CELL DEATHS IN NORMAL VERTEBRATE ONTOGENY

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I. INTRODUCTION

During normal vertebrate ontogeny a number of transient structures are formed which subsequently regress completely, as, for instance, the tail of anurans, or which are partially transformed into persisting organs, as the mesonephros. The regression and often the transformation of these tissues involve the resorption of numerous cells. Cell death during normal ontogeny is, however, not restricted to these rudimentary or transient formations. It is found also during a number of embryological processes, such as invaginations and evaginations, separation of parts from each other, migration of rudiments, closure of tubes, vesicles, etc. Observations of this type have been reported by numerous authors, but there are few systematic studies of these phenomena.

Rabl (1900) and other embryologists (Jokl, 1918, 1920) found it difficult to accept the idea of cells dying during embryogenesis and particularly in actively growing regions. They therefore interpreted the 'granules' (i.e. pycnotic nuclei) as 'mitotic metabolites', and such an interpretation was accepted and defended by Peter as late as 1936. It is, of course, a surprising fact that embryonic cells in actively growing regions die, and it is difficult to see what may cause the death of these cells. To produce cells which are subsequently discarded appears as an extravagant waste

on the part of the organism, and this fact represents a challenge to any deterministic view of ontogeny.

The aim of this article is to stimulate interest in the study of these cellular degenerations as one of the mechanisms of the integration of cells into tissues and organs, to survey some of the reports which deal more specifically with these phenomena, and to examine some of the hypotheses put forward. Since the morphological appearance of degenerating cells has often been misinterpreted, it is necessary to deal first with the morphology of cell death. The second part of the article summarizes the incidence of cell deaths during the various embryonic processes in vertebrates and analyses the conditions under which degenerations occur, while the final part surveys the various hypotheses and interpretations of degenerative phenomena.

II. THE MORPHOLOGY OF CELL DEATH DURING NORMAL VERTEBRATE DEVELOPMENT

The terms cellular degeneration, cellular disintegration, necrobiosis and others are used to describe the process of slow cell death in contradistinction to the instantaneous cell death produced by histological fixatives. Death produced by good fixatives should not grossly distort the structures of the living cell, while the slow death of cells induces intravital changes in their structure. The particular way in which cells die varies with their degree of specialization and with the agents causing the death. Experimentally, cells can be killed in various ways: radiations, for instance, cause cells to break down during mitosis or while attempting division, and the process of degeneration concerns mainly the chromatic parts of the nuclei resulting in chromatopycnosis. Interference with the blood supply, on the other hand, causes a process of necrosis which is characterized by the loss of staining power of the nucleus, i.e. predominantly a process of autolysis leading to karyolysis and cytolysis.

The degree of specialization of the cell is responsible for the rather different mode of the death of a cartilage cell by 'hydropic swelling', the sarcolytic process in striated muscle fibres or the death by keratinization of epidermal cells. Degenerate cells often contain fat, glycogen, mucin, or, in amphibia especially, they are laden with pigment (Kremer, 1927, 1930*a*, *b*).

The form of cellular death most frequently encountered in normal vertebrate embryos is characterized by a number of nuclear changes: (1) the initial stage, chromatopycnosis, consists in the separation of the chromatic from the non-chromatic material of the nucleus and the precipitation and coalescence of the former into larger granules and finally into a single mass. The non-chromatic material seems to liquefy and to form confluent vacuoles. (2) These nuclear changes result in the appearance of a single chromatic mass sitting as a cap on the vacuole formed by the non-chromatic material. This stage is described as hyperchromatosis of the nuclear membrane. Both the nucleus and the cytoplasm, which becomes liquefied or undergoes a fatty change, shrink by the loss of fluid. (3) After gradual

shrinkage a mere chromatic granule persists and is surrounded by a liquefied or fatty zone. The granule loses its affinity for nuclear stains, becomes Feulgen-negative, breaks up and disappears: this is chromatolysis.

These three stages have been described in detail (Glücksmann, 1930*a*; Spear and Glücksmann, 1937). They may occur in the isolated cell or inside another cell, that is, the degenerating cell may be phagocytosed by a neighbour, and the last stage in particular is often found to proceed within the resorbing cell.

There is a number of variations of this process: for instance, a cell breaking down in prophase forms bands of chromatin rather than granules, and these bands subsequently conglomerate to form the hyperchromatic stage (Spear & Glücksmann, 1937). A cell breaking down during meta-, ana- or telophase may do so by a process of coagulation of the chromosomes and then pass directly into the stage of chromatolysis (Glücksmann, 1940, 1947). In these instances mitotic abnormalities precede cell deaths by chromatolysis. If there are numerous dying cells close to each other they tend to become confluent (Glücksmann, 1930*a*) and to form a large fat globule (Froboese, 1926) or they may form a liquefied zone (Glücksmann & Tansley, 1936).

During the embryonic development of individuals of certain genetic constitutions, vascular damage occurs at certain stages of development with consequent karyolysis, karyopycnosis or karyorrhexis; in the first process the nuclei retain their volume, but lose definition of internal structure and staining power. The second process involves a shrinkage of the nucleus and the condensation of the chromatic material. The third process consists of the breaking up of the nucleus into pycnotic granules (Hoepke, 1931). The cytoplasm shrinks and may undergo either liquefaction or condensation. Cherry (1950) has found that the nuclear changes are to some extent independent of the cytoplasmic processes. These forms of cell deaths are observed in necrotic areas rather than in the normally developing regions of a 'normal' embryo. In the latter the degenerations most frequently seen are those described as chromatopycnosis, hyperchromatosis of the nuclear membrane and chromatolysis, and it is these processes which will be referred to subsequently as cellular degenerations.

In cells exposed to injurious agents mitochondria often show early and irreversible changes indicative of processes leading to subsequent cell death (Ludford, 1935; Foggs & Warren, 1937; Zollinger, 1948*a*, *b*). In embryological material examined by the usual histological methods, the mitochondria are only rarely seen and their degenerative changes have not been reported as systematically as the more obvious nuclear reactions.

Degenerating cells are often phagocytosed, and if the resorbing cells divide, remains of dying cells are found even in mitotic cells. This phenomenon led to two generalizations: (*a*) that resorption by adjacent cells is necessary for chromatolysis and (*b*) that granules produced by degeneration are really mitotic metabolites. Gräper (1914) maintained that only enzymes of normal cells are capable of the digestion of chromatin, and that the process of chromatolysis is observed usually, if not exclusively, after the resorption of the dying cell by a normal neighbour. This

statement is supported to some extent by the fact that in the whole body the rate of autolytic processes is considerably greater than in isolated sections (Mönninghoff, 1939), but is at variance with the finding that, for instance, in irradiated tissues degenerating cells may proceed to complete chromatolysis and cytolysis without being resorbed, and that cells extruded into the lumen of embryonic organs (lens, neural tube etc.) undergo chromatolysis without being resorbed.

The fact that degeneration granules are found in mitotic cells led Rabl (1900) and more recently Peter (1936) to interpret them as mitotic metabolites. Neither author has explained how these Feulgen-positive granules come to be formed during mitosis. There is overwhelming experimental evidence that degenerating cells in the process of chromatopycnosis and chromatolysis are resorbed by cells which may subsequently divide (Spear & Glücksmann, 1937; Dustin, 1947), and no evidence to show that these 'mitotic metabolites' are derived from chromatic material of a normally dividing cell. The proof that the described degenerative processes lead to the death of the cell rests on the analysis of transitional stages up to final cytolysis in normal material (Glücksmann, 1930*a*), in material exposed to radiation injury (Glücksmann & Tansley, 1936; Spear and Glücksmann, 1937), and on the identity of these processes with those observed under pathological conditions (v. Szily, 1912; Froboese, 1926; Ernst, 1926, 1929).

That the degeneration granules are derived from nuclear material is shown by their initially positive reaction with Feulgen's stain (Bartels & Voit, 1931; Jacobson, 1932; Bieling, 1937; Glücksmann, 1940). Similar forms of degenerations are produced at certain intervals after exposure to ionizing radiations (Strangeways & Oakley, 1923; Glücksmann & Tansley, 1936; Spear & Glücksmann, 1937) or to karyoclastic poisons (Dustin, 1947). In such experiments the appearance of the various stages can be clearly identified up to the complete resorption of the dead cells, and the sequence of stages can thus be clearly established. The same processes have been observed directly in tissue cultures during the death of individual cells (A. Fischer, 1930; Levi, 1934; Fell & Andrews, 1927; Fell, 1939). Tissue-culture experiments have shown, furthermore, that degenerating cells stain supravitally with neutral red, toluidin blue and other vital stains (A. Fischer, 1930; van Weel, 1948). Gräper (1935), Bieling (1937) and Stockenberg (1937) used supravital staining with neutral red for the identification of areas of degenerations in embryos, and Bieling could identify in the fixed specimen the stained regions with areas of cell degenerations.

The appearance of cell deaths may be restricted to a developmental stage of short duration, and consequently their presence may be easily overlooked. During the development of the frog tadpole retina, for instance, cellular degenerations occurred in three distinct waves of varying duration (Glücksmann, 1940). The first of these degeneration waves was noticed within 1 day of hatching, and about 50% of cell deaths remained visible for about 7 hr. The process of degeneration may, however, be considerably shorter. Thus a short wave of degeneration followed the exposure of tadpoles to radium, and during that wave the whole process of degeneration must

have been completed within 1–3 hr. (Spear & Glücksmann, 1937). Similar experiments on chick fibroblasts in tissue culture showed that some cellular disintegrations were completed in 0·5–1 hr. (Strangeways & Oakley, 1923). Direct observations of tumour cells *in vitro* (Jensen rat sarcoma) by Fell & Andrews (1927) indicated that some degenerations took about 7 hr., and a similar figure was obtained by calculation for the duration of cell death in the Jensen rat sarcoma *in vivo* (Lea, Lasnitzki & Glücksmann, 1944). When, however, numerous cells of a tissue or organ die simultaneously, the time taken for clearing away the debris may be extended to days rather than hours. A physiological example is the time taken for the resorption of the tail during the metamorphosis of anurans, and an experimental one is the time taken for the clearance of extensive degenerations induced by radium in the retina of the 2-day-old rat (Glücksmann & Tansley, 1936).

III. THE INCIDENCE AND LOCALIZATION OF CELL DEGENERATIONS DURING NORMAL VERTEBRATE DEVELOPMENT

Observations of degenerate cells during embryogenesis and organogenesis are recorded in Table 1. The number of dead cells varies in individuals of approximately the same stage, and so to some extent does the process of degeneration found in different species. Since cell deaths are linked with certain developmental processes rather than with the 'age' in days of the embryo, the time of occurrence is given in terms of developmental stage. The appearance of dying cells in lumina often drew attention to them, i.e. after the dying cells had become separated from their normal epithelial surroundings. Very frequently the process of degeneration has started before the extrusion of these cells, as, for instance, in the lens vesicle. In explanation of the tabulated reports the following remarks are pertinent.

(1) *Embryogenesis*

This term includes early embryonic stages up to the neurula and processes involving the detachment of the embryonic disk from the yolk sac or its equivalents, and the formation of an approximately cylindrical body.

Before and during gastrulation some yolk endoderm cells degenerate and are found either in the subgerminal or endodermal lumen (I, II)*. These deaths are presumed to play some role in the metabolic breakdown of yolk.

In the chameleon a transient circumscribed thickening of the amnion (III) is characterized by the presence of pigment and chromatin granules. Peter (1935) suggested that this structure by secreting a hormone or in some other unspecified manner promotes embryonic development.

Stockenberg (1937) observed cell deaths in the primitive node (V) and at other localizations (X, XVI, XXIV, XXX) of supravitally stained embryos. Degenerating cells and yolk metabolites are found to stain strongly and thought to be analogous structures and concerned with the induction of organs. No evidence for this assumption is adduced.

* Serial numbers refer to Table 1.

Table 1. *Localization of embryonic cell deaths*

No.	Site, tissue and reference	Stage or process	Animal
		Embryogenesis	
I	Yolk and endoderm (7, 13, 33, 47)	Blastula	*Torpedo*, *Acanthias*, *chick*
II	Endoderm (3, 91)	Gastrula	*Triton cristatus*, *Tropidonotus*
III	Thickening of amnion (70)	Folding of amnion	Chameleon
IV	Presumptive neural tissue (47)	Primitive streak	Chick
V	Primitive node (86)	Primitive streak	Chick
VI	Mid-ventral region (13, 22)	Detachment and folding of embryonic disk	Vertebrates*
VII	Epidermis, mesenchyme and mesothelium of mid-ventral line (16)	Union of body halves before formation of sternum	Chick, sparrow, budgerigar
		Organogenesis: central nervous system	
VIII	Neural plates (47)	Before invagination	Chick
IX	Neural groove (34)	During invagination	Chick
X	Neural tubes (6, 13, 24, 34, 72, 86, 88)	Before and during invagination, closure and detachment from ectoderm	Vertebrates
XI	Forebrain (13)	Evagination of optic vesicle	Mole, rabbit
XII	Roof and floor of 4th ventricle and recessus interventricularis (13)	Before vascularization	Fish, mammals
XIII	Region of paraphysis (13, 22)	Vestigial formation	Mouse, pig
XIV	Spinal cord, anterior horn (13, 22, 28)	Vascularization	Duck, mammals
XV	Spinal ganglia (13, 28, 35, 41)	Differentiation of early neuroblasts	*Lacerta*, chick duck, mole, mouse, rabbit, pig, man
XVI	Ganglia of branchial nerves (13, 22, 72, 86)	Metamorphosis	*Torpedo*, *Rana*, *Alytes*, *Lacerta*, birds, mammals
		Sense organs: eye	
XVII	Optic vesicle (6, 13, 22, 24, 64, 74, 88, 89)	Before, during and after invagination to optic cup	Vertebrates
XVIII	Retina (29)	Differentiation of the three cell layers	Frog
XIX	Lens (13, 24)	Before and during invagination	Vertebrates
XX	Lens (13, 20, 24, 51, 52, 74, 92)	Detachment from ectoderm	Vertebrates
XXI	Lens (25)	Regression of peridermal plug	Fish, mole, pig, cat, rabbit, sheep, man
XXII	Conjunctival papilla (67)	Form changes and regression	Chick
XXIII	Mesenchyme of conjunctiva (67)	Prior to formation of scleral bone	Chick
		Sense organs: ear	
XXIV	Auditory vesicle (6, 13, 24, 34, 72, 86)	Before and during invagination and detachment from ectoderm	Vertebrates
XXV	Octocyst (13)	Fusion with acusticus ganglion	Fish, birds, mammals

* The term vertebrates covers the following Anamnia and Amniota:
Anamnia: (fish) *Torpedo*, *Pristiurus*, *Scyllium*, trout, (amphibia) *Triton*, *Salamandra*, *axototl*, *Rana*, *Alytes*.
Amniota: *Lacerta*, (birds) duck, sparrow, chick, *Melopsittacus*, (mammals) mole, bat, cat, guinea-pig, rabbit, mouse, sheep, pig, man.

Table 1 (*cont.*)

No.	Site, tissue and reference	Stage or process	Animal
		Sense organs: nose	
XXVI	Olfactory pit (6, 13, 22, 24, 26, 65, 79)	During invagination and detachment from palate	Amniota
XXVII	Olfactory epithelium (13)	During neurotization	Mammals
XXVIII	Nose plug and membrana buccnasalis (26, 65, 83, 84)	Regression	Amniota
XXIX	Stratified cuboidal epithelium of nose (26)	Formation of ciliated epithelium	Amniota
		Epidermis and transient ectodermal structures	
XXX	Pharyngeal and cloacal membrane; branchial membrane (13, 72, 86)	Regression Regression	Vertebrates Amniota
XXXI	Proliferated epidermal seams of lids, lips, preputium, anus, vulva, urethral orifice and external auditory meatus (73, 83, 84)	Regression	Man
XXXII	Apical epithelial papilla of limb buds (27)	Formation, regression	*Lacerta*, birds, Amniota
		Digestive tract	
XXXIII	Endoderm (13)	Closure, formation of thymus, thyroid and parathyroids	Vertebrates, mammals
XXXIV	Tongue (38, 39, 40)	Formation of fungiform papillae	*Rana sylvatica*
XXXV	Salivary glands (8)	Solid anlage	Mouse
XXXVI	Maxilla and branchial arches (6, 13, 22)	Undifferentiated mesenchyme	Mole, rabbit, pig, mouse, man
XXXVII	Oesophageal glands (80)	Solid anlage	Chick
XXXVIII	Duodenum and small intestine (49)	Regression of folds	Man
XXXIX	Colon (50, 94)	Regression of folds	Guinea-pig, man
XL	Intestinal tract (56)	Shortening during metamorphosis	Anurans
		Respiratory tract	
XLI	Larynx (13, 22)	First appearance	Amniota
XLII	Trachea and oesophagus (13)	Separation of anlagen	Amniota
XLIII	Lung	Branching of pneumonomeres	Man
		Urogenital tract	
XLIV	Pronephros (2)	Regression	Man
XLV	Mesonephros (2, 13, 22)	Prior to regression and during regression of cranial tubules	Pig, mouse, man
XLVI	Metanephros (2)	Regression of first generation of secretory tubules	Man
XLVII	Orifice of ureter (13)	Separation from urogenital sinus	Mouse
XLVIII	Müllerian duct (2, 68)	Partial regression	Man, hamster (males)
XLIX	Wolffian duct (13, 68)	Partial regression	Mouse, hamster (females)
L	Vagina (54, 90)	Solid stage	Man
		Vascular system	
LI	Vascular rudiments (72)	Prior to lumen formation	Man
LII	Aorta (44)	During bifurcation and shortening	Chick
LIII	Ductus arteriosus (44)	Regression	Chick

Table 1 (*cont.*)

No.	Site, tissue and reference	Stage or process	Animal
	Apparatus of locomotion: (*a*) notochord		
LIV	Notochord (13)	Detachment from endoderm	Fish, rabbit
LV	Notochord (2)	Partial regression	Vertebrates
	Apparatus of locomotion: (*b*) Somites		
LVI	Cranial and caudal somites (13)	Abortive segmentation	Mole, mouse, rabbit, man
LVII	Sclerotome in mid-line of future vertebra (13, 27, 28, 46)	Prior to cartilage formation	Birds, mammals
LVIII	Myotome (27, 28)	Formation of second muscle type and of permanent musculature	Teleosteans, Amphibia, Amniota
LIX	Dermatome (27, 28)	Dissolution of medial part	Amniota
LX	Mesenchyme between myotome and spinal ganglion (13)	Prior to differentiation	*Melopsittacus*
	Apparatus of locomotion: (*c*) Chondro- and osteogenesis		
LXI	Mandible, vertebrae and long bones (13, 15, 27, 28, 46)	Dense prechondral mesenchyme	Birds, mammals
LXII	Protochondral tissue (77)	Prior to formation of matrix	Cyclostomes
LXIII	Hypertrophic cartilage (15, 22, 46)	Prior to ossification	Chick, mouse, pig, man
LXIV	Osteoblastic tissue of mandible (22, 46)	Formation of matrix	Mole, mouse, rabbit, pig, man
LXV	Dense mesenchyme of hind limbs (13)	Regression of pentadactylous primordium	Birds
	Apparatus of locomotion: (*d*) Morphogenesis of muscles and skeleton		
LXVI	Mandible, mid-line (48)	Union of bilateral anlagen	Chick
LXVII	Mesenchyme of mandible (48)	Before ingrowth of myogenic tissue	Chick
LXVIII	Proximal osteogenic centres of mandible (48)	Before subdivision	Chick
LXIX	Mid-ventral body wall (16)	Before ingrowth of dorso-lateral tissue	Chick, sparrow, budgerigar
LXX	Knee-joint ('opaque patch') (18)	Before joint formation	Chick
LXXI	Sarcolysis (32, 59)	Early differentiation of myoblasts	Mammals
LXXII	Sarcolysis near tendons (76, 78)	Remodelling of insertions	Man
LXXIII	Sarcolysis of rump muscles (28, 57, 58, 62)	Metamorphosis	Amphibia
LXXIV	Sarcolysis in tongue (11)	Before expansion of serous glands	Rat

The gradual folding of the embryonic disk and the closure of the ventral body wall are accompanied by numerous cell deaths in the mesenchyme and epidermis (VI, VII). Epidermal ridges are formed during this process and regress by cell deaths (Fell, 1939).

Cell degenerations occurring in the unsegmented cranial and caudal mesenchyme of higher vertebrates have been interpreted as representing abortive somites (LVI).

(2) *Nervous system*

During the transformation of neural plates into grooves and tubes and during the subsequent detachment of the neural tube from the ectoderm numerous cell

deaths are found (VIII, IX, X). They precede the formation of the groove but are still present in this stage, particularly near the ventral midline. Later they are found mainly in the middle parts of the tube, and finally they are scattered in the dorsal region and in the stalk which connects the neural tube with the ectoderm. The ectoderm overlying the neural tube is thickened and contains degenerations before, during and after the detachment of the neural tube. The connecting stalk degenerates, and remains of dying cells are found between the closed tube and the ectoderm. The number of cell deaths varies over the length of the tube and is particularly great in the region of the anterior neuroporus (X). These cell degenerations occur prior to changes in the shape (Glücksmann, 1930*a*; Bieling, 1937; Stockenberg, 1937), i.e. prior to groove formation, to the folding and closing of the tube, and to the separation of the neural tube from the ectoderm. Though they persist until after these organs have completed their change in form, they cannot be caused by the detachment or any other of the involved morphological processes as suggested originally by Ernst (1926), since they precede them.

In anurans, particularly, the metamorphotic processes in the branchial region are reflected in the appearance of degenerations in the ganglia of branchial nerves (XVI). These are obviously concerned with the removal of cells rendered superfluous. Cell deaths in smaller numbers are found in the same localization in Amniota, in which only abortive branchial organs are formed and consequently there are fewer cells to be removed.

Ernst (1926) reported considerable numbers of cell deaths in the spinal ganglia (XV) of the limb regions which he related to the plexus formation and the disturbance of the original segmental arrangement. Glücksmann (1934*c*) found such cell deaths also in the spinal ganglia of the thoracic and upper cervical regions, particularly in birds and lower mammals, at the onset of skeletal ossification. They follow on cell deaths in the anterior horn of the spinal cord, while no significant numbers of degenerations were found in the posterior horns of the spinal cord. The occurrence of cell deaths in spinal ganglia outside the limb regions makes their relation to plexus formation rather doubtful.

Hamburger & Levi-Montalcini (1949) studied the degenerations in the spinal ganglia of chick embryos in greater detail by quantitative and experimental methods. They found that the spinal ganglia which serve the limbs are larger than adjacent ganglia, and this size difference can be correlated with a greater mitotic incidence and an absence of degenerations in the spinal ganglia of the limb region. In adjacent ganglia cell deaths abound particularly during the 5th and 6th day of incubation, while they are rare in ganglia of the limb region. If, however, the limb bud is removed at $2\frac{1}{2}$ days, early neuroblasts of the spinal ganglia of the limb region degenerate in great numbers, particularly on the 5th and 6th day of incubation.

Ernst (1926) suggested that cell deaths in the anterior horn of the spinal cord and in the 4th ventricle occur just prior to vascularization and are an expression of nutritional insufficiency (XII, XIV). This suggestion requires further substantiation.

Cell degenerations in the brain of mouse and pig embryos in a region homologous

to that of the paraphysis of lower vertebrates are interpreted by Hoepke (quoted by Foboese, 1926) as the vestigial primordium of the paraphysis (XIII), i.e. cells determined to form the paraphysis die before they begin their morphological and histological differentiation. This finding and its interpretation have been confirmed by Ernst (1926).

(3) *Sense organs*

(*a*) *Eye*

Cell degenerations in the optic vesicle prior to, during and after the invagination of the optic cup have been described as blood cells by Mann (1928) and Kornzweig (1942) and as mitotic metabolites by Rabl (1900) and Jokl (1918, 1920). von Szily (1912) recognized them as cell deaths, and correlated them with the formation of nerve fibres. He suggested that the dying cells exert a chemotactic influence on optic nerve fibres which grow in channels formed by the resorption of dead cells. This interpretation is at variance with the fact that cell deaths occur even before the opticus ganglion cells begin to differentiate and are present after the nerve fibres are formed. Ernst (1926) correlated these cell degenerations with the process of invagination, i.e. he thought that cells were displaced during invagination, damaged or rendered superfluous and removed.

In a detailed study of the exact localization of mitotic and degenerate cells in the optic vesicle and the optic cup (XVII) it was found (Glücksmann, 1930*a*) that both these activities occur in places which subsequently change their shape, i.e. that morphological changes of the eye vesicle are brought about not only by cell movements but also by the mitotic multiplication and by the resorption of cells. The contribution which the various cellular activities make to the shaping of epithelial organs varies to some extent with the species and in particular with the yolk content of the cells.

In mammals (mouse and man), for instance, the volume of the eye and the number of its cells increase considerably during the relevant stages while the cell size remains constant. In the frog tadpole, on the other hand, the volume of the eye remains fairly constant during comparable stages, the number of cells increases while with the resorption of yolk the cell volume decreases (Glücksmann, 1940). In the mammals the cells are closely packed so that little space is left for lateral cell movement and mitosis is restricted to the free outer surface. In the tadpole retina cells can move freely owing to their decrease in size and divide even in the middle layers of the retina. In the mammalian retina the cells of the outer part are arranged as a regular columnar epithelium with their nuclei and long axes orientated at right angles to the free surface. The corresponding cells of the tadpole retina are almost round and irregularly arranged. As invagination proceeds and the cells decrease in volume, they assume a cone-like shape and arrange themselves into a regular pattern. In the mammals numerous dead cells are found and their resorption allows of cell movements and of modelling the shape of the eye, while hardly any cell deaths occur at this stage in the tadpole retina.

Three distinct waves of cell degeneration are found subsequently during the

differentiation of the tadpole retina (XVIII). The first is due to the death of dividing cells of the central region of the eye and is followed by the appearance of ganglion cells in this region. The mitotic cell count at this period shows a marked decrease, and subsequently mitotic cells are seen almost exclusively in the peripheral germinative zone of the eye (near the iris). The second wave of degeneration occurs at a period of relatively great mitotic activity and affects resting cells of the middle layers of the central region which is immediately afterwards occupied by the inner nuclear layer. The number of cell deaths seems to vary considerably in different individuals. The degenerative process is sometimes associated with pigment formation which renders the dying cells very conspicuous, as the other cells of the central region no longer contain pigment. The pigment epithelium also shows numerous degenerate cells at the same time and in the same regions. The third wave of cell deaths concerns the border of the central differentiated and the peripheral germinative zone of the retina in regions destined to form the outer nuclear layer.

The coincidence of each wave of degeneration with the onset of a new type of differentiation seems highly significant and might be interpreted as follows: factors responsible for the differentiation of opticus ganglion cells in the central part of the retina inhibit cell division and cause the death of (?partly inhibited) cells which have embarked on mitosis. Similarly, cells already embarked on differentiation to opticus ganglion cells are unable to respond to a stimulus for the differentiation of inner nuclear cells and die on reaching the end of their life span. A similar condition obtains for cells which have begun to differentiate in the direction of inner nuclear cells and are unable to follow an impulse for the differentiation to outer nuclear cells.

These degenerations in the frog tadpole retina are not found in the mammalian eye. Thus, the retina of the 2-day-old rat contains merely a few dead cells at the border of the inner differentiated and the outer undifferentiated zone of the inner nuclear layer. This differential incidence of cell deaths is undoubtedly linked with the fact that the tadpole retina differentiates and even functions while still rapidly growing, whereas in the mammalian eye cell multiplication almost ceases when the inner and outer nuclear layers differentiate. The difference in the relation of growth to differentiation of the tadpole and of the mammalian retina is also reflected in the distribution of mitotic cells which in the tadpole are restricted to the germinative zone between the iris and the differentiated part of the retina, while they occur in the outer layers of all parts of the still growing, undifferentiated mammalian retina.

Changes in the shape of the lens during the invagination, the formation of a vesicle and the detachment from the ectoderm (XIX, XX) are correlated with mitosis, cell movements and degenerations in the same way as described for the optic cup. In addition to such morphogenetic cell deaths other degenerations occur in connexion with the regression of the lens plug, i.e. of a peridermal overgrowth which appears in some species (XXI) as the result of a coincidence of periderm formation and of lens induction. This peridermal proliferation accounts for the primary solid anlage of the lens in fish and for the appearance of plugs in some mammals.

The conjunctival papilla (XXII) is a transient structure in birds and undergoes some changes in shape prior to complete regression. Murray (1943) described morphogenetic degenerations involved in these changes. Between the region of the papilla and that of the future scleral bone, cell deaths are found in the mesenchyme and related to them collagen fibres are formed (XXIII).

(*b*) *Ear*

The cell deaths observed before, during and after formation of the otocyst and its separation from the ectoderm (XXIV) are analogous to those described for the eye and lens and belong to the group of morphogenetic degenerations (XXIV).

(*c*) *Nose*

The degenerations in the olfactory plates, pit and groove are related to morphogenetic processes (XXVI).

Degenerations in the olfactory epithelium and the olfactory nerve like those found during the fusion of the otocyst with the acusticus ganglion (XXV) are thought to be related to the neurotization of these organs (Ernst, 1926).

As the formation of the palate progresses the epithelial wall connecting oral and nasal cavities regresses by degeneration of cells or by their parakeratosis which is a form of keratinization. In the latter case they may appear as horn pearls in the mesenchyme of the palate (Peter, 1913).

In most Amniota the nares are occluded for some time by a peridermal plug (XXVIII) which represents a proliferation of the invaginated oral cavity epithelium and which increases in volume with the amount of invaginated epithelium, i.e. towards the opening of the nares. This solid mass of epithelial cells is canalized by their degeneration and resorption, or by their keratinization and desquamation. In some species part of the basal layers of the epithelial mass is used subsequently for the formation of the ciliated epithelium (XXIX). In the human, for instance, cuboidal cells of the more cuticular layers are transformed into columnar ciliated cells, while others degenerate. In the mouse, on the other hand, most of the basal and all of the cuticular cells die, and the ciliated epithelium is formed by the proliferation of some remaining undifferentiated cells of the basal layer. In the mole and in birds basal and cuticular cells left over after the regression of the plug form at first a regular stratified cuboidal epithelium which subsequently changes into the ordinary type of ciliated epithelium.

(4) *Epidermis and transient ectodermal structures*

The closure of the body in the mid-ventral line (VI, VII) is facilitated by the proliferation of the joining edges. Fell (1939) described the appearance of an epidermal ridge after closure which later is resorbed. Similar temporary proliferations occur (XXXI) when other organs are formed by the joining of opposing halves; the lens, nose and ear have already been mentioned.

Cell deaths as well as parakeratotic processes are involved in the regression of some hypertrophic epidermal seams (XXXI), such as those closing temporarily the lids, lips, choana, external auditory meatus, and of epithelial proliferations in the genital region, as, for instance, on the urethral orifice, glans, preputium, vulva and on the anus. Steiner (1930, 1931) traces these proliferations to a type of embryonic epidermis which tends to grow in depth rather than in the other two dimensions. These excrescences may, however, be accounted for by the absence or by a greatly reduced rate of desquamation rather than by increased cell proliferation, and a similar explanation may hold for the originally solid anlage of the vagina and of the initial mammary gland bud. It is noteworthy that during these periods there is little evidence of keratinization or of parakeratosis.

Some openings are temporarily closed by the formation of membranes (XXX) in connexion with the junction of parts; the pharyngeal and cloacal membranes, for instance, are thus formed and regress by cell degeneration.

In Amniota a transient apical ectodermal ridge appears at the tips of the limb buds (XXXII) which becomes a fold in reptiles, thus indicating its homology with the epithelial fold which appears during the development of fins in fish. In mammals this papilla is a mere thickening, though cell degenerations reveal an attempt at forming a fold. The actual folding process in birds involves the appearance of morphogenetic degenerations, while cell deaths and resorption bring about the regression of the ridge. Associated with these epithelial degenerations are degenerations in the underlying mesenchyme. Saunders (1948) showed that this ectodermal ridge is necessary for the normal development of the wings, and Zwilling (1949) found this ridge absent in wingless chicks.

(5) *Digestive tract*

The epithelial occlusions of the intestinal tract (XXXVIII, XXXIX) which occur in almost all vertebrates in various regions and to a varying extent are usually hollowed out and canalized by cell degeneration. Similar processes as well as cell movements account for the formation of a lumen in the solid rudiments of a number of glands associated with the intestinal tract (XXXV, XXXVII).

Cell deaths have been observed in Amniota during the formation and the subsequent changes of the branchial region, of its membranes and its derivatives (XXXVI). Some of these degenerations occur a long time before the regressive changes (Froboese, 1926), others are probably linked with an abortive metamorphosis.

(6) *Respiratory tract*

Unpublished observations on human embryonic lungs grown in tissue culture (XLIII) confirmed Heidenhain's (1921, 1923) and Bender's (1925) description of the hammer-like spreading and subsequent division of pneumonomeres and showed that mitosis and cell degenerations as well as cell movements bring about these alterations in form.

(7) *Urogenital tract*

Cell degenerations have been reported during the various stages of development and regression of pro-, meso- and metanephros as well as during the transformation of the Wolffian and Müllerian ducts in males and females respectively (XLIV–XLIX). Very little attention has, however, been paid to their specific time of appearance and their roles in the process of development.

(8) *Vascular system*

Hughes (1943) found cell deaths at the bifurcation of the aorta in 5–7-day chick embryos. These cells appear to be rendered superfluous by the shortening and dichotomy, and their appearance is followed shortly by that of elastic fibres.

During the regression of thin-walled vessels of the branchial region of chick embryos endothelial cells migrate into the mesenchyme and dedifferentiate into mesenchymal cells (Hughes, 1943), while the regression of the thicker walled ductus arteriosus (LIII) involves some cell deaths.

(9) *Locomotory apparatus*

(*a*) *Notochord*

Cell deaths occur during the separation of the notochord from the endoderm (LIV, LV) and are of the usual morphogenetic type. The regressive changes in the notochord and those leading to the formation of nuclei pulposi are well known.

(*b*) *Somites*

The principal task of somites in vertebrates is to produce the muscles and skeletal parts of the rump. This function persists during the phylogeny and ontogeny of vertebrates, though its realization undergoes some modifications; thus the relative extent of the sclerotome is increased in higher vertebrates at the expense of the myotome. The role of the dermatome too is modified, though it retains its main function to act as a germinative zone for the myotome (Glücksmann, 1934*c*).

Comparative embryological studies show that in the Amniota the locomotory apparatus passes through three stages of development. The first is formed by the notochord and by layers of muscles not connected with it. The second stage is represented by the cartilaginous skeleton and by a musculature which is still mainly built up of layers but which has formed secondarily connexions with the skeleton by the development of connective tissue sheaths and of pseudotendons. These are due to the ingrowth of mesenchymal cells around vessels into the myosepta. At this stage for the first time muscles are also present as individual organs, for instance in the head and limb regions. The third stage is formed by the ossified skeleton and by individual muscles which have continuous connexions with the skeleton through true tendons. These three stages are best seen during the development of anurans where the change from the second to the third stage is effected during the metamorphosis. In selachians the second stage persists, while in teleosteans head and fin muscles reach the third stage and there is also some ossification of the skeleton.

In Amniota, on the other hand, the first two stages in the development of the locomotory apparatus are abortive and, as regards the muscles, are often represented by the appearance of degenerate cells at the appropriate stages (LVIII, LIX, LXXIII). Two waves of degeneration occur: one at the beginning of the formation of the second muscle type which coincides with the onset of chondrification, and one at the end of this stage when the transient formations are replaced by the persisting types of tissue, i.e. at the onset of ossification. The number of degenerations of these two waves varies with the species; mole, mouse and bat, for instance, have many cell deaths during the first wave and only few during the second, while in *Lacerta* and pig only few cells die during the first and very many during the second wave. In birds and man intermediate conditions obtain.

The process of degeneration differs in the two waves; in the first, undifferentiated cells die, while during the second partly differentiated muscle fibres regress by a process of sarcolysis. In Anamnia the first wave of cell deaths is completely missing and instead a fully functioning musculature develops. The second wave occurs in teleosteans and amphibia, in the latter during metamorphosis, and involves progressively the whole of the body musculature. The process has been described as partial sarcolysis, as the fibres break down into a multinucleate symplasma which forms the blastema for the permanent muscle tissue. v. Gehlen (1937) described a similar process in mammals under experimental conditions.

In Anamnia the medial parts of the epithelial dermatome act as a germinative zone for the already differentiated myotome, and particularly for the formation of the second muscle type. The lateral parts only contribute initially to the formation of loose connective tissue. In Anamnia the specialization of the dermatome proceeds without cell deaths. In Amniota many cells die in the medial parts of the dermatome at the onset of chondrification when the second muscle type is formed by the ingrowth of cells.

The correlated changes in the skeleton, such as the regression of the notochord and of the cartilaginous skeleton, do not require special description. It should, however, be noted that in the sclerotome and in the dense precartilaginous mesenchyme of Amniota (LXI) cell deaths are very frequent, while they are rare in comparable stages of Anamnia.

(*c*) *Chondrogenesis and osteogenesis*

Two types of cell degenerations have been assumed to be instrumental in producing cartilage ground substance: the death of early cartilage cells (LXII) and the very numerous cell deaths in the dense prechondral mesenchyme (LXI). These latter degenerations are said to be the more numerous the longer the cartilage persists (Jacobson, 1932) and are assumed to represent a chemical step in the formation of the ground substance. Since they occur mainly in Amniota, they may be a means of reducing the size of the primordium rather than fulfil a chemical function in cartilage formation. The degeneration of hypertrophic cartilage cells (LXIII) and its relation to ossification have been described by Fell (1925); Fell & Canti (1934).

Ernst (1926) related cell death in the mesenchyme of the hind limbs of birds to the regression of a pentadactylous primordium (LXV).

(*d*) *Morphogenesis and histogenesis of muscles and skeleton*

The development of the sternum follows the folding of the embryonic disk. In chick, budgerigar and sparrow embryos (LXIX) the original loose mesenchymal tissue of the ventral body wall degenerates prior to its replacement by the down-growing tissue from the dorsolateral part of the thoracic wall which includes muscle, connective tissue and the sternal plates (Fell, 1939). Where the streams from both body halves meet in the mid-line a sharply defined median zone of degenerations appears which persists until the sternal plates and pectoral muscles have assumed their final position. These cell deaths are involved in the shrinkage of the ventral body wall which also finds expression in deep folds of the mesothelium and the epidermis of the mid-line. The mesenchymal cell degenerations are followed by the appearance of collagenous fibres running at right angles to the epidermal plane. Fell (1939) relates both these waves of cell deaths to the movement of the sternal plates and adjacent tissues. The degeneration of the loose original mesenchyme allows of the ingrowth of the dorsolateral tissue. The movement of the latter is brought about by the streaming of undifferentiated cells, and without degenerations this continued influx of undifferentiated material would lead to an enormous cell accumulation in the mid-line. The degeneration and shrinkage of the ventral body wall is, furthermore, a secondary mechanism helping to bring about the union of the sternal plates. These cell deaths occur in the explanted sternal tissue as well as in the intact embryo.

In vivo as well as in tissue cultures cell deaths occur in the undifferentiated mesenchyme of the chick mandible (LXVII) prior to the ingrowth of myogenic tissue for which space is thus provided. Prior to the converging of the two halves of the mandible (LXVI) degenerations appear in the mid-line of the jaw and also when the proximal osteogenic centres of the mandible subdivide into three parts (LXVIII).

In the region of the chick embryo knee joint an 'opaque patch' composed of degenerating cells has been described by Fell & Canti (1934). This area takes up vital stains (van Weel, 1948) and is a useful marker for experimental dissection. Its role in the development of the limb is quite obscure.

The death of early myoblasts in some mammals (Godlewski, 1902; Kulczycki, 1931) is probably linked with the replacement of the second muscle type. The sarcolytic processes (LXXII) described by Schaffer (1893, 1933) in human embryos of 10–16 weeks may bear some relation to the removal of the second muscle type, though the suggestion that they serve to remodel muscle and tendon insertion on the changing skeleton deserves further investigation.

Sarcolytic processes in the rat tongue (LXXIV) allow the ingrowth of the rapidly expanding serous glands.

IV. CLASSIFICATION OF CELL DEGENERATIONS ACCORDING TO THEIR DEVELOPMENTAL FUNCTIONS

In many instances embryonic cell deaths play a significant role in developmental processes (Kallius, 1931), while no functional significance can be assigned to others. The processes in which degenerations appear as constant or even necessary phenomena may be distinguished as morphogenetic, histiogenetic and phylogenetic, i.e. as concerned with changes in form of organs or tissues, with the differentiation of tissues and with the formation and regression of such structures which though merely transitory in higher vertebrates have definite functions in lower vertebrates.

Table 2 is an attempt at classifying degenerations according to their developmental functions. No specific significance can at present be ascribed to another group of cell deaths: those occurring during early embryogenesis (Table 1, I–V), the 'opaque patch' of the knee joint region (Table 1, LXX), the degenerations in the larynx (Table 1, XLI), and those in the mesenchyme between the spinal ganglion and the myotome (Table 1, LX).

The distinction between phylogenetic and histiogenetic cell deaths is often very difficult. Histiogenetic degenerations related to the differentiation of functioning organs of lower vertebrates may appear during the formation and regression of the vestiges of these organs in higher vertebrates and are then classed as phylogenetic degenerations. The sarcolytic processes in amphibia and the comparable degenerations in the development of Amniota illustrate this point. A similar difficulty is encountered in the distinction of morphogenetic and phylogenetic degenerations. In the folding of the apical ridge of the limb buds in reptiles morphogenetic degenerations play a role, while in mammals such degenerations are almost indistinguishable from those leading to the regression of the apical thickening. The appearance of vestigial formations may have some significance for the development of the embryo. Some of them may have the function of inducing organs, viz. the apical ridge on the limb bud of chick embryos, while others may be merely by-products of development and testify merely to the incomplete repression of a particular genetic system. Discussion of this problem is, however, outside the scope of this article, and the description of formations as phylogenetic does not imply that they have no function in the ontogeny of higher vertebrates.

Some transitory structures, such as the plugs of the lens, nares, the seams of lips and lids, are, however, definitely by-products, and the cell deaths involved in their regression are considered as morphogenetic degenerations as they help to form a lumen.

(1) *Morphogenetic degenerations*

These appear in epithelia during invaginations and evaginations, during seam formations and separations of rudiments. The cell deaths usually precede alterations in shape but persist during the actual change in form and may be found even afterwards. They occur when free cell movements are restricted by the regular arrangement of cells and their close packing, and are absent when resorption of yolk

and the concomitant decrease in cell volume allows epithelial cells to move freely. One of the important functions of morphogenetic cell deaths is, thus, to facilitate

Table 2A. *Morphogenetic degenerations*

Site and tissue	Process	No. in Table 1
Cell deaths related to changes in form of organs		
Neural plate, groove and tube	Invagination and closure	VIII, IX, X
Forebrain	Evagination of optic vesicle	XI
Optic vesicle	Invagination to cup	XVII
Lens plate	Invagination and closure	XIX
Auditory plate	Invagination and closure	XXIV
Olfactory plate	Invagination	XXVI
Endoderm	Closure of tube	XXXIII
Lung	Branching of pneumonomeres	XLIII
Midventral region	Folding of embryonic disk	VI
Conjunctival papilla	Formation	XXII
Epithelial papilla on limbs	Formation	XXXII
Tongue and fungiform papillae	Formation	XXXIV
Cell deaths related to ingrowth of tissue		
Mandible, mesenchyme	Ingrowth of myogenic tissue	LXVII
Mid-line of anterior body wall and mesenchyme	Ingrowth of dorsolateral tissue	LXIX
Tongue muscles	Growth of serous glands	LXXIV
Cell deaths related to the union or detachment of parts		
Mid-ventral line, epithelium, mesenchyme and mesothelium	Union of body halves	VII
Neural tubes and ectoderm	Detachment of neural tubes	X
Lens vesicle and ectoderm	Detachment of lens vesicle	XX
Otocyst and ectoderm	Detachment of otocyst	XXIV
Olfactory tube and palate	Detachment of olfactory tube	XXVI
Mandible and mid-line	Union of halves	LXVI
Mandible and proximal osteogenic centres	Subdivision	LXVIII
Notochord	Detachment from endoderm	LIV
Trachea and oesophagus	Separation of rudiments	XLII
Orifice of ureter	Separation from urogenital sinus	XLVII
Aorta	Bifurcation	LII
Cell deaths related to the formation of lumina in solid or partly occluded organs		
Neural tubes of selachians	Lumen formation	X
Salivary glands	Lumen formation	XXXV
Oesophageal glands	Lumen formation	XXXVII
Duodenum	Lumen formation	XXXVIII
Colon	Lumen formation	XXXIX
Vagina	Lumen formation	L
Vascular rudiments	Lumen formation	LI
Lens and peridermal plug	Regression	XXI
Nose and peridermal plug	Regression	XXVIII
Membrana bucconasalis	Regression	XXVIII
Pharyngeal, branchial and cloacal membranes	Regression	XXX
Proliferated epidermal seams on lids, lips, external auditory meatus, anus, urethral orifice, preputium and vulva	Regression	XXXI

the movement of cells. The changes in form and shape of organs are brought about by an integration of cell divisions, cell deaths and cell movements.

Table 2B. *Histiogenetic degenerations*

Site and tissue	Process	No. in Table 1
Cell deaths related to the differentiation of tissues and organs		
Retina	Differentiation of three cell layers	XVIII
Spinal ganglia	Differentiation of neuroblasts	XV
Nose epithelium	Differentiation of ciliated epithelium	XXIX
Hypertrophic cartilage	Ossification	LXIII
Muscle fibres	Remodelling of insertions	LXXII
Metanephros	Replacement of first generation of secretory tubules	XLVI
Müllerian duct	Differentiation of male organs	XLVIII
Wolffian duct	Differentiation of female organs	XLIX
Ganglia of branchial nerves	Metamorphosis	XVI
Intestinal tract	Metamorphosis	XL
Body muscles of Amphibia	Metamorphosis	LXXIII
Cell deaths related to the formation of matrix and fibres		
Sclerotome	Cartilage formation	LVII
Prechondral mesenchyme	Cartilage formation	LXI
Protochondral tissue	Cartilage formation	LXII
Osteoblastic tissue of mandible	Ossification	LXIV
Mesenchyme of conjunctiva	Formation of argyrophile fibres and scleral bones	XXIII
Mesenchyme of mid-ventral body wall	Formation of argyrophile fibres	VII
Mesenchyme of aortic bifurcation	Formation of elastic fibres	LII
Lens stalk	Formation of anterior vitreous body	XX
Cell deaths possibly related to organ development		
Fourth ventricle and recessus interventricularis	Vascularization	XII
Spinal cord, anterior horn	Vascularization	XIV
Otocyst	Neurotization	XXV
Olfactory organ	Neurotization	XXVII

Table 2C. *Phylogenetic degenerations*

Site and tissue	Process	No. in Table 1
Cell deaths representing vestigial organs		
Brain	Rudiment of paraphysis	XIII
Myotome, dermatome and early muscle fibres of Amniota	Rudiment of second muscle type	LVIII, LIX, LXXI
Cranial and caudal mesenchyme	Segmentation	LVI
Mesenchyme of hind limbs	Rudiment of pentadactylous primordium	LXV
Cell deaths during regression of larval organs		
Notochord	Partial regression	LV
Ganglia of branchial nerves of Amniota	Abortive metamorphosis	XVI
Mesenchyme of branchial arches of Amniota	Abortive metamorphosis	XXXVI
Pronephros	Regression	XLIV
Mesonephros	Partial regression	XLV
Conjunctival papilla	Regression	XXII
Epithelial papilla on limbs	Regression	XXXII
Ductus arteriosus	Regression	LIII

The removal of superfluous cells after the joining of parts and the closure of openings is another example of morphogenetic degenerations. As a rule, parts which are destined to form a seam are thickened, and very frequently—if not invariably—too many cells have been produced, particularly when a sort of stalk precedes the final closure of an opening, as, for instance, in the lens vesicle. The cells of the stalk as well as of the thickened insertions of the stalk in the ectoderm and the lens become superfluous and die.

The formation of lumina in solid glands or in temporarily occluded organs, such as the duodenum and colon, may be brought about by the death of central cells. The removal of peridermal plugs and of transitory membranes has already been mentioned as falling into this category.

In the mesenchyme, too, morphogenetic degenerations are encountered; death and removal of cells in the mesenchyme of the mandible precede the ingrowth of myogenic tissue, and similarly degenerations in the mesenchyme of the mid-line of the ventral body wall prepare for the influx of dorsolateral tissue and indeed make such ingrowth possible. These examples are analogous to cell deaths in epithelial organs preceding and facilitating cell movements.

Another type of mesenchymal morphogenetic degeneration is found in the cell deaths which bring about the subdivision of the osteogenic centres in the mandible.

(2) *Histiogenetic degenerations*

These are related to the differentiation of tissues and organs. In the retina of the frog tadpole, for instance, cell deaths coincide with the differentiation of the three cell layers and probably represent cells unable to undergo this particular process of specialization because they are already specialized in another direction. The partial sarcolysis of the body muscles during the metamorphosis of amphibia represents the histiogenetic degeneration of fully differentiated systems (i.e. the muscle fibres), though it does not invariably lead to the death of the component cells, which are able to dedifferentiate and to form a blastema for the permanent muscles. Another example of histiogenetic degenerations is seen in the atrophy and resorption of parts of the Müllerian and Wolffian ducts in males and females respectively at the onset of sex differentiation of the individual.

Whether cell deaths are really necessary to provide the material for myelin (Spatz, 1918), the anterior vitreous body (Watzka, 1935), of cartilage and bone ground substance is doubtful. These degenerations may, however, play some part in the initiation of these processes by releasing some enzyme or substrate. Brachet (1947) attributes an evocatory function to ribonucleic acid and believes that the liberation of ribonucleic acid from dead cells may account for the strong quantitative correlation between cytolysis of implanted material and the induction of neural tissue in amphibia (Holtfreter, 1945). Though ribonucleic acid may stimulate evocatory processes, it is unlikely to exert a specific evocatory action. Similarly, cell deaths may contribute unspecifically to the initiation of processes resulting in the formation

of fibres (Fell, 1939; Murray, 1943; Hughes, 1943), of myelin and of cartilage and bone ground substance.

The presumed relation of cell deaths to neurotization of the otocyst and the olfactory epithelium and to the vascularization of the spinal cord and the fourth ventricle needs further investigation before this interpretation can be accepted.

(3) *Phylogenetic degenerations*

These are of two types: those which represent a vestigial organ and those involved in the regression of larval organs. The difference between them is merely the stage which a particular structure has reached before it regresses. The best examples for the first group are the degenerations representing the paraphysis in higher vertebrates and the degenerations in the myotome and medial part of the dermatome in Amniota which replace the formation of the second muscle type.

Whether the cell deaths preceding the formation of a cartilaginous skeleton in Amniota, and those appearing in the anterior horn of the spinal cord and the spinal ganglia, belong to the same category, remains to be further investigated. There is also need for supporting evidence that degenerations in the mesenchyme of the hind limbs of birds represent the anlage of a pentadactylous primordium.

Cell deaths during the regression of the pronephros and mesonephros in higher vertebrates, of ganglion cells in the branchial region, of the conjunctival papilla are examples of the second group of phylogenetic degenerations.

V. DISCUSSION

There can be no doubt that cell deaths occur regularly at certain developmental stages of all vertebrate embryos. They cannot be considered as artefacts due to fixation or other conditions of handling (Stieve, 1926), since they occur in the best fixed specimens and can also be detected in the living embryo (Fell & Canti, 1934). The reported observations were made by various authors on embryos obtained from mixed stocks, and it is thus most unlikely that these cell deaths represent genetically conditioned anomalies of pure lines.

Some of the degenerations might be expected on general principles, i.e. those concerned with the removal of larval organs or with changes in the shape of the body, viz. during the metamorphosis of amphibia. It might be assumed that organs rendered superfluous by the development of other organs would be resorbed (Huxley, 1942), though there remain the problems of why only some of the constituent cells are preserved for further use, why some parts of these structures persist as vestigial organs (e.g. Wolffian duct remnants in the ovary), and why others are transformed. Furthermore, while organs may become superfluous, their cells are not necessarily rendered superfluous provided they are able to dedifferentiate and to differentiate in another direction. Examples are found in the partial sarcolysis in amphibia (Table 1, LXIII), in the regression of smaller branchial vessels (Table 1, LIII) and during the formation of the ciliated epithelium of the nose (Table 1, XXIX).

But even if cells have become superfluous, that statement by itself does not explain why they die. Ernst (1926) suggested that endogenous as well as exogenous factors might cause the death of embryonic cells. He thought that the life energy of embryonic cells might become exhausted like that of adult blood cells, epidermal cells, etc., and die. This comparison is not very apt, since embryonic cells are able to rejuvenate themselves by division while the differentiated adult cells are incapable of division. If Ernst's conception is modified to mean that in the absence of stimuli for division an embryonic cell ages and on reaching the end of its life span dies, it could be used to account for some of the phylogenetic, histiogenetic and morphogenetic degenerations. Experimental embryological and genetical observations suggest that certain stimuli for the proliferation and differentiation of organs are active for limited periods only, and it is thus reasonable to assume that when these stimuli cease, cells fail to divide and complete their specialization and thus age and subsequently die.

As exogenous factors of cell deaths Ernst (1926) considered the pressure of neighbouring mitotic cells and the displacement of cells by changes in the shape of organs (invaginations, etc.) which may lead to nutritional disturbances and result in death. While such causes may be operative in a few instances, they certainly would not account for the majority of morphogenetic degenerations. The fact that these appear prior to changes in form and thus to displacement (Glücksmann, 1930*a*; Bieling, 1937; Stockenberg, 1937; Maurer, 1936; Schneider, 1935) and that they appear in the same place and approximately in the same amount in the vascularized tissue of the intact embryo and after explantation to tissue cultures without a vascular supply (Fell & Canti, 1934; Fell, 1939; Jacobson & Fell, 1941) rules out such an explanation. (Degenerations due to adverse conditions occur frequently in tissue cultures; these are, however, quite distinct from the cell deaths discussed here.) The pressure due to adjacent mitotic cells (i.e. the pressure due to the increase in volume of the dividing cell as distinct from the 'growth' pressure in a tissue due to an increase in cell numbers) is unlikely to cause many cell deaths and certainly does not account for cells dying during mitosis, as, for instance, in the frog tadpole retina.

The causation of numerous morphogenetic degenerations is quite obscure. They precede form changes and thus cannot be caused by displacement of cells or by impairment of nutrition. They occur frequently in the most actively growing regions, and this fact led to their initial interpretation as mitotic metabolites. Pressure or other injurious effects can almost certainly be excluded as causes of degeneration in the loose mesenchyme of the mid-ventral body wall.

Cell deaths undoubtedly play an important role in the morphogenesis of various organs by facilitating cell movement in epithelial and mesenchymal tissues, by bringing about the separation of organs, etc. Gräper's (1935) suggestion that cell deaths in Amniota replace the yolk of Anamnia and have a metabolic function prior to vascularization may apply to some extent to early embryonic stages but does not cover the degenerations which occur in Anamnia and Amniota after vascularization. Though there is no evidence to support Stockenberg's (1937) suggestion that cell

deaths may play a role in the induction of organs, Brachet's (1947) hypothesis deserves further attention, and so does the role which cell deaths may play in the formation of fibres, of cartilage and bone ground substance.

Embryonic cell deaths might be interpreted on the lines that the development of organs proceeds on a sort of statistical basis, i.e. a differentiation impulse expands over a field of cells, some of which respond fully to the stimulus and form the organ while others are late or unable to respond and thus after partial differentiation cease to proliferate and to complete their differentiation, age and die. Such an interpretation could be applied to most of the phylogenetic and histiogenetic degenerations which occur almost haphazardly in a field of differentiation. It might even account for some of the morphogenetic degenerations, for instance, for those in the mesenchyme of the body wall and the mandible and to those in epithelial stalks and seams. It is difficult to see, however, how such a view could account for the distinctly localized degenerations which precede form changes in epithelial organs and which seem to be integrated with the incidence and orientation of mitosis as well as of cell movements. The causation of these latter processes is, however, equally obscure as that of the associated cell deaths.

Cell degeneration is undoubtedly an important means of integrating cells into tissues and organs or, expressed in alternative terms, an important means by which the developing embryo marshals its cells into organs. These alternative terms reflect the view of the cell theory that the individual cells by their inherent potentialities and interactions build up the embryo, and the holistic view which considers the cells as the medium in which the developing embryo realizes its potentialities. Discussion of these alternatives, though tempting, is clearly outside the scope of this review.

Paraphrasing Adrian (1949) one might say that while it is satisfactory to find embryology establishing relations with biophysics and biochemistry, it is only natural that such work has little obvious contact with the study of integrated cell behaviour. In analyses of such integration of cellular activities to form organs and organisms, particularly from the viewpoint of comparative embryology, it appears worth while to pay attention to cell degenerations as indicators not only of phylogenetic steps but also of the mechanism of differentiation.

VI. SUMMARY

1. Degenerations of embryonic cells have either been reported as such or have been misinterpreted by various authors as 'mitotic metabolites' or blood cells.

2. There is ample support for the morphological identification of dying cells from the following considerations: the degeneration 'granules' are initially Feulgen-positive and have thus originated from nuclear constituents; the stages of cell deaths seen in normal embryos are identical with those produced experimentally and with those observed directly in tissue cultures; degenerating cells react in the same manner to supravital stains *in vivo* and *in vitro*.

3. The process of degeneration varies with the degree of specialization of the cell, with its functional state (e.g. mitosis), with the type of animal and under experimental conditions with the causative agents.

4. Cell death may take from less than 1 hr. to about 7 hr. when only a small proportion of a living tissue dies, but may be prolonged to days when numerous cells die simultaneously and their resorption is delayed.

5. Degenerations have been found during the normal development in embryos of all vertebrate animals examined. The occurrence of necrosis in embryos of pure genetical lines is excluded from this article.

6. The incidence of embryonic cell deaths according to site, tissue, developmental stage or process and type of animal is summarized in Table 1.

7. While some degenerations have no obvious function in embryonic development, others seem to play a significant role in embryonic processes, e.g. the morphogenesis and histogenesis of tissues and organs, and the representation and regression of phylogenetic steps (Table 2).

8. Morphogenetic degenerations precede changes in the form of epithelial organs, e.g. during the invagination of the optic cup, the formation of the crystalline lens, the olfactory pit, the neural tube, etc. They bring about the separation of rudiments such as that of the neural tube and the lens from the ectoderm. They reduce the excessive thickening of uniting edges such as those of the body wall and of the mandibles. They are involved in the production of lumina in the solid rudiments of glands and the intestinal tract. In the mesenchyme they precede and make possible the influx of specialized tissue such as the sternal plates or the ingrowth of myogenic tissue in the mandible.

9. Histiogenetic degenerations are related to the differentiation of tissues and organs. The differentiation of the three cell layers of the frog tadpole retina, for instance, is accompanied by three waves of degeneration. Similar cell deaths of early neuroblasts are found in the spinal ganglia outside the limb regions. In amphibia a partial sarcolysis during metamorphosis provides a blastema for the permanent musculature. Sex differentiation of the individual involves the partial degeneration of the Müllerian or Wolffian ducts. Cell deaths also occur in relation to fibre formation and to the appearance of bone and cartilage matrix. Their role in these and in evocatory processes needs further elucidation. Whether cell deaths in the central nervous system and the sense organs at the time of vascularization and neurotization are related to these phenomena remains to be further investigated.

10. Phylogenetic cell deaths are of two types: those which represent a vestigial organ such as the paraphysis or the second muscle stage in higher vertebrates, and those concerned with the regression of larval structures such as the conjunctival papilla, parts of the ganglia of branchial nerves, of the pro- and mesonephros. Some of these larval organs have a function in embryonic development, viz. the apical ridge on the limb buds.

11. The causation of the distinctly localized morphogenetic degenerations is obscure. Vascular or nutritional disturbances are unlikely to be responsible for these

cell deaths which precede changes in form and appear in the same localizations and amounts in the vascularized tissue of the intact embryo and after explantation in tissue cultures.

12. Most of the histiogenetic and phylogenetic cell deaths, as well as some of the not strictly localized morphogenetic degenerations, may be due to the fading out of stimuli for their proliferation or for the completion of their differentiation. If such cells fail to divide, they age and die on reaching the end of their normal life span. This conception assumes that stimuli for the formation of embryonic tissues and organs act for limited periods only and extend over a field of cells. Some of these cells respond fully to stimulation, while others are late to react or do so only partially or receive only a fraction of the whole stimulus. The partial differentiation of cells unfits them for division, for dedifferentiation and redifferentiation in another direction.

13. The localized morphogenetic degenerations are correlated with the incidence and orientation of mitosis and of cell movements, and changes in the form of embryonic organs are brought about by the integration of these three cellular activities. Cell deaths are abundant wherever the regular arrangement and close packing of cells prevent free cell movements; they are rare or absent when, as, for instance, in the tadpole eye, a loose arrangement of cells and a decrease in cell volume (by resorption of yolk) allow of free cell movements.

14. Cell degeneration in vertebrate ontogeny is an important mechanism of integration of cells into tissues and organs by helping to shape the form of organs, by the removal of superfluous cells or by the preparation of a dedifferentiated blastema in histio- and phylogenesis.

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