DEVELOPMENT OF THE CAUDAL AXIS OF THE BRACHYURY MUTANT OF THE MOUSE.

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I. General Survey of the Literature.

A. HISTORICAL REVIEW

Much progress has been made in our understanding of the biology of the gene (Creighton and McClintock, 1931; Beadle and Tatum, 1941; Watson and Crick, 1953; Jacob and Monod, 1961 and Clever, 1964). A wide gap still exists, however, between our knowledge of genetics on the molecular level and the events intervening between gene action and morphogenesis.

Investigations of the mechanisms of gene action in development date back to Wilson (1898), Boveri (1902), Morgan (1919), and Goldschmidt (1927, 1938). From the time when Goldschmidt (1927) stated in his Physiologische Theorie der Vererbung that "we know next to nothing of the action of the hereditary material in controlling development" to the present day, a large body of information on the subject has been accumulated.

When studies on the developmental basis of inherited mammalian abnormalities were started about 50 years ago (Wright, 1917, 1941), their primary aim was to obtain insight both into the mode of gene action and into mechanisms of normal development. The focus of attention changed from the means of genetic transmission to the way in which genes operate.

The analysis of changes in developmental patterns leading to the abnormalities in selected mutants became the method by which classical developmental geneticists attempted to identify the mechanisms of gene action in development (Gluecksohn-Waelsch, 1951). This classical approach did not fulfill the initial expectations but did serve to confirm in mammals the existence of many developmental mechanisms demonstrated previously by experimental procedures in other vertebrates.

Demerec (1934, 1936) and Poulson (1940, 1945), in describing the effects of deficiencies on the embryonic development of Drosophila melanogaster, were among the first to attempt to examine the early deviations from normal development caused by genetic mutations in an organism whose genetics was very well known.

However, developmental geneticists turned increasingly to investigations of birds and mammals where an increasing number of mutants became available for study that affected the morphogenesis of a variety of structures such as the skeletal, nervous, sensory and pigment systems (Landauer, 1932, 1934; Gluecksohn-Schoenheimer, 1938, 1943, 1944, 1945; Dunn, 1939, 1941, 1942, 1956; Russell, 1949, 1954; Gluecksohn-Waelsch, 1951, 1954; Grüneberg, 1952, 1955a, 1955b, 1958a, 1958b, 1961; and Carter, 1954).

The vertebrate skeleton particularly became a favorable subject for studies of abnormal development because of the susceptibility of the vertebral column to developmental accidents, and because in the mouse over 70 simple Mendelian mutations have been discovered that affect the morphogenesis of the axial and appendicular portions of the skeleton.

B. REVIEW OF MUTANTS AFFECTING THE MORPHOGENESIS OF THE AXIAL SKELETON OF THE MOUSE.

Genetic analysis of the house mouse has revealed a large number of loci that control the morphogenesis of the posterior axis (reviewed by Grüneberg, 1963). Based on extensive studies of pathogenesis, most of these skeletal mutants may be classified into four categories according to the type of disturbances by which the development of the vertebral column is interfered. These include (1) disturbance of

the unsegmented paraxial mesoderm, (2) interference with segmentation, (3) interference with sclerotome differentiation, and (4) suppression of notochord-mesoderm interaction.

(1) Examples of anomalies of the unsegmented paraxial mesoderm are provided by the mutations Vestigial tail (vt) on chromosome 7 and by Bent tail (bt) on the X chromosome. In both of these mutants the vertebrae of the tail are reduced in number and are abnormally formed. Grüneberg (1957) states that in vestigial tail the unsegmented paraxial mesoderm "seems to be more loosely knit than that of normal embryos" causing constrictions to occur in the tail. Moreover, the distal part of the tail, which is destined to regress, lacks segmentation altogether while the somites proximal to the constriction of the tail tend to be irregular in shape and size. No involvement of the notochord has been noted in this mutation. In Bent tail (Grüneberg, 1955b), the tail skeleton consists of a mixture of normal and abnormally formed vertebral elements and the total number of caudal vertebrae are reduced. The conclusion has been reached by Grüneberg (1963) that this mutant gene interferes with the supply of raw material from which the caudal segments are formed.

(2) Disorders of segmentation of caudal mesoderm are responsible for the abnormalities of the tail structure of the mutants pudgy (pu), Loop-tail (Lp), curly-tail (cu), Crooked-tail (Cd), Fused (Fu), and Kinky-tail (Fu^{Ki}). In pudgy (on chromosome 1), the entire axial skeleton is disrupted leading to a highly disorganized, irregularly segmented, and shortened vertebral column (Grüneberg, 1961). In Loop-tail (on chromosome 13), and curly-tail (on chromosome 3), somites are small and irregular in size (Stein and Rudin, 1953; Smith and Stein, 1962). In Crooked-tail (on chromosome 7) somites are of unequal size and are frequently fused with one another

(Theiler, 1956; Matter, 1957). The genes for Fused and Kinky-tail (both on chromosome 9), also disrupt the segmentation pattern of caudal mesoderm leading to a shortened and kinked tail (Theiler and Gluecksohn-Waelsch, 1956). Anomalies of the neural tube, such as the formation of deverticula or double neural tubes in the tail, accompany the skeletal abnormalities in these two mutants.

(3) Interference with sclerotomes is responsible for the anomalies of the mutants tail-kinks (tk) on chromosome 3, undulated (un) on chromosome 5, and flexed-tail (f) on chromosome 14. According to Grüneberg (1955b) each of these mutant genes affects a somewhat different aspect of the process of sclerotome differentiation. In tail-kinks, portions of some sclerotomes are defective (Grüneberg, 1955a) and produces a tail consisting of a mixture of normally and abnormally formed vertebrae. In the mutant undulated, the normal number of vertebrae is present but the distal tail vertebrae are progressively shortened. According to Grüneberg (1963) the shortening of the individual vertebrae is due to a "reduction in the size of the condensations of mesenchyme cranial to the sclerotome fissure" and to the retention of the material that normally forms the posterior part of the vertebrae within the intervertebral disc. In flexed-tail, a mutation that merely affects the shape of the tail but not its length, ankyloses of adjacent vertebrae are apparently the result of abnormal development of intervertebral discs (Kamenoff, 1935).

(4) Suppression of the notochord-mesoderm interaction seems to be involved in four extensively analysized mutants that affect tail length and shape. These mutants are: Danforth's short-tail (Sd) on

chromosome 5, Brachyury (T) on chromosome 9, Pintail (Pt) on chromosome 8, and truncate (tc) on chromosome 11. In these cases, reduction in tail length is preceded by extensive notochordal abnormalities occuring during the early development of the body axis.

In the development of embryos heterozygous for Danforth's short-tail (Sd) (Dunn, Gluecksohn-Schoenheimer, and Bryson, 1940), the earliest deviation from the normal path of development involves the reduction in length of the notochord (Gluecksohn-Schoenheimer, 1945; Grüneberg, 1958a). These authors reasoned that the absence of the notochord in the tail region is the primary cause for the extensive cell pycnosis that takes place in surrounding somites and neural tube. This cellular degeneration leads to the regression of the distal region of the tail in Sd/+ individuals and of the whole tail in Sd/Sd embryos (Grüneberg, 1958a).

In Pintail (Hollander and Strong, 1951; Berry, 1960), the tail develops a tip which is thin and filamentous. The caudal vertebrae align in a faulty manner so as to cause distal kinks. The notochord is also much reduced and terminates proximal to the tip of the tail. In truncate (Theiler, 1957, 1959), the vertebral column terminates near the base of the tail leaving only a thin filament. The earliest deviation from normal development that has been observed in truncate is an abrupt termination of the notochord at the level of the posterior neuropore. Extensive pycnosis occurs in the surrounding somites leading to regression of the distal portion of the tail.

The most extensively investigated of the many known mutations affecting the axial skeleton in the mouse is the "short-tail" or Brachyury mutant, first discovered by Dobrovolskaia-Zavadskaia in 1927. There is an

extensive literature on its embryology and genetics (for a review see Bennett, 1964).

The T gene is dominant and seems to be part of a "complex locus" extending over some length of the ninth chromosome. A number of different complementation groups have been identified having alleles that affect the early organization of the embryo, chiefly the axial structures (Bennett, 1964). Recessive alleles of T, t^n (n standing for a numbered series), lead to taillessness in T/t^n combinations, with +/t^n heterozygotes being phenotypically normal (Dunn, 1964). The homozygotes of many of the t-alleles (t^n/t^n) are early embryonic lethals (Bennett, Dunn, and Badenhausen, 1959).

In 1932, Chesley found that phenotypic Brachyury mice are actually heterozygous for the T gene and that inter se mating (T/+ X T/+) results in a ratio of two Brachyury animals to one normal animal rather than a 3:1 or 1:2:1 ratio as might be expected. Chesley determined that the mutant homozygotes are a homogeneous group that die in utero between the 10th and 11th day of gestation (Figure 1).

Viable T/+ heterozygotes show a shortened and malformed tail. Most investigators who have studied the early developmental history of the T/+ and the T/T embryos are agreed that the notochord is the structure primarily affected by the T gene. The regression of the tail tip in the heterozygote has been interpreted by Chesley (1935), Gluecksohn-Schoenheimer (1938), Grüneberg (1958a), and by Bennett (1964) as being a secondary consequence of the disturbance of the notochord.

By 8½ days of gestation abnormalities of the notochord and neural tube are evident histologically. The notochord develops lumina through much of

its length and in some areas becomes incorporated into neighboring structures, such as the cloaca or neural tube. The distal filament, which develops presumably as a consequence of notochordal interaction in that region, eventually drops off leaving a shortened and a malformed tail. Besides resulting in a shortening of the tail, the T/+ embryo also develops a number of gross structural defects in the architecture of the remaining caudal vertebrae (Dobrovolskaia-Zavadskaia, 1934).

In embryos homozygous for the T gene the destructive processes start much earlier and are far more extensive. The entire posterior body behind the forelimb fails to develop. Although the notochord forms initially throughout the length of the mutant animal, it rapidly becomes disorganized and incorporated into neighborning structures such as the gut and neural tube. The embryo dies at about 10 3/4 days because the allantois fails to meet the maternal blood supply (Grüneberg, 1958b).

Grobstein and Holtzer (1955) have shown that the somites of mouse embryos will form cartilage only under the influence of an inducer, the most effective one being the ventral spinal cord from embryos of 12-24 somite stage. Using this information, Bennett (1958) has shown that normal ventral spinal cord is unable to induce cartilage in somites from T/T animals in vitro. However, ventral spinal cord of T/T embryos is still capable of inducing cartilage in normal somites.

These experiments indicate that the tail mesoderm of mice homozygous for the Brachyury mutant is defective to the extent that it is incapable of undergoing normal histogenesis.

II. Introduction to the Present Study.

Previous investigators (Chesley, 1935; Grüneberg, 1958a; Dobrovolskaia-Zavadskaia, 1934; and Kobozieff, 1935) have concentrated mainly on studies of the early development of the T/T and T/+ embryos. The present study is primarily concerned with the subsequent pathology initiated in the tail mesoderm presumably as a consequence of the failure of normal notochordal inductive interaction.

It has been established that in the T/+ embryo, during the 2nd trimester of gestational life, a constriction develops toward the tip of the tail. Tissues distal to this constriction regress, producing a thin filament. Soon after birth this filament drops off leaving the remaining tail structure in the "Brachyury" condition that gives this mutation its name.

The superficial similarity between tissue destruction taking place in tails of embryos heterozygous for the T gene with the involution of larval tail structures observed during amphibian metamorphosis (Weber, 1964, 1967) suggested the possibility that similar mechanisms may be operating in both processes.

Interest in the mechanism of tissue involution has been stimulated by the emergence of the lysosome concept (reviewed by de Duve and Wattiaux, 1966). This hypothesis proposed, among other ideas, that lysosomes because of their content of lytic enzymes, are the tools of cell autolysis and that cell death may be the result of the release of lysosomal enzymes into the cytoplasm.

It seemed of interest, therefore, to survey the ultrastructure of tails of Brachyury (T/+) embryos and compare them with those of normal littermates in order to determine whether the tissue destruction of the

tail of T/+ mutants resembles the picture that has been described for involution of other structures such as the larval tadpole tail during metamorphosis.

The study was extended to include a histochemical assay of acid phosphatase, a marker enzyme for lysosomes, and to compare the appearance of this enzyme in the regressing tails of T/+ embryos with that of littermates carrying the normal alleles (+/+).

The phenotypic effect of the T/+ mutant is not limited to resoption of varying portions of the caudal axis but includes the development of vertebral anomalies in the remaining tail stump. A second objective was, therefore, to study some of the morphogenetic changes which occur in the surviving portions of the tail mesoderm of T/+ mutants in hopes of understanding how the mesoderm is transformed into abnormal and malformed vertebrae.

The expression of the T gene may be altered under the influence of different genetic backgrounds. Dunn (1942) has demonstrated that in T/+ mice which were backcrossed to the Bagg albino strain, the manifestation of the T gene was reduced. A similar shift toward a more normal tail length has also been reported with a DBA strain by C. V. Green (1936). The present study includes an attempt to identify some of the processes of the developmental pathology in the Brachyury mutant which are subject to modifier influences on different genetic backgrounds. Specifically, the purpose was to find out whether all the manifestations of the Brachyury syndrome are affected in their expressivity by a given set of modifier factors and whether the factors influencing expressivity of the T gene also influence the penetrance of that gene. Finally, an effort was made to

ascertain the time and stage of embryogenesis when the morphogenetic effects of modifier action of genetic backgrounds on the phenotypic expression of the T gene (T/+) becomes apparent.

III. Histology and Ultrastructure of the T/+ tail and +/+ tail.

A. METHODS

Black spot Brachyury mice (T/+), obtained from the laboratory of Dr. D. Bennett and from the laboratory of Dr. L. C. Dunn, were mated either inter se or to normal (+/+) Swiss albino females and at 17 days of gestation embryos were removed in cold saline solution. The progeny were separated by phenotype into those with normal tails representing the (+/+) genotype and those with short tails, including a tail filament representing the (T/+) genotype. Tails were dissected from the body of the specimens and transferred to cold 3% glutaraldehyde in Millonig's (1961) phosphate buffer (pH 7.3) and fixed for two hours at 4° C.

The specimens were then washed four times in 0.1 M Millonig's buffer with 2 mM $CaCl_2$ and 0.3M sucrose. The specimens were postfixed ice-cold in 1% osmium for two hours, washed in cold distilled water, processed in a graded series of alcohols and embedded in Epon.

Sectioning was done with glass and diamond knives on a Porter-Blum MT-1 ultramicrotome. "Thick" sections (1μ) stained with Azure II and 1% methylene blue (modified after Richardson, et. al., 1960) were prepared for light microscope histological examination. Thin sections were mounted on uncoated copper grids and stained with uranyl acetate and lead citrate. Microscopy was performed using Philips EM 300 and Zeiss EM9S electron microscopes.

Four tails from Brachyury embryos and two tails from normal embryos, obtained from two different litters, were sectioned and observed. Sections were made at several different levels of the filament and also at the prospective adult tail tip (Figure 2).

B. RESULTS

1. Light microscopy of the tails of normal (+/+) embryos 17 days of gestational age.

As indicated in Figure 3, a semithin cross-section (about 1 μ) through the distal portion of the mouse tail at 17 days of gestational age shows a thin epithelium enveloping the tail. The oval profile of the neural tube, consisting mainly of columnar cells, is located more apical to the circular, solid section of the notochord. Dark-staining erythrocytes of various shapes are seen in blood vessels next to the neural tube and near the periphery of the tail (Figure 3). Within the cytoplasm of the numerous loosely-packed mesenchyme cells are found occasional circular dense-staining granules. This histological pattern is typical of sections taken throughout the normal mouse tail.

2. Light microscopy of the tails of Brachyury (T/+) embryos 17 days of gestational age.

The tail filament of the Brachyury embryo is surrounded by a thin epithelium and erythrocytes are seen in vessels (Figure 15). In contrast to the normal tail, however, no neural tube or notochord is found in the tail filament of the Brachyury embryos. The central core of the filament, composed of presumably mesenchyme cells or phagocytes, or both, contains dense bodies occurring singly or in clusters (Figure 15).

3. Electron microscopy of the tails of normal (+/+) embryos 17 days of gestational age.

Epithelium. The epithelium consists of large cells, typically arranged in two or three layers (Figures 4 and 6). The intercellular spaces appear more prominant than commonly encountered in epithelia generally, so that they may have arisen from artifactual changes during glutaraldehyde

fixation. The apical surface of the epithelium may appear as a narrow cytoplasmic isthmus or extension of cells whose cytons are out of the same section (Figures 4 and 6), or it may have a juxtanuclear association (Figure 5). In the nucleus of epithelial cells may be found dense, irregular chromosomal patches at the periphery, and a dense, circular nucleolus near the center. The cytoplasm of epithelial cells is characterized by sparse granular endoplasmic reticulum, numerous polyribosomes, circular or elongate profiles of mitochondria containing few cristae, Golgi bodies and vesicles.

Neural Tube. The neural tube consists of closely-apposed, elongated cells with surface cytoplasmic flaps projecting into the tubular lumen and into intercellular spaces (Figure 7). Neural tube cells contain Golgi saccules and associated vesicles, mitochondria, few cisternae of the rough endoplasmic reticulum and numerous polyribosomes.

Blood Elements. Blood vessels contain erythrocytes in various stages of development. A large population of the blood cells are normoblasts, some with their nuclei still present and others with their nuclei extruded (Figure 8). Numerous polyribosomes, some vesicles and occasional mitochondria are encountered in young red blood cells. Other blood cells found include polymorphonuclear leucocytes with characteristic surface villi, and anucleate thrombocytes containing vesicles, rough endoplasmic reticulum and mitochondria.

Subepithelial Tissue. Mesenchyme cells appear to predominate in the subepithelial spaces of the normal tail (Figures 9, 10, 11, and 12). The typical architecture of these cells consists of a large nucleus, few cisternae of the rough endoplasmic reticulum, numerous polyribosomal aggregates, Golgi complexes and associated vesicles, and circular and oval

profiles of mitochondria. A few dense lysosome-like bodies and membrane-limited vacuoles presumably containing cell debris are encountered occasionally within or near some cells (Figures 13 and 14).

4. Electron microscopy of the tails of Brachyury (T/+) embryos 17 days of gestational age.

Epithelium. The architecture of epithelial cells of the tail filament of Brachyury embryos appears similar to that described for the normal tail (Figures 16 and 17).

Blood Elements. No morphological difference could be discerned between erythrocytes, thrombocytes and endothelial cells in mutant tails (Figures 18 and 19) compared to these cell types found in normal tails. Images of polymorphonuclear leucocytes are not available thus far to compare with those found in blood vessels of the normal tail.

Subepithelial Tissues. In contrast to the picture presented by the subepithelial cells of the normal tails, the morphology of comparable cells sectioned at levels A, B, and C (Figure 2) of the tail filament of Brachyury embryos displays a dramatic difference. Their cytoplasm contains numerous dense membrane limited vacuoles, which, because of their morphological characteristics (Scharrer, 1964, 1966; Weber, 1967) are referred to as lysosomes (Figures 20 to 36).

Some of the cells containing these dense lysosomal bodies are probably mesenchyme undergoing necrosis, and others are probably phagocytes charged with ingesting remains of morbid cells. Similarities in the morphology of the nucleus, granular endoplasmic reticulum, polyribosomes, Golgi bodies and mitochondria between these two cell types make it difficult to distinguish between the two cell populations. Interestingly, the

presence of mitotic figures (Figures 22 and 23) suggests that continued cell division of some mesenchyme cells appears to accompany disintegration or phagocytosis of others.

Lysosomal bodies of the mutant tail illustrate a variable morphology in that they may contain (1) a dense matrix of uniform density (Figures 33 and 35), (2) a "dense core" in a matrix of lesser density (Figures 32 and 36), and (3) scattered particulates (Figures 24, 27, 29, and 35).

Capture and engulfment of cell debris following cell death is suggested by profiles of certain circular or ovoid membrane-bounded vacuolated bodies in intercellular spaces (Figures 20, 21, 30, 31, and 34) and within the cytoplasm of presumed phagocytes (Figure 21). Presumed phagocytosis of such cell debris is shown in Figures 20, 34 and 36. Moreover, the apparent ingestion by one or two phagocytes of an entire morbid cell containing a nucleus and lysosomes is suggested by Figure 35.

A majority of cells surveyed from sections cut at the base of the tails from T/+ embryos proximal to the filament (level X, Figure 2) contains no lysosomal bodies within their cytoplasm, but resemble in morphology the cells found throughout the length of the normal tails. The few cells containing lysosomal bodies found in this area (Figures 39 and 40) are as infrequent in number as those found throughout the normal (+/+) tails (Figures 9 to 14).

C. CONCLUSIONS

In the present study, morphological evidence at the electron microscope level suggests that (1) lysosome-like cell organelles are occasionally found in the normal mouse tail (Figures 13 and 14), and in the more proximal non-regressing portion, that forms the stump of the Brachyury tail (Figures 39 and 40), and (2) numerous lysosome-like bodies are encountered with great frequency throughout the filamentous end of the Brachyury tail where extensive resorption is taking place (Figures 20 to 36).

In the filament of the Brachyury tail stained with methylene blue and examined at the light microscope level, the intensely-stained cytoplasmic granules are presumably equivalent to many or all of the dense lysosomal bodies observed in the electron micrographs.

Although the actual formation of the electron dense bodies described in the preceding section could not be followed, there is justification for calling these structures found so abundantly in the T/+ tail filament either "lysosome-like" bodies or "autophagic vacuoles." As defined by de Duve (1963), lysosomes are membrane-bounded cytoplasmic inclusions containing hydrolases for the digestion of endogenous and exogenous cell constituents. In some sections (Figures 24 and 35) it appears that a membrane surrounds these bodies.

Essner and Novikoff (1961), Novikoff (1963) and others have shown that dense reaction products accompanying acid hydrolase activity are reliable markers for identifying lysosomes with the electron microscope. Assay for acid phosphatase within the dense granules of the T/+ tail filament by electron microscopic histochemistry would provide the most critical test for identifying them as lysosomes. However, the demonstration by light microscope histochemistry that the T/+ tail filament contains scattered areas of intense staining for the enzyme (see Section IV) lends further support to the claim that the dense bodies and vacuoles observed in the tail filament of the T/+ embryos are probably lysosomes of cells that are in terminal or near terminal stages of involution or digestion.

The implied role of lysosomes in the regressing Brachyury tail adds to the findings of other workers who have studied the cytology and biochemistry of degenerating tissues in invertebrates and vertebrates. As an example of work in invertebrates, Scharrer (1966) found that lysosomes play a decisive role in the autolysis of the regressing prothoracic gland of blattarian insects. She noted that lysosomes appear in active glands, but undergo a significant increase in numbers during early phases of regression. Autophagic vacuoles, some with recognizable cytoplasmic constituents,

also abound. Additional ultrastructural and cytochemical studies of Osinchak (1966) confirmed Scharrer's morphological evidence that the dense bodies of insect prothoracic glands are indeed lysosomes.

Among vertebrates, the regressing tissues in amphibians have been studied extensively. Using Xenopus, Weber (1964, 1967) reported that the earliest ultrastructural signs of connective tissue and muscle degeneration in regressing tails is an autolytic process which apparently is independent of enzymes from preformed lysosomes. Furthermore, he observed that in later stages of involution, macrophages with acid phosphatase-rich organelles appear to play a role in phagocytosis and catalysis of tissue debris (Figure 41).

Lysosomal enzymes have also been implicated in the involution of other vertebrate tissues. Scheib (1963) found that regressing tissues of Müllerian ducts of chick embryos of chick embryos contain acid hydrolases in lysosomal particles. Jurand (1965) reported strong acid phosphatase reactions in protoplasmic organelles of cells undergoing necrosis in mouse and chick limbs. Saunders (1966) and his co-workers have shown a correlation between distribution of cell deaths and sculpturing of the contours of the wing bud in chick embryos. Their findings of "macrophages" containing large phagosomes (Figure 42) resemble closely the ultrastructural picture present in the T/+ tail filament.

It is interesting to recall the thoughts of Holt (1963) on the possible origin and role of macrophages in regressing tissues. Recognizing that macrophages may invade tissues, he proposed that they may also arise via transformation from other cell types. Among possible evidence for transformation he cited the above work by Weber and that of Miss Kathleen Ballard in his laboratory who has "strong evidence suggesting that mesenchymal cells in the rat foetus can be transformed into macrophages as a result of the proximity of cells dying during regressive changes." A similar transformation may occur in regressing Brachyury tail tissue of the mouse fetus, as suggested by the fact that it is difficult to distinguish between mesenchyme cells and phagocytes on a morphological basis unless pseudopod-like extensions (Figures 20, 34, and 36), presumably extended in acts of engulfment, may be interpreted as belonging to phagocytes.

The electronmicrographs of the Brachyury tail obtained so far do not permit one to decide with confidence whether cells containing the lysosomal dense bodies are autocatalytic or whether they are invading macrophages, or whether both cell types contribute to the resorptive process in tails of T/+ embryos. Macroscopic observations reveal numerous hematomas in the tips of the tails

of T/+ embyros. The hematomas are probably due to fragmented capillaries and ruptured large blood vessels that possibily could be a source of macrophages in the core of the tail filament. Futhermore, the presence of cell debris in the Brachyury tail (Figures 20, 21, 22, 34, and 36) is suggestive of cell necrosis, possibly of autophagic origin. Thus, it seems reasonable to assume that both macrophagic and autocatalytic cells participate in the resorptive processes of tail tissue in the T/+ filament.

The present study implies that cell degeneration in the T/+ filament most probably follows the same model found applicable to other systems of tissue involution.

The processes of cell death and tissue involution during vertebrate embryology and insect and amphibian metamorphosis occur in a well defined and predictable manner. It is likely that degeneration during normal development is under genetic control, and it is not surprising, therefore, that there are mutant genes that express themselves by modifying normal patterns of cell death or by causing involution of tissues in which cell death does not normally occur.

The observation that degenerative processes are operating not only in tail tissues of mutant T/+ embryos but are taking place also along the tail axis of the normal (+/+) embryonic littermates, suggest that the extent of cellular dgeneration occuring in normal development of the tail skeleton is merely exaggerated in the T/+ mutant. Similarly, Zwilling (1942, 1964) found that the mutant phenotype of "rumpless" in the hen results from the extension of regularly occuring regions of degenerating cells in the tail bud. The extent

of necrosis is directly correlated with the degree of tail reduction.

The Brachyury mouse is not the only abnormal phenotype in which cell death is one of the stages by which a mutant gene gains phenotypic expression. Observations by Loewenthal (1957) on the genetically "creeper" chick embryo and by Milaire (1962) on mice with oligosyndactylism show a similar sequence of events leading to cell destruction in the apical ectodermal ridge.

It remains for future research to determine the underlying causes and events that precede and initiate cell death.

IV. Histochemistry of the T/+ and +/+ tails.

A. METHODS.

The simultaneous coupling azo dye methods of Burstone (1958) and Barka (1960) were used for the demonstration of lysosomal acid phosphatase activity. Embryos between the ages of 15 to 20 days of gestational age were obtained from Swiss albino female mice mated with Black Spot Brachyury heterozygotes. Seven tails from normal (+/+) and 12 from Brachyury (T/+) littermates obtained from four different litters were removed in cold (4° C) saline solution (0.09%). The tails were drained, frozen on dry ice, and sectioned in the cryostat at 10-12 μ. Longitudinal and cross-sections were thawed onto warm coverslips and stored at -15° C prior to processing.

Sections of normal and Brachyury tails were fixed simultaneously ("back-to-back") in the same dishes of cold (4° C) filtered calcium formol. After rinsing in cold distilled water, sections were incubated at 20° C, "back-to-back", in the Barka (1960) medium containing the substrate sodium naphthyl acid phosphate (Sigma) and diazotized pararosanilin in veronal acetate buffer, pH 6.5. The Burstone (1958) medium contained naphthol AS-TR phosphate as substrate, diazotized pararosanilin, and veronal acetate buffer, pH 5. Sections were counterstained in methyl green (pH 4), dehydrated in a graded series of ethanols, and mounted in synthetic resin.

B. RESULTS.

1. Normal (+/+) tail.

With the methods employed in this study, acid phosphatase acitivity was confined to the cytoplasmic portions of cells. Nuclei displayed no activity and were visualized only with the methyl green counterstain. Observations on material incubated in both the Barka (1960) and Burstone (1958) media were similar.

Epidermal cells displayed low levels of diffuse cytoplasmic acid phosphatase activity, but occasional cells contained small clusters of particles which were enzyme-active and hence bright red in color. Cells in the dermis were negative for acid phosphatase activity, but in the fibrous elements immediately beneath the stratum germinativum, activity was moderate and diffuse.

The central core of the tail consists of closely packed, concentrically arranged, flattened notochordal cells ventral to the neural tube. The cytoplasm of the cells display diffuse and light acid phosphatase activity. In some such cells, however, intensely active granule clusters are encountered.

Peripheral to this dense axis loosely packed clusters of cells originating from mesodermal somites are found. The cells, presumably sclerotome centra, display only light to moderate diffuse activity, as do some of the loosely packed mesenchyme cells that fill the remaining area between dermis and central axis. Occasionally, some of the positive staining cells within the mesenchyme are characterized by prominent intensely active clusters of cytoplasmic granules that exhibit the enzyme activity (Figure 43).

2. Brachyury (T/+) tail.

In the Brachyury tail immediately proximal to the resorbing filament (level X, in Figure 2), the dense notochordal axis, the neural tube and the accompanying sclerotomic mesenchymal condensations are absent. Instead, the subdermal core of the tail is filled with loose mesenchymal and fibroblast-like elements and possibly macrophages, all of which display only slight (background) diffuse staining (Figures 45 and 46).

Along the length of the filament distal to the point of constriction (levels A, B, and C in Figure 2), the frequency of cells staining intensely for acid phosphatase activity increases markedly. This activity is particularly evident in the mesenchyme where numerous islands of cells are encountered which display clusters of granules staining intensely for this enzyme (Figures 44, 45, and 46). The identity of cells exhibiting the positive enzyme reaction cannot be determined with certainty, but most likely both mesenchyme and macrophages contribute to it.

In addition, there are distributed throughout the base and filament of T/+ tails, but not of normal (+/+) tails, cells approximately 10-15 μ in diameter whose cytoplasm is filled with brown granules. These granules display no acid phosphatase activity but resemble those that have been described for mast cells.

C. CONCLUSIONS.

The demonstration of intense acid phosphatase activity in filaments of tails of Brachyury embryos and its relative absence in corresponding sections of tails of normal embryos is offered as supporting evidence for lysosomal activity during the process of tissue resorption that leads to the shortening of the tail in this mutant.

V. Pathology of the T/+ mutant revealed by macroscopic measurements and observations.

A. METHODS.

For the observation of skeletal morphology, one and ten-day old mice were sacrificed, fixed in 95% alcohol for 24 hours, and whole mounts prepared according to Dawson (1926). The clearing and staining of specimens was carried out according to the techniques of M. C. Green (1952) and Burdi (1965). For the demonstration of the bony skeleton, specimens were skinned and eviscerated, then placed in 1% fresh KOH solution for 24 hours then transferred to glycerin-KOH (1:9) mixture with a few drops of 0.5% alizarin red solution added, and after several changes of glycerin-KOH stored in pure glycerin for observation. To demonstrate cartilage, specimens were transferred, after clearing in KOH, into a 0.06% toluidine blue solution made in 70% alcohol and were allowed to remain there until a blue color indicating presence of cartilage appeared.

Morphological observations were carried out with the use of a binocular dissecting microscope. Specimens were examined for abnormalities of the caudal skeleton. Counts of caudal vertebrae were made using the sacral vertebrae as a reference point (Cook, 1965). The vertebra posterior to the fourth sacral was designated as the first caudal.

In order to determine whether the mesoderm that ultimately remains to form the much reduced and frequently malformed tail remnant in the T/+ mice is capable of chondrification and ossification, a pilot project was set up which involved growing mesoderm obtained from distal portions of tails of T/+ embryos in organ culture.

The grid technique described in part by Paul (1960) and modified by

Yoshihara (1966) was employed. According to this modification, explants of tail segments prepared under sterile conditions were removed in Hank's balanced salt solution and the explant was placed onto a sterile, stainless steel grid which was placed into a plastic well-dish filled with medium. The medium consisted of 88 ml of Trowell's T8 medium, 10 ml of fetal calf serum, and 2 ml of streptomycin.

Tail rudiments of 16-day old embryos were chosen for culture because Yoshihara (personal communication) found that tail rudiments of 16-day old embryos develop typical vertebral structures in culture. Explants, obtaining 4 to 8 prospective vertebral elements, were carried in culture for five days with one medium change after 2.5 days and incubated at 30°C in 10% CO_2 + 90% gas phase. Tail rudiments of seven Brachyury embryos from two different litters and tail rudiments from eight normal embryos obtained from three different litters were used for culturing.

In order to determine the state of maturation reached by tail segments of 16-day old embryos grown in vitro for five days, the explants were compared at the end of the culture period with corresponding tail segments of newborn mice or of mice whose gestation period was terminated at 21 days. For this purpose whole mounts of tail segments from five-day old cultures and from newborn mice were stained with toluidine blue for cartilage and with alizarin red for bone, or sectioned at 10μ, stained with hematoxylin and eosin, or by the tri-chrome method, and examined microscopically for the appearance of cartilage or bone.

B. RESULTS.

1. Morphology of the caudal axis of T/+ embryos.

Embryos heterozygous for the T gene obtained from crosses of ♂Black

Spot (T/+) X ♀Black Spot (T/+) can easily be distinguished from their normal littermates by the 11th day of gestation by the possession of a tail filament. At that time the T/T embryos have died and the expected 2:1 ration of heterozygotes to normal prevails.

2. Postnatal morphogenesis of the caudal axis of T/+ mice (birth to 10 days).

By the tenth day of postnatal age, two basic characteristics distinguish the heterozygote from that of the normal littermate: (1) a much reduced length of the tail (Table 1 and Figure 63), and (2) structural abnormalities of caudal vertebrae in the distal portion of the tail (Figures 47 to 55).

Whole mounts of skeletons, stained for cartilage and bone respectively, of ten-day old heterozygotes and normal specimens were compared with respect to (1) the number of caudal vertebrae, and (2) the morphology of vertebral elements forming the tail. Essentially, three types of defective processes were identified contributing to the abnormal morphology of the tail characteritic of Brachyury animals. These were:

(1) Reduction in number of tail vertebrae. As shown in Table 2, the mean number of caudal vertebrae in normal phenotypes of all strains tested is between 26-27 at one day of age. This number does not change any more between one day and tend days of postnatal age (Figures 64 and 65).

The number of caudal vertebrae found in Brachyury mice varies between 15-18 at one day and 16-23 at ten days of age.

(2) Fragmentations. Fragmented vertebrae representing incompletely formed centra, were found mainly at the distal tip of the tail. The vertebral fragments were mainly represented by cap-like wedges or ill-defined slips of bone that were oriented at an angle to one another (Figures 49, 50 and 54).

(3) Ankylosis. These represented fusion occurring between two adjacent normally shaped vertebral processes or between vertebral fragments. Such fusions always occurred at margins and the angles formed from such ankyloses gave the tail a bent and crooked appearance (Figures 49, 50, 53).

3. Maturation of caudal vertebrae in vitro.

Examination of whole mounts and of histological sections stained for cartilage and bone of five-day organ cultures of tail segments obtained from 16-day old normal embryos revealed that the more anterior tail vertebrae that were already pre-formed in cartilage at explantation either ossified or maintained the cartilaginous states in culture, while mesenchymous segments taken from the tips of tails of normal embryos chondrified in culture. This pattern of chondrification and ossification observed in normal littermates was the same in explants of mesenchyme obtained from tails of T/+ embryos (Figures 56 to 61).

C. CONCLUSIONS.

The anomalies of the Brachyury syndrome can be resolved into three major categories: (1) the reduction in number of caudal vertebrae leading to shortening or loss of the tail, (2) fragmentation of vertebrae, and (3) fusion and ankylosis of vertebrae or vertebral fragments.

The reduction in number of caudal vertebrae is mainly responsible for the shortening of the posterior axis, while fusion and fragmentation of vertebral processes lead to structural defects of the remaining tail axis, and probably account for the kinks and the blunting of the Brachyury tail.

The nature of the pathology of the caudal mesoderm that must precede the subsequent abnormal vertebral morphogenesis remains unidentified. The development of T/+ and T/T mice has been studied by Chesley (1935),

Grüneberg (1958a) and Theiler (1961). Based on studies of the early embryology of T/+ mutants these authors agree that the ultimate fault lies with the notochord. In T/+ embryos the notochord of the tail tends to become incorporated wither into the neural tube or the tail gut and eventually ceases to function as an inductor of adjacent neural tube or mesoderm. The portion of the tail lacking a functional notochord becomes manifest by a constriction along which all existing tail structures break down by a process of cell degeneration.

The findings presented here contrast to those of Chesley (1935), Grüneberg (1958a), and Theiler (1961) who studied T/+ between 10-20 days of gestational age, in that they are based on observations of the postnatal development of this mutant. It is suggested that the loss of degenerating distal tail mesoderm, while it may account for reduction in length and extent of the caudal column, does not adequately explain the abnormal morphogenesis of the remaining tail structure that is part of the T/+ syndrome.

The various structural defects in the architecture of the vertebral column that becomes manifest in T/+ postnatal mice suggest that the tail mesoderm that survives to form the blunted, often kinked, looped or bent tail of Brachyury mice must contain some intrinsic pathological defect or reduced growth potential that expresses itself in the faulty morphogenesis of the caudal vertebrae. This may explain the observation that in Brachyury heterozygotes distal caudal vertebrae are still formed and added on until some time between 5-10 days of postnatal age, while in normal littermates, formation of caudal vertebrae has been completed at birth. It may also explain the observation that most of the morphological tail defects such as fusion, fragmentation and angulation occur mainly along the more distal portion of the caudal axis,

and invariably involve the very tip of the tail. Along this gradient of pathology, the most distal tail mesoderm may be completely resorbed leading to the shortening and stunting of the tail in T/+ mice. Proximally, resorption and tissue destruction may be less drastic and severe and becomes visible by the incomplete vertebrae that are formed at the tip of the shortened tail of Brachyury mice.

The limited results obtained from the in vitro experiments can be interpreted to mean that the surviving caudal mesoderm in Brachyury heterozygotes is fully capable of undergoing histological differentiation to form cartilage and bone. The incidence of incompletely formed vertebrae in the distal portion of the tail of T/+ mice is, therefore, most likely not the result of competence of distal tail mesoderm to undergo chondrification or ossification, as was shown to be the case for T/T mesoderm (Bennett, 1958).

The events responsible for the specific malformations of caudal vertebrae of T/+ tails can only be speculated upon. The fragmentation of distal tail vertebrae, a major morphological anomaly of the Brachyury syndrome, seems to represent either incompletely formed centra or centra lacking typical processes. Possibly, the fragmentation is due to insufficient mesenchymal aggregation rather than secondary tissue destruction of initially normally formed structures.

It is interesting that no other mutant in the mouse whose axial skeleton is deformed has such extensive fragmentation. The literature makes no mention of the possible mechanisms that might be involved in the formation of this type of abnormality.

A possible mechanism causing fusion and ankyloses of adjacent skeletal

elements may involve a disturbance in resorptive processes. This hypothesis is speculative but deserves attention because it has been advanced as a general mechanism in the shaping of skeletal parts (Saunders and Fallon, 1966). These authors have shown that selective cell deaths may play a role in the sculpturing of limb parts in birds to the extent that a programmed death of cells along a margin may account for the morphological carving out of digits. In line with this idea, it is proposed that the ankyloses between vertebrae may be the result of the suppression of a portion of the death program of cells which in normal conditions may bring about the contouring of adjacent somites to form the typical row of vertebrae.

VI. Analysis of penetrance and expressivity of the T/+ gene.

A. METHODS

A stock of Brachyury (T/+) mice of the Black Spot strain obtained from the laboratory of Dr. D. Bennett and Dr. L. C. Dunn, inbred for many generations, was used. The C57bl/6, DBA/2 strains maintained at the Roscoe B. Jackson Laboratory, Bar Harbor, and the Swiss albino strain, obtained from Charles River Laboratories, served as genetic backgrounds for outcrossings. The choice of these strains was suggested by older reports of Green (1936) and Dunn (1939) showing that the expression of some tail mutations differ in the DBA strain, and by more recent reports in the literature that there exist differences in response to the action of some teratogenic agents known to interfere with skeletal development when these strains were used as genetic backgrounds (Hamburgh and Callahan, 1967; Smithberg, 1967). Tested heterozygotes for the Brachyury gene were reciprocally outcrossed to these strains.

Mutant and normal phenotypic littermates obtained from each genetic background were analyzed with respect to tail length, skeletal morphology, and counts of caudal vertebrae. Measurements of crown-rump length and total linear tail length were carried out with the use of a millimeter ruler on all F_1 progeny. Specimens were prepared for demonstration of vertebral morphology by the techniques employed in Section V.

B. RESULTS.

1. Influence of different genetic backgrounds on the expressivity of the T/+ gene.

At 16 days of gestational age, the embryos heterozygous for the T gene could easily be distinguished from their normal littermates by possession of a tail filament. At one day of age, mice heterozygous for the T gene could be easily distinguished from their normal littermates by their shortened tails, but the degree of shortening was the same on all backgrounds tested (see Table 1). At five and ten days of age, the differences in tail lengths between mutants from each strain became more apparent (Table 1). Whole mounts of skeletons obtained from mice of different strains and stained for cartilage and/or bone revealed, however, that at one day of age, heterozygotes for the T gene on the Black Spot background had the lowest number of caudal vertebrae, while mutant offspring from ♂ Black Spot (T/+) X ♀DBA (+/+) crosses had the greatest number of vertebrae. Offspring from the other crosses tested had intermediate values (Table 2).

In offspring of three crosses ♂ Black Spot (T/+) X ♀DBA (+/+) the number of caudal vertebrae present in mice carrying the T gene almost approached the number present in normal littermates at ten days of postnatal age (see Table 2).

The distribution of morphological defects of the vertebrae also varied on the backgrounds tested, but to a much lesser extent, than did vertebral numbers.

The frequency of fragmentation was somewhat higher in Brachyury mice of the Black Spot strain and lower in Brachyury mice bred into the DBA strain. (Table 3).

The frequency of vertebral ankyloses, the third type of defect found in T/+ heterozygotes was the same in the Black Spot C57B1/6 and Swiss albino F_1 hybrids but in the DBA hybrids, the expression of this aspect of the Brachyury gene was reduced to 0. (Table 3). A small number of reciprocal crosses revealed that the shift toward the more normal phenotype exerted by the DBA background on the Brachyury gene occurred only if the DBA genotype was contributed by the female parent. The frequency of fragmentation formed an exception to this rule (see Table 3).

2. Influence of genetic background on penetrance of the T/+ gene.

Outcrossings of Black Spot (T/+) mice to C57B1/6 and Swiss Albino strains resulted in numbers of progeny conforming to the expected ratio of 1:1, one normal to one Brachyury animal. A deviation from this ratio occurred only in crosses of Black Spot (T/+) to DBA strain. The progeny obtained from crosses between ♂Black Spot (T/+) X ♀DBA (+/+) yielded twice as many phenotypically normal appearing animals as was the case between crosses of ♂Black Spot (T/+) X ♀Black Spot (+/+) or ♂Black Spot (T/+) X ♀C57B1/6 (+/+) and ♂Black Spot (T/+) X ♀Swiss albino (+/+) (see Table 4). Reciprocal crosses did not change these ratios.

C. CONCLUSIONS

This study tends to confirm similar observations reported by Green (1936) that the expressivity of the T gene can be suppressed on the DBA background. Although this sample is admittedly small, it appears that not all the morphogenetic effect of the T gene are modified to the same extent in the DBA F_1 hybrids.

Unlike in the study reported by Green (1936), in this study the expressivity of the T locus was not changed if the DBA background was provided by the male parent, but a change in expressivity of the T gene was noted in a small sample of reciprocal crosses with DBA females.

It is unfortunate that the number of crosses between DBA/2 females and T/+ males was so small, because breeding difficulties were experienced with DBA/2 females due to periods of infertility which occur not infrequently in this strain. In spite of the limited number of the sample, differences in phenotype between T/+ heterozygotes obtained from reciprocal outcrosses to a DBA/2 father as compared to a DBA/2 mother consistently reappear when different age groups were examined or where different parameters such as tail length and caudal vertebrae count are studied (see Table 1 and 2, column 3b, 4b).

Maternal environment or cytoplasmic inheritance are usually invoked to account for genotypic or phenotypic differences obtained from reciprocal crosses, and I have at the present no basis for choosing between either of these alternatives.

In the small sample examined, the modification of the T gene on the DBA background resulting in a shift toward a more normal tail become progressively more apparent during early postnatal development (see Tables 1,2).

This suggests to us that "modifier action" may be operating through some kind of "repair" or "correcting" mechanism that tends to restore the caudal vertebral axis in some Brachyury mice so that it appears almost normal by the tenth day of postnatal age. Some evidence that ability to repair damage of embryonic tissue is indeed under genetic control was presented by Hamburgh and Callahan (1967).

In contrast to the modifiers protecting against expressivity of the T gene the factors that tend to increase or decrease the penetrance of that gene seem to operate in a different manner, because the penetrance of the T gene on the DBA background was reduced to the same extent in offspring obtained from reciprocal crosses between T/+ and +/+ DBA parents.

The assumption is sometimes made that penetrance may be a relative term reflecting failure of the investigator to detect some subtle expression of the gene and should, therefore, be equated with (0) expressivity. Observations from this study may tend to strengthen the interpretation that penetrance may be the result of a very different mechanism than that operating to change expressivity of a gene. Expressivity may involve the operation of some factors repairing and correcting at different rates and effectiveness, the pathological processes occurring during development while penetrance may involve total repression of an operon by repressor genes, which themselves may be part of the chromosomal complex.

VII. General Discussion.

In the following section I wish to add a few general comments about some of the more fundamental problems of Developmental Biology and Developmental Genetics to which the results of this study relate and the lines of further investigation that are suggested by the findings present here.

The results of the electronmicroscopic and histochemical observations of T/+ embryos seem to imply that differential tissue regression mediated either by autolytic processes or macrophage digestion or both leads to the formation of the "short-tailed" condition of the Brachyury phenotype.

That phenomena of cell death and resorption of cells are important aspects in growth and development of tissues and organs is attested by several examples. Necrosis is a normal part of most morphogenetic movements such as invaginations, evaginations, separations and fusion of embryonic parts, migrations of rudiments, closure of tubes, formation of lumina, and changes of shape (Glucksmann, 1951).

The "utility" of cellular death in regression of transient and obsolete tissues and organs is also obvious (Saunders, 1964; Glucksmann, 1951). Examples include regression of Müllerian ducts in male embryos and the change from a pronephros or mesonephros to a metanephros in higher vertebrates.

Cellular degeneration participates in histological differentiation as well. Studies by Hamburger and Levi-Montalcini (1949) on chick nervous sytem have shown that lack of sufficient peripheral load causes death of related dorsal root ganglial cells. Cell death occurs prominently in association with differentiations of skeletal parts of the limb (Fell, 1925; Fell and Canti, 1934). Recently, Saunders (1962, 1966)

and his collaborators have shown a correlation between distribution of cell deaths and sculpturing of the contours of the wing bud in chick embryos.

The close relationship in many instances between phagocytosis and lysosomes has suggested (de Duve, 1959; Hirsh, 1964) that these bodies act as "suicide bags", with their rupture leading to autodigestion and cell death. This somewhat simplistic view has been challenged by an increasing number of investigators who have contributed evidence for the conclusion that the release or activation of hydrolases is more probably the result rather than the cause of cell death (de Duve, 1966; Saunders, 1966; and Weber, 1967) and that the release of lysosomal contents in cells which are destined to die is probably triggered by both intrinsic and extrinsic factors.

Scharrer (1966) has shown that during regression of the prothoracic gland of blattarian insects, lysosome-like bodies appear and acid phosphatase activity increases considerably. In advanced stages, hemocytes invade areas of cellular atrophy in great numbers and appear to engulf debris. However, considerable changes occur in the nucleus and cytoplasm of the gland prior to phagocytic activity.

In studies of the posterior necrotic zone (PNZ) of the wing of the chick, Saunders (1962, 1966) has shown that no sign of cell degeneration occurs prior to the onset of phagocytosis and that no distinctive ultrastructural changes become apparent before the state at which the "death sentence" becomes irrevocable.

These examples indicate that cell death and tissue involution do not result from the activation of cellular "suicide bags" but rather from other processes which render it morbid and ready for lysosomal autolysis or

engulfment. The T/+ mutant may contribute a favorable system to follow both the ultrastructural and biochemical changes accompanying tissue involution during embryogenesis and because tail resorption in this mutant is under the control of a single Mendelian gene, this syndrome may also lend itself for studies of the nature of the genetic control mechanism operating in tissue resorption and cell degradation.

While the ultrastructural changes and synthesis of acid phosphatase observed in tail filaments of T/+ mutant cells are indicative of degradation these observations do not identify yet the mechanism that triggers cell death of the tail mesenchyme.

Because manifestations of degradation are not usually seen until actual cytolysis is well under way, investigations should proceed to earlier stages in the sequence of events that lead to cell death and address themselves to identification of other macromolecular by-products of necrotic cells and their possible stimulation of phagocytic or autolytic activity in neighboring cells.

Another problem that should lend itself for study is the question of whether tissues of the Brachyury tail, whose cell death presumably results from genetic instruction, can escape the "death sentence" when transplanted to animals that do not carry the T allele. Ephrussi (1935) has shown that T/T embryonic tissue cultured in vitro live past the time at which tissues die in vivo in the homozygote. However, he did not include in his study tissues of either neural tube, notochord, nor somites from the T/T embryos, nor did he extend the investigation to any of the tissues of the T/+ embryo.

The finding that the expression of the T gene is extremely variable

and subject to modifiers action of genetic background was not surprising and merely confirmed similar reports in the earlier scientific literature (Dunn, 1942; Green, 1936). The question of specificity of action of a set of background modifiers is invariably raised. Will the same set of modifiers triggered into action by one gene react with any other gene having a similar phenotypic effect? The DBA background not only reduces the expressivity of the T/+ heterozygote, but according to Smith (personal communication) also reduces the expressivity of another tail mutation, Looptail (Lp). In the case of Sd/+ and T/+ mutants, Dunn (1942), on the other hand, has shown that the Bagg albino strain modifies the expressivity of both these genes, acting to increase the expressivity of Danforth's short-tail (Sd) and decrease the expressivity of Brachyury (T).

These findings would argue against the idea that modifiers act with a high degree of specificity. Since, on the other hand, the shift toward a more normal tail accomplished in offspring carrying the T gene on the DBA/2 background is not accompanied by a corresponding increase in number of caudal vertebrae or increase in length of tail in "normal" (+/+) littermates, there is merit in the argument that modifiers exert their influence only in the presence of the T gene, a conclusion that has been reached by Dunn (1942) in the system that he investigated (Bagg albino).

The two arguments may be resolved by the suggestion that perhaps the modifiers suppressing T gene expressivity operate by introducing repair mechanisms. On this assumption one would expect that modifiers will only reveal themselves in the presence of the T gene. The normal (+/+) littermates of the DBA background, while possessing these same repair or corrective mechanisms in their cytoplasm, will never mobilize them because of the absence of the T gene in their genotype, and hence the presence of

"modifiers" is not revealed. Interference with normal morphogenetic processes either brought about through environmental agents or through the action of a deleterious gene, may activate those repair processes that are eventually recognized by the observer as expressivity of a structural gene.

VIII. Summary.

The development of the posterior axial structure leading to the abnormalities of the heterozygous Brachyury (T/+) mutant was studied on the macroscopic, the ultrastructural and the histochemical level, and the influence of different genetic backgrounds on expressivity and penetrance of the T gene was analyzed.

The Brachyury mutant in heterozygous condition is dominant, non-lethal and interferes with the normal morphogenesis of the caudal axis by (1) resorption of the distal structures of the tail leading to shortening or absence of this structure and (2) by causing many of the remaining distal vertebral processes to align abnormally resulting in blunting and kinking of the tail.

The present study revealed that the extensive cell death that occurs during tissue regression of the distal portion of the tail of the Brachyury (T/+) mutant is accompanied by considerable phagocytic and/or autophagic activity. The increase of lysosomal dense bodies and/or autophagic vacuoles in regressing tails of the Brachyury mutant is correlated with a significant rise in acid phosphatase activity in the tail filament of the T/+ embryos.

Study of the developmental changes encountered in T/+ mice from the 16th day of gestation until the tenth day of postnatal age revealed, furthermore, that three different processes contribute to tail abnormalities; these are: (1) reduction in number of tail vertebrae, (2) fragmentation of vertebral elements, and (3) fusion of adjacent vertebrae.

Organ culture of tail segments obtained from 16-day old embryos of T/+ heterozygotes and their normal littermates has shown that within a five-day culture period chondrification and ossification of caudal axial

explants can occur in vitro. The appearance and extent of vertebrae formed in vitro from tail rudiments of T/+ embryos was indistinguishable from those obtained from normal phenotypes.

The expressivity and penetrance of the T gene in heterozygous condition was tested on four different genetic backgrounds provided by the Black Spot strain, the C56bl/6 strain, the DBA/2 strain, and the Swiss albino strain.

On the DBA/2 strain, the expressivity of the T gene was considerably modified in the "normal" direction only when the DBA background was provided by the female parent. The penetrance of the T gene was also shifted from the expected 1:1 ratio to a 2:1 ratio in both reciprocal outcrossing to the DBA strain.

These latter results are interpreted to mean that some of the modifiers that shift expressivity of the T gene are probably cytoplasmic in origin and act by initiating "repair" processes, whereas the factors responsible for penetrance of the T gene are probably provided by more orthodox nuclear genes.

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Table 1. Tail length of normal and Brachyury mice at 16 days gestational and one, f
ten days postnatal age.

Cross*	At 16 Days Gestational		At One Day Postnatal	
	Normal Tail Length (cm) (a)	Brachyury Tail Length (cm)** (b)	Normal Tail Length (cm) (a)	Brachyury Ta Length (cm) (b)
1. ♂Blk Spt (T/+) X ♀Blk Spt (T/+)	0.93±.03 N=3	0.80±.05 N=9	1.3±.05 N=9	1.0±.04 N=6
2. ♂Blk Spt (T/+) X ♀C57bl/6 (+/+)	---	---	1.5±.02 N=30	1.1±.03 N=31
3. ♂Blk Spt (T/+) X ♀DBA/2 (+/+)	1.03±.03 N=3	0.79±.07 N=6	1.5±.0 N=23	1.1±.03 N=20
4. ♂DBA/2 (+/+) X ♀Blk Spt (T/+)	---	---	1.4±.01 N=83	1.0±.03 N=45
5. ♂Blk Spt (T/+) X ♀Swiss alb (+/+)	---	---	----	----

t-tests of means of tail lengths at 16 days gestational and
ten days postnatal age.

t-test between cross numbers	p at 16 days gestational age	p at one day	p at five
1 (b) and 2 (b)	----	.05 not signif.	.005 si
1 (b) and 3 (b)	.9 not signif.	.05 not signif.	.001 si
1 (b) and 4 (b)	----	.05 not signif.	.1 no
2 (b) and 3 (b)	----	.05 not signif.	.2 no
2 (b) and 4 (b)	----	.05 not signif.	.005 si
3 (b) and 4 (b)	----	.05 not signif.	.005 si
3 (a) and 3 (b)	.02 signif.	.005 signif.	.005 si
1 (a) and 1 (b)	.05 not signif.	----	----
1 (a) and 3 (a)	.10 not signif.	----	----

*Blk Spt= Black Spot
**measurements made from base of tail to tip of tail,
not including distal tail filament.

mice at 16 days gestational and one, five and

Gestational	At One Day Postnatal		At Five Days Postnatal		At Ten Days Postnatal	
Brachyury Tail Length (cm)** (b)	Normal Tail Length (cm) (a)	Brachyury Tail Length (cm) (b)	Normal Tail Length (cm) (a)	Brachyury Tail Length (cm) (b)	Normal Tail Length (cm) (a)	Brachyury Tail Length (cm) (b)
0.80±.05 N=9	1.3±.05 N=9	1.0±.04 N=6	2.3±.07 N=9	1.5±.07 N=14	3.4+.19 N=8	2.2+.02 N=14
---	1.5±.02 N=30	1.1±.03 N=31	2.4±.05 N=12	1.9±.11 N=6	3.4+.12 N=13	2.7+.08 N=15
0.79±.07 N=6	1.5±.0 N=23	1.1±.03 N=20	2.5±.00 N=2	2.1±.08 N=3	3.3+.14 N=9	3.1+.13 N=4
---	1.4±.01 N=83	1.0±.03 N=45	2.2±.05 N=42	1.7±.08 N=22	3.0+.02 N=45	2.1+.03 N=19
---	----	----	---	---	3.4+.07 N=30	2.0+1.1 N=27

ail lengths at 16 days gestational and one, five, and
e.

e	p at one day	p at five days	p at ten days
	.05 not signif.	.005 signif.	.001 signif.
	.05 not signif.	.001 signif.	.001 signif.
	.05 not signif.	.1 not signif.	.01 signif.
	.05 not signif.	.2 not signif.	.01 signif.
	.05 not signif.	.005 signif.	.001 signif.
	.05 not signif.	.005 signif.	.001 signif.
	.005 signif.	.005 signif.	.001 signif.
	----	----	----
	----	----	----

of tail,

Table 2. Mean Numbers of Caudal Vertebrae of Normal and Brachyury Specimens at One and Ten Days of Age.

Cross*	One Day (a) Normal	One Day (b) Brachyury	Ten Days (a) Normal	Ten Days (b) Brachyury
1. ♂Blk Spt (T/+) X ♀Blk Spt (T/+)	27.0±.00 N=1	15.2±.56 N=4	27.5±.30 N=8	18.8±.20 N=14
2. ♂Blk Spt (T/+) X ♀C57bl/6 (+/+)	26.7±.08 N=25	17.3±.20 N=21	27.3±.12 N=13	20.8±.17 N=15
3. ♂Blk Spt (T/+) X ♀DBA/2 (+/+)	27.4±.07 N=13	18.6±.24 N=11	26.9±.10 N=9	23.7±.50 N=4
4. ♂DBA/2 (+/+) X ♀Blk Spt (T/+)	27.3±.05 N=35	17.7±.13 N=24	26.6±.02 N=45	18.8±.23 N=19
5. ♂Blk Spt (T/+) X ♀Swiss alb (+/+)	---	---	27.3+.04 N=30	16.1+1.0 N=27

*Blk Spt=Black Spot

T-Test of Mean Number of Caudal Vertebrae at One and Ten Days.

t-test between cross numbers	p at one day	p at ten days
1 (b) and 2 (b)	.001 significant	.001 significant
1 (b) and 3 (b)	.001 significant	.001 significant
1 (b) and 4 (b)	.001 significant	.9 not significant
2 (b) and 3 (b)	.001 significant	.001 significant
2 (b) and 4 (b)	.1 not significant	.01 significant
3 (b) and 4 (b)	.005 significant	.01 significant
3 (a) and 3 (b)	.001 significant	.001 significant

Table 3a. Summary of malformations at one day of postnatal age.

Cross Number	Cross*	Total number of vertebrae obtained from (T/+) offspring	Total number of fragmented vertebrae	% of fragmented vertebrae	Total number of fusions	% of vertebrae fused
1.	♂Blk Spt (T/+) X ♀Blk Spt (T/+)	61	6	9.8	1	1.6
2.	♂Blk Spt (T/+) X ♀C57bl/6 (+/+)	311	19	6.1	2	0.7
3.	♂Blk Spt (T/+) X ♀DBA/2 (+/+)	167	8	4.7	0	0.0
4.	♂DBA/2 (+/+) X ♀Blk Spt (T/+)	389	23	5.9	1	0.3
5.	♂Blk Spt (T/+) X ♀Swiss alb (+/+)	---	--	--	-	---

*Blk Spt= Black Spot

Table 3b. Summary of malformations at 10 days of postnatal age.

Cross Number	Total number of vertebrae of (T/+) offspring	Total number of fragmented vertebrae	% of fragmented vertebrae	total number of fusions	% of fused vertebrae	Total number of angulations	% of angulations
1.	264	51	19.3	6	2.2	19	7.2
2.	334	34	10.1	6	1.8	4	1.3
3.	95	14	14.7	0	0	0	0
4.	358	26	8.1	7	1.9	7	1.9
5.	412	56	13.8	8	1.9	21	4.8

Table 4

Penetrance of the T gene at one day of post-natal age.

Cross*	Number of litters	Total number of offspring	Number of Brachyury phenotype	Number of normal phenotype	Expected ratio B:N**	Observed ratio B:N**
♂Blk Spt (T/+) X ♀Blk Spt (T/+)	8	38	25	13	2:1	2:1
♂Blk Spt (T/+) X ♀C57bl/6 (+/+)	17	109	54	55	1:1	1:1
♂Blk Spt (T/+) X ♀DBA/2 (+/+)	13	66	19	57	1:1	1:2
♂DBA/2 (+/+) X ♀Blk Spt (T/+)	16	128	40	84	1:1	1:2

Penetrance of the T gene at ten days of post-natal age.

♂Blk Spt (T/+) X ♀Blk Spt (T/+)	5	22	14	8	2:1	2:1
♂Blk Spt (T/+) X ♀C57bl/6 (+/+)	5	28	15	13	1:1	1:1
♂Blk Spt (T/+) X ♀DBA/2 (+/+)	3	13	4	9	1:1	1:2
♂DBA/2 (+/+) X ♀Blk Spt (T/+)	9	64	19	45	1:1	1:2
♂Blk Spt (T/+) X ♀Swiss alb (+/+)	6	57	27	30	1:1	1:1

* Blk Spt = Black Spot.
** B:N = Brachyury phenotype to normal phenotype.

Figure 1. Inheritance and effects on development of the Brachyury mutation in the house mouse. Results of an inter se cross between two animals heterozygous for the T gene (from Sinnot, Dunn, and Dobzhansky, "Principles of Genetics", p. 349). The illustration at 16 days of gestational age shows the filament completely resorbed. However, on the background strain used in the present study (Black Spot), the filament usually remains until birth.

Inheritance and effects on development of the Brachyury mutation in the house mouse. Note, in the 11-day heterozygous embryo, the constriction that marks the end of the notochord. The tail beyond this point has been resorbed in the 16-day embryo.

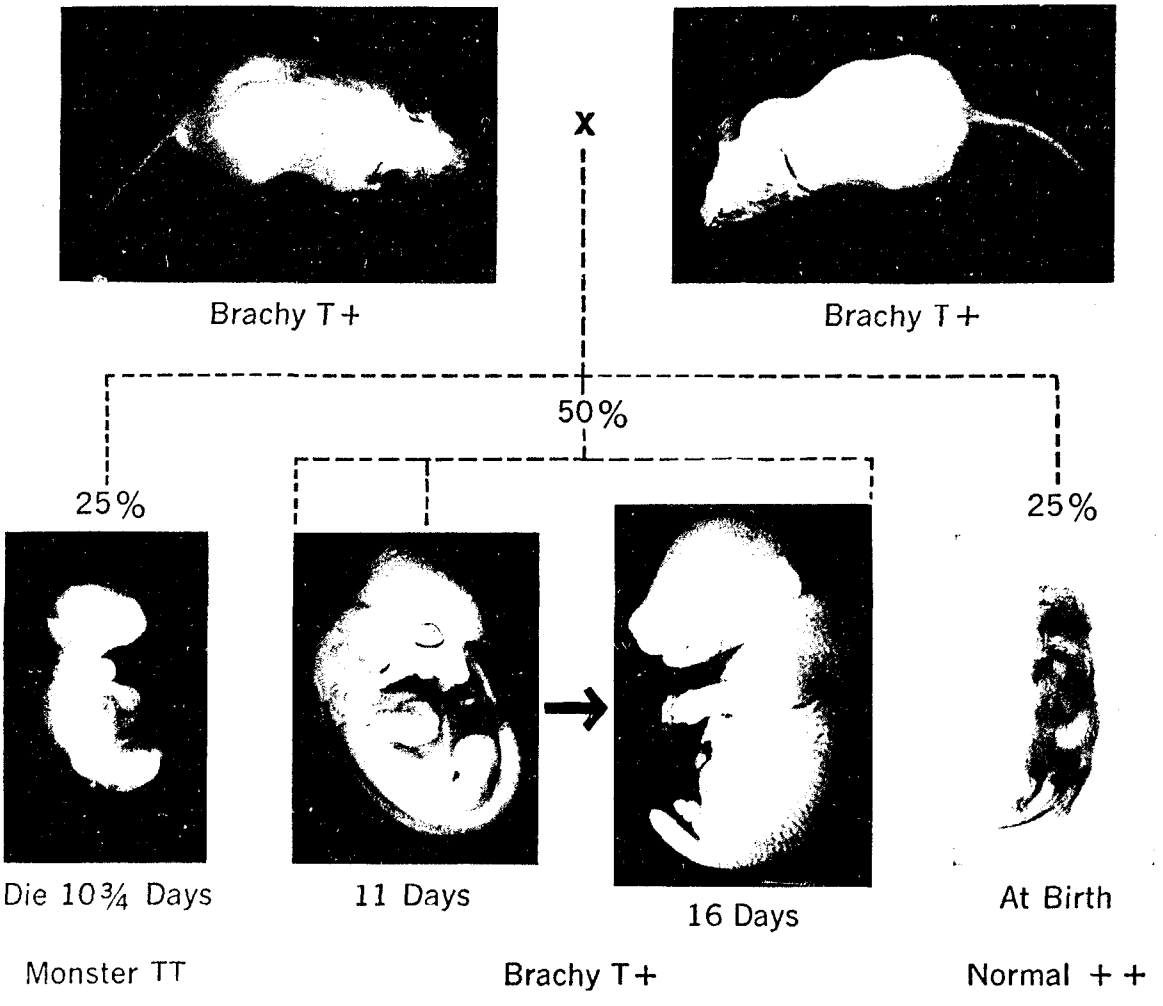
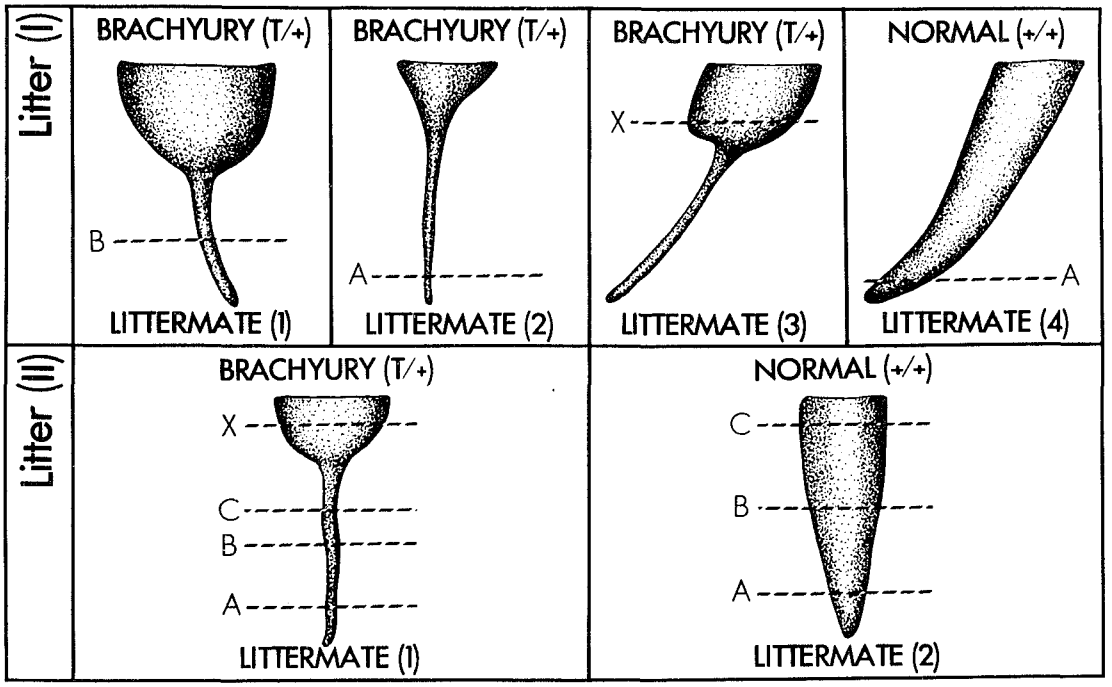


Figure 2. Drawings of tails of normal (+/+) and Brachyury (T/+) embryos at 17 days of gestational age prepared for light and electron microscopy. The letters "A, B, and C" represent levels of sectioning in either the Brachyury filament or the normal tail tip. The letter "X" represents the level of sectioning in the prospective adult Brachyury tail tip. Magnification X6.


Litter (I)
BRACHYURY (T/+)
B
LITTERMATE (1)
BRACHYURY (T/+)
A
LITTERMATE (2)
BRACHYURY (T/+)
X
LITTERMATE (3)
NORMAL (+/+)
A
LITTERMATE (4)
Litter (II)
BRACHYURY (T/+)
X
C
B
A
LITTERMATE (1)
NORMAL (+/+)
C
B
A
LITTERMATE (2)

2

List of abbreviations for light and electron micrographs, (Figures 3 to 40).

BL	basement lamina	mb	membrane
Cd	cell debris	me	mesenchyme
ce	centriole	N	nucleus
Ch	chromosome	Ne	nucleus of an endothelial cell
cm	cilium		
D	desmosome	Nd	notochord
db	dense body	Nm	morbid nucleus
dc	dense core	Nt	neural tube
Ec	endothelial cell	nu	nucleolus
e	epithelium	p	polyribosomes
er	endoplasmic reticulum	pd	pseudopod
F	surface flaps	Ph	phagocyte cytoplasm
G	Golgi complex	PL	polymorphonuclear leucocyte
is	intercellular space		
L	lumen	r	ribosomes
Ly	lysosome-like body	rbc	red blood cell
M	morbid cell cytoplasm	ss	subepithelial space
m	mitochondria	T	thrombocyte
		ve	Golgi vesicle

Figure 3. Transverse section (1 μ) through a normal (+/+) tail tip (level A) at 17 days of gestational age, stained with methylene blue for light microscopy. Beneath a thin epithelium (e) there is an ovoid neural tube (Nt) and a circular notochord (Nd). Nuclei (N) of mesenchyme cells often contain an acentric nucleolus (nu). Observe the rare presence of circular cytoplasmic dense bodies (db). (rbc) red blood cell, or normoblast. Magnification X415.

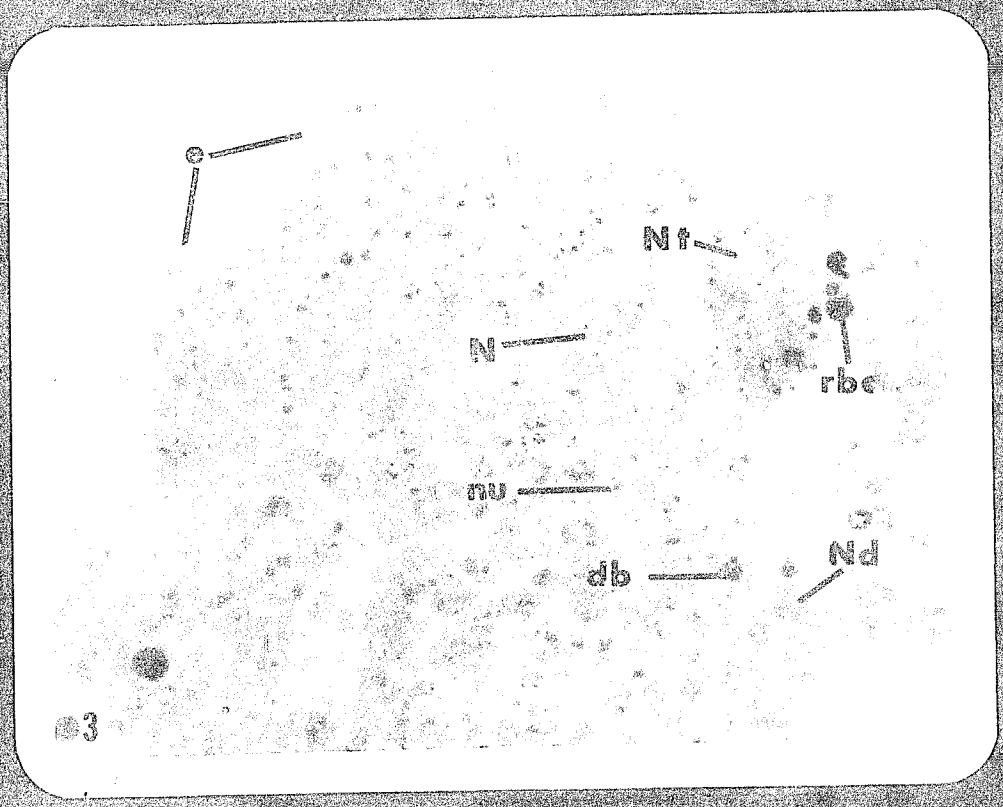

e
Nt
N
rbc
nu
db
Nd
3

Figure 4. Electron micrograph of epithelial cells of the normal tail (level A). The apical surface (black wedges) is presumably a cytoplasmic extension of a cell body whose nucleus may not be in this section.

The cells are separated by wide intercellular spaces (is), and have a glycocalyx-like basement lamina (BL) of moderate density. Other structures seen are nuclei (N), nucleolus (nu), sparse endoplasmic reticulum (er), mitochondria (m), polyribosomes (p) and subepithelial spaces (ss). Magnification X 11,400.

Figure 5. Epithelial cells of the normal tail (level B). Perinuclear cytoplasm of one cell serves as the outermost surface layer in this region (left black wedge), whereas the nucleus of the apical cytoplasm marked by the right black wedge is apparently out of this section. (G) Golgi complex. Magnification X11,400.

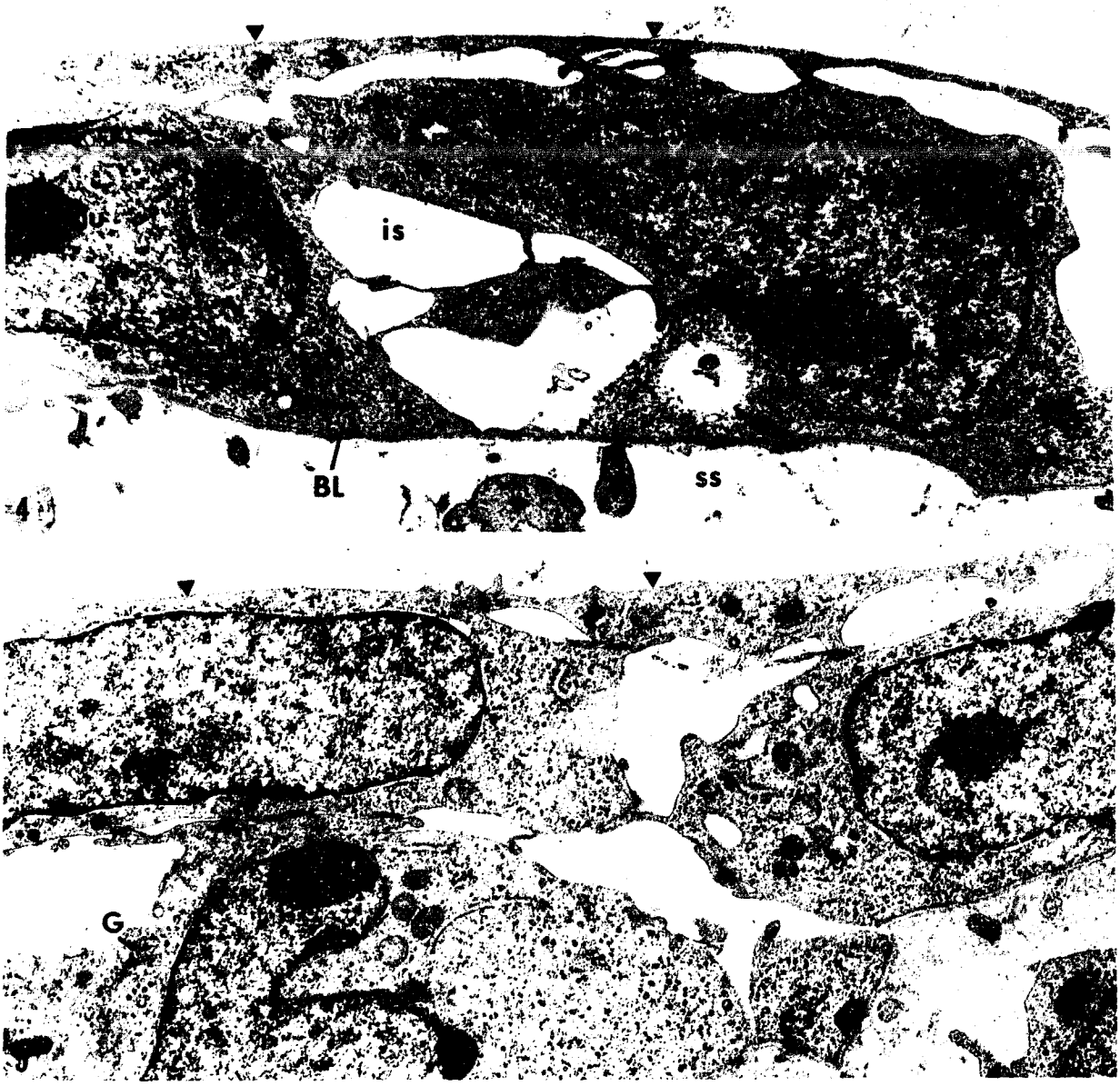
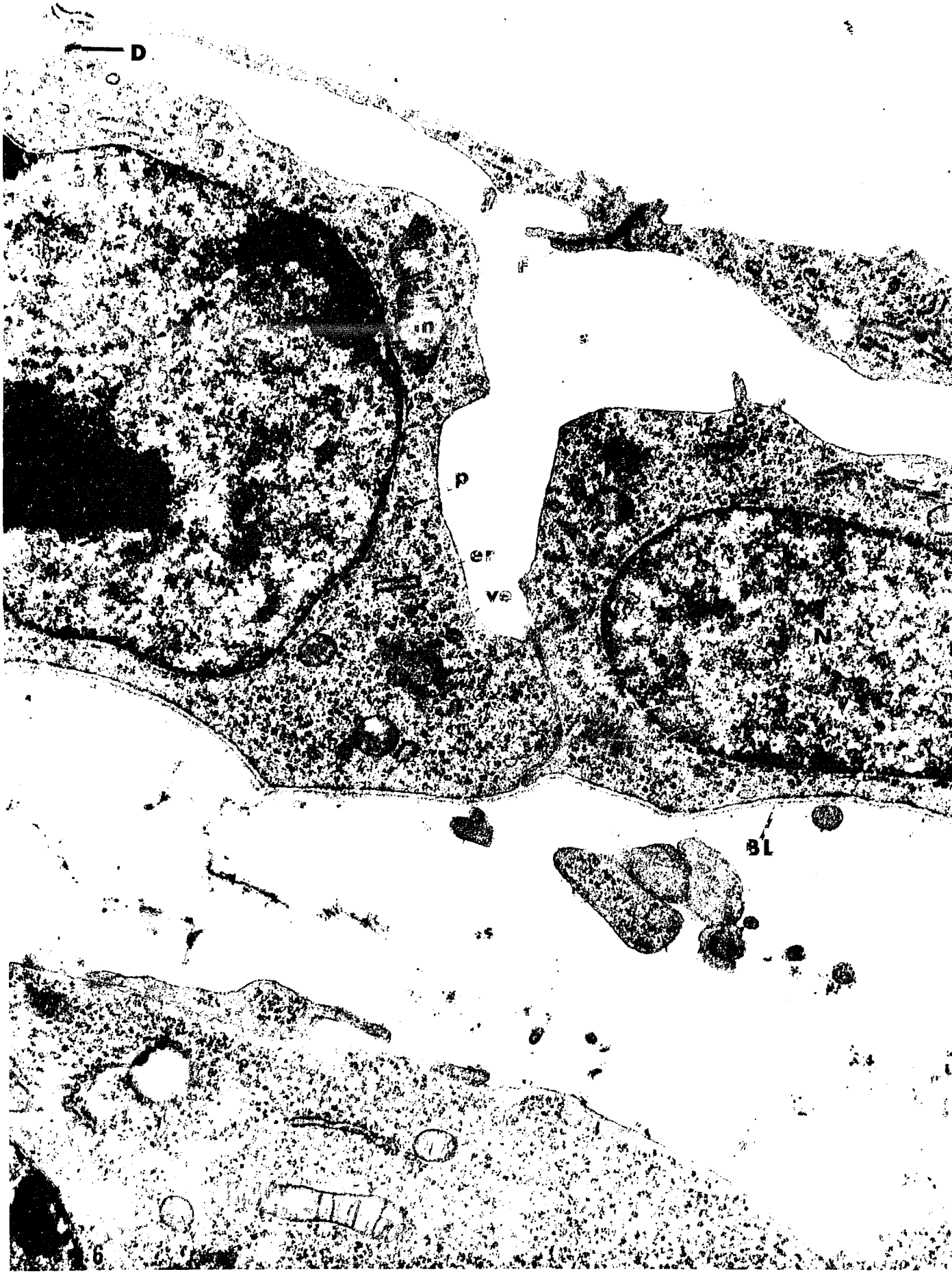

is
BL
ss
G

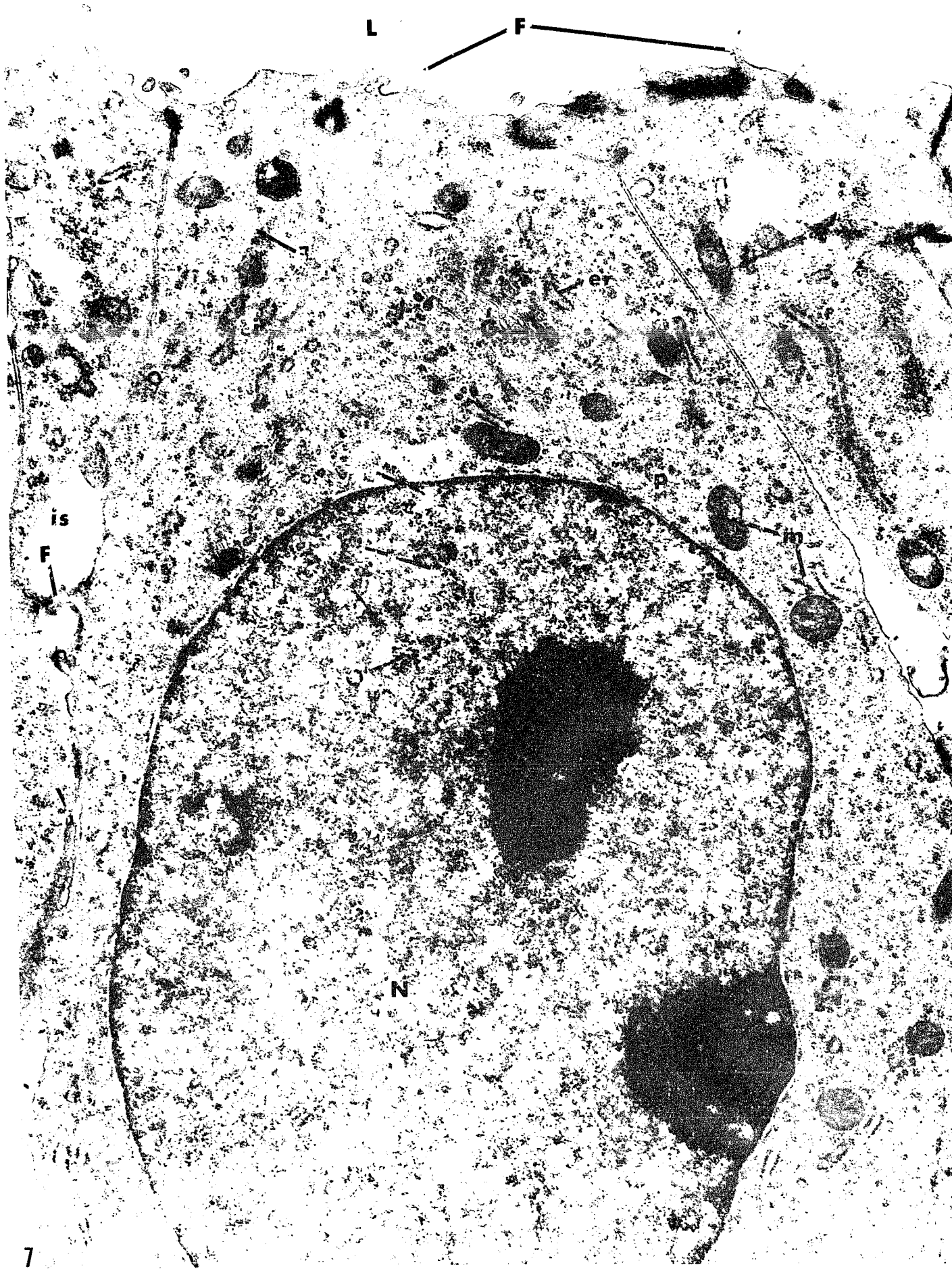
Figure 6. Two neighboring epithelial cells from normal tail tip (level A).

Note dense vesicles (ve) presumably part of a Golgi complex (G) and the numerous clusters of polyribosomes (p) and few cisternae of rough endoplasmic reticulum (er). Other structures seen are intercellular space (is), subepithelial space (ss), basement lamina (BL), mitochondria (m), and nucleus (N). Magnification X17,100.



6

Figure 7. Columnar neural tube cells from normal tail tip (level A). The large nucleus (N) contains dense, presumably chromosomal patches in a less dense nucleoplasm. The cytoplasm is characterized by Golgi vesicles and saccules (G), mitochondria (m), granular endoplasmic reticulum (er), polyribosomes (p), and surface flaps (F) extending into the lumen (L) of the neural tube or intercellular spaces (is). Magnification X17,100.



7

Figure 8. Profiles of red blood cells (rbc) with and without a nucleus from normal tail tip (level B). Red blood cells with nuclei are probably normoblasts. Numerous polyribosomes give these cells a speckled appearance. Vesicles (ve) containing particulates, and mitochondria (m) occur in erythrocytes. Neighboring cells are a polymorphonuclear leucocyte (PL) with surface flaps, and anucleate thrombocytes (T) with mitochondria (m) and vesicles (ve). (N) nucleus. Magnification X9,700.

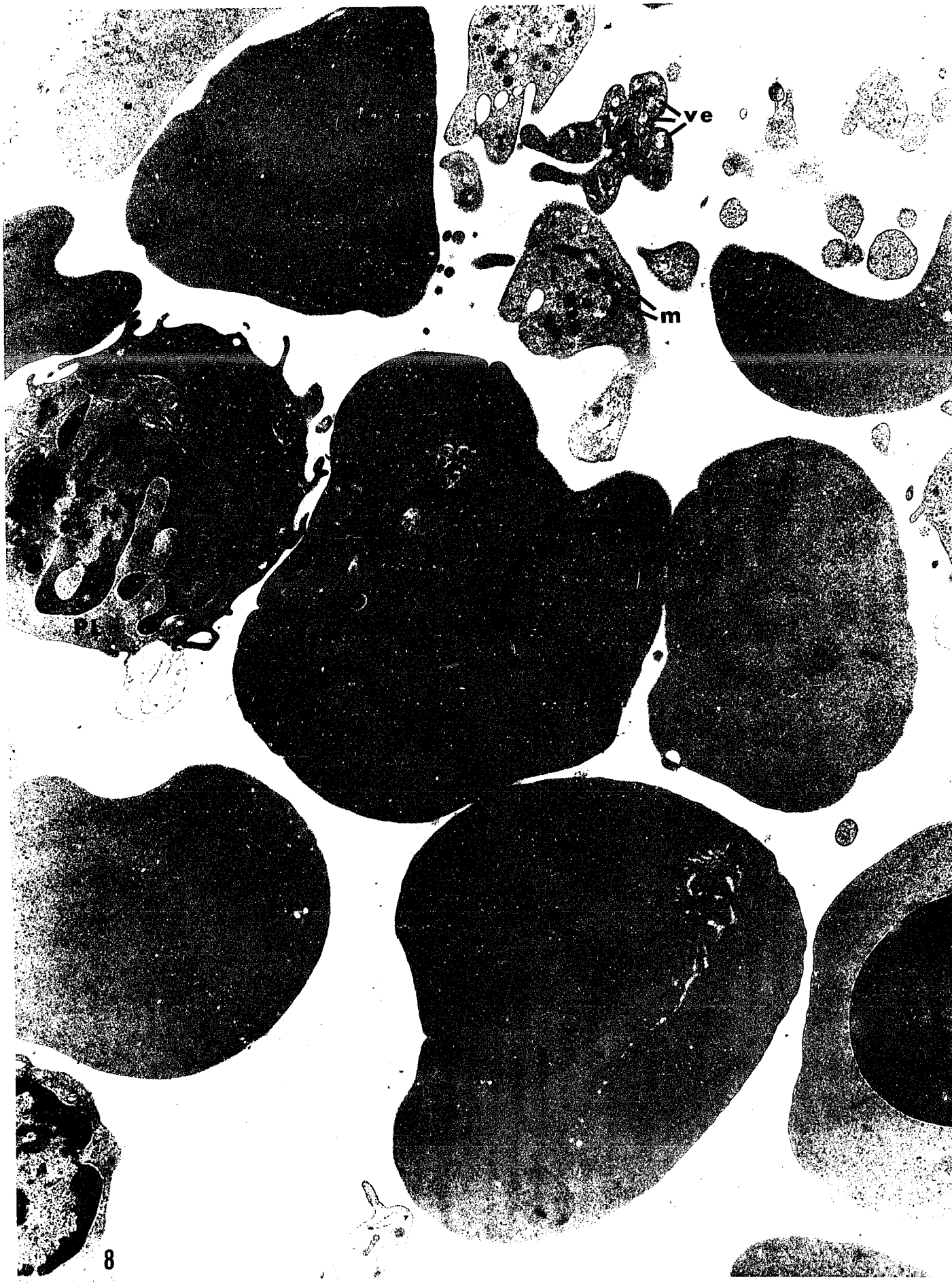
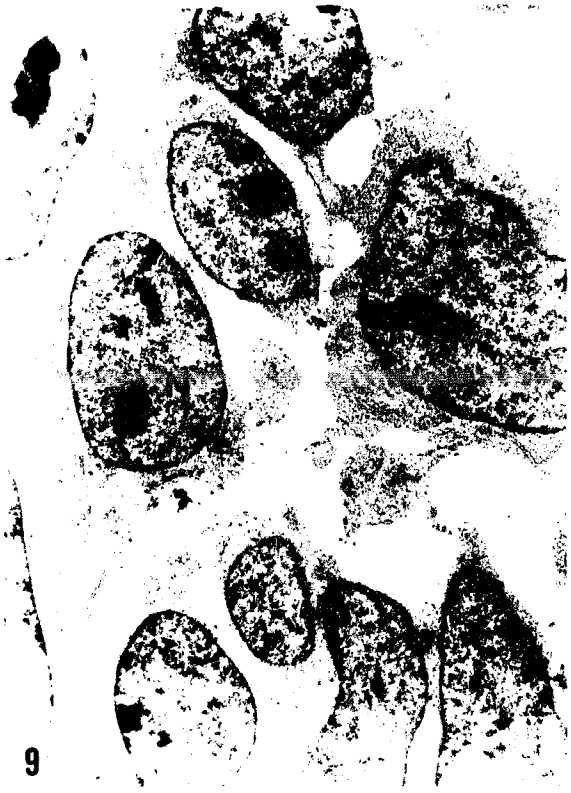

ve
m

Figure 9. Mesenchyme cells from normal tail tip (level C) illustrate typical morphology encountered in most cells of this type. (N) nucleus surrounded by cytoplasm containing mitochondria, polyribosomes, and endoplasmic reticulum. Magnification X3,190.

Figure 10. Mesenchyme cells from normal tail tip (level B) showing large nucleus (N) and mitochondria (m). Magnification X8,100.

Figure 11. Mesenchyme cells from normal tail tip (level B) showing large nucleus (N) surrounded by cytoplasm containing mitochondria, polyribosomes, and endoplasmic reticulum. Magnification X10,260.

Figure 12. Mesenchyme cells from normal tail tip (level A) showing nucleus (N), mitochondria (m), polyribosomes (p), and endoplasmic reticulum (er). Magnification X15,960.



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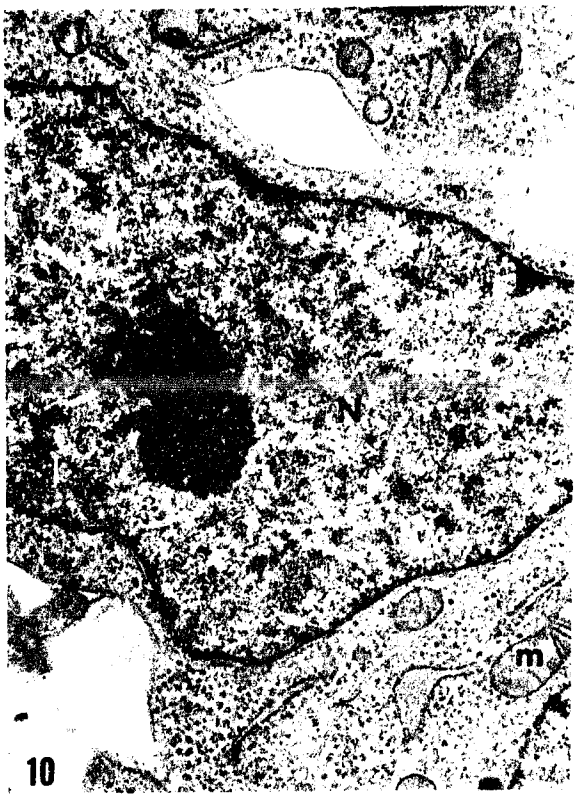

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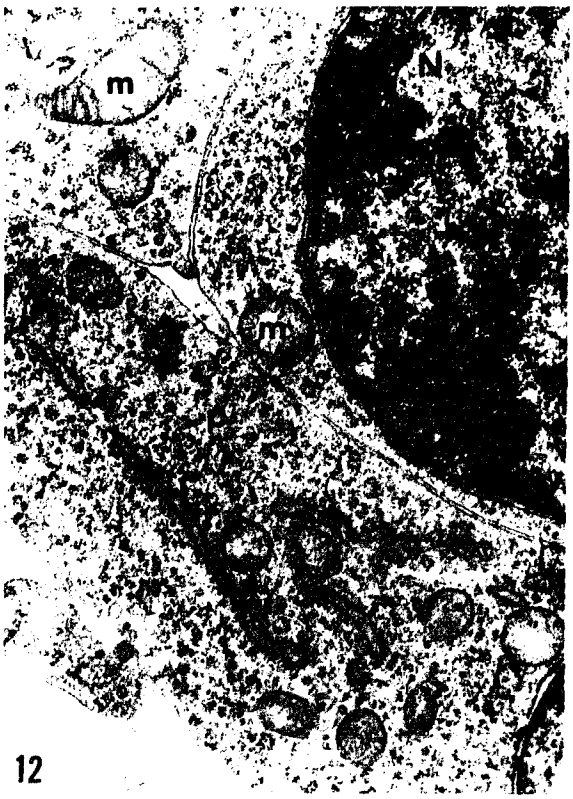

12

Figure 13. Mesenchyme cells from the normal tail tip (level B). Note one of the rare mesenchyme cells which contains a lysosome-like body (L) adjacent to a similar cell that is devoid of such a body. Magnification X4,560.

Figure 14. Dense bodies (db) found in the normal tail tip (level B), similar to these are rarely encountered within or outside of mesenchyme cells of the normal tail. Magnification X5,590.

13

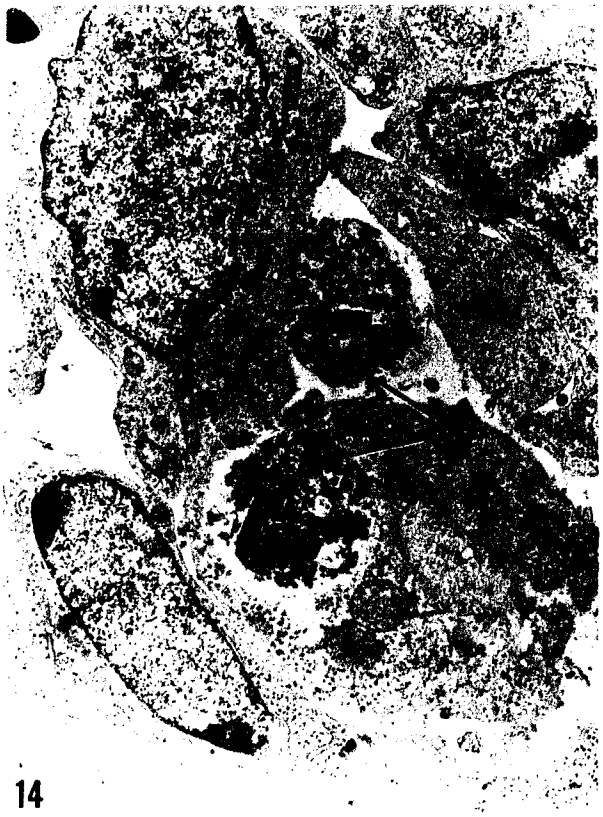

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Figure 15. Transverse section (1 μ) through a Brachyury (T/+) tail filament (level A) stained with methylene blue for light microscopy. An epithelium (e) composed of stellate cells arranged in two layers surrounds cells which are presumed to be mesenchyme and phagocytes, many which contain dense bodies (db). Neural tube and notochord are absent. Note nucleoli (nu) within nucleus (N). (rbc) red blood cells, or normoblasts. Magnification X415.

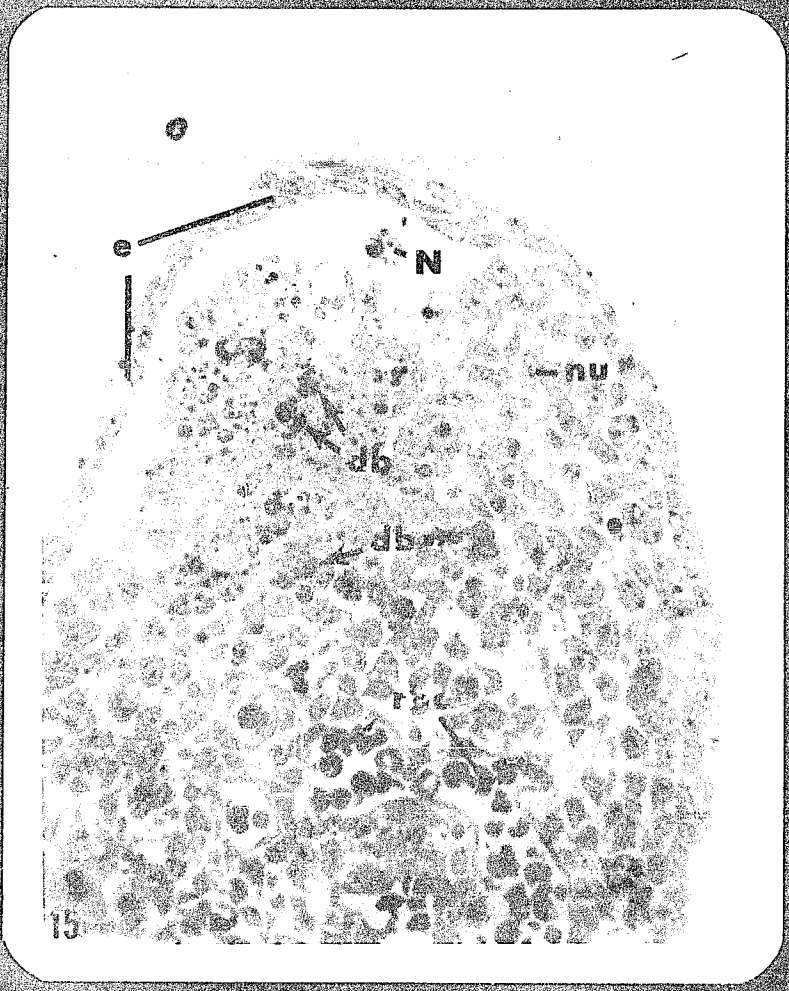
e
N
nu
db
15

Figure 16. Epithelial cells from the filament of a Brachyury (T/+) tail (level C) resembles epithelium of the normal tail tip (+/+). Note the intercellular (is) and subepithelial (ss) spaces. Magnification X3,190.

Figure 17. Typical epithelial cell of the Brachyury filament (level C). Note the Golgi complex (G), mitochondria (m), and intercellular space (is). Magnification X8,100.

Figure 18. Red blood cell (rbc) with a nucleus is probably a normoblast. This cell is found within a vessel of the Brachyury filament (level A) and appears similar to erythrocytes of the normal tail tip. Note fusiform nucleus (Ne) of endothelial cell and desmosome (D). Magnification X8,100.

Figure 19. Thrombocyte in blood vessel of Brachyury filament (level B) features an endoplasmic reticulum (er), mitochondria (m), glycogen particles (g) and other structures similar to those described by Wetzel, et. al. (1967) in mammals.

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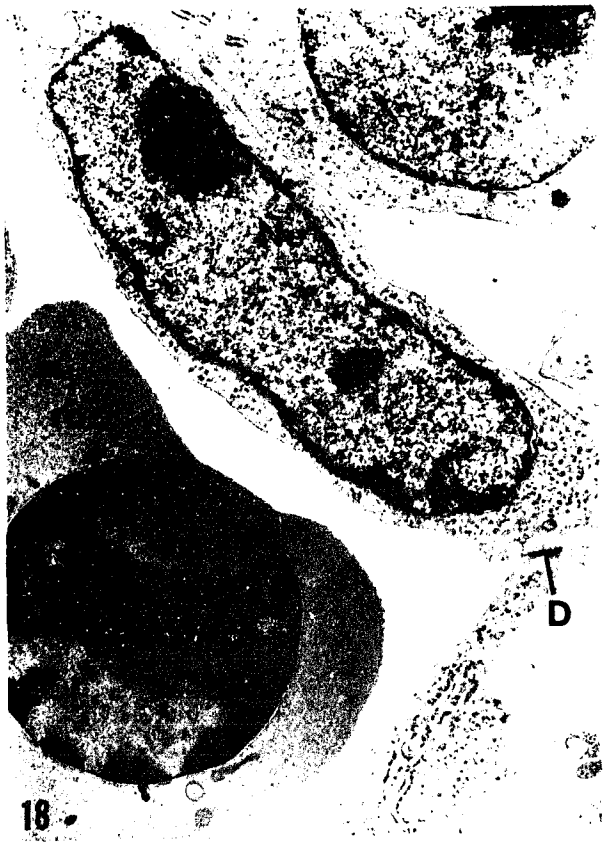

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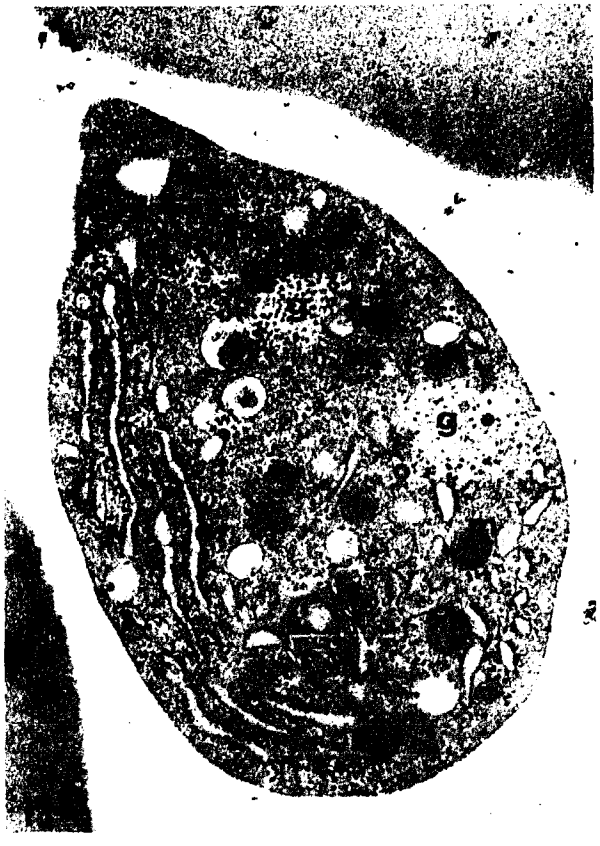

Figure 20. Section through tail filament (level A) of a Brachyury embryo containing mesenchyme cells and phagocytes. Cell debris (Cd) suggest the occurrence of widespread necrosis in the vicinity of endothelial cells (Ec). Cell debris (Cd) appears as extracellular material containing a granular substrate and vesicles of varying density. The apparent phagocytosis of a mass of cell debris is suggested by pseudopod-like extensions (arrows) of a phagocyte. This phagocyte contains a dense, stellate lysosome-like body (Ly) and a centriole (ce) from which emanates a cilium (cm). Other structures present include Golgi complex (G), nucleus (N), mitochondria (m), and endoplasmic reticulum (er). Magnification X7,890.

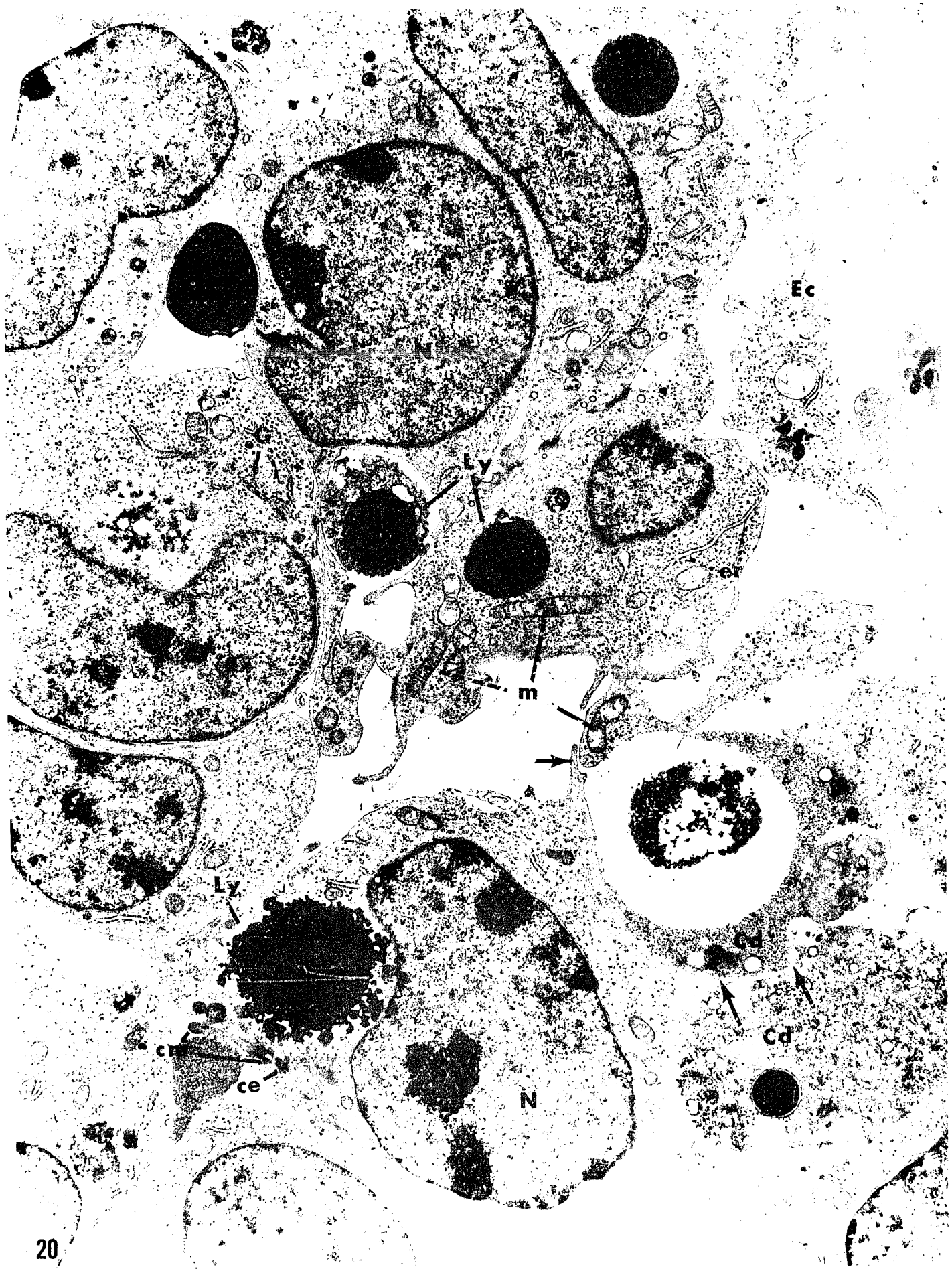
Ec
Ly
er
m
Ly
Cd
Cd
cm
ce
N

Figure 21. Section through tail filament of Brachyury embryo (level A). Perivascular cells, which are presumably phagocytes, partaking in necrosis suggested by several dense lysosome-like bodies (Ly), some with a "dense core". Note cell debris (Cd) both in intracellular and extracellular locations. Other structures present is a red blood cell (rbc) with its nucleus, probably a normoblast and endothelial cells (Ec). Magnification X7,980.

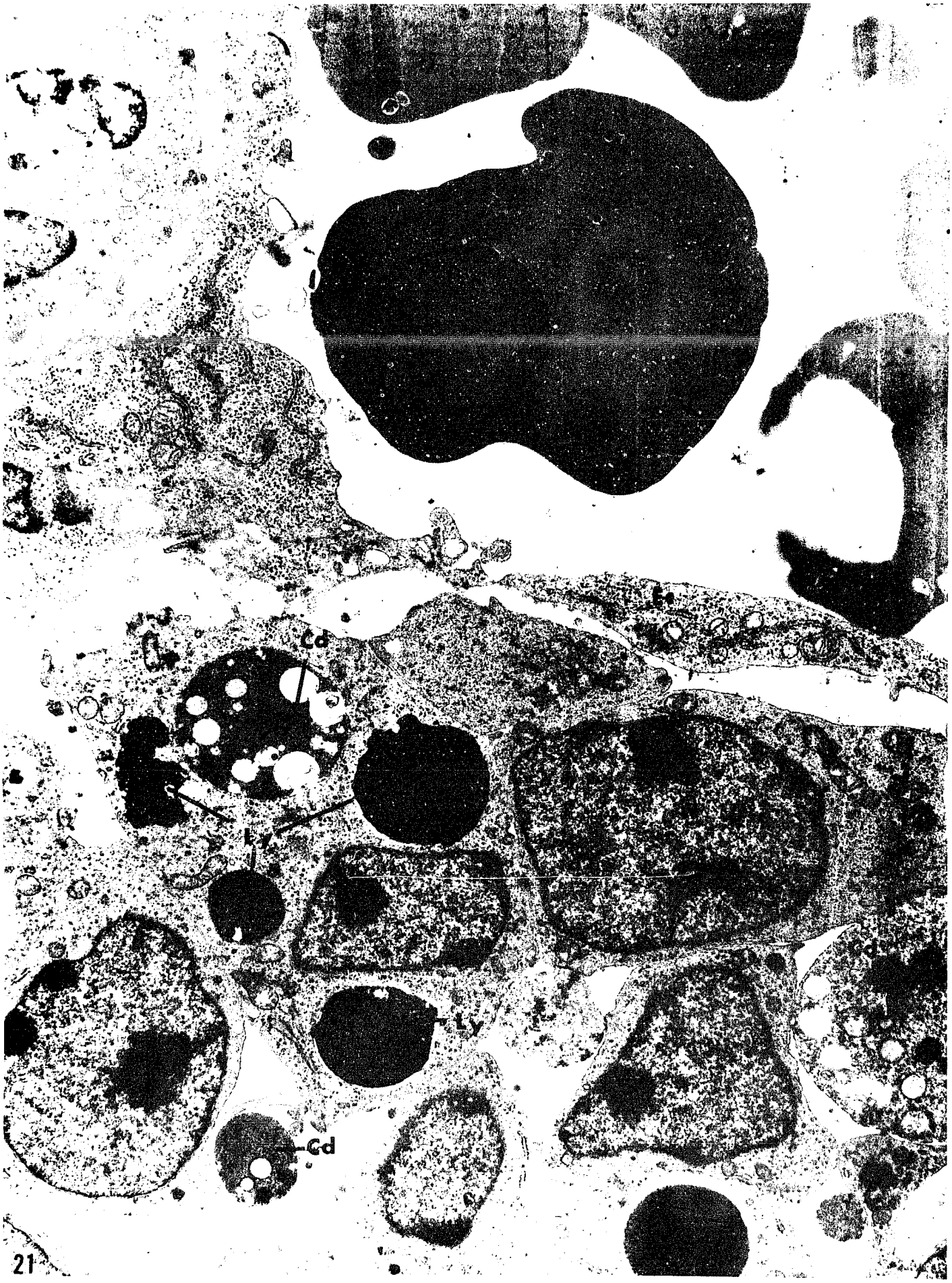

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Figure 22. Section through tail filament (level A) of a Brachyury embryo containing mesenchyme cells and phagocytes. Lysosome-like bodies (Ly) are found in both intracellular and extracellular locations. The apparent phagocytosis of a mass of cell debris (Cd) is suggested by pseudopod-like extensions (arrows) of a phagocyte. Note one mesenchyme cell (me) apparently in cell division, with its chromosomes (Ch) evident. Magnification X7,890.

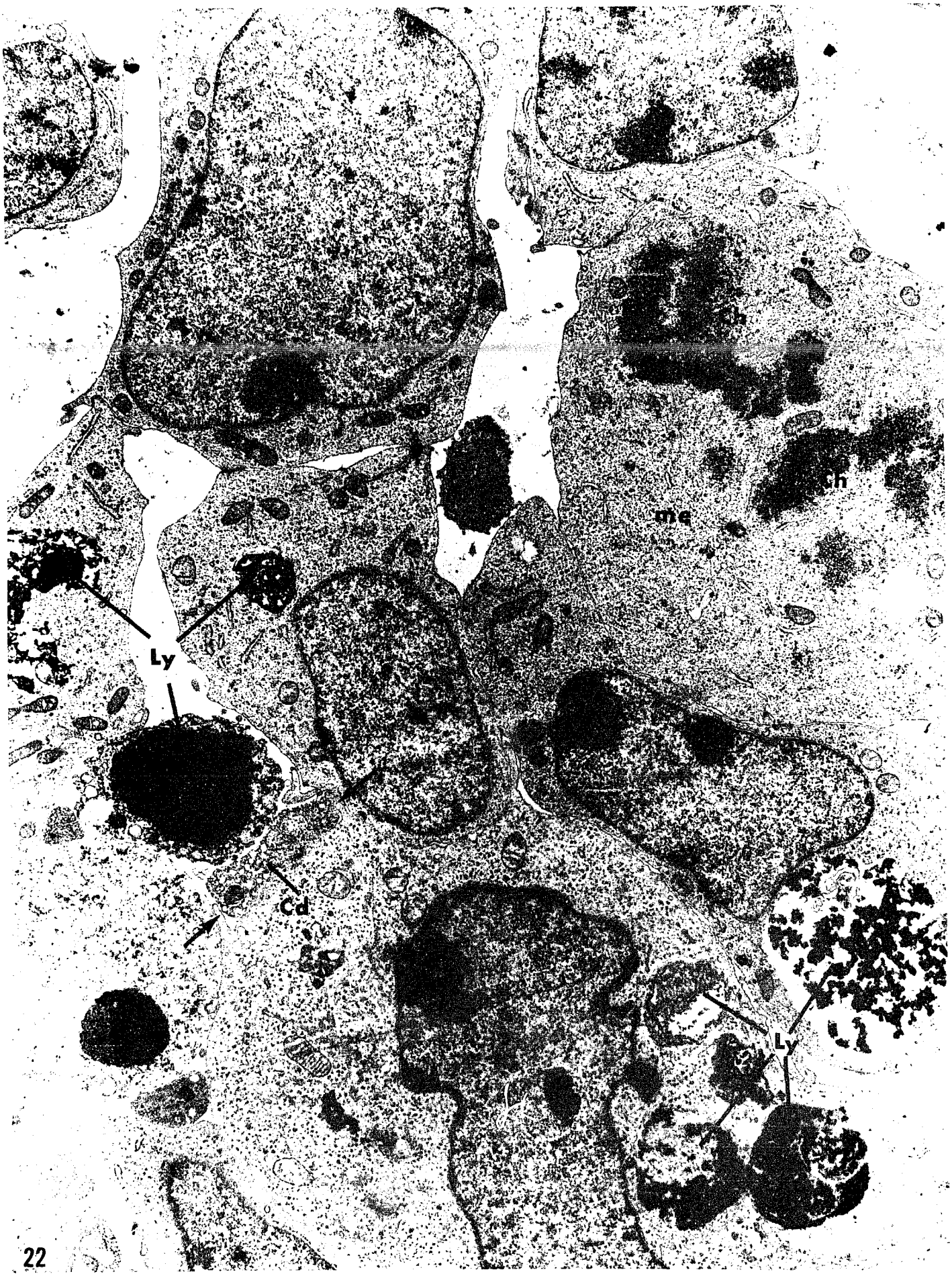
Ly
Cd
Ly

Figure 23. Section through tail filament of Brachyury embryo (level B). Note cell apparently in cell division containing chromosomes (ch) in mitosis, adjacent to cells undergoing involution and containing lysosome-like bodies (Ly). Magnification X5,490.

Figure 24. A cell containing a large, membrane-bounded vesicle and necrotic material taken from a Brachyury tail filament (level B). Magnification X10,260.

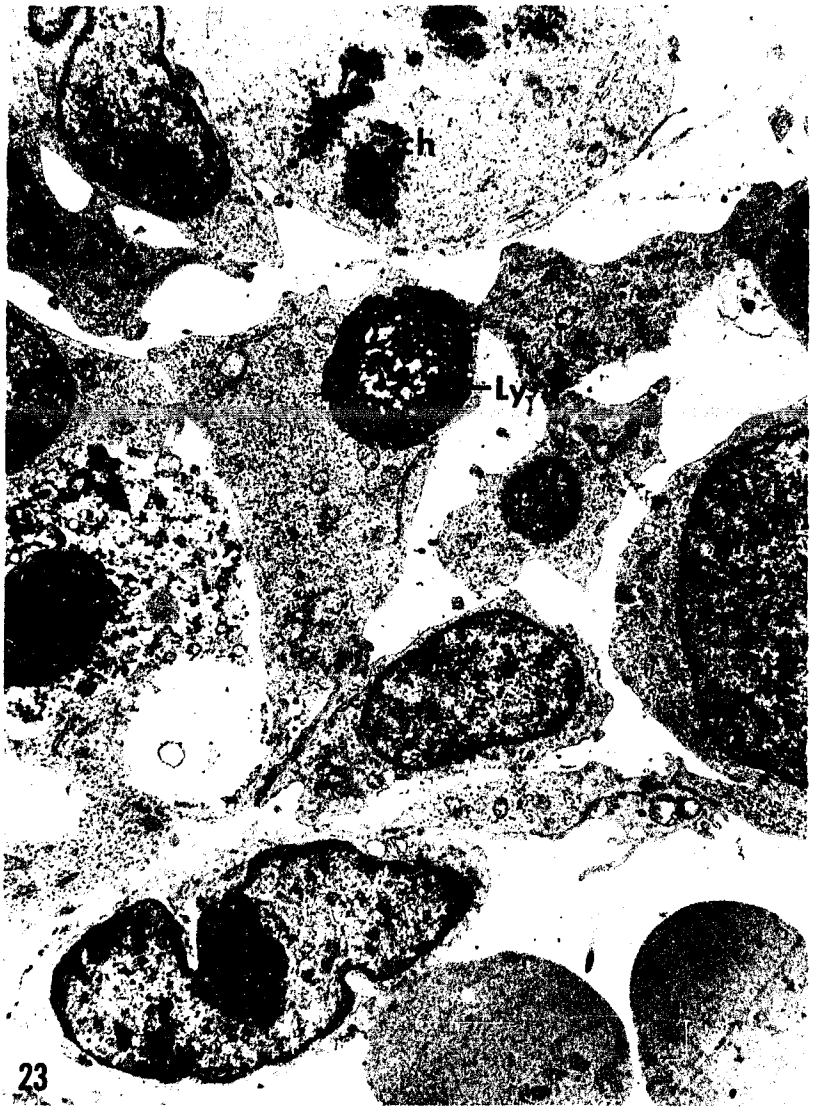

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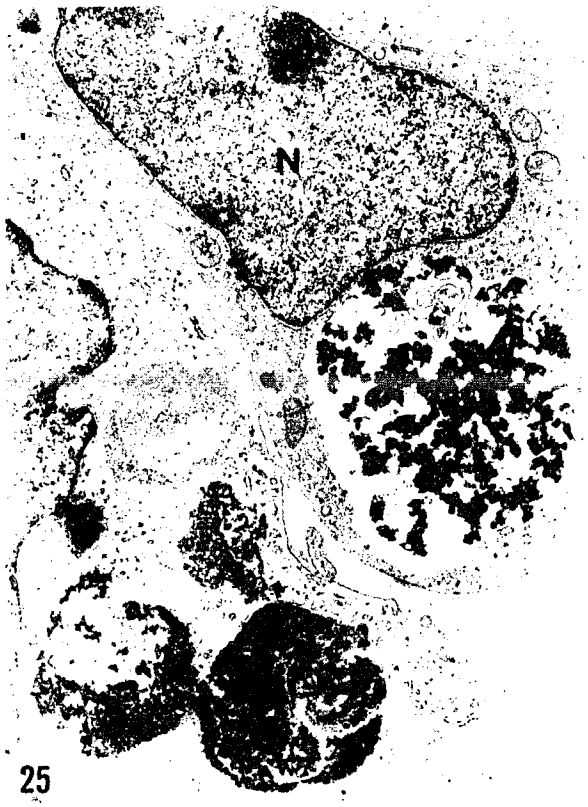
Figure 25. Section through Brachyury tail filament (level A) containing cells with lysosome-like bodies. Note the indented nuclear margin with adjacent nucleus (N). Magnification X8,100.

Figure 26. Section through Brachyury tail filament (level B) containing a cell with a lysosome-like body. Note that the cytoplasm contains polyribosomes, endoplasmic reticulum, and mitochondria. Magnification X10,260.

Figure 27. Section through Brachyury tail filament (level B) containing lysosome-like bodies and cell debris. Magnification X10,260.

Figure 28. Section through Brachyury tail filament (level C) with a lysosome-like body having a variety of densities and particulate material. (N) nucleus. Magnification X10,260.



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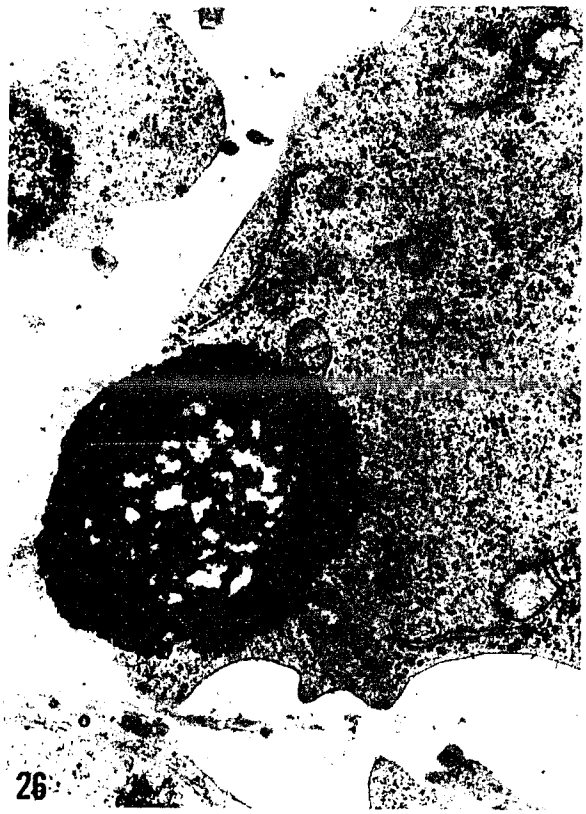

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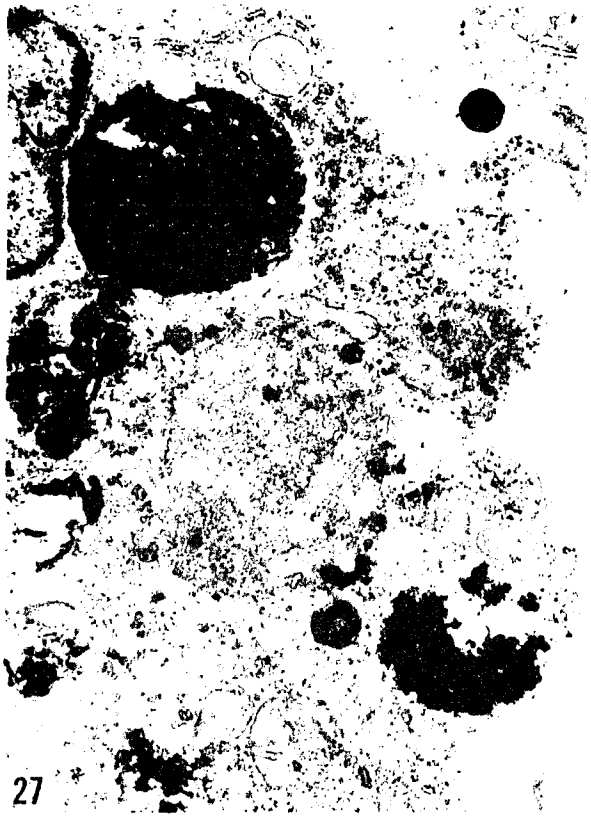

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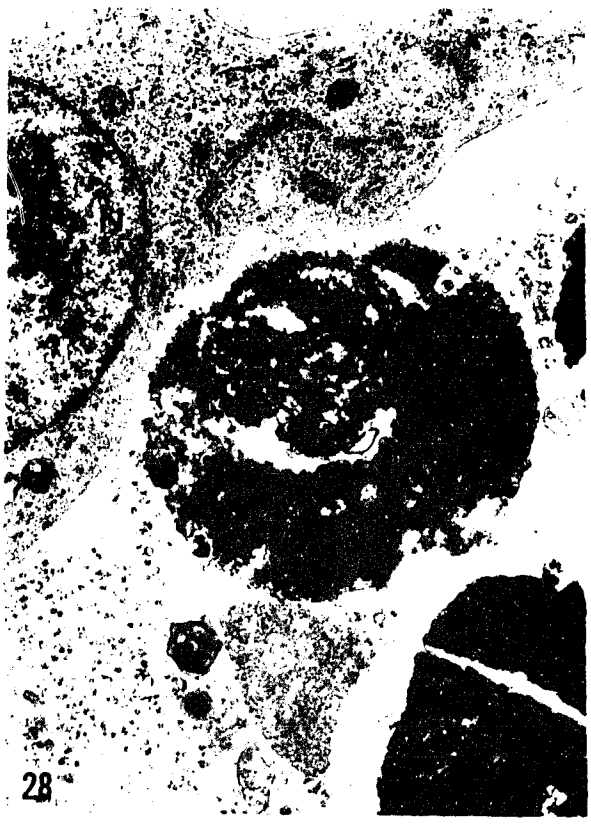


28

Figure 29. Section through the tail filament of a Brachyury embryo (level B). Note Golgi complex (G) next to dense, lysosome-like (Ly) body, some of whose dense material may been extracted during preparation. Magnification X 25,650.

Figure 30. Section through the tail filament (level B) of a Brachyury embryo. Note the indented nuclear margin with adjacent dense, lysosome-like body (Ly). (N) nucleus, (Cd) cell debris. Magnification X 7,975.

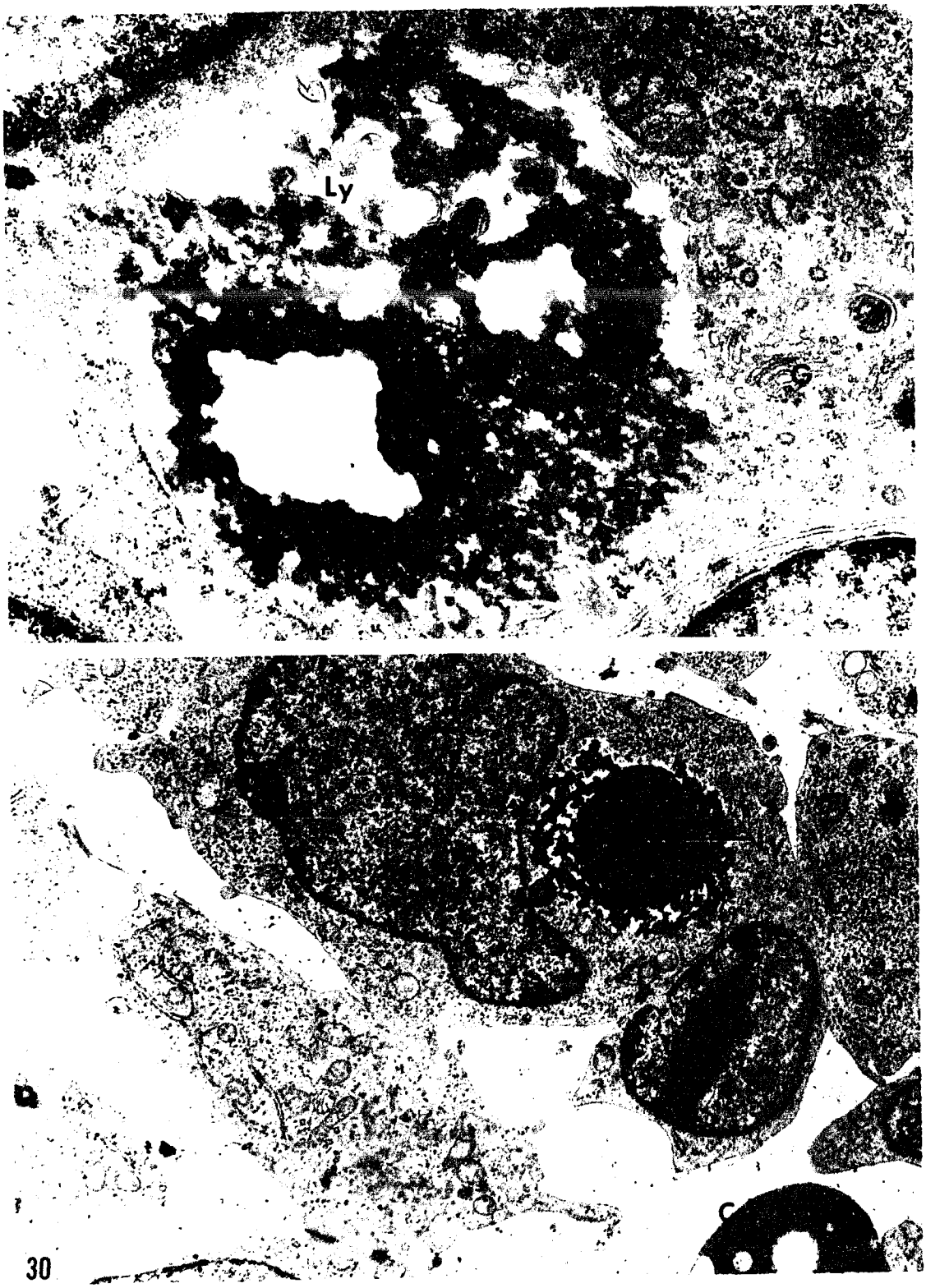
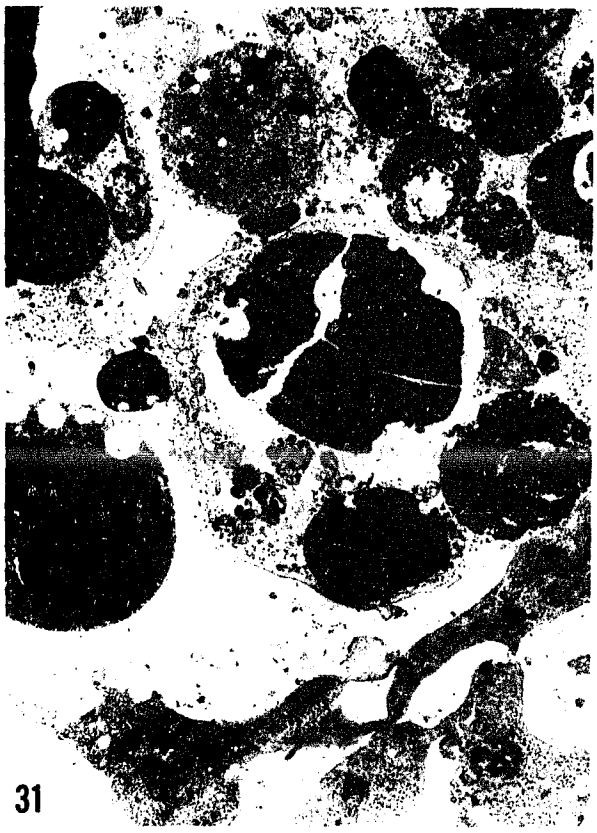

Ly
C

Figure 31. Section through the tail filament (level C) of a Brachyury embryo. Note large central cell containing prominant dense bodies and cell debris, and also an extracellular mass of cell debris (Cd). Magnification X3,880.

Figure 32. Section through the tail filament (level C) of a Brachyury embryo. Note the dense body with a "dense core" adjacent to a nucleus (N). Magnification X6,840.

Figure 33. Section through the tail filament (level A) of a Brachyury embryo. The dense lysosome-like body near a nuclear (N) indentation is found adjacent to a Golgi complex (G) and cilium (cm). Magnification X8,100.

Figure 34. Section through the tail filament (level A) of a Brachyury embryo. The central cell appears to be in the act of phagocytosis as suggested by pseudopodial (pd) capture of cell debris (Cd). Note the typical location of the lysosome-like body in the indented margin of an adjacent nucleus (N). Magnification X8,100.



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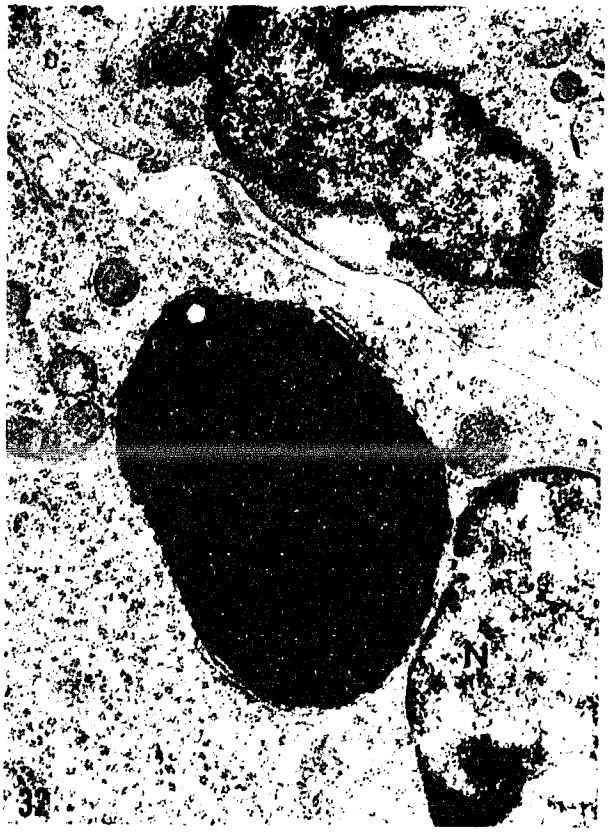

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Figure 35. Section through the tail filament (level B) of a Brachyury embryo. A phagocyte appears to be in the act of entraping a cell containing a morbid nucleus (Nm) and four dense, lysosome-like bodies (Ly). Inner arrows mark the plasma membrane of the entrapped cell (M), and the outer arrows mark the plasma membrane of the phagocyte. Two of the lysosome-like bodies (Ly) can be seen to have distinct membranes (mb). The phagocytic cell nucleus (N) is fusiform and is found in a cytoplasm (Ph) containing mitochondria (m), polyribosomes (p), and endoplasmic reticulum (er).
Magnification X17,000.

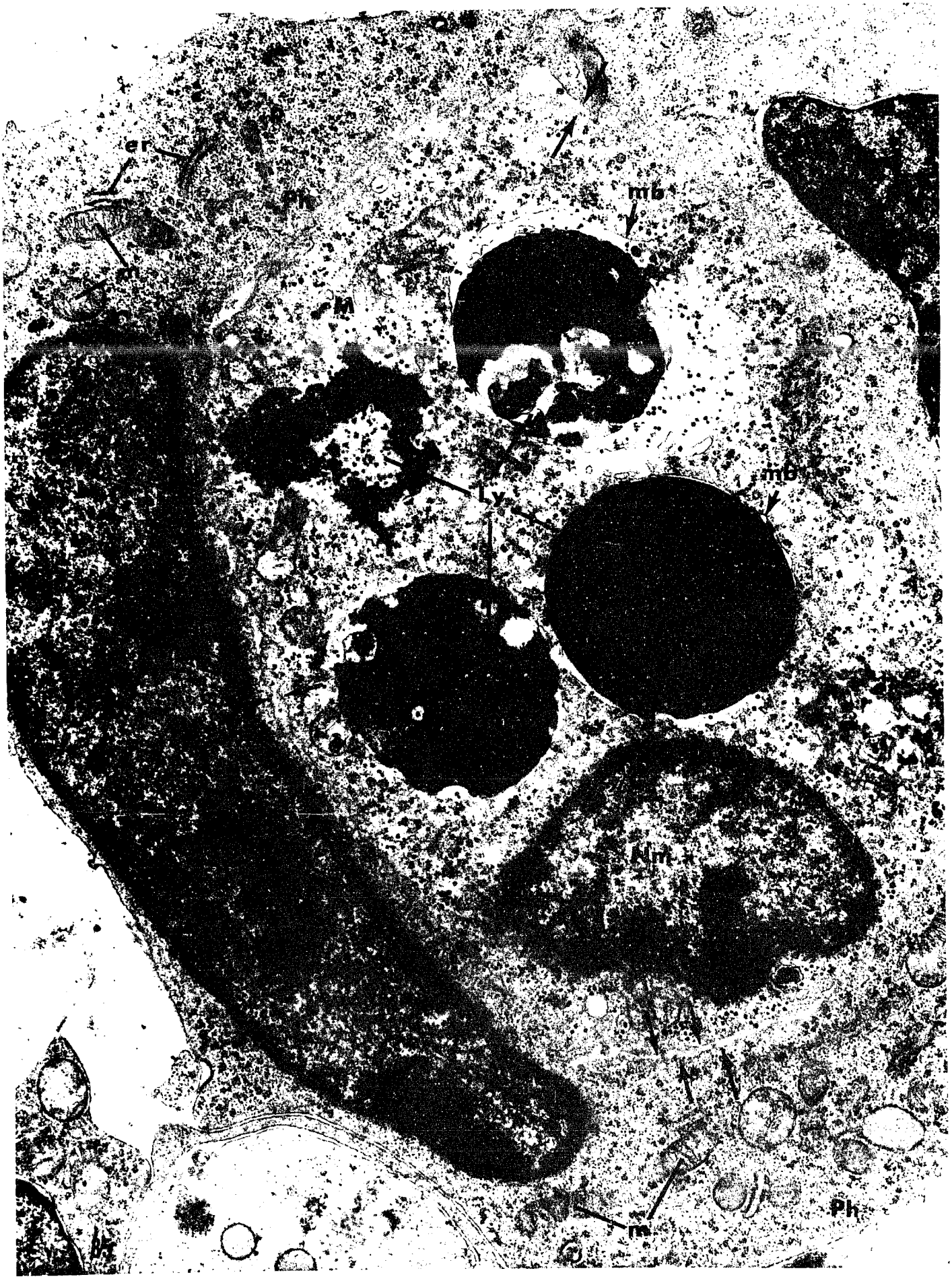

er
Ph
m
M
mb
Ly
mb
Nm
m
Ph

Figure 36. Section through a tail filament (level A) of a Brachyury embryo. Phagocytosis of cell debris (Cd) is suggested by pseuodopod-like (pd) extensions from the phagocyte. Subcellular particles seen in cell debris at lower right include mitochondria (m), and free ribosomes (r). Other structures seen include lysosome-like body (Ly) with a dense core (dc), endothelial cells (Ec), red blood cells (rbc), and a thrombocyte (T). Magnification X20,200.

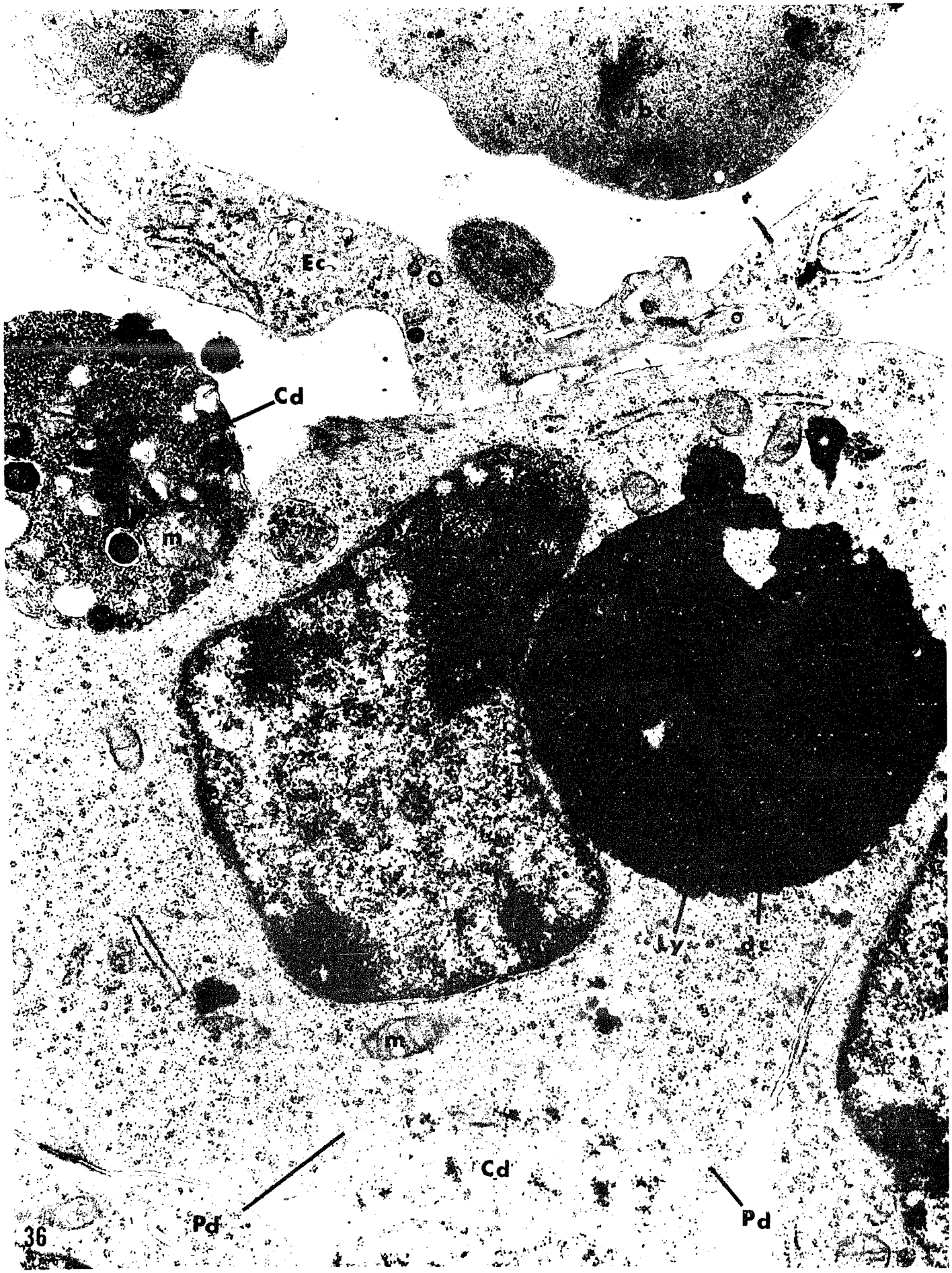
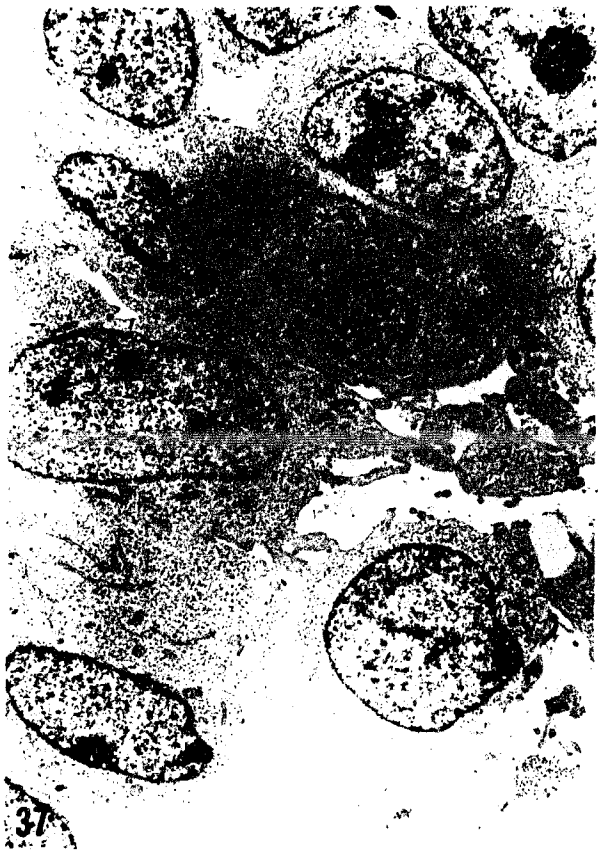

Ec
Cd
m
Ly
dc
m
Cd
Pd
Pd

Figure 37. Section through the prospective adult tail tip (level X) of a Brachyury embryo. Area of mesenchyme cells which appear similar to normal (+/+) mesenchyme cells. Magnification X3,190.

Figure 38. Section at level X of Brachyury tail proximal to the constriction which forms the tail filament. These mesenchyme cells are in the region of a red blood cell. Magnification X3,190.

Figure 39. Section through the prospective adult tail tip (level X) showing an example of a dense body found in a subepithelial cell. No lysosome-like bodies are seen in the majority of mesenchyme cells in this region. Magnification X15,650.

Figure 40. Section at level X of Brachyury tail proximal to the constriction which forms the tail filament. Another rare occurrence of a dense body in a subepithelial cell of this region. Note nucleus (N) and mitochondrion (m). Magnification X15,960.



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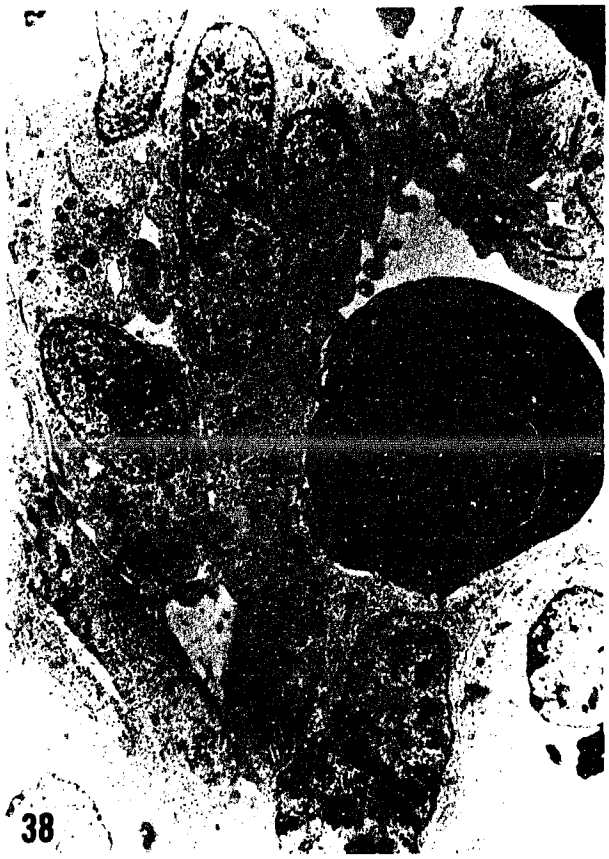

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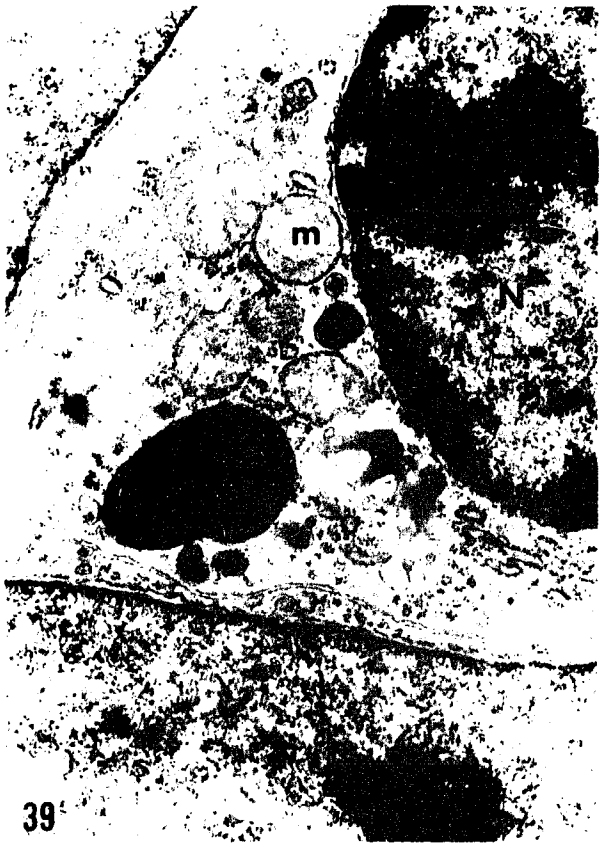

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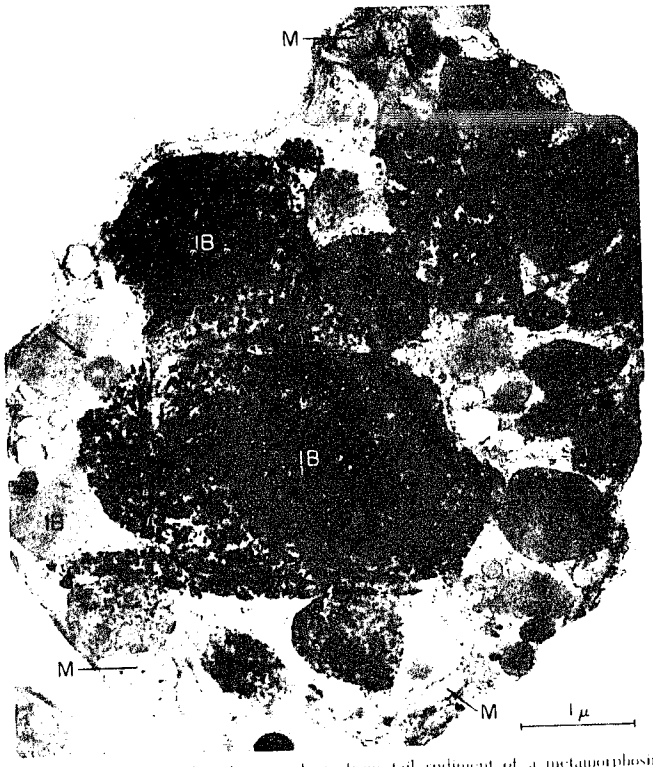
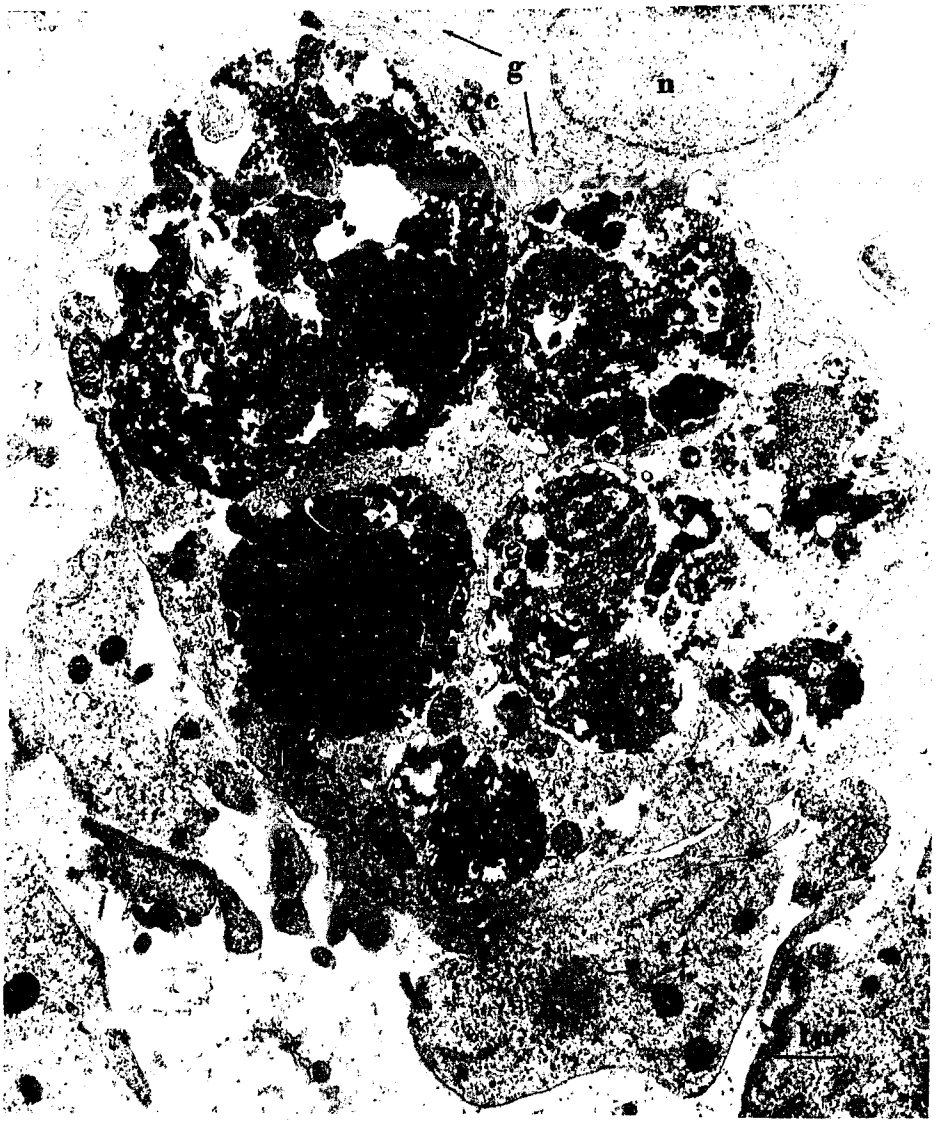
Figure 41. Electron micrograph of a macrophage from a tail rudiment of a metamorphosing Xenopus larva (from Weber, 1964; p. 485).



Well-developed macrophage from tail rudiment of a metamorphosing *Xenopus* larva. Black lead deposits demonstrate acid phosphatase activity, which appears confined to cytoplasmic inclusion bodies (IB) or "phagosomes"; some inclusion bodies also contain myelin figures (↑). (M) = mitochondria. From Weber (1964).

Figure 42. Electron micrograph of a phagocyte engorged with cellular debris, taken from the posterior necrotic zone of a stage 24 chick embryo wing (from Saunders and Fallon, 1966; p. 310).



Phagocyte engorged with cellular debris in various stages of degeneration. Typical of these cells is the abundant Golgi (g) lying near the nucleus (n) and the centriolar apparatus (c). Photograph by Mr. Donald Heinkel.

Figure 43. Cross-section through the tip of a normal (+/+) embryo, 17 days of gestational age, prepared by the Burstone method (1958) for the demonstration of acid phosphatase activity. Sites of enzyme activity are indicated by arrows. (e) epithelium, (Nd) notochord, (ms) mesoderm. Magnification X435.

Figure 44. Cross-section through the filament of a Brachyury (T/+) embryo, 17 days of gestational age, prepared by the Burstone (1958) method for the demonstration of acid phosphatase activity. Sites of enzyme activity are indicated by arrows. (e) epithelium, (ms) mesenchyme and presumably macrophages. Magnification X435.

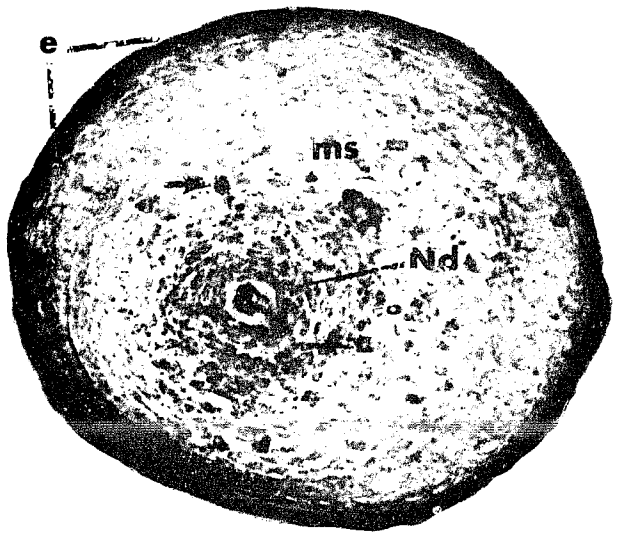


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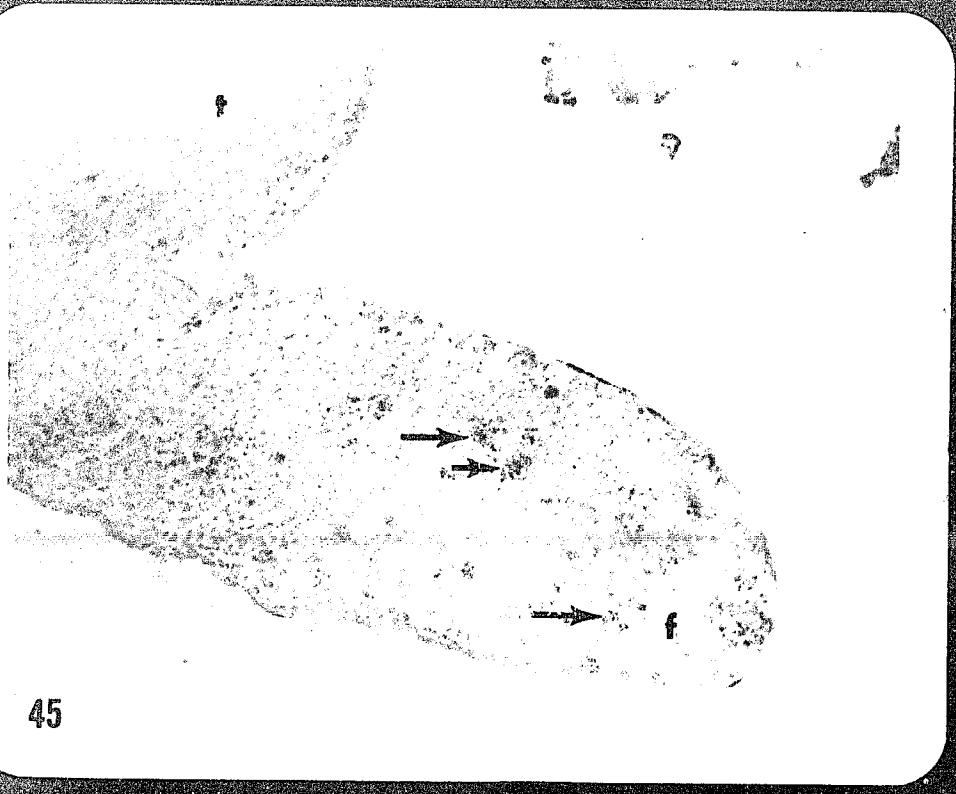
Figure 45. Longitudinal section through the tail of a Brachyury (T/+) embryo, 17 days of gestational age, prepared by the Burstone method (1958) for the demonstration of acid phosphatase activity. Note the intense enzyme activity (arrows) present in the filament portion (f) and the relative lack of such activity in the prospective adult tail tip (t). Magnification X135.

Figure 46. Longitudinal section through the tail of a Brachyury (T/+) embryo, 17 days of gestational age, prepared by the Burstone method (1958) for the demonstration of acid phosphatase activity. This specimen is from a different litter than the embryo in Figure 45. Magnification X135.



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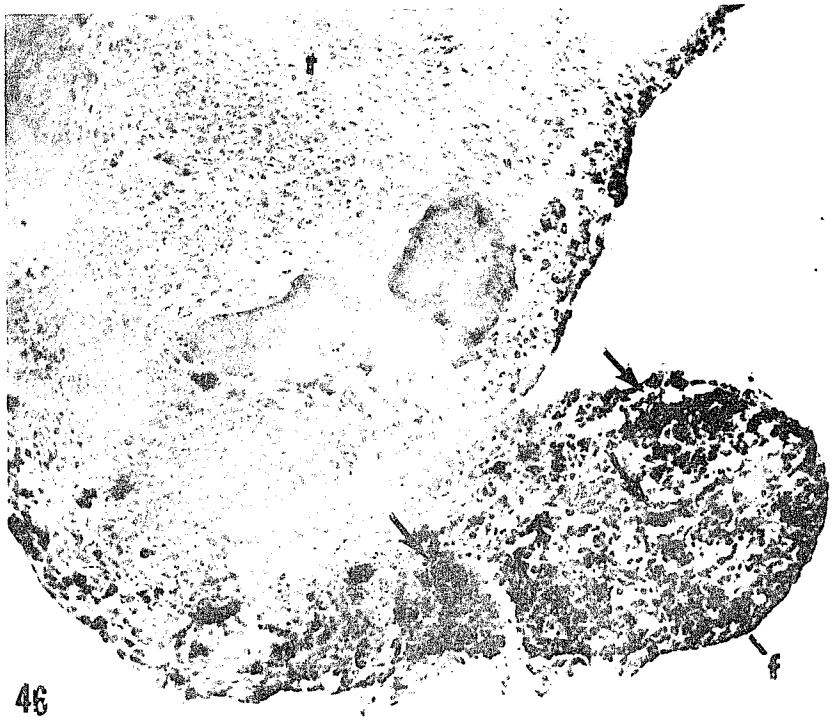

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Figure 47. Drawings of whole mounts of tails from mice of Brachyury phenotype, at one day of postnatal age. Stained with toluidine blue after clearing in KOH and glycerin.

(a) tail tip of a Brachyury mouse obtained from cross ♂ Black Spot (T/+) X ♀ DBA/2 (+/+). Note partly formed vertebra (arrow) fused to the preceding vertebral element.

(b) tail tip of a Brachyury mouse obtained from cross ♂ DBA/2 (+/+) X ♀ Black Spot (T/+). Note partly formed vertebra (arrows) fused to the preceding vertebral elements.

(c) tail tip of a Brachyury mouse obtained from cross ♂ Black Spot (T/+) X ♀ Black Spot (T/+). Note unusual fusion of cartilage in a "lamelar" arrangement (arrow).

(d) tail tip of a Brachyury mouse obtained from cross ♂ Black Spot (T/+) X ♀ C57bl/6 (+/+). Note vertebral elements at the tip of the tail (arrows) are fragmented.

(e) portion of the tail proximal to the tip of a Brachyury mouse obtained from cross ♂ Black Spot (T/+) X ♀ C57bl/6 (+/+). Note fusion between adjacent vertebrae (arrow).

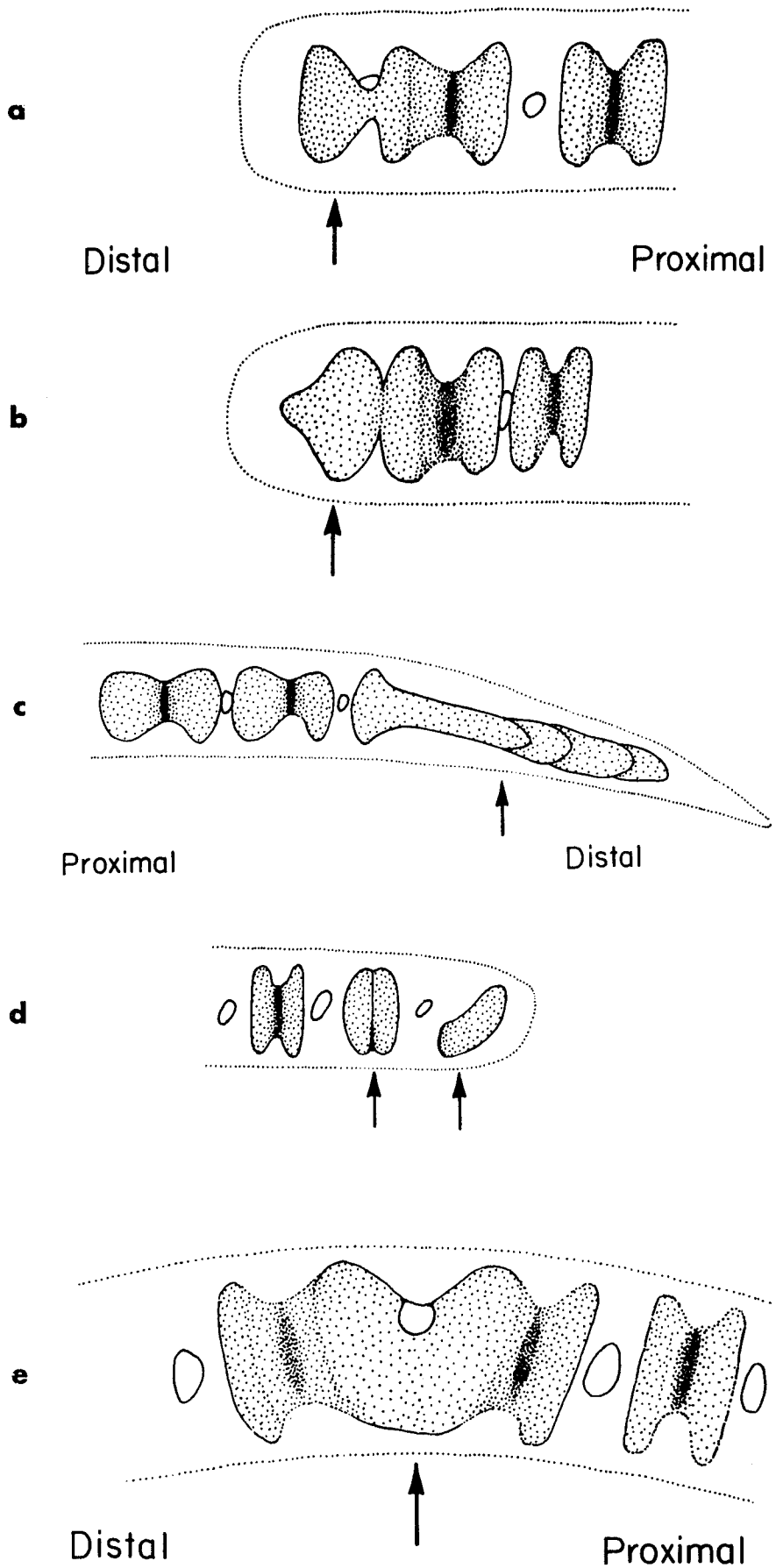

a
Distal
Proximal
b
c
Proximal
Distal
d
e
Distal
Proximal

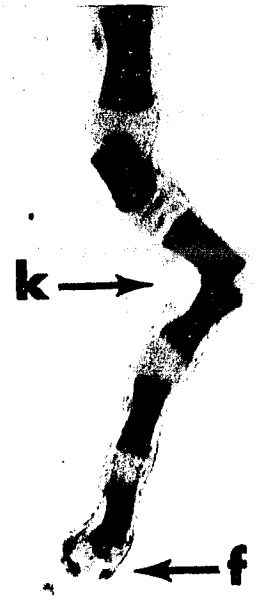
Figure 48. Tail tip of a mouse, 10 days of postnatal age, of normal phenotype obtained from cross ♂ Black Spot (T/+) X ♀ Black Spot (T/+). Stained with alizarin red after clearing in KOH and glycerin. Magnification X2.

Figure 49. Tail tip of a mouse, 10 days of postnatal age, of Brachyury phenotype obtained from cross ♂ Black Spot (T/+) X ♀ C57bl/6 (+/+). Prepared as above. Note the kink (k) formed by the fusion between adjacent vertebrae, and the fragmented and malformed vertebrae (f) at the tip of the tail. Magnification X2.

Figure 50. Tail tip of a mouse, 10 days of postnatal age, of Brachyury phenotype obtained from cross ♂ Black Spot (T/+) X ♀ Black Spot (T/+). Prepared as above. Note fragmented vertebral elements at tail tip (f) and the wedge-shaped vertebra (w) located more proximally. Magnification X2.

Figure 51. Tail tip of a mouse, 10 days of postnatal age, of Brachyury phenotype obtained from cross ♂ Black Spot (T/+) X ♀ C57bl/6 (+/+). Prepared as above. Note two kinks (k) caused by the fusion of adjacent vertebrae. Magnification X2.

48



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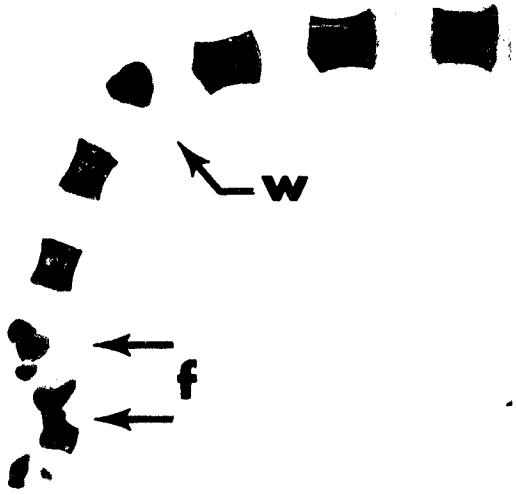

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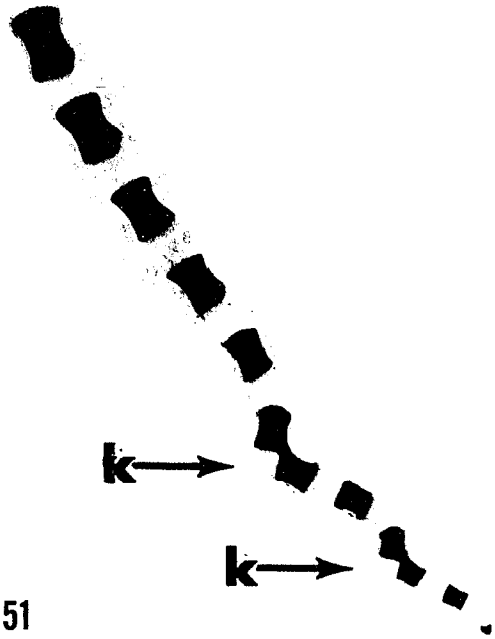

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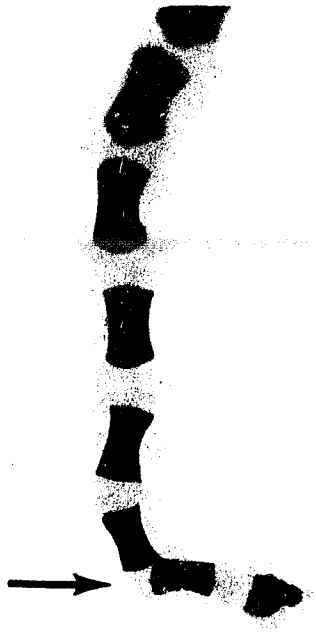
Figure 52. Tail tip of a mouse, 10 days of postnatal age, of Brachyury phenotype obtained from cross ♂ Black Spot (T/+) X ♀ Black Spot (T/+). Prepared as above. Arrow shows presence of a malformed vertebral element which has fused with the preceding vertebra at the tip of the tail. Magnification X2.

Figure 53. Tail tip of a mouse, 10 days of postnatal age, of Brachyury phenotype obtained from cross ♂ Black Spot (T/+) X ♀ Black Spot (T/+). Prepared as above. Note the severe kink (arrow) caused by the fusion of adjacent vertebrae. Magnification X2.

Figure 54. Tail tip of a mouse, 10 days of postnatal age, of Brachyury phenotype obtained from cross ♂ Black Spot (T/+) X ♀ DBA/2 (+/+). Prepared as above. Note multiple vertebral fragments (arrow) at the tip of the tail. Magnification X2.

Figure 55. Tail tip of a mouse, 10 days of postnatal age, of Brachyury phenotype obtained from cross ♂ DBA/2 (+/+) X ♀ Black Spot (T/+). Prepared as above. Note the presence of ring-like (r) and a cap-shaped vertebra (c). Magnification X2.

52



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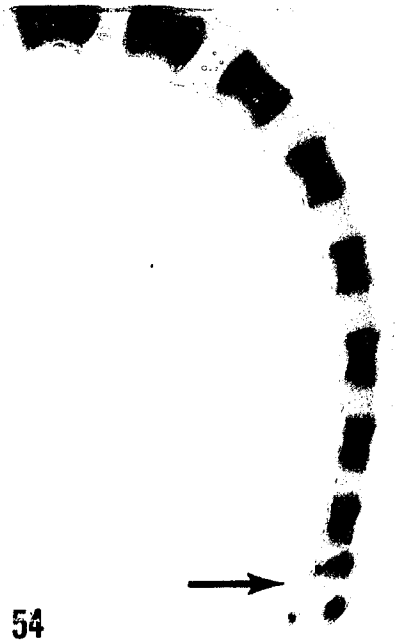

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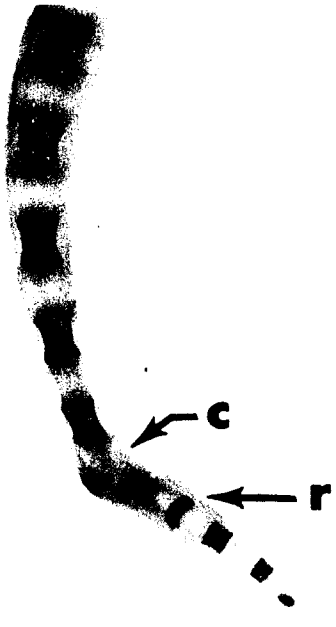

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Figure 56. Cross-section through the tail tip of a Black Spot embryo of normal phenotype at 16 days of gestational age. Note mesenchyme cells (M) surrounding the notochord. Magnification X200.

Figure 57. Cross-section through the tail tip of a Black Spot embryo of normal phenotype at birth. Note cartilage cells (C) surrounding the notochord. Magnification X200.

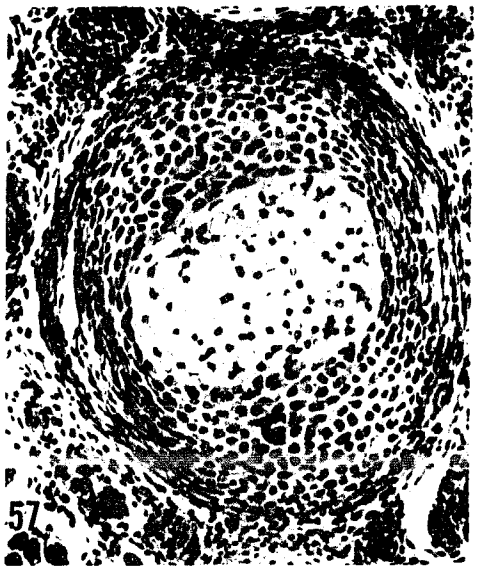
Figure 58. Cross-section through the tail tip of an organ culture from a mouse embryo of normal phenotype at 16 days of gestational age, and maintained in culture for five days. (C) cartilage cells. Magnification X275.

Figure 59. Cross-section through the tail tip of an organ culture from a mouse embryo of Brachyury phenotype at 16 days of gestational age, and maintained in culture for five days. (C) cartilage cells. Magnification X275.

Figure 60. Whole mount of an organ culture of the distal portion of the tail from a mouse embryo of Normal phenotype at 16 days of gestational age, and maintained in culture for five days. Vertebrae (arrows) appear well formed and in normal spatial orientation to each other. Magnification X70.

Figure 61. Whole mount of an organ culture of the distal portion of the tail from a mouse embryo of Brachyury phenotype at 16 days of gestational age, and maintained in culture for five days. Vertebrae (arrows) appear well formed and in normal spatial orientation to each other. Magnification X70.


M
56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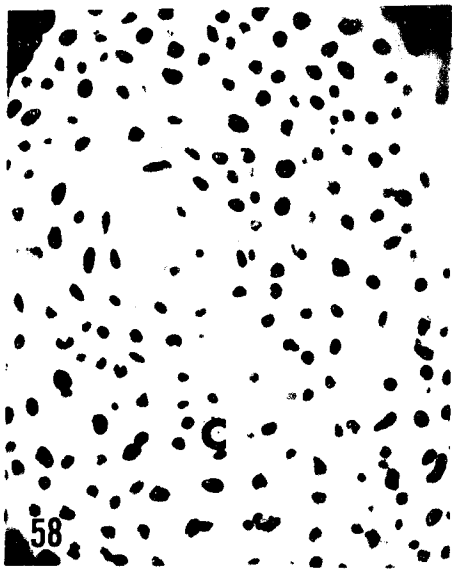
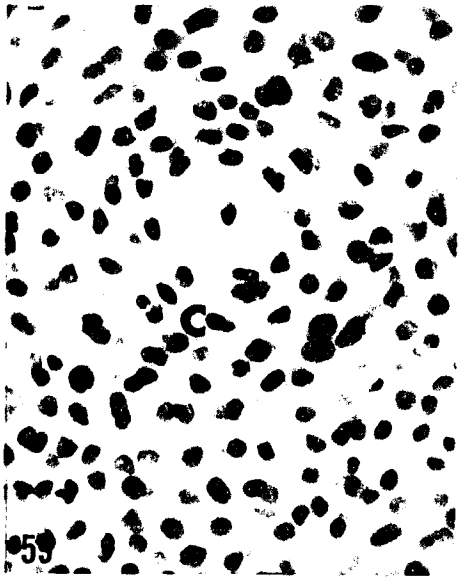

C
58


C
59


60

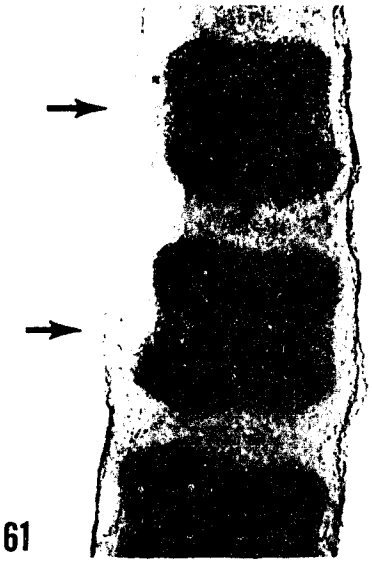
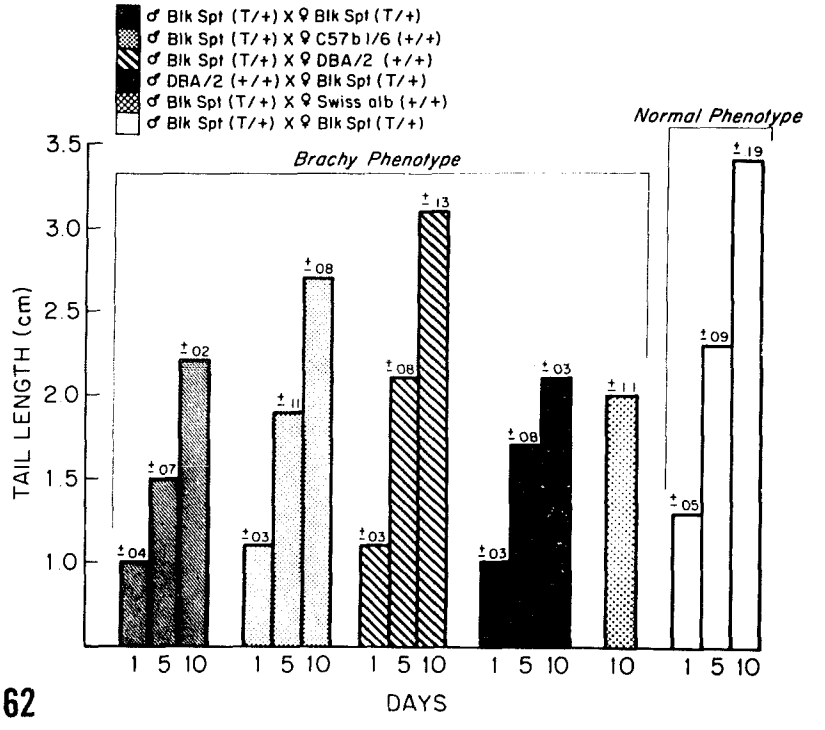

61

Figure 62. Mean tail length of normal and Brachyury phenotypes at one, five, and ten days after birth. Over each bar is placed the standard error of the mean.

Figure 63. Mean tail length of normal and Brachyury phenotypes at 16 days of gestational age. Over each bar is placed the standard error of the mean

MEAN TAIL LENGTH OF NORMAL AND BRACHYURY PHENOTYPES
AT ONE, FIVE, AND TEN DAYS AFTER BIRTH



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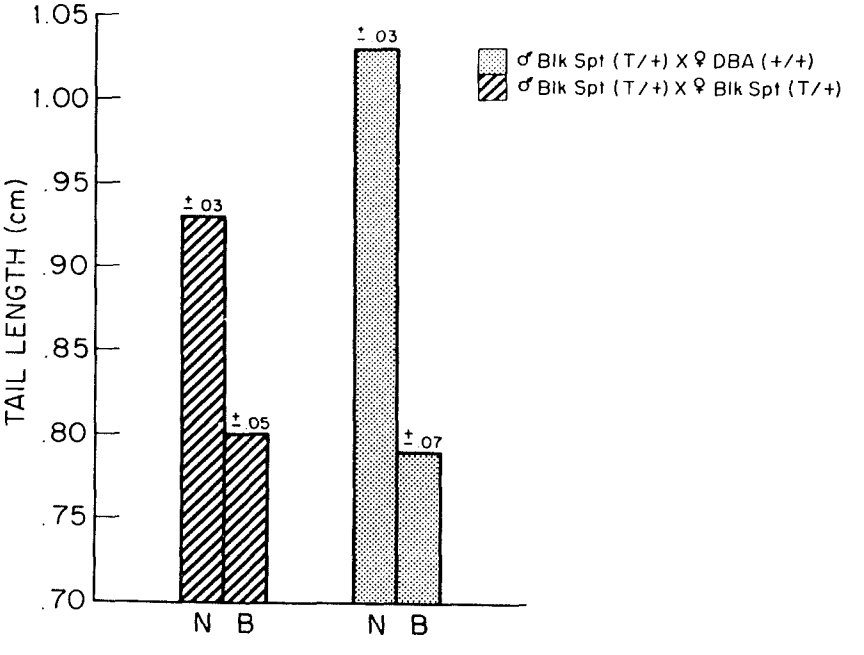
MEAN TAIL LENGTH OF NORMAL AND BRACHYURY
PHENOTYPES AT 16 DAYS OF GESTATION



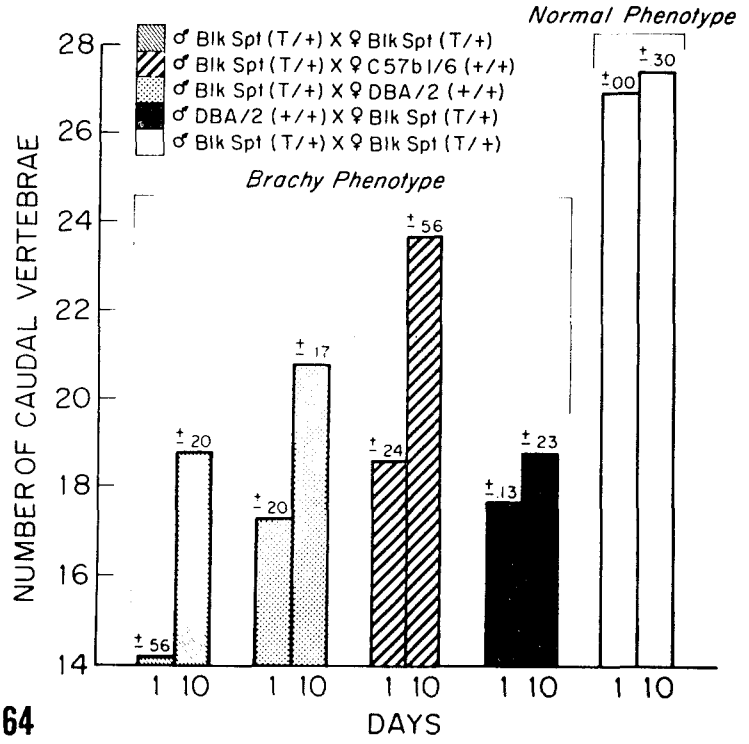
N - Normal (+/+) Phenotype
B - Brachy (T/+) Phenotype

63

Figure 64. Mean numbers of caudal vertebrae of normal and Brachyury phenotypes at one and ten days after birth. Over each bar is placed the standard error of the mean.

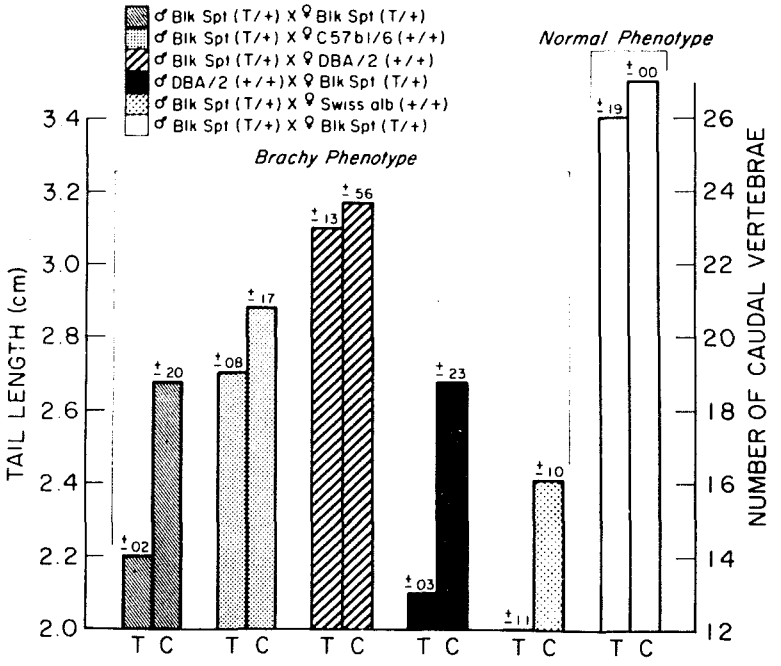
Figure 65. Comparisons between mean tail length and mean number of caudal vertebrae at ten days after birth. Over each bar is placed the standard error of the mean.

MEAN NUMBERS OF CAUDAL VERTEBRAE OF NORMAL AND BRACHYURY PHENOTYPE AT ONE AND TEN DAYS AFTER BIRTH



64

COMPARISONS BETWEEN MEAN TAIL LENGTH AND MEAN NUMBER OF CAUDAL VERTEBRAE AT TEN DAYS AFTER BIRTH



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T – TAIL LENGTH
C – CAUDAL VERTEBRAE

Autobiographical Statement

EDUCATION

Public School 104, Bronx, New York.

Macomb's Junior High School, Bronx, New York.

William Howard Taft High School, Bronx, New York.

The City College of New York (B. S. in Biology, June 1963).

TEACHING EXPERIENCE

Lecturer (part-time), The City College of New York (9/63-6/65); laboratory instructor in general biology, comparative anatomy, and developmental biology; lecturer in general biology.

Instructor, Hudson Valley Community College (9/68-8/69).

Assistant Professor, Hudson Valley Community College (9/69 to present); laboratory instructor in anatomy/physiology and microbiology; lecturer in anatomy/physiology.

RESEARCH EXPERIENCE

Trainee, National Institutes of Health (7/65-7/67); in the laboratory of Dr. Max Hamburgh, The City College of New York.

Beginning Investigator (6/68-9/68); in the laboratory of Dr. John W. Saunders, Jr., The Marine Biological Laboratory, Woods Hole, Mass.

Investigator (6/69-9/69); at the Marine Biological Laboratory, Woods Hole, Mass.

AWARDS

Traineeship, National Institute of Child Health and Human Development, under the direction of Dr. Max Hamburgh (1965-1967).

AWARDS (continued)

Faculty fellowship and grant-in-aid, The Research Foundation of The State University of New York (1969-1970).

PUBLICATIONS

Krupa, P. L., G. L. Cousineau, S. Inoue, and K. Wittman. 1969. Electron microscopy of colloid adsorption by the surface of a playthelminth parasite. Biol. Bull. 137: 406 (abstract).

Wittman, Karl S. 1966. Effect of genetic background on expressivity and penetrance of the "Brachyury" gene in the mouse. Amer. Zool. 6: 511 (abstract).

Wittman, Karl S. 1969. Protein synthesis during hydranth regeneration in Tubularia. Biol. Bull. 137: 415 (abstract).

Wittman, Karl S. and Max Hamburgh. 1968. The development and effect of genetic background on expressivity and penetrance of the Brachyury mutation in the mouse: A study in developmental genetics. J. Exper. Zool. 168: 137-146.

PAPERS PRESENTED

"Effect of genetic background on expressivity and penetrance of the Brachyury gene in the mouse", at the December 1965 meeting of the American Association for the Advancement of Science, Washington, D. C.

"Protein synthesis during hydranth regeneration in Tubularia", at the August 1969 general meeting of the Marine Biological Laboratory, Woods Hole, Mass.

EXTRA-CURRICULAR ACTIVITIES

The Biological Society of the City College of New York (1959-1963); President and editor of the club journal.