27*) assumes that the neoplastic imaginal disc cells receive gene products that they lack from the wild-type cells.

## Malignant Blood Cell Neoplasms

A third type of tumor mutation induces malignant neoplastic transformation of the presumptive blood cells in the larval blood-forming organs—commonly called the lymph glands. Three blood tumor mutants have been isolated. The mutant genes are located on chromosomes 1, 2, and 3 and are designated as lethal (1) malignant blood neoplasm [*l(1)mbn*] (*28*), lethal (2) malignant blood neoplasm [*l(2)mbn*] (*29*), and lethal (3) malignant blood neoplasm [*l(3)mbn*] (*30*) (Table 1).

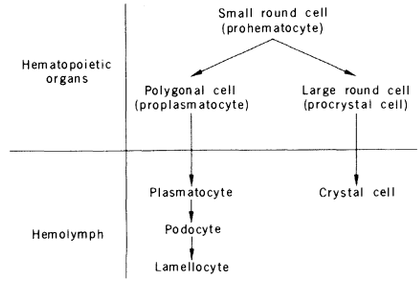


Fig. 5. Tentative diagram of the hematopoietic pathway in the larva.

Rizki (*31*) studied the cell types in wild-type larval hemolymph and found two main types of blood cells: the plasmatocytes and the crystal cells (Fig. 4a). Plasmatocytes possess no humoral immune response and should not be confused with plasma cells of vertebrates. The plasmatocytes can transform into podocytes and these in turn can change into lamellocytes (Fig. 4, b and c). This transformation occurs at a high rate at the end of larval life and extends beyond puparium formation.

The defense mechanisms in *Drosophila* are mainly cellular and include phagocytosis and encapsulation (*32*). Small particles are phagocytosed by podocytes. Larger sized bodies are encapsulated, mainly by lamellocytes, and rendered harmless by being melanized.

The crystal cells are large, round, fragile cells which sporadically contain crystalline inclusions consisting of tyrosine (Fig. 4a) (*33*). Crystal cells rupture easily upon the wounding of the animal and expel a sticky material. Their function is not well understood but they seem to be involved in melanin synthesis, coagulation of the hemolymph, and agglutination of bacteria and yeasts during metamorphosis (*19, 34*).

The larval blood cells originate in the hematopoietic organs (*35, 36*). The organs consist of four to seven pairs of lobes located along the dorsal heart vessel in the fourth to seventh larval segments (Fig. 4d, *A*). The larval hematopoietic pathway has not been well investigated. By means of phase contrast (*37*) and electron microscopy (*38*) the cell types in the hematopoietic organs have been compared with the free blood cell types in the hemolymph and a tentative hematopoietic pathway has been suggested (Fig. 5). Within the hematopoietic organs three cell types could be detected: (i) polygonal cells (proplasmatocytes), (ii) large round cells (procrystal cells), and (iii) small round cells (prohematocytes). Polygonal cells seem to be the precursors of the plasmatocytes. The large round cells with crystalline inclusions give rise to crystal cells. The polygonal cells as well as the large round cells seem to originate from the small round cells or prohematocytes. All cell types divide.

The developmental defects in the hematopoiesis of the three blood tumor mutants are as follows. The most obvious change in the mutant larvae is a manyfold increase in the number of free blood cells in the hemolymph (Table 2) (*37*). There are also profound differences between the blood cell types in the hemolymph of the three blood tumor mutants and those found in the hemolymph of a third-instar wild-type larva. The *l(1)mbn* blood cell population contains the same cell types as the wild type but in much higher numbers (Table 2). Furthermore, the blood cell composition resembles more closely that of a prepupa than of a third-instar larva. In contrast to the wild-type larva, where the majority of the free blood cells are plasmatocytes (*31*) in *l(1)mbn* podocytes and lamellocytes constitute approximately 95 percent of the blood cell population and plasmatocytes and crystal cells the remaining 5 percent. The *l(1)mbn* podocytes also show an overwhelming diversity in size and shape when compared with their wild-type counterparts (Fig. 4e).

The *l(1)mbn* blood cells are not only morphologically differentiated, they also show functions which are typical of wild-type blood cells, such as phagocytosis and encapsulation. However, *l(1)mbn* blood cells phagocytose and encapsulate their own tissues and resemble thus the blood cell behavior of melanotic pseudotumor mutants (*39*). Mutant blood cells often encapsulate and melanize the posterior fat body which leads to the formation of melanotic masses.

The hemolymphs of the *l(2)mbn* and the *l(3)mbn* mutants exhibit, in great abundance, actively dividing, small round cells which are plasmatocyte-like (Fig. 4, f and g, see connecting lines). Besides these one finds a prominent sickle-like to boat-like cell type with a V-shaped profile and an irregularly structured cell surface (Fig. 4g). This cell type vaguely resembles the lamellocytes. Very few crystal cells can be observed.

In older mutant larvae the individual lobes of the blood-forming organs have broken up and many new nests of hema-

topoiesis can be seen distributed throughout the body cavity (Fig. 4f). The cells in these nests and the plasmatocyte-like cells around are engaged in vigorous mitosis. The blood cells of all three mutants invade the imaginal discs and destroy their epithelium (Fig. 4h). In *l(2)mbn* and *l(3)mbn* the malignant blood cells eventually destroy every tissue in the body. Just before death the larvae appear as sacks entirely filled with blood cells.

The *l(1)mbn* hematopoietic organs remain mostly intact and they enlarge, often exceeding 300 to 400 times the volume of a wild-type lobe. In the mutant larvae one finds three to four large prominent tumors (Fig. 4d, *B*) which, in older larvae, become detached from the heart. Histological preparations of such hematopoietic tumors show that they contain the same three cell types which are present in the wild-type blood-forming organs (*37, 38*) (Fig. 4, i and j). However, the amounts of the different cell types and their degree of differentiation differ drastically from the wild type. Histological preparations and phase contrast observations of the cells in vitro reveal that in the wild-type larval hematopoietic organs the majority of the cells are primordial blood cells and only very few are differentiated podocytes and crystal cells (*37, 38*) (Fig. 4j). In the mutant hematopoietic organs, in contrast, at least 50 percent of the cells are fully differentiated podocytes and lamellocytes (*37, 38*) (Fig. 4i). This explains the large amounts of free podo- and lamellocytes in the mutant hemolymph. The remaining cell types in the mutant hematopoietic organs are proplasmatocytes and procrystal cells. In autoradiographic preparations 30 percent of the mutant cells incorporate [$^3$H]thymidine, whereas only 15 percent of all cells are labeled in wild-type cells.

During development of the wild type the release of blood cells from the hematopoietic organs is strictly regulated. For instance, a massive release of blood cells takes place at the end of the second and third larval instars while during the third larval instar blood cells are not released from the hematopoietic organs in detectable amounts. In contrast, the *l(1)mbn* hematopoietic organs produce throughout the third larval instar new blood cells which they emit continuously into the hemolymph. These results show that the *l(1)mbn* mutation affects the mechanism which controls the production of blood cells in the hematopoietic organs and not blood cell differentiation. The *l(2)mbn* and the *l(3)mbn* mutant genes, in contrast, seem to interfere with blood cell differentiation. In the hemolymph of these mutants one finds numerous nests of hematopoiesis with prohematocyte-like cells (Fig. 4f) and an enormous number of free, undifferentiated plasmatocyte-like cells filling the body cavity.

Table 2. Blood cell counts in the larval hemolymph of wild-type *Drosophila* and three blood-tumor mutants. The blood cell counts for each individual group were compiled from the counts on five mature larvae.

| Designation | Cells in hemolymph (No./mm$^3$) |
|---|---|
| Wild-type | 1,600 |
| Lethal (1) malignant blood neoplasms | 12,500 |
| Lethal (2) malignant blood neoplasms | 39,000 |
| Lethal (3) malignant blood neoplasm | 225,200 |

The capacity for autonomous neoplastic growth of the presumptive blood cells in the hematopoietic organs of the three blood tumor mutants were further investigated in transplantation tests. Hematopoietic organs of *l(1)mbn* larvae implanted into female adult abdomens grew rapidly and invasively and killed 50 percent of the hosts in 9 days. Autoradiographic studies of the transplanted cells showed numerous labeled cells (*38*). The *l(1)mbn* blood cell neoplasm is transplantable for numerous transfer generations in female flies. Figure 4k shows a portion of a cross section through a female adult abdomen where the malignant blood cells have invaded and destroyed almost completely the left ovary and individual egg chambers. The malignant blood cells invade also the thoracic muscles and the head capsule. Histological and phase contrast preparations reveal that the cells constituting the neoplastic growth are exclusively plasmatocyte-like cells (Fig. 4k). Crystal cells are not found in the transplanted growth. These results indicate strongly that the plasmatocyte-like cells are the malignant component, which grow in an autonomous way, while the crystal cells do not participate in the growth after transplantation. Control implants of wild-type hematopoietic organs do not grow and do not have any effect on the development of the adult fly.

Implantation of pieces of the *l(1)mbn* hematopoietic tumor into different larval stages was also harmful to the hosts (*38*). Young third-instar larvae receiving *l(1)mbn* implants usually failed to pupate and died as larvae. The few which did pupate died shortly thereafter. Histological preparations of such hosts revealed clearly the cause for the lethality. The mutant blood cells continued to divide vigorously; they invaded the imaginal discs and destroyed partially or completely their epithelia. Implants into larvae, a few hours before puparium formation, affected adult development by interfering with the differentiation of adult tissues such as the muscles, and thus causing the death of the developing imago. All of these results show that the *l(1)mbn* plasmatocyte-like cells are neoplastically transformed and behave in situ, as well as after transplantation into wild-type adults and larvae, in a malignant fashion. They also show that the mutant blood-forming organs in situ represent transplantable, malignant hematopoietic neoplasms.

In the remaining two blood tumor mutants, *l(2)mbn* and *l(3)mbn*, the transplantation test was negative. Portions of mutant hematopoietic organs implanted into adult flies did not proliferate and were not lethal to the host, thus indicating that the neoplastic state of the mutant blood cells in situ is not autonomous, but may result from abnormal factors elsewhere in the animal.

A similar drastic increase of the blood cell population is regularly observed in the temperature-sensitive, recessive-lethal mutants *l(1)mad$^{ts}$* (*mad*, mitotic arrest in development; *ts*, temperature-sensitive) and *l(1)213$^{ts}$* after temperature treatment (*19*). Exposure to a temperature of 29°C for 5 days, in addition to causing morphological and developmental changes in the imaginal discs, the central nervous system, and other organs, also results in an enormous increase in the blood cell population. Blood cell counts reach numbers almost as high as in the *l(3)mbn* mutant. The blood cell types are also very similar to the blood cell types in the *l(2)mbn* and *l(3)mbn* tumor mutants. It is possible that in these mutants blood cell numbers increase not as a primary action of the mutant gene in the blood cells but as a response to abnormal developmental processes elsewhere in the larva, such as in the imaginal discs. This intriguing possibility that blood cell malignancies may be the result of an external pathological stimulus somewhere else in the body should be further investigated.

## Conclusions

More than 70 years of intensive genetic research with *Drosophila* has created a wealth of information which no other eukaryotic organism can surpass (*40*). The isolation of specific mutations on all four chromosomes has become a

routine procedure (*41*). Among the recessive-lethal mutations isolated in my laboratory, about 0.3 percent caused malignant neoplasms in various larval tissues (*13*). More such single gene tumor mutations may also be found in the embryo and in the developing adult. Thus, it appears that contrary to man and other mammalian systems, where two or more defective genes have been postulated before a cell can become malignant (*42*), *Drosophila* shows a direct relation between a mutated gene and the neoplastic transformation of a specific cell type. This fact may prove extremely valuable for the elucidation of the mode of gene function in each specific case of neoplasm.

In this respect, temperature-sensitive tumor mutations, which can also routinely be produced in *Drosophila*, should be of value (*43*). Here lies a further advantage of *Drosophila*. Temperature-sensitive tumor mutations should prove useful in studying the time of gene activity or the time when specific gene products are used during normal development and the resulting molecular disorders leading to cancer.

With the help of powerful developmental genetic and surgical methods, such as the somatic recombination and gynander tests (*14, 44*) and a number of microinjection methods (*45*), it should be possible to study the state of determination, the developmental capacities, and the reversibility of tumorous tissues.

In vertebrates, tumor implants are often rejected by immunological host reactions, which make the transplantation test unreliable. In *Drosophila*, which lacks transplantation immunity, tumorous implants are never rejected. This makes the transplantation test a most useful criterion for the characterization of a malignant neoplastic growth, and represents another advantage of this system.

The diploid chromosome set of *Drosophila* contains only eight chromosomes which renders karyotype investigations an easy task. In addition, somatic pairing resulting in polyteny of the chromosomes in some larval cells, such as the salivary gland cells, allows visualization and mapping of genes (*46*), the study of gene activity and its control (*47*), and the identification of chromosomal rearrangements (*48*). Since vertebrate karyotypes with their numerous small chromosomes often present great difficulties, *Drosophila* also offers an advantage here.

A most pertinent problem in cancer research is the reversibility of the neoplastic condition of cells to the differentiating state. Tumorous cells of genetic as well as of epigenetic origin in *Drosophila* are capable of reversion (*13*). Studies of the reversion processes and the intrinsic and external factors involved in it may prove possible in the future with *Drosophila*.

Large numbers of viruslike particles are found regularly in the nuclei of all neoplastic cells of *Drosophila*. In wild-type cells they are present only sporadically and in very small numbers (*20*). Their function is not known. It would be of great interest to determine their viral nature. They may possess regulatory gene functions, and may be responsible for genetic instability and certain types of mutations (*21, 49*). *Drosophila* is easy and inexpensive to rear in the laboratory and has a short generation time (11 to 12 days at 25°C). Its homologous chromosomes do not undergo crossing-over in the germ cells of the male, techniques are available for cell culture in vitro (*50*), and much published information on the genetics and development of *Drosophila* is available. Two major centers house thousands of mutant stocks readily available to all *Drosophila* workers (*51*).

The only disadvantage that still restricts research with *Drosophila* is its small size, which makes it difficult to obtain large amounts of cellular material for biochemical studies. Such difficulties may, however, be overcome with the development of better culture methods and the selection of suitable temperature-sensitive mutations.

Genetic factors seem to be of great importance in cancer etiology (*42*). *Drosophila* chromosomes contain genes which, when mutated, cause malignant and intermediate neoplastic growth in specific tissues and cells of the larva. These tissue-specific genes have during normal development profound effects on the growth and differentiation pattern of the cells. In this sense tumor genes in *Drosophila* represent nothing more than fundamental development genes which, during normal development, affect the proliferation rates and the differentiation of cells.

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# Hubris in Science?

Lewis Thomas

Everyone says that the root cause of society's diminishing confidence in science is the failure of scientists to explain what they do with their lives, and I agree with this. But I do not see this as an easy problem to solve, not so much because of any inarticulateness on the part of the scientists, and not so much because of deficiencies on the part of the professional journalists who devote their careers to science, but because of the sheer, overwhelming enormity of the field. The enterprise of biomedical research in the United States has expanded in scale and scope so greatly in the past 30 years that no one can begin to keep up with the reading of it. It used to be that a working immunologist could keep abreast of his field by covering three or four professional journals, plus *Nature* and *Science* for the first accounts of new observations. Now there are ten times that number of journals, each containing papers on immunology that cannot be overlooked, plus any number of monographs, review volumes, national and international symposium reports, and even a few newsletters. The journals are themselves five times their former size, with briefer articles and smaller print.

It is the same for all the other fields of biology and medicine. The literature has become too vast to be comprehended. And, to make matters even more difficult, most of the published work is good. The papers that one ought to be reading are important and interesting. The quality of the science, despite its enormous bulk, is really better today than at any time in the past. It is intricate and complicated, and much of it is difficult to grasp even for the workers in closely neighboring fields, but it is filled with meaning.

So, communication has become a serious problem not only between the scientists and the public, but among the scientists themselves. How do the investigators cope with the problem? Not, I think, by relying on computerized library services, although increasingly clever systems for retrieving more or less current information have come into existence in recent years. Nor are the journals themselves used as extensively as they used to be as sources of new information.

What is happening is that there is much more reliance on word of mouth for the transmission of scientific data than ever before in my memory. And, despite the literature problems that I have just been citing, I have the impression that the people doing the work are really better informed about what is going on in other laboratories than ever before. There is a new system at work, which I do not understand. I have the impression that a great body of information is getting around by a mechanism that can only be termed gossip.

The telephone has become an indispensable scientific instrument. Laboratories in New York are in touch with Dallas, La Jolla, Boston, and Paris, all on the same day. By the time papers are published in the *Journal of Experimental Medicine*, most of the people working in that particular field are already familiar with the general drift of the work. If a group in Edinburgh is getting close to solving a special problem, the other laboratories all around the world seem to know about it, and in fine detail. And the information travels almost with the speed of light. A corridor conversation in a research institute in Cambridge will be reported almost instantaneously in Pasadena.

The most surprising thing about the system is that it seems to be functioning with considerable accuracy and reliability. It is also surprising that there is so much openness and candor. It used to be thought that scientists tend to be rather secretive, hiding their data away from each other in order to be sure of priority for the published manuscripts; but these

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