ASPECTS OF GAMETOGENESIS AND RADIATION PATHOLOGY IN THE ONION FLY *HYLEMYA ANTIQUA* (MEIGEN) II. RADIATION PATHOLOGY

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CONTENTS

1. INTRODUCTION

The onion fly, *Hylemya antiqua* (Meigen), is the key pest in onion crops in The Netherlands. This is one of the reasons why this species has been chosen to serve as an example of insect control by the Sterile Insect Method (SIM). An alternative for purely chemical control of this pest was considered to be necessary after repeated resistence to insecticides had been observed and in view of the economic importance of the crop. The possibility to apply the SIM was enhanced by the concentration of onion growing in certain areas of the country.

The potential feasibility of using the SIM to control *H. antiqua* was recognized by NOORDINK and preliminary research started in 1965. General considerations on this subject have been published by DE FLUITER et al. (1967), TICHELER (1967, 1968) and NOORDINK and THEUNISSEN (1971). During the first years of laboratory research an artificial meridic diet was developed (TICHELER, 1971), the proper sterilization dose determined (TICHELER and NOORDINK, 1968; NOORDINK, 1971) and radiation pathology studied (THEUNISSEN, 1971). In 1970 preparations were made to start a series of four consecutive yearly small scale field trials. This progress was also made possible by the work of NOORLANDER (not published) who developed rearing methods which permitted to carry out these field trials, and LOOSJES (1976) who studied vital aspects of the ecology, developed a suitable trap and devised computer simulation models on the dispersion of the fly. Other model studies were performed by FRISSEL and WIJNANDS-STÄB (1973) and WIJNANDS-STÄB and FRISSEL (1973). Aspects of the gametogenesis of this fly were studied by THEUNISSEN (1973 a, b; 1974 and 1976) to meet the demand for an egg chamber stage classification and basic information on gametogenesis. NOORDINK (1971) developed radioisotope applications for use both in the field and in the laboratory (THEUNISSEN and NOORDINK, 1976). In the meantime the cytogenetics and the induction and selection of chromosome translocations of *H. antiqua* were studied to provide background information for the development of the SIM (VAN HEEMERT, 1973 a, b; 1974 a, b; 1975) WIJNANDS-STÄB and VAN HEEMERT, 1974). Results of the series of field trials have been frequently reported (THEUNISSEN et al., 1973, 1975; TICHELER et al., 1974). Additional information on both laboratory and field research is found elsewhere (ANONYMUS 1965–1975).

The priciples of the SIM are generally known. They have been discussed a.o. by LACHANCE (1967) and LACHANCE et al. (1967). GROSCH (1962) reviewed radiation effects on insects as determined by genetic and physiological standards, while PROVERBS (1969) discussed the properties and use of sterilized insects. The entire field of genetical control has been reviewed by DAVIDSON (1974) in its two main aspects: the SIM (synonyms: Sterile Male Technique, SMT and Sterile Insect Technique, SIT) and the more purely genetic

methods. The latter are based on inherited sterility (IS): cytoplasmic incompatibility, chromosome translocations and hybrid sterility. Insect species of both agricultural and medical importance to be controlled by genetic methods have been discussed. Publications of the International Atomic Energy Agency and the World Health Organization contain much information on the development and use of genetic control techniques against harmful insects.

The present study has been carried out within the frame of the development of a SIM technique to control *H. antiqua*. Irradiation effects are usually determined according to genetical or/and physiological criteria. Sterilization doses which cause a sufficiently high degree of sterility with a minimum of adverse side-effects are commonly found in an empirical way. It has been considered to be necessary, however, to study the internal irradiation effects in *H. antiqua* in order to apply irradiation in a well considered manner and to avoid undesirable side-effects. Effects of irradiation with hard X-rays on the tissues and organ systems have been studied mainly histologically. As has been pointed out earlier (THEUNISSEN, 1971) at the dose levels used irradiation effects have been observed only in the gonads. This observation necessitated a closer study of normal spermatogenesis and oogenesis (THEUNISSEN, 1976) to provide a scientific base for histopathological studies on treated flies.

In the second part of this study histopathological symptoms in the gonads of *H. antiqua* are described following irradiation with 3 kR, the sterilizing dose, after a preceding defining of the used terminology. The pathogenesis of these symptoms as well as the symptoms after irradiation with lower and higher doses are discussed. A short account of a quantitative approach to evaluation of radiation effects is added to this survey on the radiation histopathology in *H. antiqua*.

2. MATERIALS AND METHODS

2.1. MATERIAL

The bionomics of the onion fly and its laboratory rearing on a meridic diet have been described earlier (THEUNISSEN, 1976). LOOSJES (1976) used *Delia antiqua* (Meigen) as scientific name of the onion fly, based on a recent revision of the Anthomyiidae (HENNIG, 1974). *Hylemya antiqua* (Meigen) is used here to maintain a consistent nomenclature in both parts of this study.

2.2. METHODS

2.2.1. *Histological and cytological methods*
The methods which were used in a standardized histological processing and in cytological investigations have been described elsewhere (THEUNISSEN, 1976).

2.2.2. *Irradiation*
Irradiation took place at the Institute of Applied Atomic Science in Agriculture (I.T.A.L.) at Wageningen.

For histopathological experiments the pupae were irradiated with hard X-rays by an X-ray machine because with this machine very accurate dosimetry was possible. The 250/25 X-ray machine operated at 250 kV, 15 mA, without extra filters, at 300 rad/min and a focus distance of 50 cm.

Large quantities of pupae for field trials were irradiated with gamma-rays by means of a ^{60}Co-source, with a dose of 3 kR (dose rate 178 kR/hour, source 17.10⁴ Ci, irradiation during 30 sec., Fricke dosimeter). Samples taken from these quantities were compared to pupae which received 3 kR of hard X-rays. No difference was observed in histopathological symptoms in both groups. Although the accuracy of the irradiation of the gamma-ray source was less when compared to the X-ray machine no significant effects of the larger margin in actually administered dose could ever be detected. This method of 'histological control' has been used as a final routine check on the quality of the irradiation of the released pupae.

Irradiation was carried out on 11 days old pupae, which were due to emerge 2–3 days later. For each dose, groups of many pupae were used in order to ensure ample material at each sampling (table 1).

2.2.3. *Sampling*
After irradiation the pupae of each dose group were placed in a cage to emerge. The emerging flies were subjected to the same conditions and provided with water, food and onion-chips. Emergence usually took place during about 4 days in a normal distribution curve. For prolonged sampling usually only

TABLE 1. Number of replicates and size of groups of pupae/irradiation dose.

dose range*	number of replicates	number of pupae irradiated/dose
0 (control)	5	450–3,000
0.5–2.5	2	450, 3,000
3.0	5	200–3,000
5.0–9.0	1	450

* dose in kR.

flies which emerged during the third day after irradiation were kept in the cages. Flies emerging earlier or later were discarded unless they were needed, for instance to study pathogenesis of the radiation syndrome.

At certain intervals flies were sampled from the cages, anaesthesized by means of CO_2, killed and processed histologically. Each sample contained at least 25 flies. Both males and females were usually sampled at the same time. Apart from a few exceptions each series of histological preparations which belong together (dose group, age group etc.) contained at least 10 comparable individuals.

In addition to the usual sampling procedure some data on performance were collected of flies which has been irradiated in lower dose ranges (0.5–2.5 kR). Every 2 days the fecundity and fertility of the flies in each cage was determined. The produced eggs were weighed. This experiment was performed in duplicate at different times. The results of the observations are summarized in table 5 (page 67).

2.2.4. *Illustrations*

Photographs and drawings were made by means of a Wild M20 microscope with photographic equipment and a drawing tube. Unless otherwise indicated the bar in photographs and drawings represents 10 μ.

3. RADIATION PATHOLOGY

An increasing interest in the possibilities of the use of ionizing radiation to control insects results in a growing knowledge of the genetical, physiological and pathological consequences of irradiation of insects. Reviews of GROSCH (1962), DAY and OSTER (1963) and MANDL (1964) deal with the older literature. HARKER (1963) cites some papers on carcinogenic radiation effects in insects. More recent reviews by DUCOFF (1972) and GROSCH (1974) show a considerable increase in activity in this field.

Some comparative research has been performed on the pathological effects of radiation versus chemosterilants, for instance on gonads of *Drosophila melanogaster* (CANTWELL and HENNEBERRY, 1963), of *Anthonomus grandis* (REINECKE et al., 1969), of *Paratetranychus citri* (BEAVERS et al., 1971) and of *Circulifer tenellus* (AMERESEKERE et al., 1971).

Research on radiation effects on various tissues, organs and their functioning in a number of mammalian species is far more advanced when compared to insects, genetical research excluded. General reviews on radiation effects have been given by ERRERA (1959), LEA (1962), BACQ and ALEXANDER (1966), TODD and TOBIAS (1974) and TOBIAS and TODD (1974). TAKETA (1971) discussed the relationship between metabolism and radiosensitivity of intestinal cells. Radiation effects on prenatal development were treated by MURAKAMI et al. (1971). A mathematical approach was mentioned by STEWARD (1974). Histopathological effects were discussed by TULLIS (1949, 1958), LIEBOW et al. (1949), HEMPELMAN et al. (1953), and LUSHBAUGH (1974). ACETO et al. (1974) discussed general physiological and pathological effects relative to the hazards of space flight.

Radiation effects on chromosome structure have been extensively studied. The ways in which chromosomes may break have been discussed by KOLLER (1954). Radiation induced chromosome breakage has been dealth with in handbooks on radiation genetics and – biology (e.g. LEA, 1962; BACQ and ALEXANDER, 1966; TOBIAS and TODD (1974a). Techniques like autoradiography (HEDDLE et al., 1969) and electron microscopy (HUMPHREY and BRINKLEY, 1969) have been used to detect the mechanism by which chromosomal damage is repaired (WOLFF, 1966; WOLFF and SCOTT, 1969). Damage to chromosome structure has been used to determine the effect of treatments. For instance, CROUSE (1950) used chromosome rearrangements, translocations and breaks as criteria to assess radiation effects in the germinal cells of *Sciara coprophila*. Radiation effects on the Y-chromosome of living spermatocytes of *Drosophila hydei* have been examined by HESS (1965).

When describing symptoms as expressions of pathological processes in insect tissues and cells it seems appropriate to define the present interpretation of the concept of pathology.

Pathology refers to a condition or process in the organism or its constituents at any level which is significantly aberrant from and principally inconsistent with the common course of the processes in the living organism. This condition or process can be specified to denote its nature. It can be variable in time, location and ultimate effects, depending on many factors which influence its etiology and pathogenesis.

Although the term 'pathology' covers the study and knowledge of disease in all its aspects, it is commonly used to indicate a situation of the organism or its parts which is different from the normal, healthy condition. This raises the question in what degree a particular situation must be different from the 'normal' one to be called pathological. A quantitative approach could result in the following description: because the 'normal' situation is standard, any significant aberration of that standard is considered to be pathological. According to this concept the degree of pathology is a matter of quantity and equilibrium. The 'normal' situation can include aberrant elements up to a certain quantity to maintain a flexible equilibrium between the limits of normality. The variability of the 'normal' situation is a general property of living matter. Hence, the need for a proper description of what is considered to be the 'normal' situation is evident in order to avoid misinterpretation of pathological and physiological phenomena and symptoms. Physiological events which commonly occur in organisms can include pathological processes at a lower level. For instance, the rather normal process of oocyte resorption in some insect species involves degeneration and lysis of the follicle epithelium cells. The level at which pathological processes take place is very important to the condition and proper functioning of the individual organism. Death at the cellular level may result in somatic death of the organism but also in nothing more than a physiological replacement of cells at the tissue or organ level. Pathology, therefore, is a flexible situation which can be limited in time, e.g. in the case of a reversible process which returns towards its normal course, in place, e.g. being localized in a particular organ, and in quantity because of the number of cells involved in the pathological processes.

Because of the considerable individual variability of the organisms, the complex nature of the 'normal' situation and the often limited number of relevant observations a useful description of a pathological state is very difficult to make.

Therefore a more structural histopathological approach seems to be appropriate. This approach deals with histopathological symptoms which reveal pathological processes. These processes are usually indicated by the term disease, sometimes by the term syndrome (DURHAM, 1960). The term '*syndrome*' can be applied to a complex of symptoms and is defined as follows:

'a syndrome is an aggregation of symptoms and physical signs that collectively constitute a clinical entity' (BAILEY, 1960). This medical definition applies also to the pathology of the invertebrates in spite of the reduced 'physical signs' which may be expected to be found.

The concept of disease is a very complex one. The most useful characterization seems to be mentioned by HOPPS (1964) as being a condition of imbalance between the subject concerned and its environment. The dynamic balance of the normal healthy condition is maintained by 'homeostasis' which 'comprises those reactions of the living system which serve to correct excess or deficiency' (WIGGLESWORTH, 1964).

BÜCHNER (1966) points to modern approaches to medical pathology in both the biological and the philosophical sense. In human pathology the structural pathology is supplemented by chemical and functional as well as by psychological aspects. In comparison to the pathology of the vertebrates, including man, the pathology of insects and other invertebrates is still rudimentary. Moreover, the practical use of the term 'insect pathology' is virtually restricted to denote diseases of insects caused by microbial and viral agents, although STEINHAUS (1963) formally called pathology the study of all aspects of disease including etiology, pathogenesis, histopathology, physiopathology etc. also when applied to insects.

Apart from diseases caused by microbial and viral agents in insects, various aspects of insect diseases caused by other intrinsic or extrinsic, biotic or abiotic factors have been comparatively little studied.

In this paper histopathological terminology will be applied in the same sense as in medecine.

One of the basic phenomena in histopathology is the death of the cell: *necrosis*. Cell death has been described and discussed thoroughly by MÜLLER (1955).

During the first stage of necrosis, necrobiosis, the first symptoms of possible cellular death become visible although no essentially irreversible processes take place. Very early degeneration symptoms have been found by ZOLLINGER (1948) by phase contrast observations of living tumour cells. Early histopathological symptoms are aberrations in staining properties of the nuclei and sometimes also of the cytoplasm. The first symptom observed is *chromatokinesis*. This term is defined and discussed on page 13 and has not been used before. *Degeneration* is a pathological, irreversible process of decreasing physiological activity of the cell caused or accompanied by changes in chemical and physical properties of the cellular material.

Degeneration at the cellular level may take place as a part of pathological or physiological processes at the tissue level.

A frequently observed degeneration phenomenon is *hyperchromatosis* which is an increased stainability of the nucleus, especially the nuclear membrane, by basic dyes (fig. 1a).

A less common symptom is *hypochromatosis* which refers to a decreased stainability of the nucleus, including the chromatin and the nuclear membrane
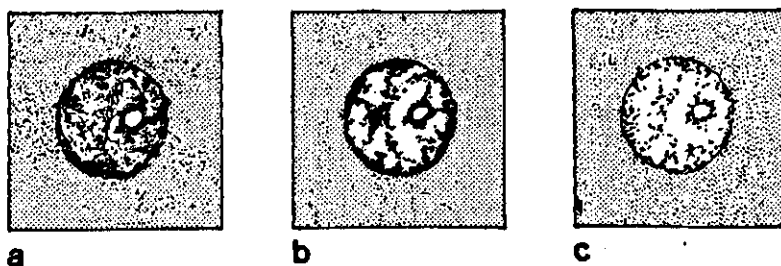
FIG. 1. Schematic representation of aberrant staining properties.
a. hyperchromatosis
b. normal
c. hypochromatosis

(fig. 1c). The cytoplasm does not show an altered stainability.

Chromatolysis is the disappearance of chromatin from the nucleus resulting in a decreased staining of the nucleus (fig. 2c). This symptom is very similar to hypochromatosis.

HOPPS (1964) mentioned a different meaning of the term necrobiosis. In more recent work it seems to refer to cell death during physiological processes
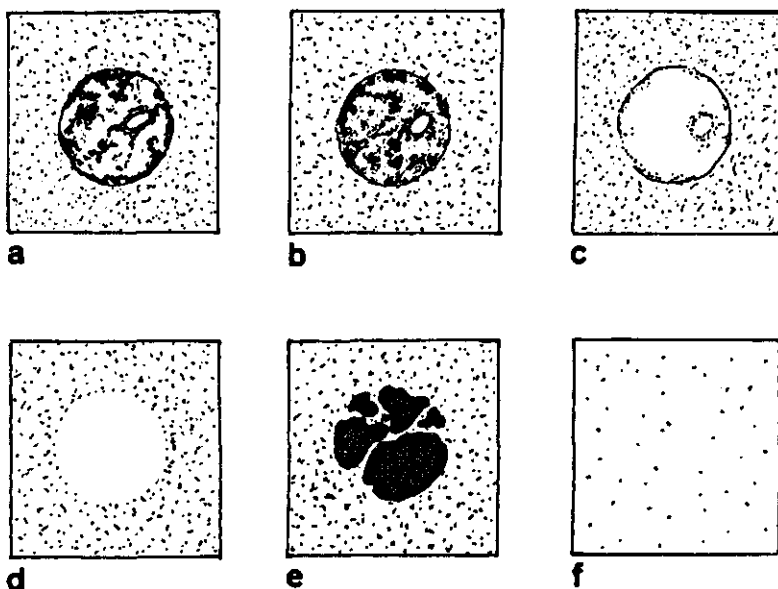
FIG. 2. Schematic representation of some degeneration symptoms.
a. normal
b. chromatokinesis
c. chromatolysis
d. karyolysis
e. karyorrhexis
f. cytolysis

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like regeneration and replacement of cell populations, for instance in intestine and skin epithelium.

The actual death of the cell may be observed by phase contrast optics (cf. ZOLLINGER, 1948). It is hard to imagine that this stage could be determined by histopathological symptoms distinguished from preceding and following events.

Necrolysis, the last stage of necrosis, comprises the irreversible processes of cellular disintegration.

Pycnosis is a progressive reduction of nuclear size due to the loss of internal structure of the chromatin which becomes massed in one large lump of an often irregular shape.

Karyorrhexis is the falling apart of the dead pycnotic nucleus into a varying number of lumps (fig. 2e). Often this symptom is not found because of earlier dissolution of the nuclear remnants by *karyolysis* (fig. 2d). When the cytoplasm disappears simultaneously with the nucleus this final stage is called: *cytolysis*. Often both last mentioned symptoms are not clearly distinguishable as the presence of cytoplasm is not always distinctly established.

Pathological symptoms concerning entire cell populations and their development are well known. They can be split up in two categories:

1. symptoms reflecting developmental abnormalities
2. symptoms related to the appearance and behaviour of cell populations.
ad. 1. Developmental abnormalities can show a varying degree of seriousness.

Agenesis is the total absence of any development of a particular organ or tissue.

Aplasia refers to a very slight development which does not differ much from agenesis from a functional point of view.

Hypoplasia is a deficient and partial development of an organ or tissue. The size of the organ is clearly below the normal dimensions. Depending on the organ or tissue concerned functioning is not always impaired within the limits set by the smaller size.

Atrophy is a gradual reduction in size of an organ or tissue after it has reached its normal size and development. It can be a pathological or a physiological process. Atrophy is an acquired condition which is due to:

a. lack of cell renewal as a result of decreased mitotic activity or
b. a shortened life span of the individual cells without adequate compensation, or
c. an accelerated rate of cell death by some cause, acting slowly but directly on the cell population, or
d. a decrease in size of the cells, or a combination of these factors.

Hyperplasia is the increase in size or volume of an organ or tissue by an increase of the number of cells involved. Hyperplasia occurs in pathological and physiological conditions and is thought to be a reaction on increased demand of the function exercised by that organ or tissue.

Hypertrophy is the increase in size or volume of an organ or tissue by an increase of the volume of the cells which are already present and available. It
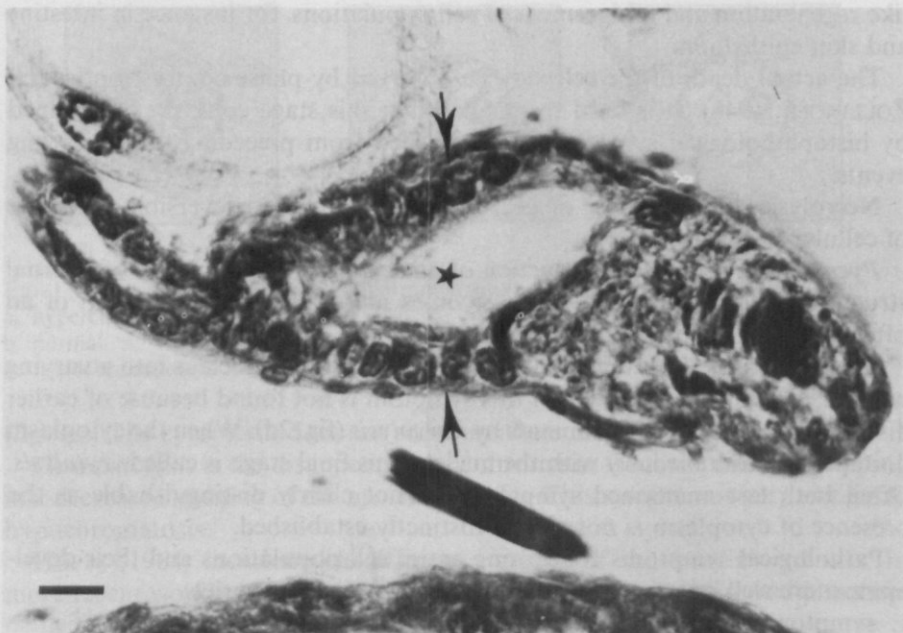
FIG. 3. Agametic testis. Case of spontaneous hypoplasia of testis in 3 days old, untreated adult. Germinal cells are absent. Somatic cells and structures are present, for instance the apical cell (right), the terminal epithelium (arrows) and the central cavity (*). Bar = 10 μ.

is observed in both pathological and physiological processes, especially in tissues which have lost their capacity of cell division. The cause of hypertrophy seems to be similar to that inducing hyperplasia. Both symptoms represent closely related processes. In this paper increase in volume of single, individual cells is not indicated by hypertrophy but is called: *swelling* as it is difficult or impossible to detect the cause of the enlargement which for instance may be due to degeneration phenomena (fig. 4).

Dystrophy refers to an acquired, pathological and irreversible decrease in size of an organ or tissue, leading to its functional elimination (fig. 5).

ad. 2. Cell populations may show various aberrations of the usual pattern of growth.

Metaplasia is a reversible replacement of a differentiated cell type by another one (fig. 6). This change usually takes place gradually and does not necessarily reflect a pathological process but a reaction to pathogenic or traumatic stimuli.

Dysplasia is a principally reversible change in a cell population as far as the size, shape and mutual orientation of the cells is concerned. The tissue shows an irregular and often distorted appearance, affecting the entire architecture of the tissue (fig. 7). The cells are variable in size, shape and stainability of both nuclei and cytoplasm. The normal nucleus/cytoplasm ratio can be changed giving the population a variable appearance (see pag. 119).

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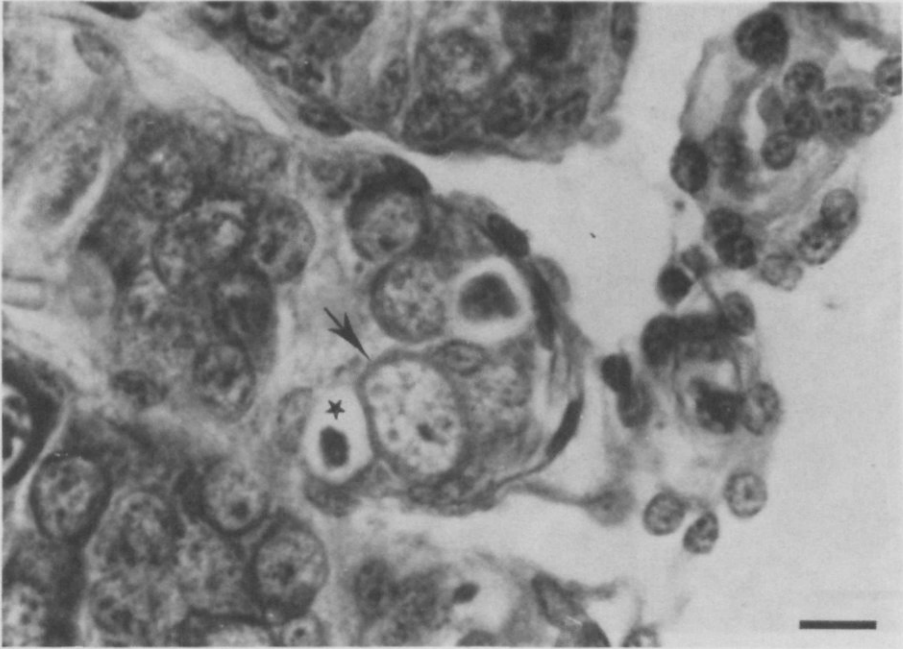FIG. 4. Hypertrophic reaction or swelling of a single trophocyte (arrow) in Sl egg chamber of newly emerged female. Oocyte (*) is laterally displaced. Dose 2 kR. Bar = 10 μ.
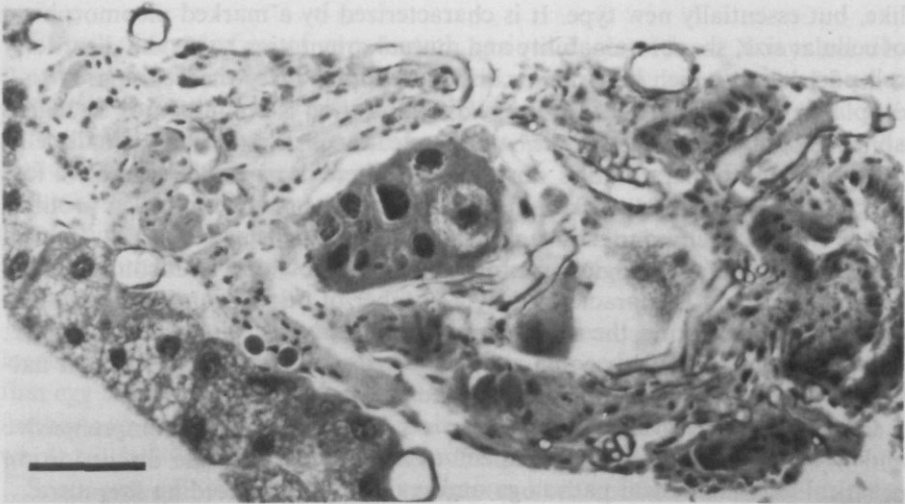

FIG. 5. Severely dystrophic ovary. Remnants of one egg chamber left showing pycnotic trophocyte nuclei. Follicle epithelium is virtually absent. In germaria far advanced cytolysis is observed. Adult 20 days, dose 2.0 kR, 23 days p.i. Bar = 50 μ.

FIG. 6. Metaplasia of follicle epithelium (arrow) of most advanced egg chamber in newly emerged adult, 3 days after irradiation with 6 kR. The spatial separation between the first egg chamber and the germarium is almost gone. Note the presence of 'diffuse' oogonia in the germarium (right).

Anaplasia is an irreversible dedifferentiation of cells to a more embryonic-like, but essentially new type. It is characterized by a marked pleomorphism of cellular size, shape, stainability and mutual orientation to yield a disorderly cell population which has a more distorted appearance when compared to a dysplastic population. The regressive differentiation is accompanied by highly abnormal forms like giant cells, multinucleate cells, abnormally small cells, cells with multipolar spindles during division etc. Anaplastic cells have lost their normal function and place in the tissue to become uncontrolled proliferating, unspecific and dangerous elements in the organism.

Neoplasia is a pathological proliferation of cells which is uncontrolled by the organism and is characterized by a number of other properties. The anaplastic cells producing the mass of freely multiplying cells which is called: 'neoplasm' or 'tumour' become neoplastic cells when breaking through naturally set boundaries like the limits of the organ.

CAMERON (1952) discussed many aspects of pathology in a comprehensive and well founded way, taking the cell as a unit to focuss the discussion on practical and theoretical pathology and reviewing the preceding literature.
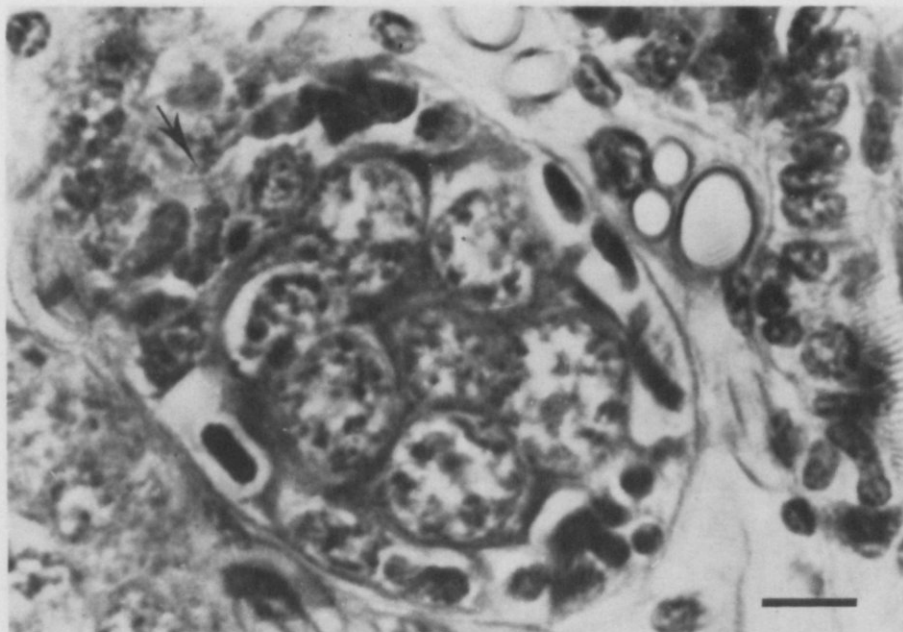
FIG. 7. Swelling and chromatokinetic degeneration of follicle epithelium of most advanced egg chamber in a newly emerged adult, 1 day after irradiation with 6 kR. Note some signs of dysplastic changes (arrow). Bar = 10 μ.

3.2. RADIATION HISTO- AND CYTOPATHOLOGY

Effects of irradiation of gonads with various doses of hard X-rays are discussed as they have been observed in *H. antiqua*. They are described from a histopathological point of view and can be recognized at several levels:

1. the cellular level which is related to the appearance and morphology of the individual cell within the different cell types,

2. the level of the cell population, which refers to the consequences of the irradiation for the existence and development of cell populations,

3. the organ level, regarding irradiation effects on the structure and functioning of the entire organ.

Obviously there can never be a complete separation between these three levels. For example, when a severe cytolysis is reported in the germaria and first egg chambers of newly emerged adults, this will automatically mean that further oocyte development is precluded on the tissue level and the ovary as a whole will be dystrophic because of diminishing size and reversed development.

Some general histopathological symptoms have not been mentioned earlier because they are not described as such in general pathology or are predominantly observed in irradiated gonads of *H. antiqua*.

Chromatokinesis is a pathological, irreversible change in affinity to dyes of

FIG. 8. Zone of chromatokinetic degeneration in germarium (arrows). Newly emerged adult, 6 days after irradiation with 2 kR. Bar = 10 μ.

nuclear material or entire cells from basophilic to acidophilic. The term refers here to a pathological affinity to eosin of chromosomes of blocked divisions, entire nuclei or cells (fig. 8). This symptom which is also observed in untreated gonads, is the very first of the degeneration symptoms which point to a pathological condition of the cells concerned. The cause of this condition is irrelevant in this respect. The term chromatokinesis has been used to avoid repeated lengthy descriptions of this symptom and to denote the shift in colour of the nuclei from blue/black to pink red. The use of this term facilitates descriptions of pathological cells and cell populations. Chromatokinesis is observed in numerous variations of colour shifts ranging from nearly normal basophilic to completely acidophilic. It generally starts in the nucleus, later extending to the cytoplasm.

Degeneration symptoms may occur simultaneously in a cell population or show a pattern in their presence and sequence. Such a pattern is associated with the development and progress of chromatokinesis. Chromatokinesis starts to appear in nuclei with a sharply delimited nuclear membrane and finely granular, regularly dispersed chromatin. The chromatin which shows an increasingly pink colour becomes organized in some rounded, progressively peripherally located concretions. The cytoplasm is also coloured pink. The nucleolus is swollen, red and somewhat irregular. The size of the nucleus decreases with increasing chromatokinesis, which accompanies a distinct

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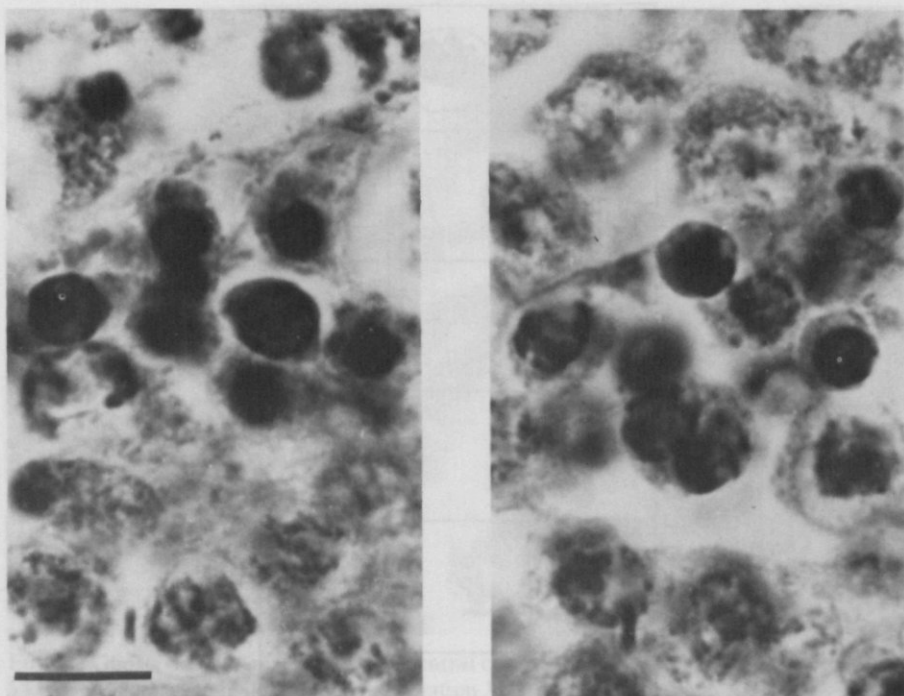
FIG. 9. Chromatokinetic degeneration. The black lumps of chromatin of testicular cell nuclei in the pictures represent a deep red colour in the preparations. Newly emerged fly irradiated with 3 kR 3 days earlier. Bar = 10 μ.

degeneration process: *chromatokinetic degeneration* (figs. 9 and 10), during which also other degenerative symptoms are observed. It is characterized by loss of structure of nucleus and cytoplasm while the nucleus gradually becomes either pycnotic and dark red or lytic causing its disappearance (fig. 10). After pycnosis the nucleus may show karyorrhexis prior to cytolysis. Another pattern is related to hyperchromatosis. *Hyperchromatic degeneration* (fig. 10) takes place when a cell degenerates by hyperchromatosis followed by a progressive reduction of nuclear size and increasing loss of internal structures and pycnosis prior to cytolysis. The cytoplasm tends to disappear soon after hyperchromatosis is evident.

A phenomenon which has been observed only in irradiated testes is the presence of hollow spheres of varying size among mainly post-meiotic cell populations. These spheres, which sometimes possess a more irregular shape, are empty nuclei which show swelling following chromatolysis and dissolution of the cytoplasm (fig. 11). The remaining nuclear membrane is normally stained. Often remnants of chromatin and nucleoli are observed in these structures. In order to adopt a short and clear designation for this commonly found symptom, these spheres have been named: *ghosts*, in analogy to the empty envelopes of bacteriophages. Depending on the cell type from which they
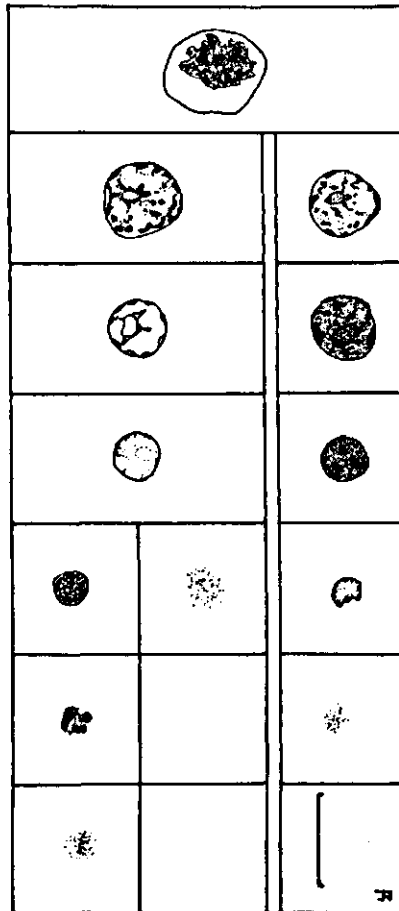
Fig. 10. Semi-schematic representation of the course of chromatokinetic (left column) and hyperchromatic degeneration of nuclei (right column). Top: the unirradiated nucleus (secondary spermatogonium) comparable with the irradiated ones (second row). The degeneration starts in the latter nuclei (downward). For further explanation, see text.

originate their size ranges from 3.5 to 11.5 μ. They are especially abundant and early observed in cysts of spermatids (fig. 12). Among spermatogonia they are rare. They seem to be present nearly exclusively among the spermatocytes and spermatids, from which they originate. The early stage of ghost formation is recognized by a stronger staining of the nuclear membrane relative to the chromatin which gradually disappears. In due course ghosts disappear by dissolution of the nuclear membrane following a decreasing stainability.

Another symptom of radiation effects in both testes and ovaries are the *blocked divisions*, which will be mentioned on page 22. They are very prominent in histological preparations by their chromatokinetic, clumped mass of chromosomal material, especially meiotic divisions. They too ultimately dissolve.

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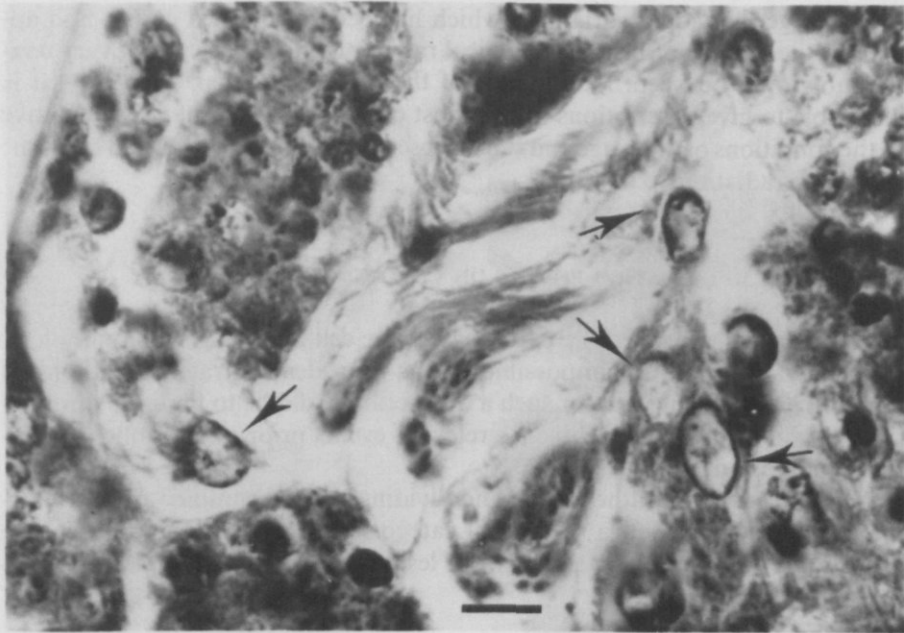
FIG. 11. Ghosts (arrow) in apical part of central cavity. Left aberrant early spermatids. Dose 3 kR, newly emerged male following irradiation 3 days earlier. Bar = 10 μ.

FIG. 12. Cyst of pycnotic early spermatids and ghosts in newly emerged adult 2 days after irradiation with 3 kR. Bar = 10 μ.

The large individual variability which has been mentioned earlier also applies to the pattern of histopathological radiation effects. They may vary considerably even between both gonads of the same individual. Therefore, it is inevitable to give descriptions of the most common pattern of effects relative to the conditions of dose, age and time elapsed between irradiation and fixation: the post-irradiation (p.i.) period.
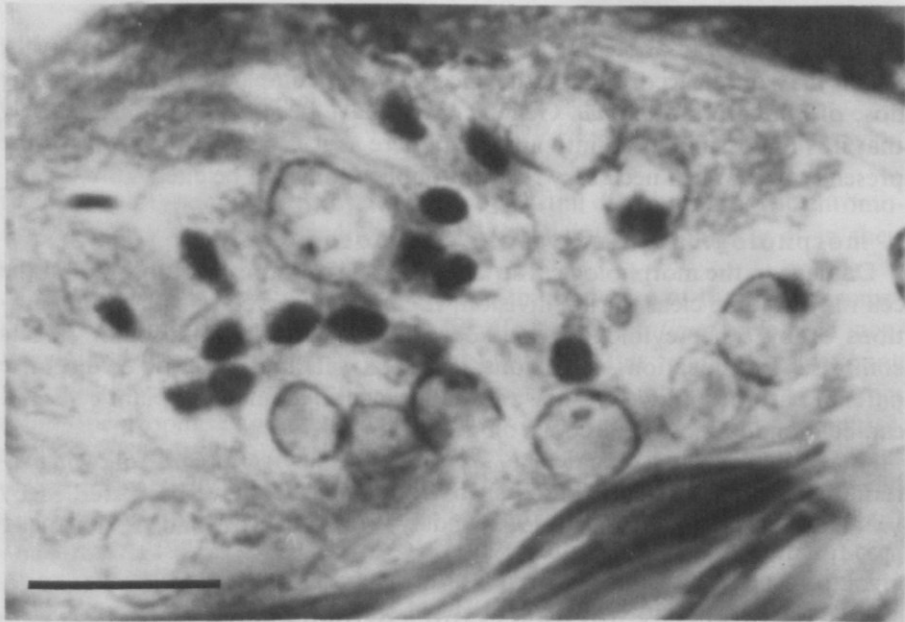
3.3. RADIATION EFFECTS ON CHROMOSOME MORPHOLOGY

To distinguish between the pathology of the cell and the general pathology of the organism is quite impossible as has been demonstrated by CAMERON (1952). The only criterium for such a distinction could be to limit the observations to the dividing cell, including relevant events prior to and following actual cell division.

When the general pathology of the dividing cell is examined from a morphological point of view a number of categories of general symptoms can be observed following the development of lesions caused by pathogenic stimuli.

a. no symptoms
The absence of morphological symptoms does not indicate the absence of any pathological effects. Depending on the quality and quantity of the pathogenic stimulus, genetic effects, for instance mutations, may be revealed after proper analysis.

Pure genetic radiation effects are obtained at relatively low dose levels, which may differ according to the species, when compared to the sterilizing dose of 3 kR for *H. antiqua*. Genetic effects have been studied so extensively that it is impossible to go into any detail here as it is beyond the scope of the present work. When necessary relevant publications will be cited.

b. morphologically analysable chromosome defects
Damage to the morphological structure of one or more chromosomes in the cell may become clear during division. It can be observed in squash-preparations or by other cytological methods. This type of chromosomal defects comprises translocations, inversions, deletions, satellites, acentric or dicentric parts of chromosomes etc. They are well known in animal and plant cells and can be found in most handbooks on genetics.

Analysable chromosome defects are found in male germ cells following irradiation with relatively low doses. In *H. antiqua* they have been studied by VAN HEEMERT (1973a, 1974a, b; 1975) and by WIJNANDS-STÄB and VAN HEEMERT (1974).

c. morphologically not analysable chromosome defects
These symptoms refer to structural defects which are so widespread, even within a single chromosome, that a clear analysis can not be obtained. They

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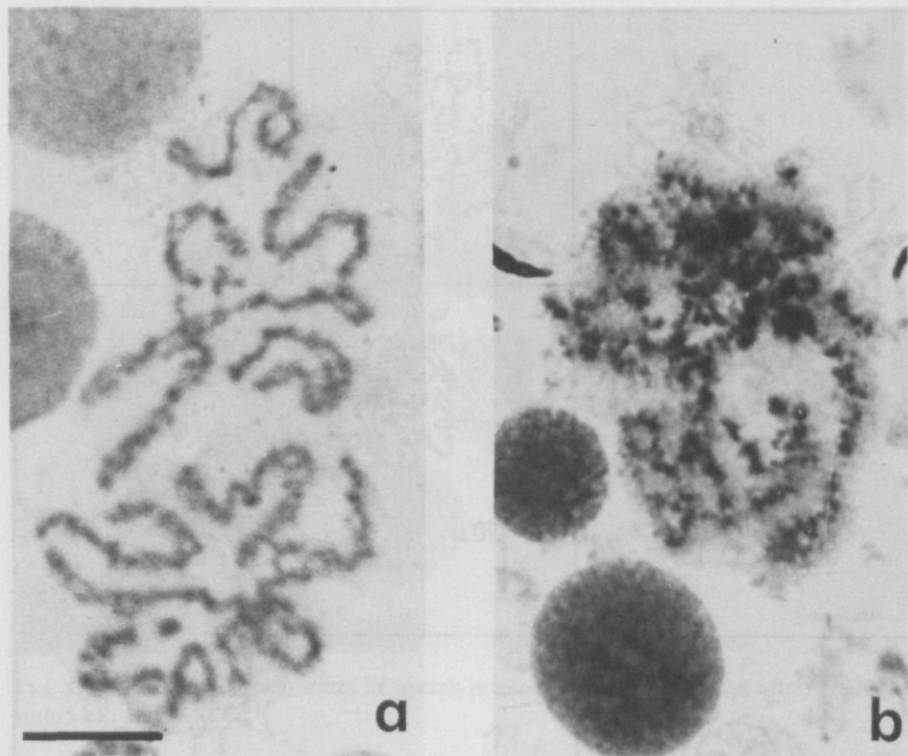
FIG. 13. Beadstring chromosomes in testicular germinal cells irradiated with different doses of hard X-rays. Bar = 10 μ.
a. meiotic prometaphase chromosomes (2 kR, 24 hours post-irradiation)
b. meiotic prometaphase chromosomes (5 kR, 24 hours p.i.)

comprise all types of fragmentation and/or partial dissolution of the chromosomes, which essentially retain their structural integrity entirely of in their separated parts.

At increasing doses the damage done to the structure of the chromosomes is becoming more serious. Some of these effects in *H. antiqua* have been mentioned and depicted earlier (THEUNISSEN, 1971). After irradiation of young adult testes with 2 kR of hard X-rays, distinct fragmentation of chromosomes in mitotic prophase and metaphase stages is observed after about 60 minutes. Cells in meiotic division show '*beadstring chromosomes*' i.e. chromosomes which are partially fragmented, resulting in an alternation of pieces of normal and reduced thickness (fig. 13). They appear like a string of beads (fig. 14a; THEUNISSEN, 1971, fig. 3) and are also observed in histological preparations. Although the normal structure of mitotic chromosomes disappears quickly after doses of 3 kR and more, the beadstring chromosomes seem to be more resistant to disintegration. They are still present 24 hours after irradiation with 5 kR of hard X-rays (fig. 14b). Entirely fragmented chromosomes are
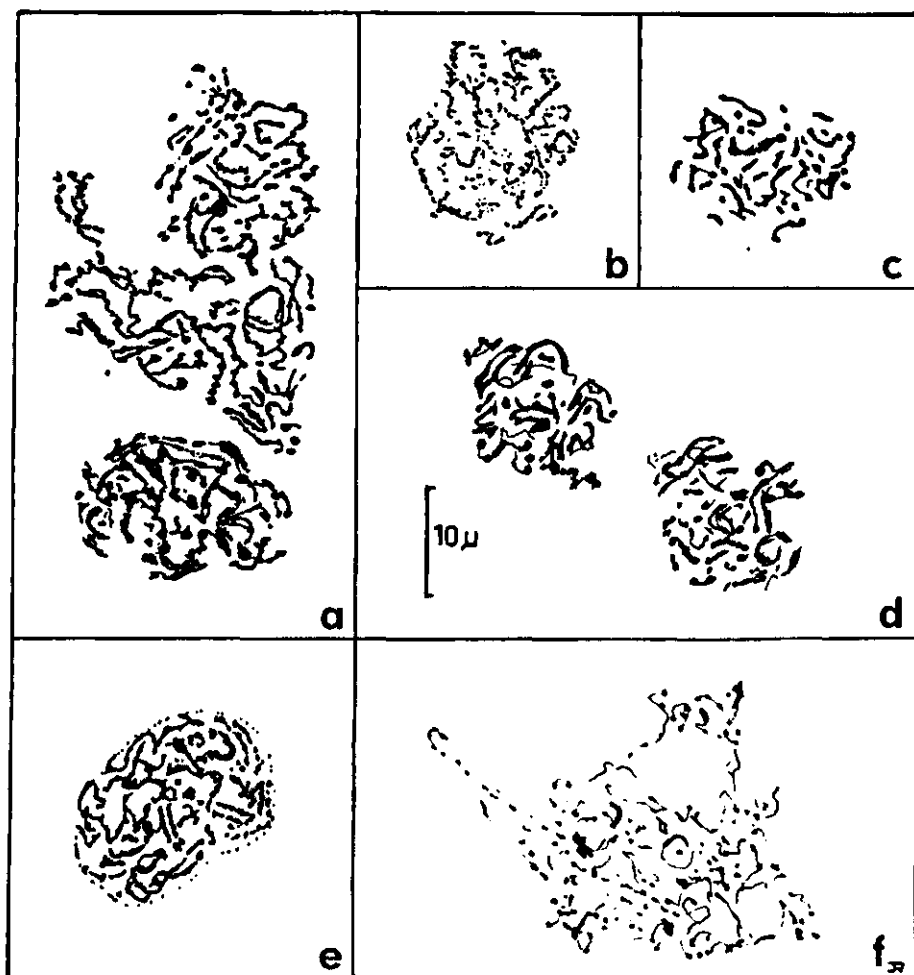
FIG. 14. Beadstring and pseudo-prophase chromosomes as examples of radiation damage to chromosome structure in male germinal cells.
a. beadstring chromosomes during prometaphase I, 75 minutes after irradiation with 2 kR hard X-rays.
b. beadstring chromosomes 24 hours after irradiation with 5 kR.
c. fragmentation of chromosomes 24 hours following irradiation with 5 kR.
d. pseudo-prophase, 20 hours after irradiation with 2 kR, showing fragments in somatic pairing.
e. prophase I 75 minutes after 2 kR irradiation showing fragmented beadstring chromosomes.
f. dissolving pseudo-prophase chromosomes, 24 hours after irradiation with 5 kR.
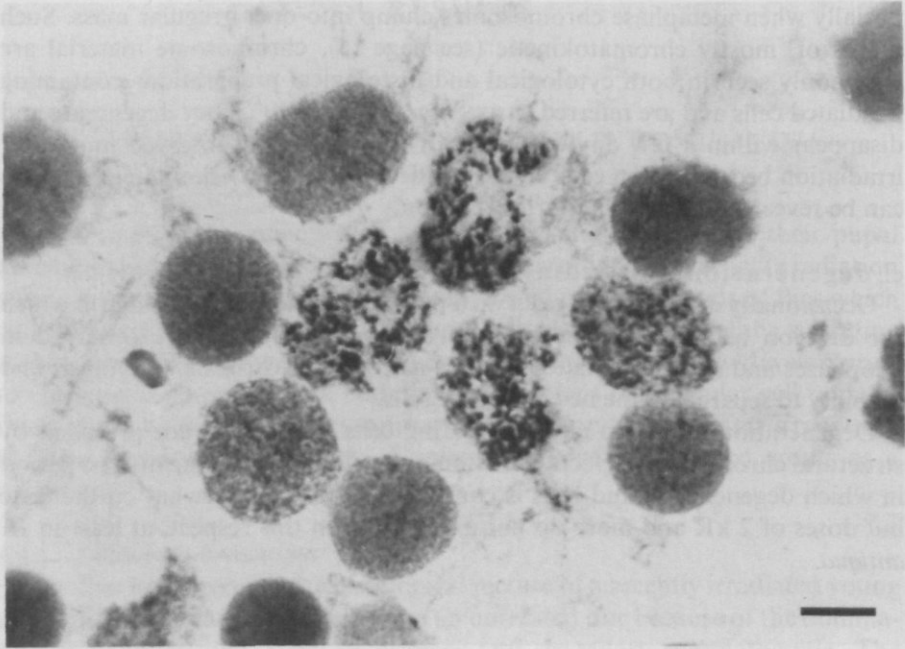
FIG. 15. Beadstring chromosomes in squash-preparation of testis 2 days after irradiation with 5 kR. Bar = 10 μ.

also relatively persistent when compared to normal ones and may be found 24 hours after administering 5 kR (fig. 14c). They often show a clew of filaments, at first sight resembling a normal prophase stage. Therefore, these generally darkly stained structures are referred to here as: '*pseudo-prophase chromosomes*' (fig. 14d). They originate from a normal prophase stage and sometimes still show fragments in somatic pairing (fig. 14c). Beadstring and pseudo-prophase chromosomes are chromosomes which already were in division during irradiation and probably do not stem from cells irradiated during interphase. Ultimately all fragmented chromosomes disintegrate within 2–3 days (figs. 14f, 15).

d. stickiness of chromosomes and their parts

These symptoms signify the irreversible loss of structural integrity and individuality of the chromosomes. Entire chromosomes or parts of them stick together and gradually degenerate. The degree of mutual attachment may vary considerably and range from very locally to the formation of an irregularly shaped lump of chromosome material in which all chromosomes are stuck together beyond recognition. Extensive stickiness is usually accompanied by chromatokinesis of the chromosome material and followed by pycnosis and subsequent karyorrhexis and/or cytolysis.

Total loss of structure show chromosomes where they stick together, es-

pecially when metaphase chromosomes clump into one, irregular mass. Such lumps of, mostly chromatokinetic (see page 13), chromosome material are commonly seen in both cytological and histological preparations containing irradiated cells and are referred to as: '*blocked divisions*'. They degenerate and disappear within a few days. Blocked divisions may be observed long after irradiation because when cells enter mitotic or meiotic division latent damage can be revealed in this form during division.

e. degeneration of dividing cells

Occasionally dividing cells react on a pathogenic stimulus at a stage in which the division nearly has been terminated. These symptoms are observed in telophases and show chromatokinesis, pycnosis, karyorrhexis, karyolysis and inability to separate of the newly formed cells.

Degeneration of the irradiated dividing cells whether or not preceded by structural chromosome defects is a common result of the treatment. The period in which degeneration and lysis is completed depends somewhat on the dose but doses of 2 kR and more do not differ much in this respect, at least in *H. antiqua*.

4. RESULTS

4.1. PATHOGENESIS OF RADIATION SYNDROME IN TESTICULAR CELL POPULATIONS

As irradiation of pupae which have reached the final part of their pupal development is the standard procedure, it is interesting to investigate radiation effects on the gonads of more and less advanced pupae. Gonads of flies which emerge 1 and 2 days after irradiation usually react differently on the sterilizing dose as compared to the gonads of flies which need more time to develop prior to emergence. Therefore, the pathological reactions of testicular cell populations of newly emerged adults, appearing 1, 2, 3 and 6 days after irradiation shall be described using testes of unirradiated, newly emerged adults as a reference.

4.1.1. *1 day post-irradiation*

The first impression of the histological picture of a recently irradiated young adult testis does not differ much from an untreated one because of the domination of 'concentrated' types of primary and secondary spermatogonia. The 'diffuse' type of secondary spermatogonia is still relatively scarce and is not present in each testis. Very conspicuous are various transitional forms of secondary spermatogonia towards the 'diffuse' type (fig. 16, 4; THEUNISSEN 1976, fig. 11).

Primary spermatogonia are seen near the apical cell. Both 'diffuse' and 'concentrated' types including a wide variety of intermediate forms are observed. In spite of the occurrence of these normal cell types, symptoms of radiation damage cause a chaotic situation which is difficult to describe exactly. Aspects are: the first signs of acute dysplasia i.e. distortion of normal spatial relations between cells, depletion of the cell population by cytolysis, chromatokinesis, hyperchromatosis and swelling of individual cells, karyolysis causing the disappearance of the nucleus from the cell.

The primary spermatogonia are bordered by the somewhat dilated apical cavities (fig. 21) which are empty or contain multinucleate cells. Both the number and the size of these cavities are larger when compared to non irradiated testes. The condition of the multinucleate cells is one of the first indicators of radiation damage. Cells which contain 3–4 nuclei seem to be very sensitive. After 1 day they are already in advanced hyperchromatic degeneration: strong hyperchromatosis, loss of internal structure, pycnosis and finally cytolysis (fig. 16, 3). The same applies to bi-nucleate cells which are also located apically of the apical cavities. Their strong hyperchromatic reaction distinguishes them easily from other cells.

Some primary spermatocytes show hyperchromatosis and a tendency to ghost formation. Normal meiotic divisions are absent, blocked ones are
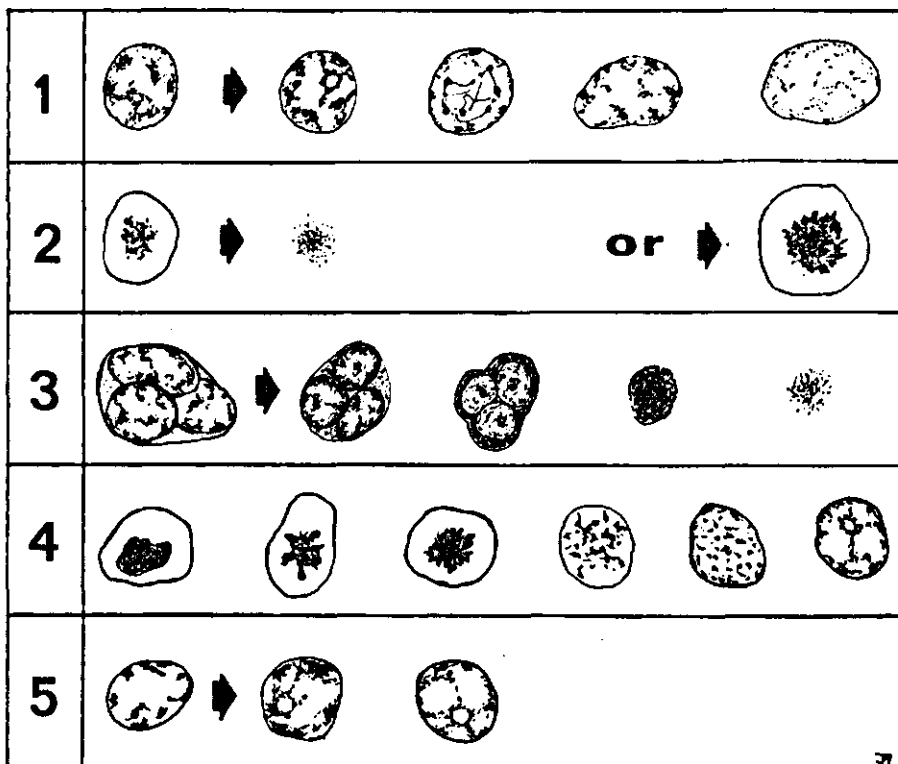
FIG. 16. Schematic drawing of normal and pathological variations in testicular cell types, varying periods after irradiation with 3 kR hard X-rays.
1. 'diffuse' primary spermatogonia. Left: the normal type in untreated testis. To the right types as they are found 1, 2, 3 and 6 days after irradiation. These types are not exclusively present at the mentioned times and nuclear swelling is more pronounced as is depicted here.
2. 'concentrated' primary spermatogonia. Left: a normal type which after irradiation seems to lyse or in some cases seems to be able to maintain its morphology up to six days after irradiation in spite of nuclear swelling.
3. multinucleate cell which degenerates by hyperchromatosis, loss of internal structure, pycnosis and cytolysis.
4. 'concentrated' secondary spermatogonia which transform via a series of types which are also present in unirradiated testes, towards the 'diffuse' type (right) which is only found in irradiated testes.
5. primary spermatocytes. Left: normal type.

numerous. Often cysts with blocked meiotic divisions also contain cells of the parental type and/or the daughter type. A mixed cyst may contain for instance primary and secondary spermatocytes apart from blocked MI divisions. Formation of such cysts is probably caused by asynchronous development of the cells at the moment of irradiation. Some cells will remain in or perhaps return to interphase, some will be blocked while dividing and some will have passed the division.
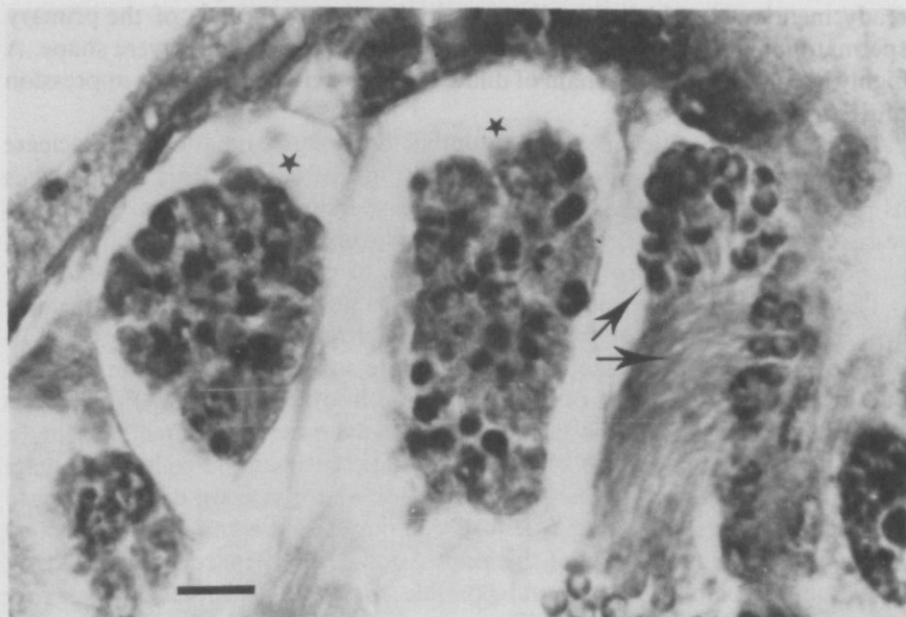
FIG. 17. Hyperchromatic and necrotic early spermatids (*) and normal looking intermediate spermatids (arrows) in newly emerged adult, 1 day after irradiation with 3 kR. Bar = 10 μ.

Early spermatids show early signs of a pathological reaction: hyperchromatosis, swelling, ghost formation and occasionally pycnosis, although normal looking cells are still present (fig. 17).

Intermediate spermatids often show vacuolization of the peripherally located chromatin, but the majority is of a normal appearance, as are the transformation spermatids and the sperm cells. The first small ghosts are seen in the central cavity, in cysts of early spermatids and rarely in cysts of primary spermatocytes.

A peculiar phenomenon is the increased irregularity of the borderline between the premeiotic populations and the central cavity, reflecting a distorted spatial interrelationship of the testicular cell populations.

4.1.2. 2 days post-irradiation

Testes of newly emerged adults 2 days after irradiation show an increased desorganization in the entire testis. frequently including a general dysplasia of the cell populations involved. Dysplasia tends to occur in particular when 'concentrated' spermatogonial populations are still large. So, the apical cell with surrounding primary spermatogonia and apical cavities can be found in the centre of the testis, bordering the central cavity. The 'diffuse' primary spermatogonia which are generally dominant in number show filamentous chromatin elements which connect granules (fig. 16, 1), causing the large nuclei to be lightly stained. 'Concentrated' types are also present in varying numbers, as are bi-nucleate cells, which are partly hyperchromatic. The symptoms al-

ready mentioned, which contribute to a chaotic appearance of the primary spermatogonial cell population are also found here in a more severe shape. A highly irregular bordering chain of dilated apical cavities adds to the impression of desorganization.

In the apical cavities a varying number of hyperchromatic multinucleate cells is seen. In this region the chaotic multitude of degenerating cells reaches its peak. Elements of chromatokinetic and hyperchromatic degeneration processes are found in a mixture of swollen, chromatokinetic, hyperchromatic, pycnotic, karyolytic, cytolytic, shrunken cells or cell groups.

The population of secondary spermatogonia may have a varied appearance depending on the relative numbers of 'concentrated' and 'diffuse' types. In some testes the 'concentrated' types dominate strongly, limiting the 'diffuse' ones to the most basal part of the population. In other testes the 'diffuse' type is the dominating one. The border between the two types often presents a confusing picture of groups of hyperchromatic or pycnotic cells, cell nuclei of varying size, chromatokinetic degeneration and a varying degree of acute dysplastic changes. Basally the 'diffuse' cell population is more homogeneous owing to a larger uniformity of this population in appearance and size. Apart from some hyperchromatosis and possible effects of a general dysplasia its appearance shows few degeneration symptoms.

Primary spermatocytes are mostly recognized by their position in the testis and their nuclear size which is slightly larger when compared to 'diffuse' spermatogonia. Occasionally strongly enlarged nuclei are found. As they can not be expected to be metabolically hyperactive, they can not be designated as hypertrophic. Many nuclei show fine filamentous chromatin structures which might be fragmented prophase I chromosomes. Blocked divisions are decreasing in numbers, mixed cysts are not. Secondary spermatocytes sometimes can be still recognized, although hyperchromatosis and other degeneration symptoms are numerous.

Spermatids react in their own way. Pycnotic cells and large ghosts are a characteristic combination in cysts of early spermatids (fig. 18). Other aberrations are hyperchromatosis, swelling, cytolysis and combinations of them (fig. 19). Normal looking early spermatids are rare. Mixed cysts of early and intermediate spermatids, which are never seen in unirradiated testes, show the same symptoms. Cysts containing only intermediate spermatids are rare. When found they react in the same way: no symptoms at all, or ghost formation, or hyperchromatosis, or pycnosis. The nuclei may vary in size. Transformation spermatids and sperm cells do not show symptoms of radiation damage.

4.1.3. *3 days post-irradiation*

When describing the pattern of radiation effects on the testicular cell populations 3 days after irradiation with 3 kR hard X-rays, the following picture emerges (fig. 20).

The testis itself does not yet show morphological changes as far as shape and size are concerned.
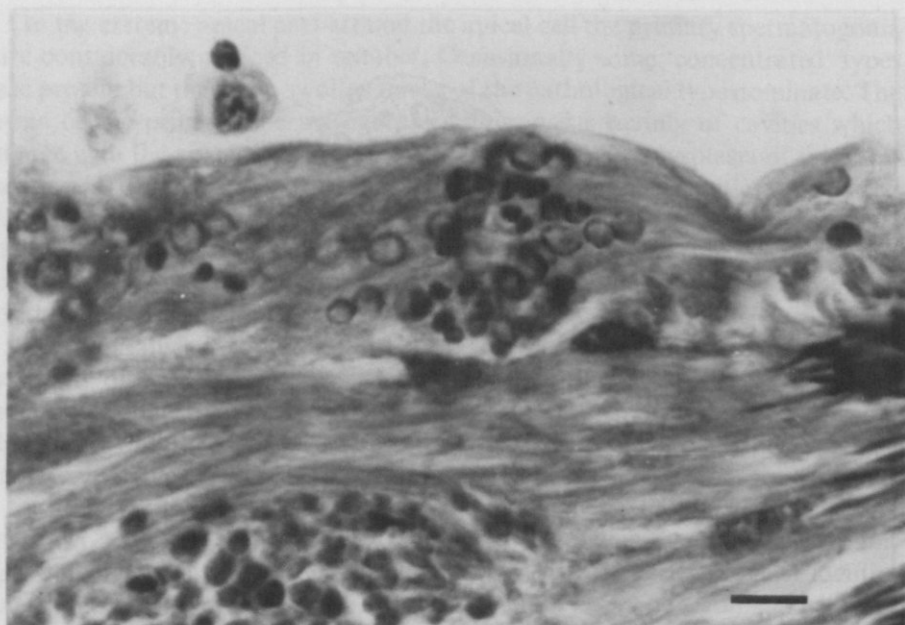
26

FIG. 18. Pycnosis and beginning ghost formation in cyst of early spermatids. Below: a part of the core of the central cavity. Newly emerged adult, 2 days after irradiation with 3 kR. Bar = 10 μ.
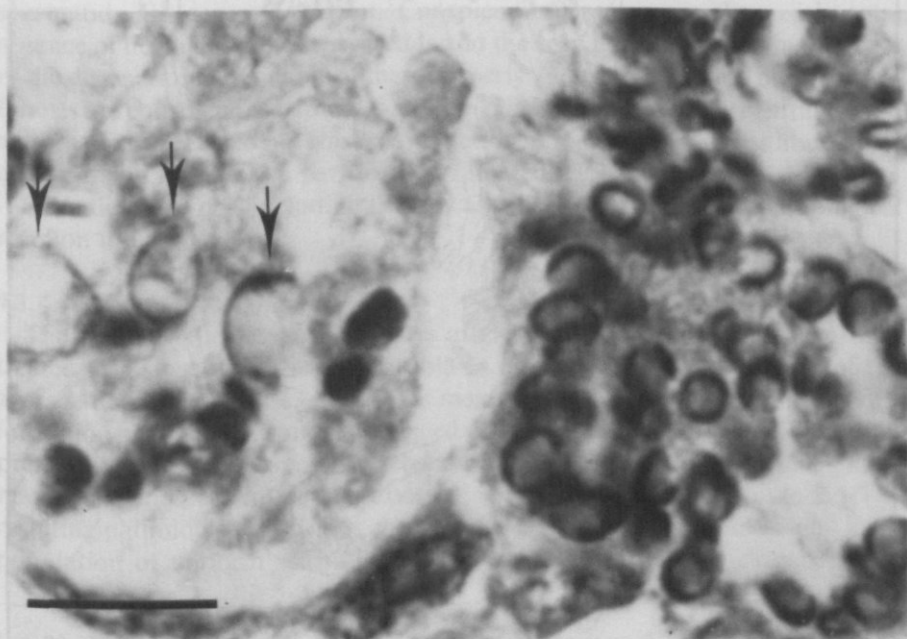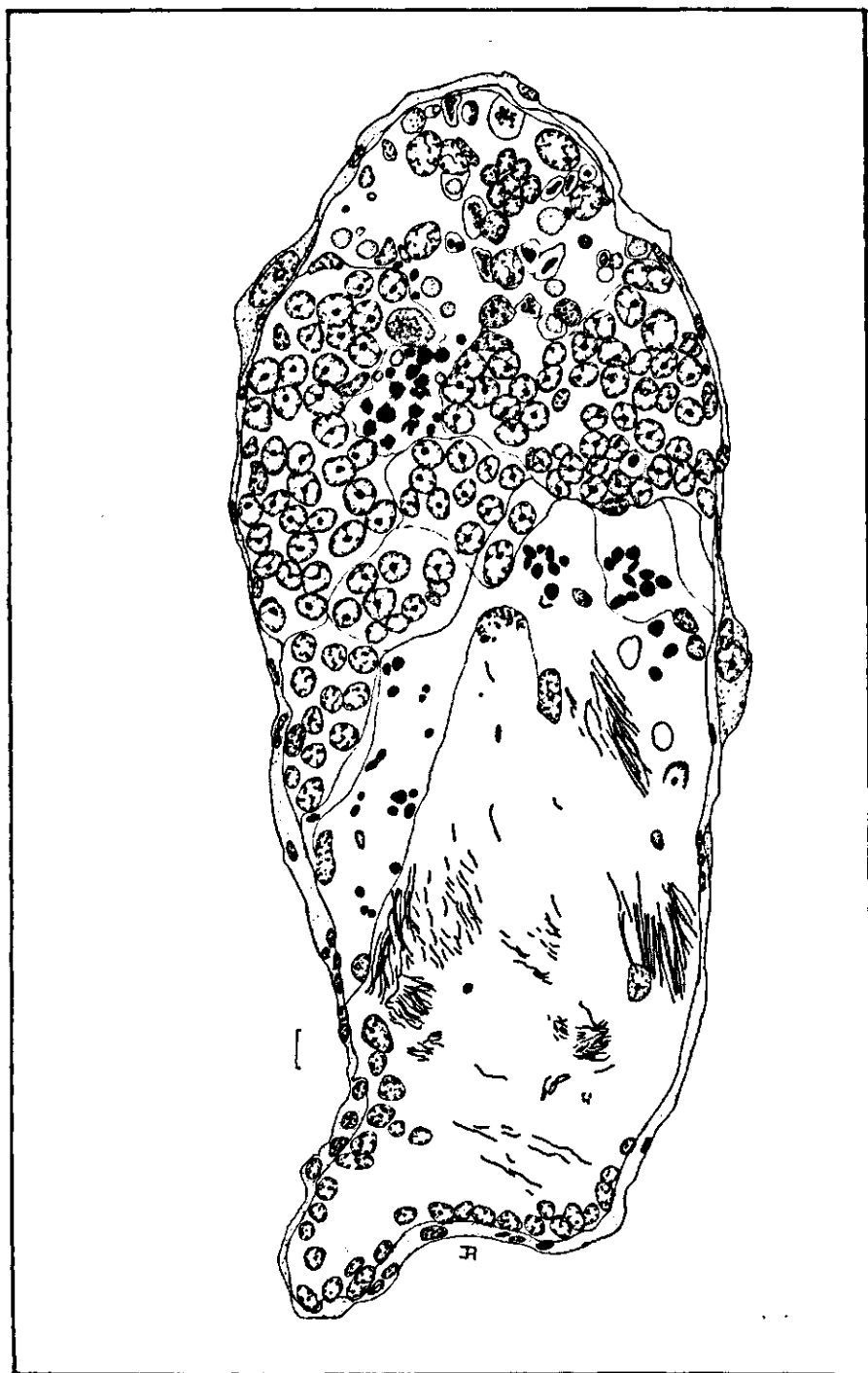

FIG. 19. Pycnotic and lytic early spermatids (left) with ghosts (arrows). Right: hyperchromatic intermediate spermatids. Newly emerged male, 2 days after irradiation with 3 kR. Bar = 10 μ.

In the extreme apical part around the apical cell the primary spermatogonia are considerably reduced in number. Occasionally some 'concentrated' types are present but the large, swollen nuclei of the pathological type dominate. The zone of the primary spermatogonia often consists mainly of cavities which merge with the apical cavities at its basal margin. Acute dysplasia of this population is regularly observed. The entire zone including the cavities is displaced laterad, sometimes basad towards the central cavity. This dislocation causes a distortion of the shape of the population on top of possible other effects which can not be isolated from direct radiation effects. The dominating impression of this part of the testis is one of emptiness and disorganization.

More basally the population of secondary spermatogonia presents usually a totally different picture. The striking uniformity of the 'diffuse' secondary spermatogonia suggests a normal, orderly development of a large population. However, mitotic divisions are absent. The main impression is perhaps best characterized by the phrase: 'law and order'.

In untreated testes young primary spermatocytes are very difficult to distinguish from secondary spermatogonia. In irradiated ones it is usually impossible to distinguish these cell types because of the morphological identity of primary spermatocytes and 'diffuse' secondary spermatogonia. The latter type is characteristic for irradiated testes.

Around the central cavity cysts or former cysts of early and transformation spermatids in various stages of degeneration are observed. Intermediate spermatids are generally absent. Conspicuous features are the nearly total absence of normal types of spermatids and the very large reduction in numbers of the degenerating remnants of their formerly and normally large populations. Ghosts are conspicuous pathological elements in and around the central cavity, whereas sperm cells do not show any reaction in morphology, gross abundance or dispersion in the basal part of the irradiated testis.

Somatic cell types, including the apical cell, do not show any pathological reaction to irradiation.

4.1.4. 6 days post-irradiation

The testes of flies emerging 6 days after irradiation took place have been treated in a stage which is comparable to one between 9 and 10 days of the average development in 13 days. This means that these testes contained transformation spermatids as most advanced cell type (THEUNISSEN, 1976) and that premeiotic cell types, especially secondary spermatogonia and primary spermatocytes, and cells in meiotic division were very dominant in number. The resulting pathological picture is characterized by the densely packed and large population of uniform 'diffuse' secondary spermatogonia which occupy the

FIG. 20. Drawing of testis of *H. antiqua* 3 days after irradiation with 3 kR hard X-rays. Note the disorganization among the primary spermatogonia, the large population of 'diffuse' secondary spermatogonia and the absence of meiotic divisions and spermatids. Compare with untreated situation in Theunissen, 1976 (fig. 55a). Bar = 10 μ.

FIG. 21–22. Pathological reactions in top of testis of newly emerged males 6 days after irradiation with 3 kR. Bar = 10 μ.
FIG. 21. Swollen primary spermatogonia (arrows) around and near the apical cell. Relatively large apical cavities (*) are observed. Note thickened testicular sheath of other testis (left).

center and lateral parts of the testis. The small but very pathological cell populations on its apical and basal borders do not correct this dominance entirely.

The apical region of the testis is very variable in size and extension because of the irregular course of the bordering apical cavities (fig. 21) and the results of a more or less severe dysplasia. distorting the population and blurring the borders of the zone. The primary spermatogonia largely conform to the description given elsewhere (page 34) but sometimes the chromatin seems to be organized in granules and in very fine filaments consisting of minute granules (fig. 16, 1). The nuclei are large and lightly stained. Another aberrant type of primary spermatogonia shows large, round nuclei in which the chromatin is concentrated (fig. 16, 2). This mass of chromatin which seems to consist of granules and small rods can be as large as an entire nucleus of the 'diffuse' secondary spermatogonia. It probably represents a 'concentrated' type of primary spermatogonia which has maintained its appearance in spite of a considerable swelling. Bi-nucleate cells which are often in hyperchromatic or chromatokinetic degeneration are numerous. Other cells in degeneration mainly show chromatokinetic degeneration, including cytolysis (figs. 22 and 23). Pycnosis and karyorrhexis are rarely observed here. The very numerous and greatly swollen apical cavities also contain many cells and groups of cells in various stages of degeneration. The border function of these cavities is evi-
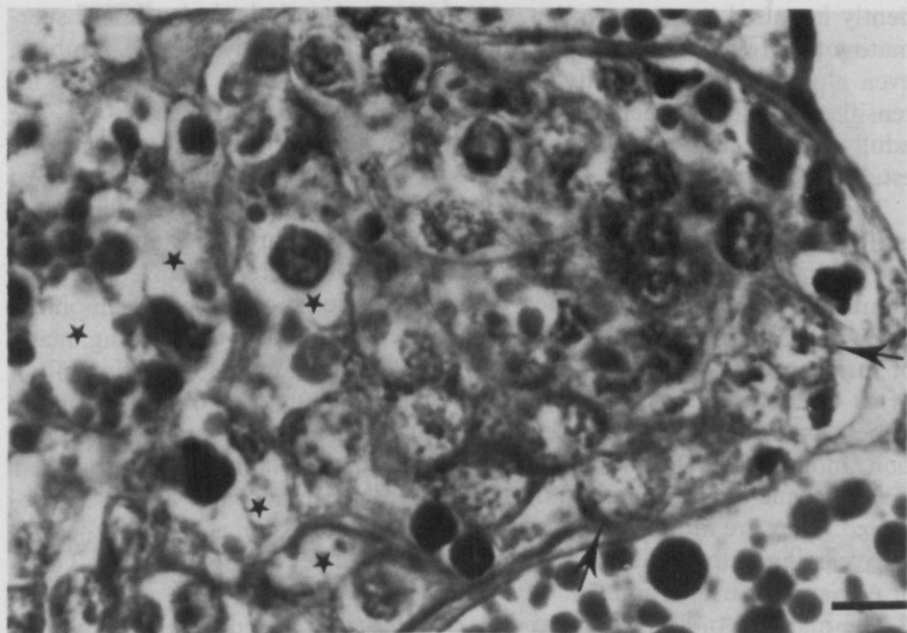
30

FIG. 22. Swollen primary spermatogonia (arrows) near apical cell. Numerous cells in chromatokinetic or hyperchromatic degeneration along the testicular sheath and in or near apical cavities (*).
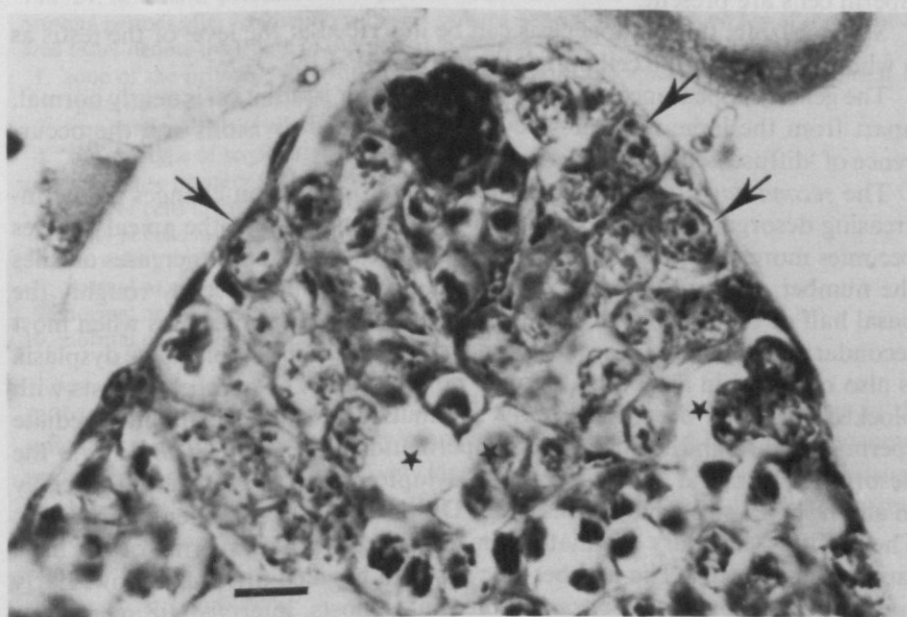

FIG. 23. Comparable untreated testis. The apical cell is surrounded by mainly 'diffuse' type primary spermatogonia (arrows). A few apical cavities are observed (*).

dently impaired because swollen, characteristical pathological primary spermatogonia are often found basally of these cavities in relatively large numbers, even along the central cavity. Dysplastic radiation effects are probably intensified by the treatment at a moment in which the central cavity and the adult spatial relationships between testicular cell populations were not yet established.

The population of 'diffuse' secondary spermatogonia is large, dense and uniform of appearance. It usually occupies the central part of the testis, apically and laterally of the central cavity (fig. 20). It is bordered basally by a large number of primary spermatocytes. As the irradiation took place at the moment when primary spermatocytes were very numerous and actively dividing, many of them are found as nuclei containing beadstring chromosomes (fig. 26, c), or as blocked divisions. Secondary spermatocytes are also observed for the same reason. They are recognized by their nuclear morphology, size and position among the other cell types. Besides the clumped metaphases of the blocked divisions, meiotic prophase and prometaphase chromosomes have been observed. They are interpreted as signs of a resumed division activity of the cells after passing of the radiation induced inhibition. They are not apparent 3 days or earlier after irradiation.

The majority of early spermatids is pycnotic and mixed with large ghosts. Sometimes normal appearing early spermatids are absent. Intermediate spermatids are rare and if found hyperchromatic, pycnotic, lytic or in ghost formation. Transformation spermatids are darkly stained, swollen and pycnotic. Sperm cells are present.

Summarizing, this pathogenesis can be described at the level of the testis as a whole of the separate cell populations (fig. 24).

The general appearance of the testis *one day* after irradiation is nearly normal, apart from the large number of cysts with blocked divisions and the occurrence of 'diffuse' secondary spermatogonia.

The *second day* after irradiation the general impression changes as the increasing desorganization of the apical part and swelling of the apical cavities becomes more prominent. The number of degenerating cells increases as does the number of 'diffuse' secondary spermatogonia which occupy roughly the basal half of the zone of secondary spermatogonia. In some cases when most secondary spermatogonia are still 'concentrated', usually severe acute dysplasia is also observed in all spermatogonial populations. The number of cysts with blocked divisions decreases. Early spermatids are abnormal, intermediate spermatids rare and transformation spermatids normal. After *three days* the desorganization and the degeneration symptoms are more severe, especially in and near the swollen apical cavities. Dysplasia is more frequently observed. The 'diffuse' secondary spermatogonia dominate. Primary spermatocytes have largely disappeared. The number of cysts with blocked divisions is low. Early spermatids are pycnotic or transformed into ghosts, intermediate spermatids extinct and transformation spermatids abnormal. *Six days* after irradiation the overall picture is dominated by the large mass of 'diffuse' secondary sper-
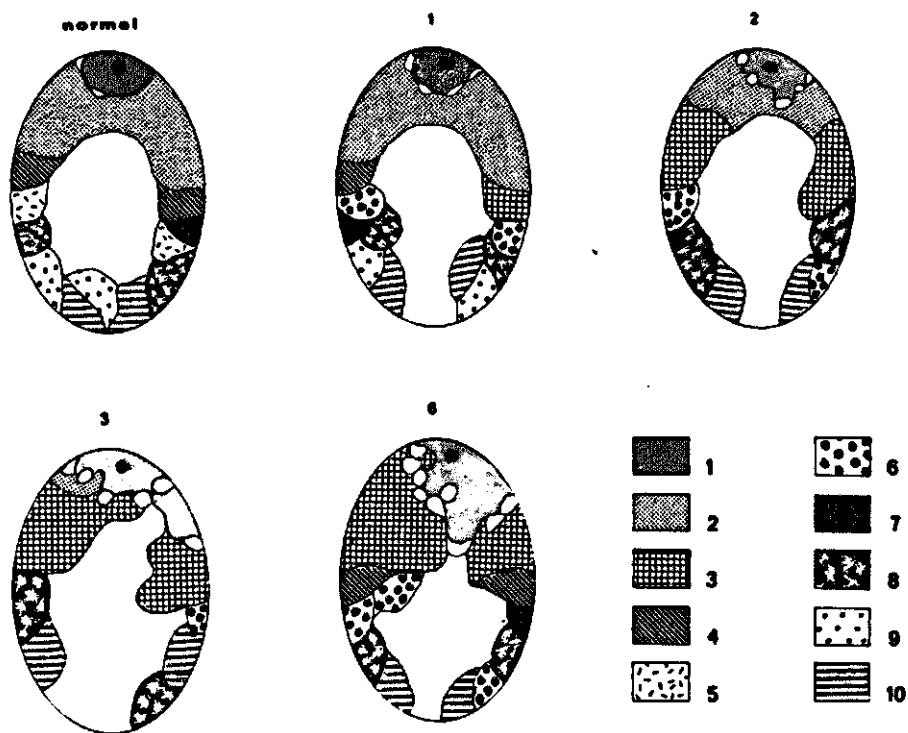
FIG. 24. Schematic presentation of general radiation effects on testicular cell populations, varying periods after irradiation with 3kR of hard X-rays. The figures on top of the schematized testes denote the length of this period in days.

1. zone of the primary spermatogonia which contains the apical cell and is bordered by apical cavities (Theunissen, 1976).
2. 'concentrated' types of secondary spermatogonia.
3. 'diffuse' type of secondary spermatogonia.
4. interphase primary spermatocytes.
5. cysts of cells in meiotic division.
6. cysts of cells in blocked meiotic division.
7. secondary spermatocytes.
8. normal or pathological early spermatids.
9. normal or pathological intermediate spermatids.
10. normal or pathological transformation spermatids.

matogonia, in spite of a considerable degeneration and acute dysplasia in the apical part of the testis. Primary spermatocytes show beadstring chromosomes and attempts to divide. A relatively large number of cysts with blocked divisions is observed. The types of spermatids are rarely normal, but usually abnormal or totally absent.

4.1.5. *Discussion*

From these descriptions it is clear that irradiation of pupal testes, which are more or less advanced when compared to the average, does not result in very

different patterns of pathological reactions beyond the differences due to the time elapsed between irradiation and emergence. These differences, therefore, may be considered to demonstrate the pathogenesis of the radiation syndrome in late pupal testes prior to emergence.

4.2. Histopathology of irradiated testes

In the following paragraphs radiation induced changes in testes and testicular cell populations are described. Histopathological reactions of the various testicular cell types in the newly emerged flies, 3 days after irradiation with 3 kR are mentioned. These characteristic abnormalities constitute the set of criteria which was used to check on proper irradiation (see page 3). Both quantitative and qualitative modifications of this radiation syndrome are found after irradiation with lower and higher doses of hard X-rays.

4.2.1. *Description of changes in male germinal cell types*

Primary spermatogonia
The primary spermatogonia react in various ways. In many testes a large part of the population has disappeared, leaving cavities which are empty or partly filled with single or multinucleate cells showing chromatokinesis, hyperchromatosis and pycnosis. Less frequently they contain cells in karyolysis or karyorrhexis. The still existing population of primary spermatogonia can have a variable appearance ranging from cells with nuclei of normal size with finely granular and regularly dispersed chromatin to cells with large, swollen nuclei with a decreased stainability (fig. 25). Nuclei with more or less centrally located flocculate/granular chromatin with some small concretions are also frequently present. The most common and clearly pathological type is the one with swollen, sometimes a little irregular nuclei containing finely granular and flocculate chromatin which is regularly dispersed. The stainability of the nucleus is reduced by dilution of the normally staining chromatin in a larger nucleus (fig. 26, a, d). When the population has been reduced and many empty or partially filled cavities take the place of the disappeared cells, the remaining primary spermatogonia nearly always belong to this type.

Sometimes acute dysplastic changes of testicular cell populations are observed, affecting in particular the primary spermatogonia. The whole population including the apical cavities can be displaced laterad and basad, thus distorting the normal spatial arrangement of the testicular cell population.

Fig. 25. Some testicular germinal cell types of a newly emerged fly (3 kR). Bar = 10 μ.
a. Swollen primary spermatogonia between cells in chromatokinetic degeneration.
b. Typical appearance of 'diffuse' type of secondary spermatogonia.
c. Primary spermatocytes, blocked divisions (arrows) and one secondary spermatocyte.
d. Secondary spermatocytes. One is hyperchromatic (arrow), others show signs of lysis.

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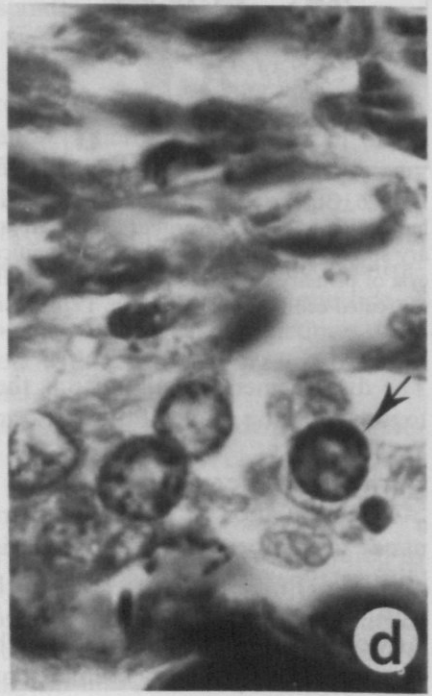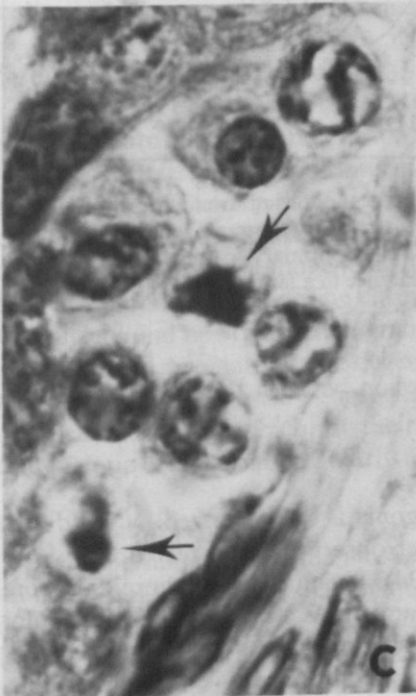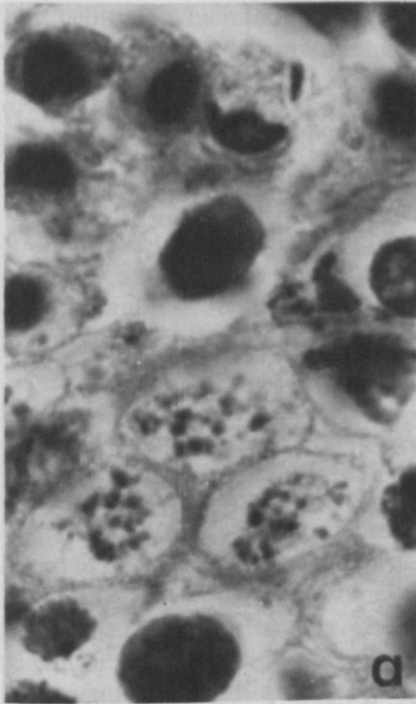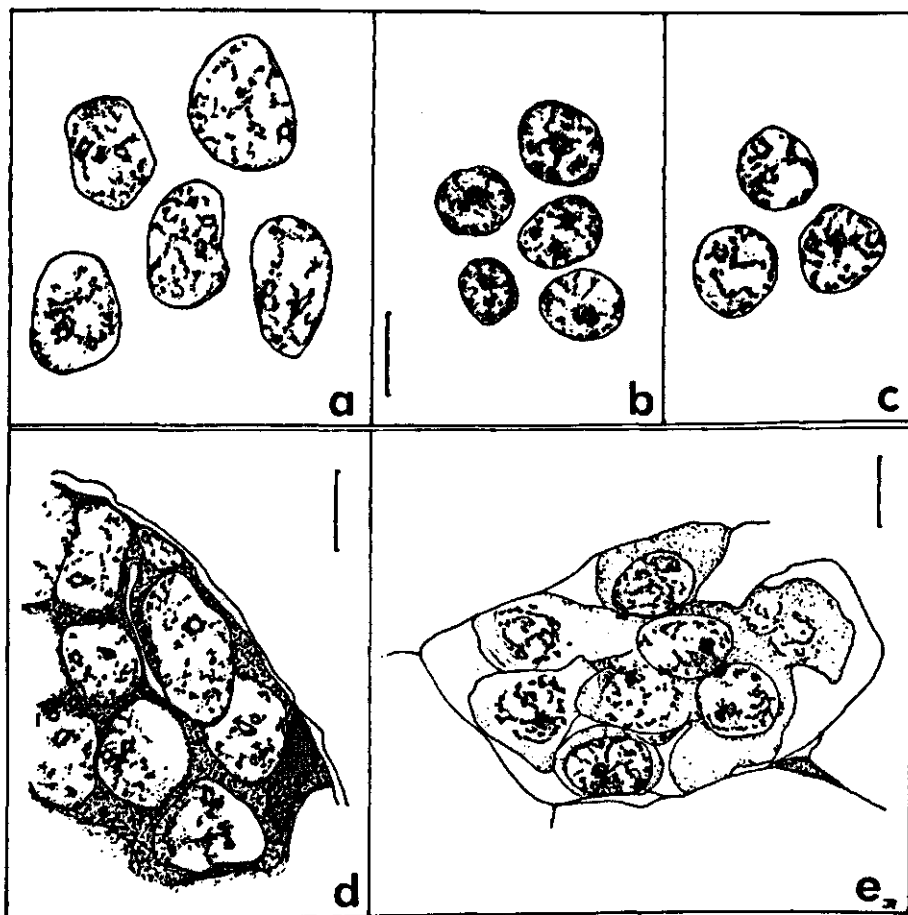
FIG. 26. Characteristic pathological testicular cell types following irradiation with 3 kR of hard X-rays. Nuclei of primary spermatogonia (a), secondary spermatogonia (b) and primary spermatocytes (c) at the same magnification. Cells of primary spermatogonia (d) and a cyst of primary spermatocytes (e) show their appearance in a larger number. Compare with untreated cell types (Theunissen, 1976). Bar = 10 μ.

The displacement usually affects the cohesion between the primary spermatogonia and the shape of the entire zone.

Secondary spermatogonia

The population of secondary spermatogonia is characterized by the dominance in numbers of a type which is never observed in untreated testes, the 'diffuse' type (cf. THEUNISSEN, 1976, page 34). The nucleus is spheroidal-ovoid with normally stained, granular and/or filamentous chromatin which is regularly dispersed (figs. 25b and 26b). The chromatin shows a slight tendency to a location on the periphery or immediately around the usually distinct nucleolus.

This cell type which looks perfectly normal is converted from 'concentrated' types of secondary spermatogonia some time after irradiation, as mitotic activity which has been terminated by the treatment prevents new cell formation. Whether or not the 'diffuse' type of secondary spermatogonia is characteristic for radiation damage is not known. In a minority of testes varying numbers of 'concentrated' types of secondary spermatogonia are still present. They form a mixture with the 'diffuse' type varying from complete absence to complete dominance of 'diffuse' cells. This phenomenon reflects the individual variability of the reactions on the same dose of radiation in identical conditions of comparable individuals. Hyperchromatosis of 'diffuse' secondary spermatogonia is occasionally observed in this stage.

Primary spermatocytes

In many testes the morphological difference between the 'diffuse' secondary spermatogonia and primary spermatocytes is virtually non existent.

In some cases disappearing nuclear membranes and beadstring chromosomes (fig. 26c, e) identify a cyst of primary spermatocytes which was possibly arrested in development by irradiation before the meiotic division could proceed as far as the production of blocked divisions. The latter ones are observed frequently. They are usually chromatokinetic and show varying degrees of stickiness.

Sometimes large ghosts are observed in a single cyst among cysts of primary spermatocytes. They seem to originate from primary spermatocytes which collectively degenerated by chromatolysis and swelling of the nucleus.

Secondary spermatocytes

This cell type is already relatively scarce in untreated testes. In irradiated ones they are rarely found. If so, they show spheroidal-ovoid nuclei of an intermediate size between those of primary spermatocytes and early spermatids. The chromatin is less peripherally located as in unirradiated ones, causing the nuclear lumen to be more darkly stained (fig. 25). Sometimes the chromatin is almost homogeneously dispersed, preceding its local concentration in small concretions. The chromatin may be shifted towards one side of the nucleus and be organized in granules of varying size. They sometimes show symptoms of chromatokinetic degeneration, with accompanying loss of internal structure. Occasionally ghosts are found amidst the cells.

Blocked divisions which, by their smaller volume, can be identified as second meiotic divisions are regularly found. They mostly show chromatokinesis.

Early spermatids

Early spermatids show the most varied reaction pattern when compared to all cell types in the testis. The majority of them is pycnotic in various forms often with vacuolated chromatin. Other nuclei are swollen and slightly stained, leading to ghost formation preceded by chromatolysis. Hyperchromatosis is relatively rarely found and morphologically normal cells are virtually absent.

A few chains of pathological reactions can be reconstructed. For instance: hyperchromatosis-concentration of chromatin to concretions-chromatolysis accompanied by swelling of the nucleus-ghost. The multitude of intermediate forms permit these reconstructions of main lines of degeneration and disappearance of cell populations. An intrigueing but unsolved problem is the reason why these genetically identical cells react in such a varied manner on the same stimulus. TATES (pers. comm.) suggests an influence of differentiation stages.

Another feature is the frequent breaking of the cyst membrane causing the dispersion of the cells outside the former cyst limits. The normal cohesion between the cells is distorted and finally lost.

Whereas no normal cells seem to be left, the number of remaining pathological types has also decreased considerably by the treatment.

Intermediate spermatids

At this stage intermediate spermatids are very rare. Cysts of presumably former intermediate spermatids only contain ghosts and cells in various advanced stages of degeneration.

Transformation spermatids

Nuclei of this cell type show a large variety of degeneration symptoms: chromatokinesis, vacuolization of the chromatin, hyperchromatosis, swelling, chromatolysis, ghost formation, abnormal dispersion of the chromatin. The usual uniformity of cells in the same cyst is distorted. Irregularly shaped nuclei, large variability in nuclear size and formation of a single unit from several nuclei are commonly seen. Limits of cysts are indistinct. The number of transformation spermatids is clearly much lower when compared to that in control testes.

Sperm cells

This cell type does not show any reaction to this dose, 3 kR, of radiation.

4.2.2. *Dose-effect relationship for pathological symptoms of irradiation*

When inducing dominant lethal mutations in sperm cells in order to obtain complete embryonic lethality of the descendants, the radiation effects caused by the sterilizing dose, 3 kR, will not be considered and evaluated separately from those brought about by lower and higher doses. In fact these effects are not different but form patterns which differ mainly in severity and ultimate result depending on age and received dose. Therefore, pathological effects of irradiation of testes with doses below and above the sterilizing level are described and discussed here in a series which reveals the actual continuity of the whole of effects and symptoms, the radiation syndrome, caused by the treatment with X-rays.

4.2.2.1. Introduction

The use of low doses here suggests the possibility to use criteria derived from

the 'normal' situation. The most obvious criterium for detecting possible radiation effects on testicular cell populations is the presence of the various cell types involved in about normal numbers. Given a certain dose and age the presence, absence or decline of normal cell populations in an irradiated testis will provide a pattern which is composed of the reactions of the assembled cell populations. This pattern can be compared with other irradiated and not irradiated testes. Since the untreated, normal testicular cell types have been defined morphologically to some extend (THEUNISSEN, 1976), and their pathological types as well (4.2.1.), it is possible to obtain this pattern by an essentially simple screening.

Histopathological reactions are characterized per age-group for each dose by a short description of the main symptoms. The most frequently observed radiation effects are described and roughly quantified because the considerable individual variability impairs a detailed account. Therefore, individual reactions may be contradictory to the described general trend. For descriptions concerning the 0 kR dose control group THEUNISSEN (1976) should be consulted.

4.2.2.2. At emergence

Pathological symptoms in testes of newly emerged flies, irradiated in the pupal stage 3 days earlier with a range of doses, are described and compared.

0 days, 0.5 kR

The reaction of each single cell population on 500 R is extremely variable, as is the appearance of the testis as a whole. Some testes already show a desorganized picture because of a moderate but acute dysplasia of cell groups and cytolysis in the zone of the primary spermatogonia. Others do hardly show any symptoms besides chromatokinesis. The primary spermatogonia are often somewhat decreased in number. In apical cavities and among secondary spermatogonia many chromatokinetic cells are found. 'Concentrated' secondary spermatogonia dominate this population in which a shift towards types with more spread chromatin (THEUNISSEN, 1976, fig. 11, left; fig. 16, 4 second from right) takes place. Both mitotic and meiotic divisions seem to proceed normally. The most conspicuous reactions are observed among the spermatids. Early spermatids show hyperchromatosis, chromatokinesis, swelling, pycnosis and ghost formation. The intermediate spermatids often are already very much reduced in numbers, as are the normal transformation spermatids which also show ghost formation. Ghosts are found in the central cavity.

0 days, 1.0 kR

Reactions are in general rather variable due to the same reasons as mentioned above. The population of primary spermatogonia decreases in numbers because of cytolysis. Many cells are chromatokinetic. 'Diffuse' type secondary spermatogonia are distinctly present as are all 'concentrated' types. The number of mitotic and meiotic divisions differs considerably per testis. The cysts of

spermatids hardly contain normal cells, showing the above mentioned symptoms, General symptoms are qualitatively the same as after irradiation with 500 R.

0 days, 1.5 kR

Variable reactions are observed in all cell populations. The primary spermatogonia decrease sharply in numbers, showing chromatokinetic degeneration. The secondary spermatogonia decrease also but are still numerous. The relative numbers of 'concentrated' and 'diffuse' types are variable. Primary spermatocytes seem to maintain themselves but show sometimes abnormal prophases and blocked divisions. The general mitotic and meiotic activity has clearly decreased when compared with both lower dose groups. No normal divisions have been observed. The spermatids are nearly extinct as for their normal morphology, while still many of them are present in some pathological form, especially as ghosts. Pathological symptoms in all cell types are essentially the same as those mentioned above, except the occurrence of a variable and irregular shape and size of spermatogonia.

0 days, 2.0 kR

The numbers of cells with a normal morphology in premeiotic cell populations have decreased generally and sharply. The primary spermatogonia have been reduced to a small fraction of the original population. Desorganization of this area in the testis is commonly found and is caused by acute dysplasia and cell depletion. In populations of secondary spermatogonia the 'diffuse' type is dominating relative to the 'concentrated' types.

As the 'diffuse' one is only found in irradiated testes, it is considered to be the 'pathological' type of secondary spermatogonia and noted as such. Including the pathological types of secondary spermatogonia and primary spermatocytes these populations are not much smaller when compared to those in control testes. The death of these cells takes place later, contrary to the primary spermatogonia. Normal spermatids are virtually extinct, the remaining ones showing various pathological symptoms already mentioned. Those of premeiotic cell types are still the same as has been described before, in general possibly a little more severe. Besides in the spermatogonial populations, the spatial relationships between other cell populations are commonly disturbed now by a general acute dysplasia.

0 days, 3.0 kR

Pathological reactions of some cell types are rather variable. In some testes primary spermatogonia are still relatively abundant, in others they have nearly disappeared. The 'diffuse' secondary spermatogonia dominate clearly over 'concentrated' types. Morphologically normal primary spermatocytes are rare and blocked meiotic divisions are regularly seen. Normal spermatids are absent. For a detailed description see 4.2.1.

	doses			
	0	**3**	**6**	**9**
p. spg				
s. spg				
p. spc				
e . spt				

FIG. 27. Schematic representation of the appearance of nuclei of some germinal cell types in testes of newly emerged flies 3 days after irradiation with various doses of hard X-rays. The cell types are primary and secondary spermatogonia, primary spermatocytes and early spermatids. Dose in kR. Bar = 10 μ. Compare also with Theunissen 1976, figs. 11, 15, 20 and 24.

0 days, 6.0 kR

Large, light nuclei of the primary spermatogonia are surrounded by little cytoplasm. The chromatin is organized in small granular elements connected by thin filaments. It is dispersed as loosely bound floccules which are connected (fig. 27). These 'diffuse' cells are dominant as all 'concentrated' types are absent (fig. 28). Chromatin in secondary spermatogonia is found as coarse granules which are mutually connected by strands and are regularly dispersed. The nuclei stain relatively dark. They can be excentrically located in the cells. This 'diffuse' type (fig. 29) is dominant but 'concentrated' types are observed as are various intermediate types. The chromatin granules in primary spermatocytes are smaller than those in untreated nuclei. Their tendency to a peripheral location enhances a clear observation of the nuclear contours. The oc-
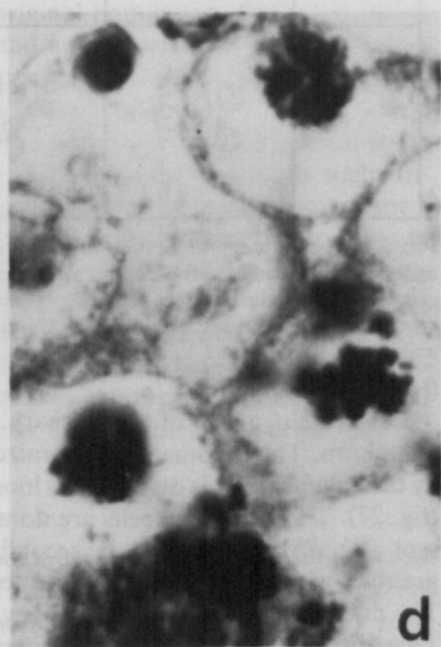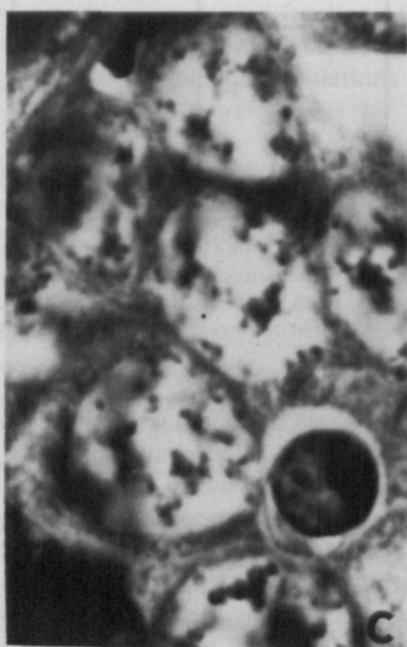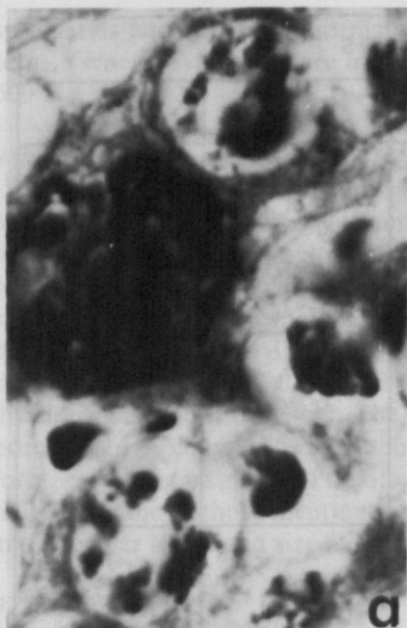
FIG. 28

FIG. 28–31. Primary and secondary spermatogonia, primary spermatocytes and early spermatids of newly emerged *H. antiqua*, 3 days after irradiation with various doses. Bar = 10 μ.
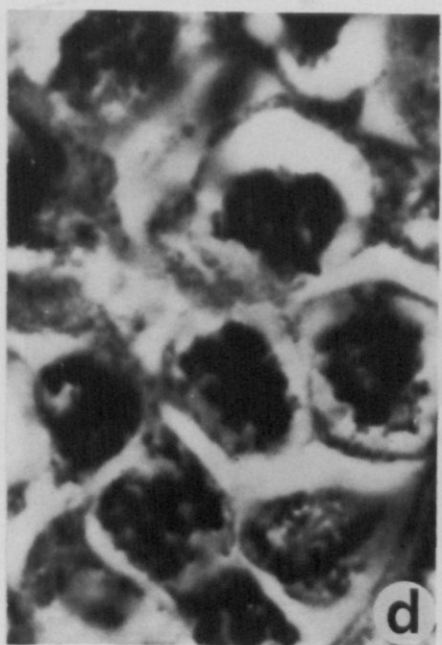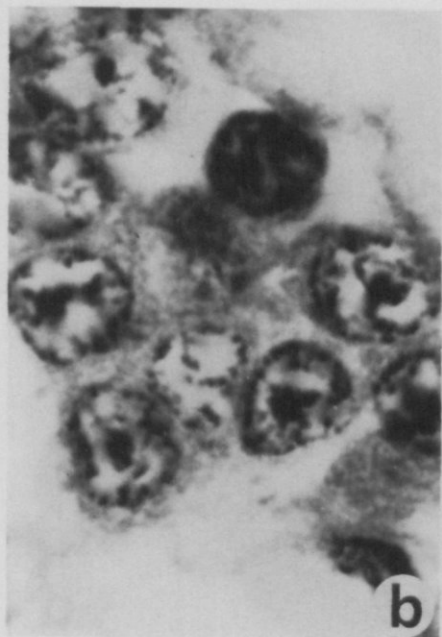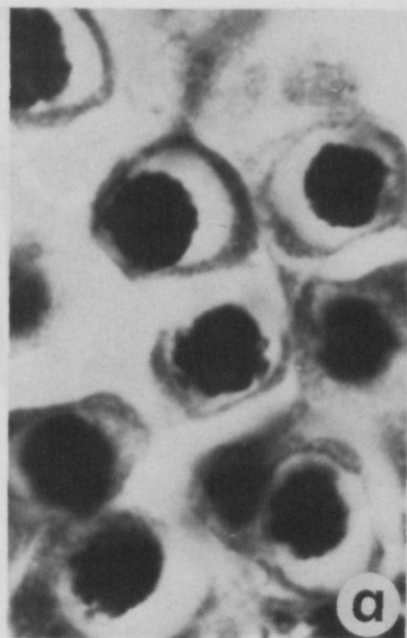
FIG. 29
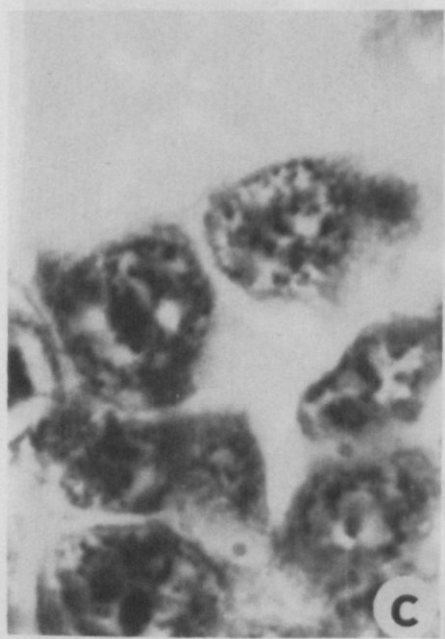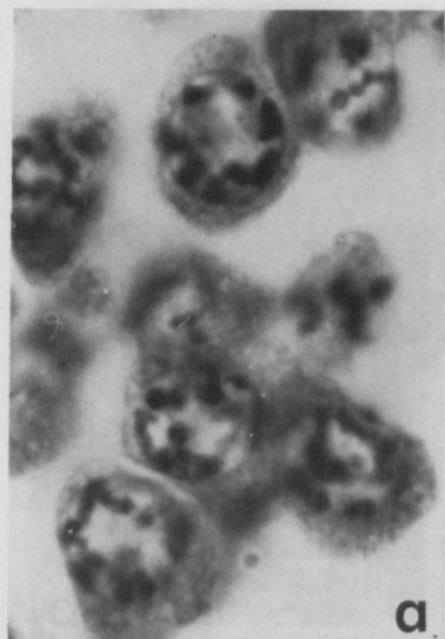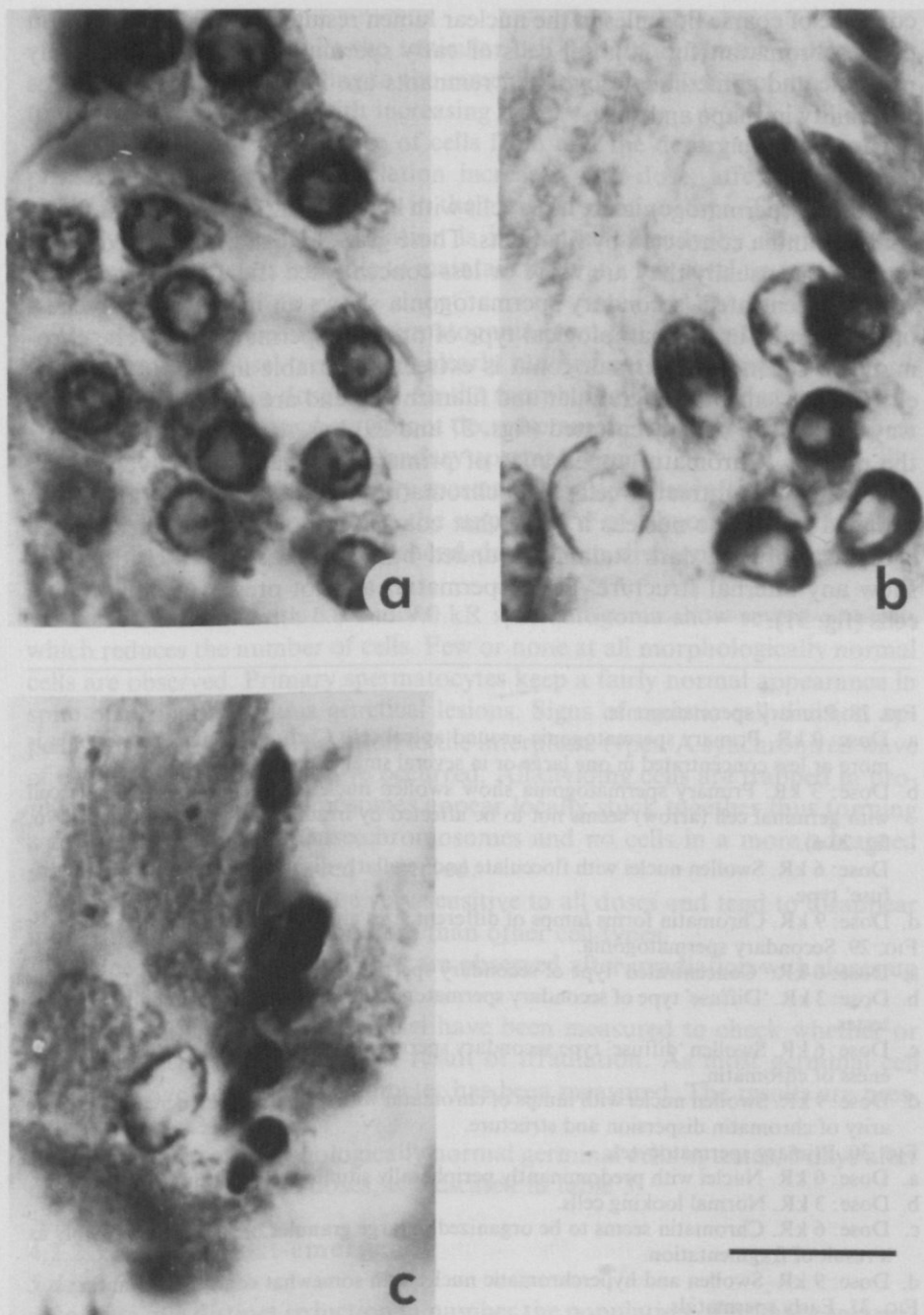
FIG. 30

Fig. 31

currence of coarse floccules in the nuclear lumen results in a regular dispersion of the chromatin (fig. 30). All cells of early spermatids are abnormal. Only pycnotic and sometimes vacuolated remnants are found which show a large variability in shape and size.

0 days, 9.0 kR

Primary spermatogonia are large cells with light nuclei. The chromatin shows coarse granula connected by filaments. These granules can be dispersed in the nucleus but usually they are more or less concentrated (fig. 27). Comparison with 'concentrated' secondary spermatogonia shows an increased coarseness of the granula in this pathological type of primary spermatogonia. The chromatin of secondary spermatogonia is extremely variable in appearance. The chromatin elements are granular and filamentous and are dispersed in various ways. They can be concentrated (figs. 27 and 29) but also evenly dispersed in the nucleus. Chromatin appearance of primary spermatocytes is very much alike as in the untreated cells. The chromatin granules are larger and more rounded giving the nucleus a somewhat coarse, granular appearance. In the cytoplasm often a dark staining, rounded body is observed which does not show any internal structure. Early spermatids are not present as identifiable cells (fig. 31).

FIG. 28. Primary spermatogonia.
a. Dose: 0 kR. Primary spermatogonia around apical cell. Chromatin in spermatogonia is more or less concentrated in one large or in several smaller concretions.
b. Dose: 3 kR. Primary spermatogonia show swollen nuclei. Association of somatic cell with germinal cell (arrow) seems not to be affected by irradiation (cf. Theunissen, 1976, fig. 20 a).
   Dose: 6 kR. Swollen nuclei with flocculate and regularly dispersed chromatin of the 'diffuse' type.
d. Dose: 9 kR. Chromatin forms lumps of different sizes and it loses its structure.
FIG. 29. Secondary spermatogonia.
a. Dose: 0 kR. 'Concentrated' type of secondary spermatogonia.
b. Dose: 3 kR. 'Diffuse' type of secondary spermatogonia, which is never found in untreated testes.
c. Dose: 6 kR. Swollen 'diffuse' type secondary spermatogonia showing an increased coarseness of chromatin.
d. Dose: 9 kR. Swollen nuclei with lumps of chromatin which vary in size. Increased irregularity of chromatin dispersion and structure.
FIG. 30. Primary spermatocytes.
a. Dose: 0 kR. Nuclei with predominantly peripherally situated chromatin.
b. Dose: 3 kR. Normal looking cells.
c. Dose: 6 kR. Chromatin seems to be organized in large granules or filaments, possibly as a result of fragmentation.
d. Dose: 9 kR. Swollen and hyperchromatic nuclei with somewhat coarser chromatin.
FIG. 31. Early spermatids.
a. Dose: 0 kR. Early spermatids in cyst. Top: some secondary spermatocytes (arrows).
b. Dose: 3 kR. Distorted, swollen and partly chromatolytic nuclei of early spermatids demonstrating 'ghost' formation.
c. Dose: 6 kR. Pycnotic nuclei of early spermatids and one 'ghost'.

Summary 0 days·

In newly emerged adults the testicular cell populations react 3 days after irradiation roughly according to the same pattern. The severity of most symptoms generally increases with increasing dose.

The degree of disappearance of cells from and the desorganization of the primary spermatogonial population increases with dose, affecting progressively also the other populations in their spatial relationships. The size of the primary spermatogonial population is influenced already by relatively low doses but nevertheless it is able to maintain itself at a low level up to the sterilising dose.

The shift of the secondary spermatogonia from the 'concentrated' types to the abnormal 'diffuse' type is very clearly running parallel to increasing dose, thus demonstrating a dose effect on the morphology of secondary spermatogonia. The absolute numbers do not decrease much, contrary to the numbers of morphologically normal secondary spermatogonia.

The presence of normal primary spermatocytes decreases after doses of 2 kR and more, concomitantly with the appearance of abnormal and blocked meiotic divisions, following a general decrease in division activity after administering 1.5 kR.

After irradiation with 6.0 and 9.0 kR spermatogonia show severe cytolysis which reduces the number of cells. Few or none at all morphologically normal cells are observed. Primary spermatocytes keep a fairly normal appearance in spite of probably serious genetical lesions. Signs of meiotic cell division appear the third day p.i. in addition to the interphase types. A synchronized wave of cell divisions seems to have occurred. All dividing cells are trapped in prophase because their chromosomes appear locally stuck together thus forming a network of long prophase chromosomes and no cells in a more advanced stage of division have been observed.

The spermatids seem to be very sensitive to all doses and tend to disappear more quickly with increasing dose than other cell types.

No reactions of the sperm cells are observed after irradiation with doses up till 9.0 kR.

The sizes of germinal cell nuclei have been measured to check whether or not large differences occur as a result of irradiation. As most germinal cell nuclei are ovoid the largest diameter has been measured. The results are presented in table 2.

The presence of morphologically normal germinal cells in testes, 3 days after irradiation with various doses, is indicated in table 3.

4.2.2.3. 5 days post-emergence
5 days, 0.5 kR

In spite of a distinct reduction in number the population of primary spermatogonia has maintained itself. It shows few changes or none at all, except a mild acute dysplasia causing only some displacement of the population. The belt of apical cavities, containing many cells in chromatokinetic degeneration,

TABLE 2. Largest nuclear diameters of male germinal cell types 3 days following irradiation with various doses of X-rays.

cell type	dose[1]			
	0	3.0	6.0	9.0
p.spg:	10.7 ± 0.98[2]	11.3 ± 1.18	11.4 ± 1.52	13.2 ± 1.31
s.spg:	9.0 ± 1.18	7.4 ± 1.10	8.5 ± 1.04	10.0 ± 1.56
p.spc:	9.0 ± 1.03	6.7 ± 0.76	8.0 ± 0.89	9.4 ± 1.47
s.spc:	5.0 ± 0.53	—	—	—
e.spt:	4.0 ± 0.61	2.8 ± 0.59	3.0 ± 0.75	—
i.spt:	3.3 ± 0.45	—	—	—

[1] dose in kR
[2] diameter in μ and n = 20 for each determination

has broadened and is often connected with the central cavity. The populations of 'concentrated' secondary spermatogonia and primary spermatocytes are drastically reduced. Normal appearing division activity has greatly diminished. Most spermatids show chromatokinesis, hyperchromatosis, ghost formation and pycnosis. Relatively few show a normal appearance, although they occasionally are present in large groups. Many ghosts are observed in and around the central cavity.

5 days, 1.0 kR

The populations of primary and secondary spermatogonia and primary spermatocytes have been reduced considerably when compared to the situation 5 days earlier. In secondary spermatogonia the 'diffuse' type is only sometimes present. Mitotic divisions are absent and normal meiotic ones are very scarce, if present at all. Most cells in meiotic division show clumped metaphases. The number of normal looking spermatids has been greatly reduced due to the pathological phenomena already mentioned. In general, the coordination between the various cell types has decreased distinctly, evident by the chaos in the testis, owing to a general acute dysplasia and the strong reduction of premeiotic cell populations. The shape and size of the organ itself have become irregular and reduced in many cases.

5 days, 1.5 kR

In comparison to 1.0 kR irradiated testes the changes are small, except a still stronger reduction of the normal populations of secondary spermatogonia and primary spermatocytes. The 'diffuse' secondary spermatogonia are the dominant type now. No normal divisions have been observed. In the apical cavities many cells in chromatokinetic degeneration are found. Very few normally appearing spermatids are found. Ghosts and chromatokinetic spermatids are abundantly present.

5 days, 2.0 kR

The number of primary spermatogonia decreases relative to less heavily irradiated testes. 'Concentrated' secondary spermatogonia are absent, leaving 'diffuse' ones in relatively large numbers. All primary spermatocytes are morphologically similar to 'diffuse' secondary spermatogonia as has been described earlier. The apparently resumed division activity results in abnormal divisions. Very small numbers of spermatids are still observed.

5 days, 3.0 kR

When compared to the situation 5 days earlier, the number of normal primary spermatogonia has decreased but a small population still exists. The secondary spermatogonia are all of the 'diffuse' type and normal looking primary spermatocytes are lacking. Blocked meiotic divisions are found. No normal spermatids can be detected.

Summary 5 days

Comparing the irradiated specimens of this age, the relatively good preservation of the primary spermatogonia should be noted. Although decreased in numbers, primary spermatogonia of a normal morphological appearance can still be found in each group.

The shift of the secondary spermatogonia from 'concentrated' types to the pathological 'diffuse' type is completed after irradiation with 2 kR. Normal division activity largely ceases after 500 R irradiation, abnormal divisions result from attempts to divide. Normal appearing early and transformation spermatids remain at very low numbers, intermediate spermatids disappear at increasing doses (table 3).

4.2.2.4. 10 days post-emergence

10 days, 0.5 kR

In particular the premeiotic cell types show a distinct repopulation of the apical part of the testis (fig. 32a), which sometimes seems to exert its influence up to the transformation spermatids. In general, however, at this age the normal looking spermatids still decrease in numbers. The extent of the repopulation process is different in each testis, but each one shows a sharp increase in numbers of primary spermatogonia and in division activity. Sometimes even meiotic divisions are observed again. As the process of repopulation will be described and discussed more detailed later, its occurrence is only established here.

10 days, 1.0 kR

Repopulation on a more limited scale and concerning only primary spermatogonia is observed in some testes, in others there is no such activity at all. The cells are 'concentrated' types of primary spermatogonia which are present in variable numbers. Blocked meiotic divisions are found but no normal ones.

10 days, 1.5 kR

Repopulation is totally absent. Only a very small number of 'diffuse' primary spermatogonia is still found in some testes, as well as tiny populations of 'diffuse' secondary spermatogonia. All other cell types except sperm cells are absent or represented by low numbers of pathological forms.

10 days, 2.0 kR

No repopulation is observed. The premeiotic cell types have disappeared, except some 'diffuse' primary spermatogonia. Even pathological types are lacking. Postmeiotic cell types are presented by pathological forms, except sperm cells.

10 days, 3.0 kR

Except sperm cells all morphologically normal cell types have disappeared.

Summary 10 days

In the lower dose groups this stage is characterized by the beginning of a repopulation of testicular cell populations. At 1.5 kR and higher doses the steady degeneration and depletion of all cell types continues, except for the sperm cells (table 3). The pathological symptoms are essentially the same as those which have been mentioned earlier in this paragraph. Only the degree of severity and the total effect on the whole of testicular cell populations is increasingly serious. All testes have a thickened, pigmented sheath, an elongated shape with an irregular, often creased apical and a widened more regular basal part. The latter is usually partly filled with a rather homogeneous mass of sperm cells, pathological forms of spermatids, cytolytic cells, chromatokinetic and swollen nuclei, cell debris etc.

4.2.2.5. 15 days post-emergence

15 days, 0.5 kR

With a few exceptions the repopulation of the testes continues, causing an impression of restored late pupal or local young adult activity. Both pre- and postmeiotic cell types are involved and often present in numbers which exceed by far those in untreated testes of the same age. The appearance of these cells is typical (fig. 32b), i.e. conform to the described cell types in young adult testes, which is not always the case in 15 days old unirradiated testes. The progress and speed of the repopulation process differ in each testis. The numbers of primary and secondary spermatogonia are large, those of primary spermatocytes and other cell types are smaller but nearly always much more numerous when compared to the unirradiated population of the same age. The number of newly formed spermatids is low and does not contribute to sperm formation as far as can be established. Only a slight division activity can be observed. Meiotic divisions are rare. Among the new cell populations, groups or single chromatokinetic cells are seen.

15 days, 1.0 kR

The repopulation of the apical part of the testis is virtually limited to the primary and secondary spermatogonia. Most testes show repopulation and the number of primary spermatogonia, usually 'concentrated' types with a large variability in appearance and size, exceeds that in untreated testes of this age. They are often mixed with 'concentrated' secondary spermatogonia which are much less numerous or even absent. In the spermatogonial populations mitotic prophases have been observed, no blocked metaphases. Whether or not mitotic or meiotic divisions were successful could not be established, with the exception of spermatogonia and sperm cells, other cell types are nearly extinct.

15 days, 1.5 kR, 2.0 kR and 3.0 kR

No repopulation at all. Except sperm cells no germinal cell types are present, not even degenerating ones. The apical cell fills the shrunken space in the apical part of the testis.

Summary 15 days

Conspicuous contrasts are observed in testes of this age-group. The repopulation phenomena, especially after 500 R irradiation result in a large restorative activity of mainly premeiotic cell populations. These populations show degeneration and premature ageing after irradiation with doses of 1.5 kR and more. The heavily pigmented testes do not contain germinal cells anymore except sperm cells (table 3). The only recognizable structure in the apical part is the apical cell which fills the narrowed interior and does not show any response to irradiation. The wide basal part contains sperm cells, a large variety of undefinable cell debris, and somatic cell types like central cavity cells.

4.2.2.6. 20 days post-emergence

20 days, 0.5 kR

Repopulation seems to have ceased rather abruptly. The apical region contains many shrunken germinal cells in hyperchromatic and chromatokinetic degeneration. These pathological cells show a very uniform appearance. Hence, they can not be identified as being former primary or secondary spermatogonia or primary spermatocytes. Large groups of pycnotic early spermatids are occasionally found basally of the degenerating mass of cells and apical in the central cavity. Sperm cells are present.

20 days, 1.0 kR

In the apical part of the testes obviously repopulation had taken place. Remnants of the germinal cell populations are found as undefinable, pycnotic, shrunken cells. Rarely some pathological spermatids can still be recognized as such.

TABLE 3. Effects of various doses of hard X-rays on germinal cell populations in the testis at different age

Dose²	0¹					0.5					1.0				
Age	0	5	10	15	20	0	5	10	15	20	0	5	10	15	20
Celltype															
p.spg	+	+	+	+	+	+	+	+	+ +	–	+ /*³	*	+ / –	+ + / +	–
s.spg	+	+	+	+	+	+	*	+ /*	+ + /+	–	+	*	*/ –	+ / –	–
p.spc	+	+	+	+	+	+	*	*	+ + /+	–	·+	*	–	–	–
s.spc	+	+	–	–	–	+	*/ –		+ + / –		+ / –	*/ –			
e.spt	+	+	+	+	+	+ /*	+ /*	*/ –	+ + / –	–	*	*	–	–	–
i.spt	+	+	+	+	+	+ /*	+ /*	–	+ + / –	–	*/ –	*/ –	–	–	–
t.spt	+	+	+	+	+	*	*	*/ –	+ / –	–	*/ –	*	–	–	–
sperm	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
M-div.	+	+	–	–	–	+	*/ –				+	0⁴			

¹ present by definition
² dose in kR
³ the variation in numbers of cells/cell type occurring within a series of testes is denoted as a combination of two signs if the indicated situation is observed in 30–50% of the testes.
⁴ a 0 indicates that blocked divisions have been observed
N.B. As secondary spermatocytes and meiotic divisions did hardly occur in 10 days old and older unirradiated testes, they have not been registered in irradiated ones.

20 days, 1.5 kR, 2.0 kR and 3.0 kR
Except for sperm cells the thick walled, heavily pigmented testes are empty.

Summary 20 days
The testes show a characteristic picture of old age. There is still sperm available but the testes of all dose groups are otherwise empty, except for somatic cell types, including the apical cell, and cell debris (table 3).

Data on the presence of morphologically normal cells per cell type, dose and age are summarized in table 3.

4.2.3. General pathological reactions of the testis

Following irradiation with relatively low doses (< 3.0 kR) the changes in external appearance of the testis are limited. In general the shape of mainly the apical part of the irradiated testis becomes irregular and shows progressing deformation with increasing age, contrary to the untreated one. An earlier and more intense pigmentation and thickening of the testicular sheath is also a morphological expression of the irradiation effect. During these processes the apical part becomes narrower and variable in diameter much earlier when compared to unirradiated testes.

In adult testes the storage function of the basal part, in particular the central cavity is becoming more important and larger with increasing age. Concomitantly with the premature disappearance of the germinal cell types from the apical part in irradiated testes and the accompanying dystrophy of this part

	1.5					2.0					3.0					6.0	9.0
0	5	10	15	20	0	5	10	15	20	0	5	10	15	20	0	0
+/*	*	*/-	-	-	*	*	*/-	-	-	+/*	*	-	-	-	*	-
+	*/-	-	-	-	+/*	-	-	-	-	*/-	-	-	-	-	*/-	-
+	*	-	-	-	+/*	-	-	-	-	*	-	-	-	-	*	*
-	*/-				*/-	-				-					-	-
*/-	*/-	-	-	-	*/-	*/-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
*/-	*/-	-	-	-	-	*/-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
*/-	0				0	0				0	0				0	0

+   'morphologically normal' cells present in about 25–100% of the usual number in untreated testes.

−   'morphologically normal' cells absent.

*   'morphologically normal' cells present in small numbers up till about 25% of the usual number in untreated testes.

+ +   'morphologically normal' cells present in numbers exceeding the usual number in untreated testes at the same stage of development.

the enlargement of the basal part is taking place earlier, contributing to the quicker ageing of these testes.

At an age of 20 days all germinal cells have disappeared from the apical part of irradiated testes, leaving a nearly empty space containing the apical cell and some somatic cells. Depending on the dose this situation may be reached at a younger age, thus causing the 'ageing effect' which is a generally recognized symptom of irradiation. In fact the ageing effect is the final result of a large number of reactions and interactions within and between germinal and probably also somatic cell populations. As the premature ageing of the testis is an end point of a development, dose effects are more exactly and reliably evaluated by examination of the preceding events which are expressed to some extent by the various cell populations.

In very exceptional cases untreated testes may be found which show some degree of desorganization and disruption of the spatial arrangement between populations of different germinal cell types or between cysts of the same population. Rarely a cell population is hypoplastic. The main part of its zone consists then of more or less empty cavities. Dysplasia is also rarely found in normal testes. But, in fact nearly all pathological phenomena mentioned can be observed when a sufficiently large number of testes is examined.

A distinct and general desorganization of the zone of primary spermatogonia is seen already 3 days after treatment with 0.5 kR. The main causes of desorganization from a histopathological view are 1. acute dysplastic changes: a difficult to describe but profound change in the architecture of testicular regions, displacement of cell populations and aberrant cellular appearance,

leading to 2. cell depletion by cytolysis. Even after irradiation with relatively low doses these symptoms seem to appear very quickly expressing the activity of processes brought about by irradiation. The frequency and seriousness of these symptoms increase gradually with increasing dose. Usually the desorganization begins in the primary spermatogonial population. Among these cells dysplasia affects the individual cells, additionally distorting their spatial relationship, accompanied by quick cytolysis which results in the formation of empty cavities in this region of formerly densely packed cells. Slightly later the secondary spermatogonia and primary spermatocytes react by degeneration processes and chromatin transformations, whether or not accompanied by dysplastic effects, which ultimately lead to cytolysis. Dysplastic changes also cause a migration of cysts of the premeiotic cell populations into the central cavity and postmeiotic populations can penetrate into the apical regions of the testis. So, sperm cells may be found amidst of primary spermatogonia where normally the apical cell is located. General cell depletion by cytolysis in due course simplifies this chaotic situation, leaving sperm cells and cell debris. In the testis of *H. antiqua* with its indistinct zonation of the various cell types, the general desorganization increases quickly with progressing age after irradiation. Already in 5 days old flies tremendously chaotic situations can be observed. It seems that the mutual cohesion between cysts is distorted seriously by the treatment, which might indicate an irradiation effect on the cyst epithelia or their outer membranes.

Chromatokinetic cells are regularly observed in low numbers in all cell populations of the untreated gonads. They probably are cells which for some reason fail to develop normally and start to degenerate. They appear as single cells but also in entire cysts. In irradiated testes the numbers of single chromatokinetic cells increase sharply, contrary to those in cysts. They are found first among the primary spermatogonia and in the apical cavities. Somewhat later other cell types are also found to be chromatokinetic.

Ghosts are found at emergence after irradiation with 0.5 kR in cysts of spermatids. Ghost formation takes place later in cysts with secondary and primary spermatocytes and rarely in secondary spermatogonia. They all disappear at about 10 days after emergence due to further lysis. The presence of ghosts is one of the first symptoms in the most sensitive cell types and they are possibly specific for radiation damage because to the authors knowledge they are nowhere mentioned in the literature concerning insect histopathology.

Also after irradiation with the relatively high doses of 6.0 and 9.0 kR desorganisation of the germinal cell populations is found. Another phenomenon is the quick cytolysis which takes place at a large scale, depleting seriously the not recovering germinal cell populations. The contents of the testis change both in location and in quantity. This results in a tendency to acquire an irregular shape, which is enhanced by gradually developing dystrophy and ageing effects. External ageing effects are premature shrinkage and pigmentation of the testis. Internally there is a quick shift in relative numbers of pre- and postmeiotic cell types, especially sperm cells. An accompanying redistribution

of the available internal space is observed increasing proportionally the basal part at the expense of the germinal cell types in the more apical part of the testis. During first 3 days p.i. the external shape changes little. At 6 days p.i. both shape and size show irregular and dystrophic changes. The 9 kR irradiated testes seem to inflate a little.

4.2.4. *Repopulation*
Recovery from damage caused by ionizing radiation on the level of the cell population is revealed by repopulation of the apical part of the testis. The extent and speed of the repopulation process, which is different in each comparable testis, is largely dependent on the dose of radiation received. Repopulation is found after irradiation with 0.5 kR and 1.0 kR hard X-rays. Differences which are due to dose effects warrant a separate description.

Repopulation following irradiation with 0.5 kR starts between 5 and 10 days of adult age and concerns a mitotic multiplication of primary spermatogonia. At 10 days a large number of primary spermatogonia, both 'diffuse' and 'concentrated' types have been formed (fig. 32a). Apically in the testis a new population of cells is clearly multiplying causing an increased cell density. At this time a few new secondary spermatogonia are already present. Between 10 and 15 days the rate of cellular development seems to increase and at 15 days relatively large populations of new premeiotic cells but also spermatids are formed (fig. 32b). In some testes new transformation spermatids are found, in others not even meiotic divisions occur at this time. The speed of development of the most advanced cells from secondary spermatogonia to transformation spermatids in 5 days conforms to the data in THEUNISSEN 1976, table 9, indicating that irradiation with 0.5 kR does not necessarily impair a normal rate of development once recovery takes place. The divergence in development, however, shows this dose to be near or at the maximum limit, apart from the sudden degeneration of all newly formed cells between 15 and 20 days. When present, meiotic divisions seem to proceed normally resulting in morphologically normal postmeiotic cell types. Some cells or groups of cells degenerate but the majority is typical. A conspicuous difference with the untreated young adult testis is an inferior and haphazard spatial arrangement of the cell populations due to a badly functioning organization of the newly formed primary spermatogonia in a coherent population. In the recovering testis new cells are formed but no new cell populations. The area where the entire repopulation takes place consists at most of the narrowed apical half of the testis. In that restricted space the consecutive cell types multiply and differentiate and are, often very irregularly, arranged against and around a newly shaped local central cavity. This cavity seems to take a shape and has a relative size in accordance with that in a normal late pupal testis (fig. 32b). The central cavity develops because of the normal tendency of cyst of spermatocytes and spermatids to prefer a peripheral location in the testis.

Repopulation after irradiation with 1.0 kR also starts between 5 and 10 days but remains restricted to the primary spermatogonia. Only about 50% of the
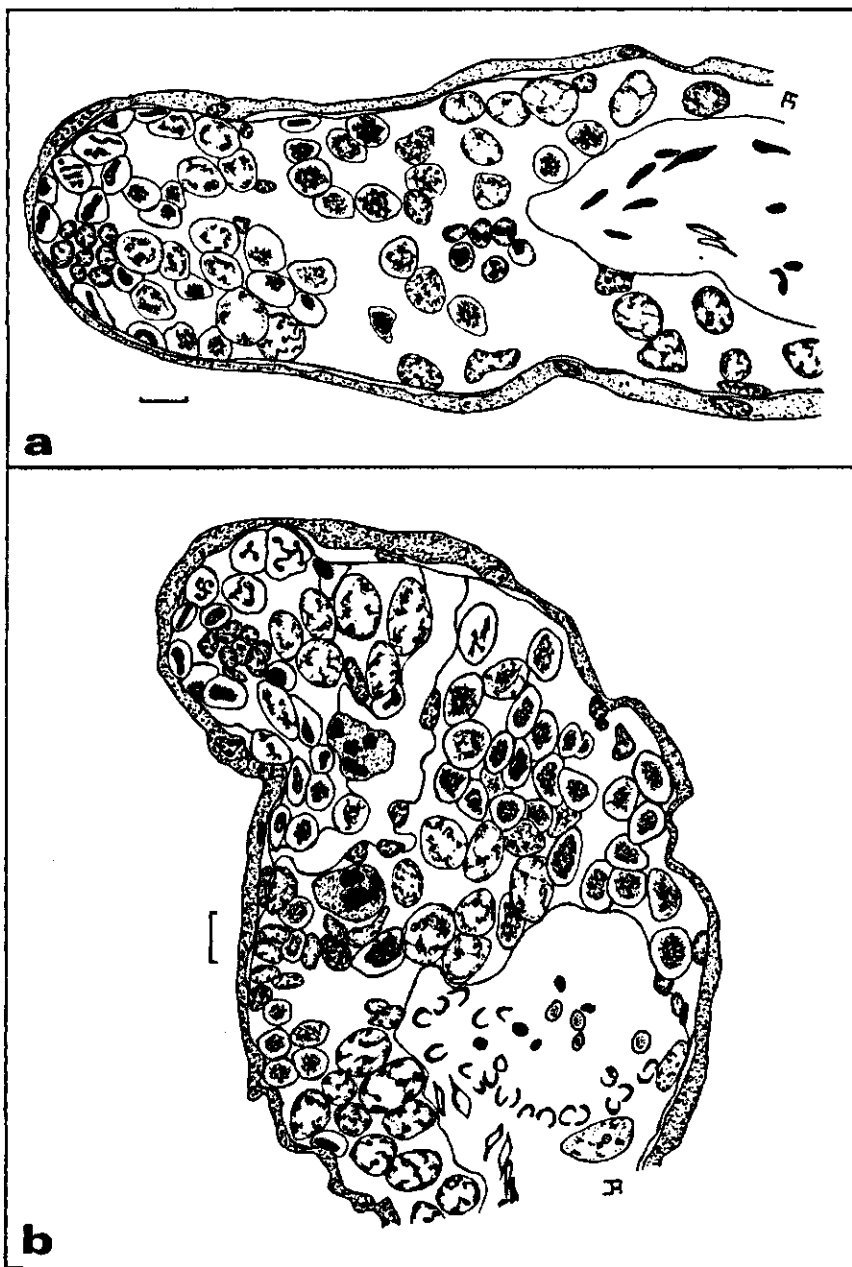
FIG. 32. Drawings of apical part of testes showing repopulation.

a. 10 days old fly, 0.5 kR. 'Concentrated' primary spermatogonia and cells in mitotic division are present.

b. 15 days old fly, 0.5kR. In the apical part a series of new cell types is seen around a new central cavity. Many 'concentrated' primary and secondary spermatogonia, primary spermatocytes and new spermatids are present. Mitotic divisions are also found.
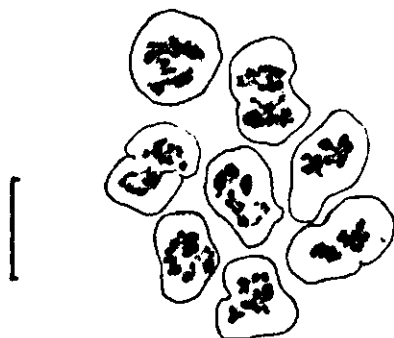
FIG. 33. Nuclei of newly formed primary spermatogonia in a repopulating testis, following irradiation with 1.0 kR of hard X-rays.

testes show signs of repopulation at 10 days, increasing to 80% 5 days later. This repopulation process is of a limited extent, qualitatively because only primary spermatogonia and at 15 days secondary spermatogonia are involved and quantitatively because relatively few cells are reproduced. In spite of their low number they equal or exceed that of spermatogonia in untreated testes of 15 days old. In testes not showing repopulation secondary spermatogonia are absent. The newly formed primary spermatogonia are somewhat atypical (fig. 33). Their nuclei are irregularly ovoid and of a small but still normal size. The chromatin is organized in granules of varying size, often it is much more dense and structureless than in normal 'concentrated' primary spermatogonia. These dense chromatin concretions can be concentrated in one or a few masses. Some nuclei show a distinct split of the chromatin material into two groups. These features suggest that these primary spermatogonia are derived from the normal 'concentrated' types of primary spermatogonia, differing in the usual density of the chromatin. Both at 10 and 15 days meiotic activity is absent. Mitotic divisions were abnormal in all cases which could be examined: no single normal mitotic division was found.

4.2.5. Discussion

Criteria used to assess radiation effects are usually of genetical or physiological origin, for instance the rate of induction of mutations in certain cell types or the lifespan of treated insects. The criteria used here are based on the reaction of the cells to irradiation as it is expressed in their cellular morphology. In this point of view there is the underlying presupposition that the morphology of the cell, including the nuclear chromatin pattern, is related to and reflects the physiological state of the cell and its changes. The criteria of histopathological origin provide a picture of the degree in which the structural integrity of the cell as it is expressed in the normal cell morphology is preserved. The presence of cells within the range of morphological types which are found in untreated, comparable testes determines the assessment of the reaction of the

testis and its germinal cell types to the treatment at given conditions of dose, age and post-irradiation period. The varied reactions of the single cell types contribute to a pattern which is characteristic for the damage expressed under the prevailing conditions (table 3). In this way a basis for comparison of various situations is created. Using this possibility *radiosensitivity* could be defined as: the degree in which the normal morphological structural integrity of the cell is lost after irradiation. Differences in radiosensitivity between testicular germinal cell types can be established at varying doses, ages and time after irradiation. In addition to this qualitative approach of assessment of radio-sensitivity, quantitative aspects have been introduced by estimating roughly the size of the cell populations concerned when compared to the controls. They indicate major changes in sizes of cell populations. These data when added to the purely qualitative ones permit a further refinement of the evaluation of radiation damage and its histopathological consequences. Based upon these combined data a ranking order of radiosensitivity of the cell types under various conditions can be established (table 4). This order is derived from data summarized in table 3. The cell type changing its morphology first is consider-ed to be more radiosensitive than that changing later after irradiation and is consequently given number 1 in the rank. When cell types disappear at the same time and rate they share a number. Table 4 shows a general pattern of com-parative radiosensitivity of germinal cell types in spite of small variations per dose and age group. Secondary spermatocytes and sperm cells are excluded from this system because the presence of secondary spermatocytes is not so much a standard of the reaction of this cell type to irradiation but more one of meiotic activity. Sperm cells do not change morphologically at the radiation doses used.

The data of the tables 3 and 4 indicate a high relative radiosensitivity of spermatids. Especially intermediate spermatids are quickly eliminated after irradiation with 1.0 kR or more. At lower doses they seem to be more resistant than transformation spermatids which are generally slightly more sensitive than early spermatids. The radiosensitivity of these cell types does not differ much when compared to other cell types. Few differences in radiosensitivity are observed between secondary spermatogonia and primary spermatocytes probably due to their close relationship and the same type of reaction to ir-radiation.

In spite of their quick and relatively violent reaction even after irradiation with low doses the primary spermatogonia as a cell population are found to be the most radioresistant cell type. They show recovery after irradiation with low doses and an intense activity while repopulating the apical testicular region following 0.5 kR irradiation. They are able to maintain themselves as a low but morphologically normal population till all other cell types degenerate and disappear, except sperm cells.

Thus, an increasing radiosensitivity is observed in germinal cells in this order: primary spermatogonia – secondary spermatogonia/primary sperma-tocytes – spermatids, a sequence which requires some attention as it shows an

TABLE 4. Ranking order of radiosensitivity of testicular germinal cell types of *H. antiqua*.

dose[1]	0.5					1.0					1.5			
age[2]	0	5	10	15	av[3]	0	5	10	15	av	0	5	10	av
celltype														
p.spg	3	4	5	4	6	4	2	3	3	6	3	3	2	4
s.spg	3	3	4	3	5	3	2	2	2	5	4	2	1	3
p.spc	3	3	3	3	4	3	2	1	1	4	4	3	1	4
e.spt	2	2	2	2	3	2	2	1	1	3	2	2	1	2
i.spt	2	2	1	2	2	1	1	1	1	1	1	1	1	1
t.spt	1	1	2	1	1	1	2	1	1	2	2	2	1	2

dose	2.0				3.0				6.0	9.0
age	0	5	10	av	0	5	10	av	0	0
celltype										
p.spg	4	3	2	4	4	2	1	4	3	1
s.spg	3	1	1	3	2	1	1	2	2	1
p.spc	3	1	1	3	3	1	1	3	3	2
e.spt	2	2	1	3	1	1	1	1	1	1
i.spt	1	1	1	1	1	1	1	1	1	1
t.spt	1	2	1	2	1	1	1	1	1	1

[1] dose in kR
[2] age in days of adult life
[3] average ranking per dose group

dose	0.5	1.0	1.5	2.0	3.0	av[4]
celltype						
p.spg	6	6	4	4	4	5
s.spg	5	5	3	3	2	4
p.spc	4	4	4	3	3	4
e.spt	3	3	2	3	1	3
i.spt	2	1	1	1	1	1
t.spt	1	2	2	2	1	2

[4] average ranking per cell type

increased radiosensitivity of cells with increasing degree of differentiation and specialization. The primary spermatogonia are considered to represent the 'stem cell' type of the male germinal line. They are comparatively flexible in their developmental activity and little specialized in spite of the ultimate destination of a part of them to produce sperm cells. It is possible that damage and stress will be repaired and compensated in such a relatively flexible cell population easier with a maximum chance on success than in populations of more specialized cells. With increasing damage the possibilities of repair may be decreasing till their limit is reached and irreversible lesions lead to cell

death. Morphologically, pathological symptoms can reflect both reversible and irreversible damage. Reversible lesions result in due course in the preservation of the normal cell types, irreversible ones continue to interfere with the structural integrity of the cells. With increasing differentiation the flexibility of the response to radiation damage seems to be restricted, resulting in a lower limit of recovery. The point at which a lesion becomes irreversible may be reached earlier, thus increasing the radiosensitivity. This working hypothesis explains the observed rank in radiosensitivity which is based on histopathological criteria.

Radiosensitivity in genetical or physiological terms is not considered here but will be discussed briefly for purposes of comparison.

The obtained data on radiosensitivity seem to contradict the law of Bergonié and Tribondeau as it has been formulated by BACQ and ALEXANDER (1966): 'The sensitivity of cells to irradiation is in direct proportion to their reproductive capacity and inversely proportional to their degree of differentiation'. Evidently, the validity of this law is determined by the definition of the term 'sensitivity' as shown here. Different criteria used to establish this 'sensitivity' may lead to different results. Important and still unexplained exceptions to the law are mentioned by BACQ and ALEXANDER (1966) (page 249), which illustrate the presence of limitations to its universal applicability. Apart from the differential radiosensitivity which is influenced by the rate of cell division, metabolic activity is an important factor in the development and expression of injury symptoms in irradiated cells. Cells which are metabolically very active during or after irradiation are subjected earlier and possibly also heavier to the development of biochemical lesions, causing symptoms and eventually cell death, than inactive cells. This generally observed phenomenon bears on the limited energy reserves of the cell and is formulated in the statement of Vintemberger, as cited by BACQ and ALEXANDER (1966): 'The duration of survival of an irradiated cell is inversely proportional to its activity after irradiation'. Thus, death of spermatogonia may take place selectively according to their mitotic activity and physiological state. Another factor to be considered in this connection is the observation of PONTECORVO (1944) of the irradiation effects on *Pediculus corporis* leading to the conclusion that cells in cysts are protected from radiation damage by their action as a syncytium. This conclusion has been confirmed for *Drosophila melanogaster* by HANNAH-ALAVA (1964). The sheltering from germinal selection of the germinal cell types which develop in cysts may explain the difference in initial reaction to irradiation between primary and secondary spermatogonia. Secondary spermatogonia develop in cysts, primary spermatogonia do not. The primary spermatogonia are exposed to the radiation effects as single cells showing a variable state of physiological activity and are killed according to their degree of activity. The individual secondary spermatogonia may show a more uniform large physiological activity but radiation induced lesions seems to be compensated by their syncytial situation. Entire cysts of secondary spermatogonia degenerate. Necrosis of individual cells is rarely found in cysts. While the population of

primary spermatogonia is severely affected by irradiation initially, the remaining cells are able to survive as morphologically normal ones during a relatively long period which exceeds that of the pathological 'diffuse' type of secondary spermatogonia.

Regardless of the dose received, division activity returns between the second and third day p.i. in particular in secondary spermatogonia and primary spermatocytes. The synchronized appearance of this wave of dividing cells is caused by stagnation in the divisions somewhere in the prophase/prometaphase stage.

Irradiation does slightly influence average sizes of germinal cell nuclei. The primary spermatogonia show a consistent tendency to swell which is most pronounced at 9.0 kR. Other cell type nuclei decrease distinctly in diameter at the sterilizing dose but tend to increase again at higher doses (table 2).

The shift of 'concentrated' types towards the 'diffuse' type of secondary spermatogonia is also observed after irradiation with 6.0 kR showing some dose dependency. In spite of some swelling the 'diffuse' type is still recognized at 6 days p.i. pointing to its relative radioresistance which has been described earlier. The quick disappearance of morphologically normal cells, however, impairs a similar determination of relative radioresistance as has been used at low dose irradiated testes. The criterium of maintained cellular integrity as demonstrated by a normal morphology is useless after irradiation with relatively high doses.

In most cases the time required to show a reaction to irradiation is less than one day as far as individual germinal cells are concerned. The reaction of a major part of a cell population is usually somewhat retarded which is probably due to the variability of the radiation damage inflicted to each cell of the population. Most populations begin to show symptoms one day p.i. with the sterilizing dose. This 'symptomless' period obviously lasts considerably shorter at higher doses although useful criteria are lacking at the moment to determine the dose dependency of this period.

Besides a decreasing visible effect of irradiation per added unit of radiation, it is evident that the histopathological pattern of testicular germinal cell populations in the dose range of 3–9 kR is more determined by the length of the post-irradiation period than by the dose received. Dose effects are relatively small as compared to those which emerge from the duration of the post-irradiation period. A higher dose speeds up the development of the pathological symptoms probably ending in instant cell death at extremely high doses. The continuity of the pathological patterns at high doses of all cell types concerned is a confirmation of those which appear at low doses. The main difference between those of low and high doses is the speed at which they develop.

A good example of the different rate of development of pathological processes at different doses as reflected by symptoms is cytolysis. At low doses cytolysis usually seems to be an end of degeneration processes e.g. chromatokinetic or hyperchromatic degeneration. Pathological effects involving cytolysis are found in a wide variety of numerous intermediate pathological

forms. At relatively high doses (6–9 kR) cytolysis proceeds so quickly that only the very fast cell depletion within one or a few days indicates this process. The speed of the cytolytic process is probably determined by the severity and nature of the cellular lesion. At a higher dose the probability of receiving an acute lethal lesion will be proportionally larger. At or below the sterilizing dose cells can also be damaged severely, triggering a quick cytolytic process which explains the increasingly serious cell depletion of testicular cell populations after receiving 1.5, 2.0 and 3.0 kR. This partly explains the decreasing visible effects of increasing doses of radiation. A slow rate of degeneration, including cytolysis, is more spectacular in histopathological terms than an unobtrusive and quick disappearance of cells. At low doses a mixture of more or less damaged cells will show both quick and slow degeneration. At increasing doses the proportion of quickly degenerating cells will increase.

Morphologically the sperm cells are radioresistant even after receiving a dose of 9 kR, although they are sensitive in genetical terms. The morphological radioresistance of sperm cells can be explained to some extent by the physiological state of chromosomes in these cells relative to their own morphological development, owing to non-functioning of the genes in sperm cells (MULLER and SETTLES, 1927; LINDSLEY and GRELL, 1969). According to LINDSLEY and GRELL (1969) the development of spermatids into sperm cells in *Drosophila melanogaster* is determined by the diploid genome of the primary spermatocytes. The genes of the spermatid and sperm chromosomes do not exert any influence on the process of sperm differentiation. When this situation also applies in *H. antiqua* the morphological insensitivity of the sperm cells may be derived from a decreased physiological expression of gene lesions due to irradiation. Non-functioning in itself obviously does not protect cells from radiation damage as spermatids are the most radiosensitive and sperm cells are the least radiosensitive germinal cells. Probably other factors contribute to the differential inhibition of morphological expression of damage in spermatids and sperm cells. If genetic damage would be the only effect of the irradiation all spermatids would differentiate into sperm cells. Evidently other processes play also a role in the development of the spermatids after irradiation. Usually pupae are irradiated at the moment when the first sperm cells have just or not yet been formed. Irradiated testes contain quantities of sperm cells which are not visibly lower than in control testes. This means a continued differentiation of spermatids into sperm cells during the first days post-irradiation, which is confirmed by the events during early pathogenesis following 3 kR irradiation. During 2 days after irradiation the transformation spermatids do not show morphological changes, whereas after 3 days this population seems to be depleted considerably. The normal period of differentiation of transformation spermatids into sperm cells is about 2 days (THEUNISSEN, 1976, table 9). If early and intermediate spermatids are in these stages during irradiation they obviously do not contribute to post-irradiation sperm cell differentiation.

The differential radiosensitivity to X-rays of the various germinal cell types

as is found here is in close agreement with numerous data concerning the radio-sensitivity of testicular cell types of *Drosophila melanogaster* which have been established using genetical criteria (cf. AUERBACH, 1954; OSTER, 1958, 1959a: FAHMY and FAHMY, 1964; SOBELS, 1966), in spite of the different criteria used. Assessment of radiosensitivity is mainly based on criteria like induction of translocations, induced mutation frequency, especially of sex linked lethals and dominant lethals. Most authors agree that spermatogonia are least sensitive to the mutagenic action of X-rays, followed by spermatocytes, sperm cells and spermatids respectively, the latter being the most sensitive type.

Criteria of physiological origin are not available to determine the differential radiosensitivity of testicular germinal cell types. As they are used when irradiation effects are evaluated on specific functions such as the ability to fly or the physical condition of the animal as a whole expressed in life span for instance, they will not be discussed here.

4.3. HISTOPATHOLOGY OF IRRADIATED OVARIES

Irradiation effects on ovaries are characterized by two main features:
1. a retarded, inhibited or dystrophic ovary development which is determined by pathological egg chamber development.
2. a pattern of histopathological reactions which is dose dependent as to the severity of its separate components (fig. 34).

Regarding the first mentioned feature, a method to establish egg chamber development and to quantify differences between individuals has already been described (THEUNISSEN, 1973a, 1974, 1976). This method has been used here to assess egg chamber development of ovaries of various ages after irradiation with various doses of X-rays. The developmental stages have been determined according to the definition given earlier (THEUNISSEN, 1973a). As in irradiated egg chambers the variability in chromatin pattern between trophocyte nuclei increases with dose, slight adaptations of the notation (THEUNISSEN, 1974) were necessary. In egg chambers a large part of the nuclei may show S2 and another part S3 characteristics for instance. This abnormal situation was noted as S2/S3. Small minorities have been ignored in this sense. In some germaria an S1 egg chamber is formed, in others of the same ovary not. The notation is accordingly G/S1. The descriptions of chromatin appearance of trophocyte nuclei and accounts on egg chamber development must necessarily be very short and generalized.

The second mentioned feature is described in the first paragraph for newly emerged adults irradiated with 3 kR. The pattern of histopathological reactions is characterized as short as possible for each of the major components of the ovariole: first and second egg chamber, trophocytes, follicle epithelium and the germarium.

In the second paragraph the reaction of the ovary to irradiation with various doses is summarized, followed by a description of the pathogenesis of histopathological changes after irradiation of ovaries with higher doses (6-9 kR).

FIG. 34. Ovary of newly emerged adult following irradiation with 2 kR. Impression of the histopathological symptoms of the radiation syndrome, i.a. swollen oogonia, chromatokinetic degeneration, acute dysplasia and general dystrophy. Bar = 10 μ.

4.3.1. Description of change in germinal and somatic elements of the ovary

To assemble the overall picture of histopathological radiation effects on the ovary the reactions of the constituing parts will be briefly described as they are observed at emergence 3 days after 3 kR irradiation of the pupa (fig. 35).

germarium

At emergence the germarium already presents a varied pattern of pathological reactions. The total number of germinal cells has clearly decreased. Widespread cytolysis is observed, leaving cavities in the germarium which are empty or filled with cell debris. Cells seem to migrate into these cavities, which contain hyperchromatic and chromatokinetic cells. Chromatokinetic degeneration is very common, just as swelling of individual germinal cells. In untreated germaria the diameter of germinal cells is less than 10 μ. In irradiated ones they may swell to about 15 μ. Most germinal cells are 'diffuse' although occasionally a few 'concentrated' types may be encountered in the most apical part. The latter are not to be confused with cells showing loss of internal structure, terminating in pycnosis. In some germinal cell nuclei filamentous ele-
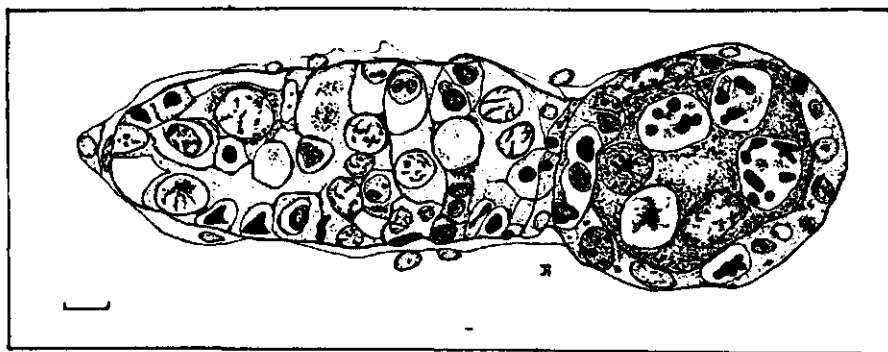
Fig. 35. Drawing of ovariole of newly emerged *H. antiqua* 3 days after irradiation with 3 kR hard X-rays. Note the desorganization and cell depletion in the germarium and the severe pathological reaction of the follicle epithelium. Bar = 10 μ.
Note: compare with fig. 75 in Theunissen (1976).

ments can be found in the chromatin, which perhaps indicates attempts to resume (endo)mitotic activity after the radiation induced inhibition.

The germarium as a whole distinctly shows a severe degree of desorganization and serious depletion of cells.

covering epithelium
The epithelium which covers the germarium is in many instances swollen, absent, vacuolated or showing various other pathological reactions.

trophocytes
The trophocytes in the most advanced (and single) egg chamber mostly show chromatin patterns which are characteristic of the S2 stage of development (cf. THEUNISSEN, 1976). In few ovaries this egg chamber is totally absent, in few others it might be advanced up to the S3 stage. The chromatin material stains darker and sometimes shows pycnosis (fig. 36). The cytoplasm is more basophil if compared to unirradiated trophocytes. It is often also more granular and/or somewhat flocculate, indicating loss of structure. The cells are sometimes deformed.

oocyte
The oocyte usually does not show any sign of abnormality.

follicle epithelium
The epithelium which surrounds the trophocytes and oocyte shows hardly a single cell with a normal appearance. Chromatokinetic degeneration is very common. The epithelium is irregular of shape, locally shrunken or swollen, containing chromatokinetic, pycnotic, lytic and a few hyperchromatic cells, presenting a striking example of advanced dysplasia. Locally the cubical epithelium shows a metaplastic change into squamous epithelium. The total pic-
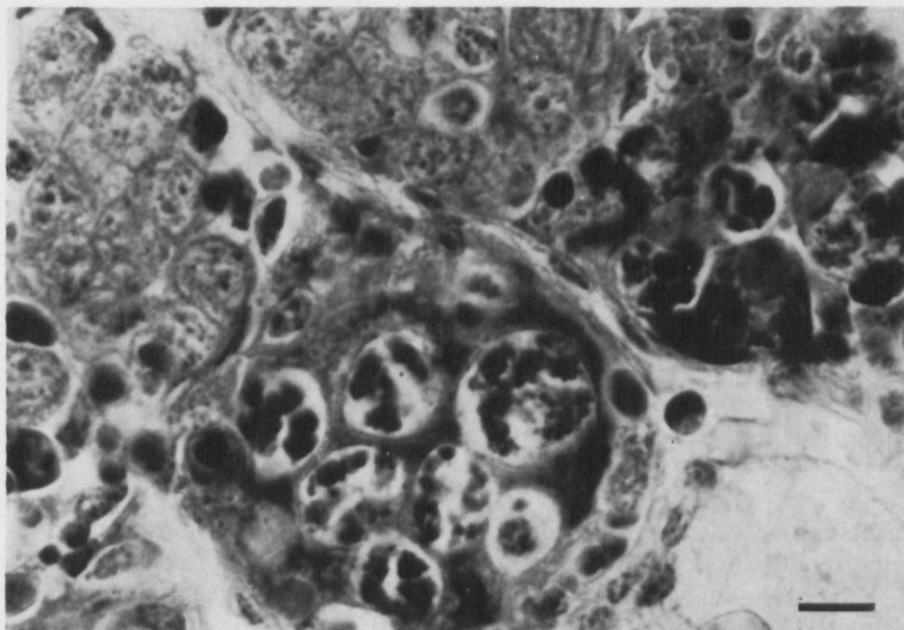
FIG. 36. S1/S2 egg chambers in ovary 3 days after irradiation with 3 kR. Local chromato-kinetic degeneration in germaria. Strong pathological reaction of follicle epithelium: swelling, pycnosis, chromatokinesis, acute metaplasia. Bar = 10 μ.

ture of the pathological reactions reflects a severe state of degeneration and malformation of the follicle epithelium.

egg chamber

The single egg chamber which is developing in each irradiated ovariole is often somewhat deformed by the combined irregularities in shape of the trophocytes and the follicle epithelium. Its average development can be determined by the mean of the egg chamber development in a number of comparable individuals, as has been discussed and applied earlier (THEUNISSEN, 1974). In this way the degree of egg chamber development of groups of females whether or not treated with various doses, at any adult age can be compared and assessed. Using this method at least one radiation effect can be determined quantitatively. In fact this radiation effect is a combined one because egg chamber development or its inhibition is the resultant of numerous, more or less interdepending processes.

Egg chamber development 3 days following irradiation with 3 kR is 1.9; in unirradiated females it is 3 for the most advanced (first) egg chamber (THEUNISSEN, 1974) and 4 when total egg chamber development is regarded (cf. page 75).

At this stage the ovary as a whole is severely dystrophic and degenerating.

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4.3.2. *Dose-effect relationship for pathological symptoms of irradiation*

In the next paragraphs the effects of irradiation with various doses on ovaries are described. Irradiation took place in the pupal stage. Summarized are the main characteristics of the resulting pathological changes in flies of several ages. In the first paragraph some physiological data on fecundity and fertility of the irradiated flies are mentioned.

4.3.2.1. Introduction

Ovaries from irradiated flies were investigated on egg chamber development and main pathological symptoms. Two experiments with low dose irradiation ($\leqslant 3.0$ kR) have been carried out. The fecundity and fertility of the flies have been recorded and relevant data are given as an average of 35 days after emergence. (table 5).

TABLE 5. Fecundity, fertility and egg weight of females irradiated with various doses[6].

dose[1]	fecundity[2]		fertility[3]		weight[4]
	I[5]	II[5]	I	II	II
0	100	100	81.4	87.8	35.1 ± 2.2
0.5	130.3	123.4	36.6	36.4	35.1 ± 1.4
1.0	58.4	39.3	9.3	7.6	38.5 ± 2.8
1.5	11.9	2.0	12.6	3.1	–
2.0	0.04	0.01	–	–	–
3.0	0	0	–	–	–

[1] dose in kR
[2] fecundity indexed, the production of the untreated control being 100
[3] fertility as percentage of hatched eggs
[4] weight of 500 eggs in mg and standard deviations
[5] I and II: the first and second experiment respectively
[6] average of the data recorded during the first 35 days of adult life

The egg production of 0.5 kR irradiated females is distinctly larger when compared to the controls. The reason for this reaction is obscure. The low figures for 2.0 kR irradiated females indicate very low numbers of produced eggs: 2 and 5 respectively. As the males also had been irradiated with the same dose the rate of egg sterility depends on both parents. The weight of the eggs shows a tendency to increase at moderate doses. Eggs of females irradiated with 1.5 kR, and in much lesser degree 1.0 kR, showed considerable variations in size, weight and shape. The number of 'brown eggs' i.e. late embryonic lethals (VAN HEEMERT, 1973a, 1974b) reached a relative maximum in the 1.5 kR group.

4.3.2.2. At emergence

0 days, 0.5 kR

The rate of egg chamber development is more variable as compared to untreated controls but stays within the limits set by the stage definitions. Subtle

differences in egg chamber development, therefore, can not be established quantitatively. As for the variations of the chromatin pattern in trophocyte nuclei within the most advanced, first egg chamber, they mainly show the chromatin transformations depicted in THEUNISSEN 1976, fig. 84. The S1 second egg chamber is formed, in spite of an occasional pathological reaction of the surrounding epithelium.
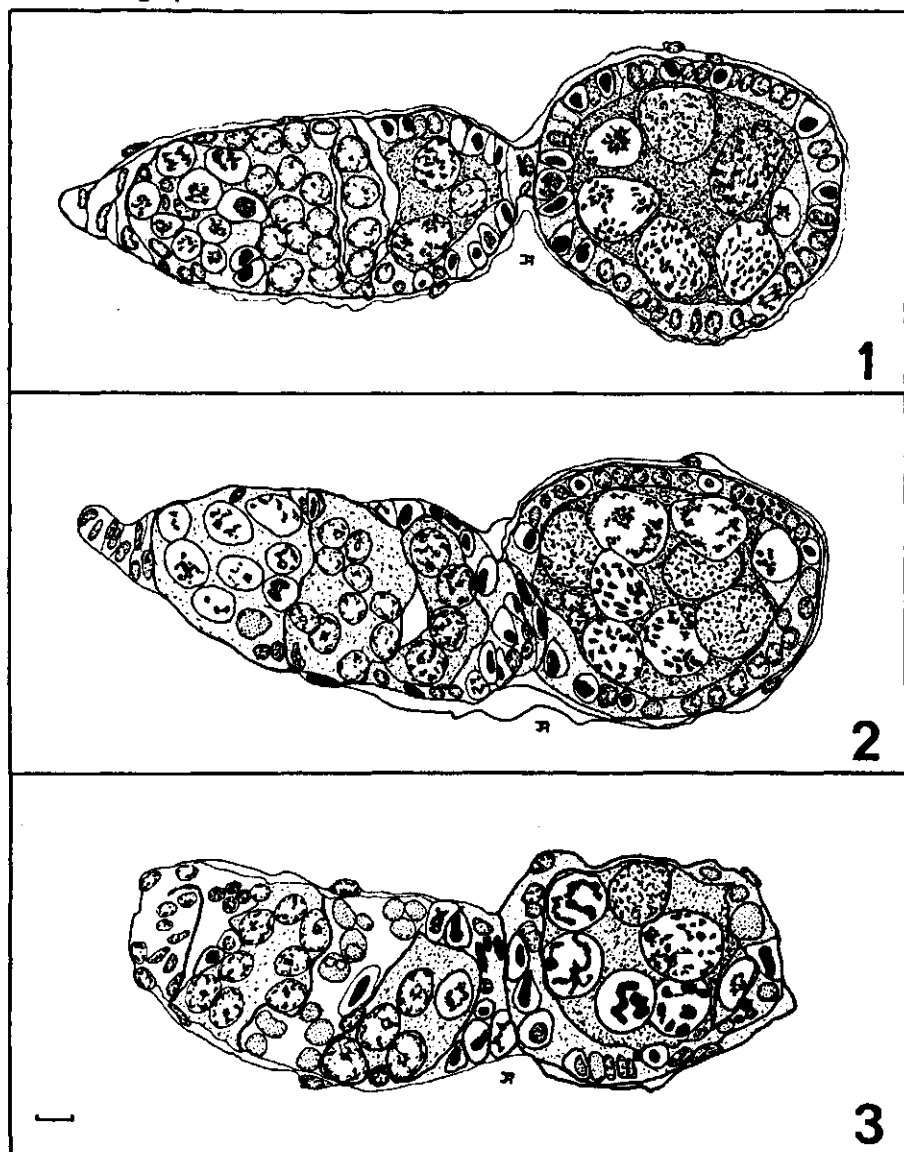

FIG. 37. Ovarioles of newly emerged flies 3 days after irradiation with 0 (1), 0.5 (2) and 1.0 (3) kR.
Compare with Theunissen 1976 (fig. 75) and fig. 40.

The most conspicuous change in the follicle epithelium is swelling of the cells, accompanied by chromatokinesis. Locally the cubical epithelium has been replaced by squamous epithelium representing acute metaplasia. Mutual orientation of the cells may be locally disturbed resulting in a stratified cubical epithelium. This phenomenon is a form of dysplasia. Around the S1 egg chamber the epithelium may show some swelling to cubical cells, especially at the border between the S1 chamber and germarium.

The germarium is clearly divided into two regions: the most basal part next to the S1 chamber and the apical part. The basal part contains 'diffuse' oogonia (fig. 37, 2), the apical part a large variety of 'concentrated' types. At close examination of a large number of comparable germaria, a whole range of cells is found which are intermediate forms between 'concentrated' types with strongly concentrated chromatin and 'diffuse' cells. This chromatin transformation (fig. 38) takes place by a decreasing concentration of the chromatin, resulting in an even dispersion of filamentous chromatin elements which produce the 'diffuse' type. The density of the chromatin elements decreases concomitantly. They are first observed as concretions, later on as floccules, granular filaments and finally filaments. This transformation is probably a radiation induced acceleration of the process which also gradually takes place in the unirradiated germarium (cf. THEUNISSEN, 1976). Germinal cells in which chromatin transformation occurs often show some swelling. Between these cells chromatokinetic cells are found, often in cavities. Dividing somatic cells are observed in the germarium and the follicle epithelium.
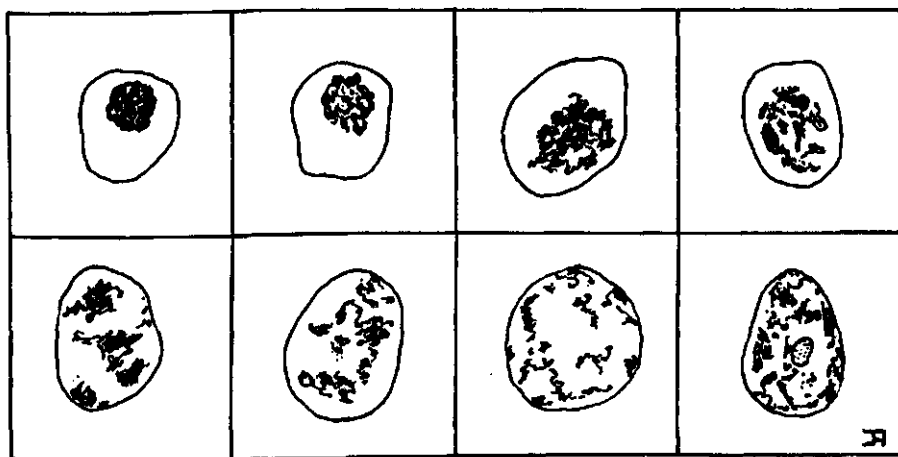

FIG. 38. Chromatin transformation in nuclei of oogonia in the germarium of newly emerged flies 3 days after irradiation with 0.5 kR hard X-rays. The typical 'concentrated' cell type (top, left) is converted into the 'diffuse' type (bottom, right). All these intermediate types can be found in the apical part of the germarium. Bar = 10 μ.

0 days, 1.0 kR

The variations in egg chamber development become larger. The trophocyte nuclei of the first egg chamber mainly show a chromatin pattern which is characterized by a regular dispersion of filamentous elements, but often also contain chromatin structures which are typical S2 (fig. 37, 3). The second egg chamber is usually developing but is occasionally absent in some ovaries or only present in part of the ovarioles. This indicates a distortion of the synchronization of the egg chamber development between ovarioles. Often the germinal cells of the S1 chamber hardly show signs of differentiation in size or chromatin pattern as compared to germarial cells.

The follicle epithelium shows a clear swelling, cytolysis, chromatokinesis, local metaplasia and dysplasia and an irregular shape. The number of b-type epithelium cells (THEUNISSEN, 1973a) has sharply decreased. Divisions are observed in about normal numbers. The epithelium covering the S1 chamber, if present, is swollen and chromatokinetic.

The separation of 'diffuse' and 'concentrated' oogonia in the germarium is also observed here. The 'diffuse' type generally shows few pathological symptoms, except occasional chromatokinesis. The 'concentrated' types, which may be absent at all, show a chromatin transformation which results in pycnosis and cytolosis (fig. 39). All stages of this transformation are characterized by an increase of coarseness of the chromatin elements if compared to the control or even to the 500 R irradiated ovary. This increase often also includes the 'diffuse' type and is a general feature of the irradiation effect at this dose and higher ones. Chromatin tends to form small concretions or large irregular granules connected by filaments. Their dispersion in the nucleus is more or less irregular. 'Diffuse' types of oogonia apparently are not formed resulting in a depletion of the cell population in the germarium by extensive cytolysis. Chromatokinetic degeneration including pycnosis is frequently observed, hyperchromatosis rarely. Dividing cells have been observed.

0 days, 1.5 kR

As for egg chamber development the variation in development of the first and second egg chambers has become larger. In the first egg chambers typical S2 but also S4 chromatin patterns in trophocyte nuclei are found. An abnormal type is characterized by narrow ribbons of chromatin with local distentions which are stained more darkly. These ribbons have indistinct outlines due to their ragged and often somewhat granular appearance. They are contracted and obviously derived from a typical S2 pattern (THEUNISSEN 1976, fig. 83, S2, 1). In some cases egg chambers contain only trophocytes of this type and are classified as S2 accordingly. S1 egg chambers are formed in all ovarioles of an ovary or not at all.

The follicle epithelium shows chromatokinesis, swelling, more frequently acute metaplasia and dysplasia, cytolysis, local and sometimes considerable variations in thickness. The mutual cohesion between trophocytes and follicle epithelium in the first egg chambers seems to decrease as the boundary be-
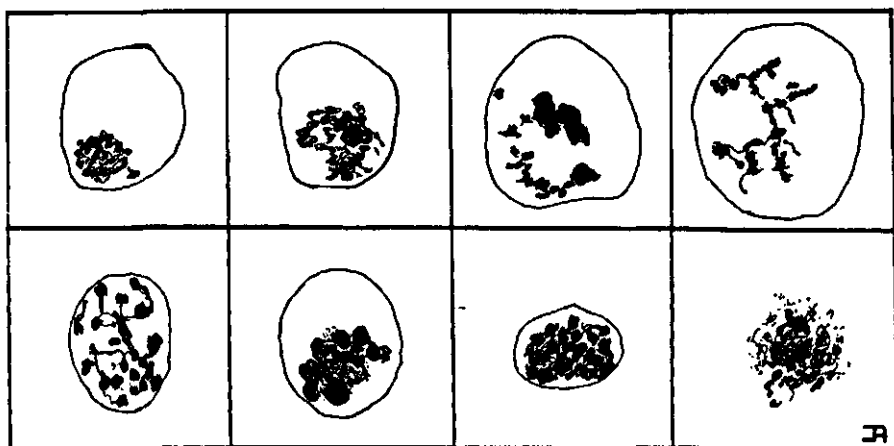
FIG. 39. Chromatin transformation in nuclei of oogonia in the germarium of flies irradiated with 1.0 kR 3 days earlier. This series of intermediates leads to degeneration and cytolysis. Note the increased coarseness of the chromatin material.

tween these populations becomes increasingly irregular and indistinct.

In the basal part of the germarium usually populations of 'diffuse' oogonia are present mixed with abnormal forms and chromatokinetic cells. The apical part is often sharply delimited and contains a variable population of cells ranging from typical 'concentrated' types to cells in cytolysis. A pathological type of oogonia observed here is characterized by an evenly dispersed, very thin filamentous chromatin. Chromatin transformation is not observed, contrary to extensive chromatokinetic degeneration ending in cytolysis. This leads to the presence of relatively large cavities (fig. 40, 1). A general picture is the progressing desorganization of the remaining cell populations in this part of the germarium. Swelling of germinal cell nuclei is generally observed, contrary to hyperchromatosis and chromatolysis. Chromatin filaments of swollen germinal cells sometimes show a cross striation of their broadened parts which is very similar in appearance to polytene chromosomes as they are observed in trophocytes.

0 days, 2.0 kR

Development of the first egg chamber shows an increased tendency to serious desorganization. The shape of the trophocytes in some egg chambers is conspicuously angular and the cytoplasm stains irregularly. The appearance of the chromatin pattern whether 'normal' or 'pathological' is decisive in determining a developmental stage according to the existing criteria. Such a determination does not necessarily mean a perfect normal morphology of the trophocytes concerned but the dominance of chromatin patterns which more or less resemble those which are typical for that stage in untreated ovaries. Development of a second chamber is inhibited.
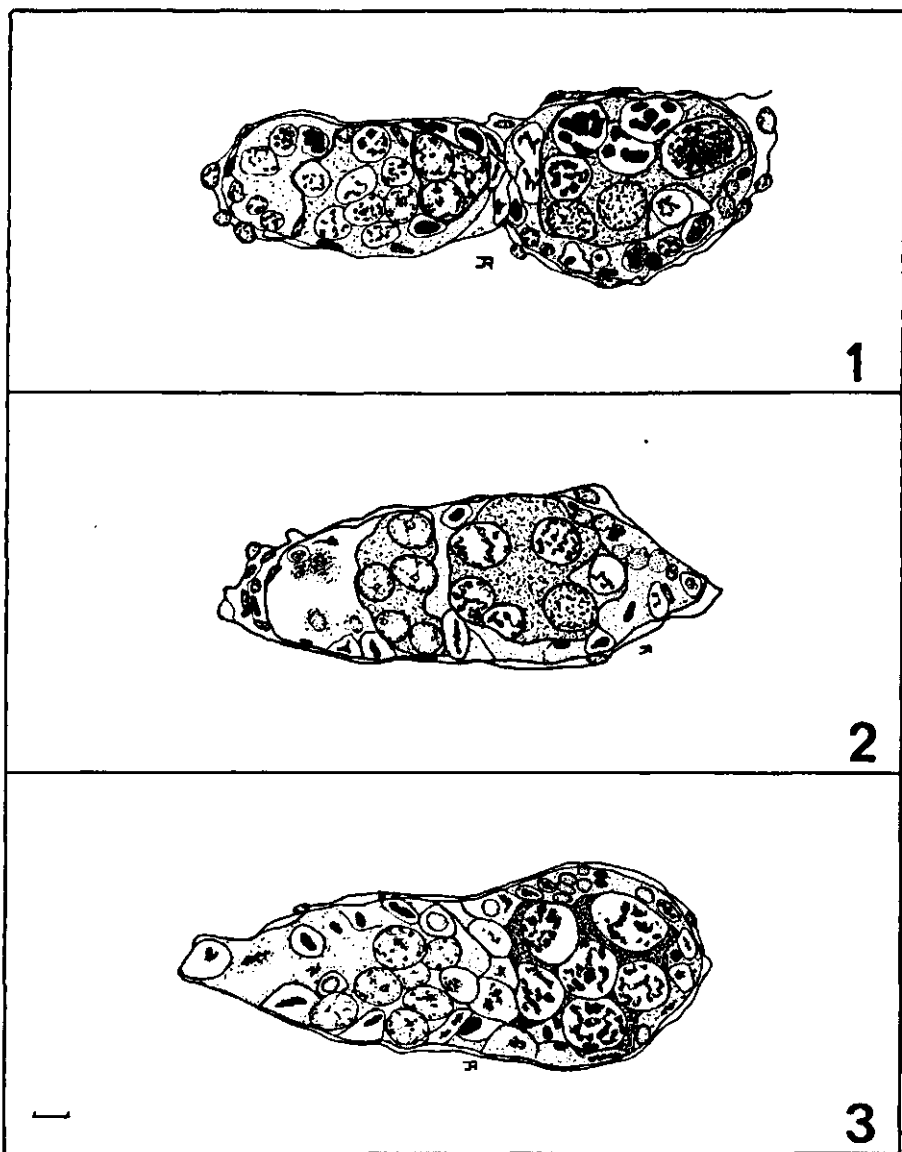
FIG. 40. Ovarioles of newly emerged flies 3 days after irradiation with 1.5 (1), 2.0 (2) and 3.0 (3) kR. Compare with fig. 37.

The follicle epithelium shows an increasing pathological reaction as compared to less irradiated ovaries (fig. 41). Its appearance is often very chaotic with locally very large swollen cells, extensive metaplasia because large surfaces of the egg chambers may be covered by squamous epithelium (fig. 40, 2), increasing acute dysplasia, chromatokinetic degeneration, karyolysis, karyorrhexis and a general desorganization. Many cells seem already to have disap-
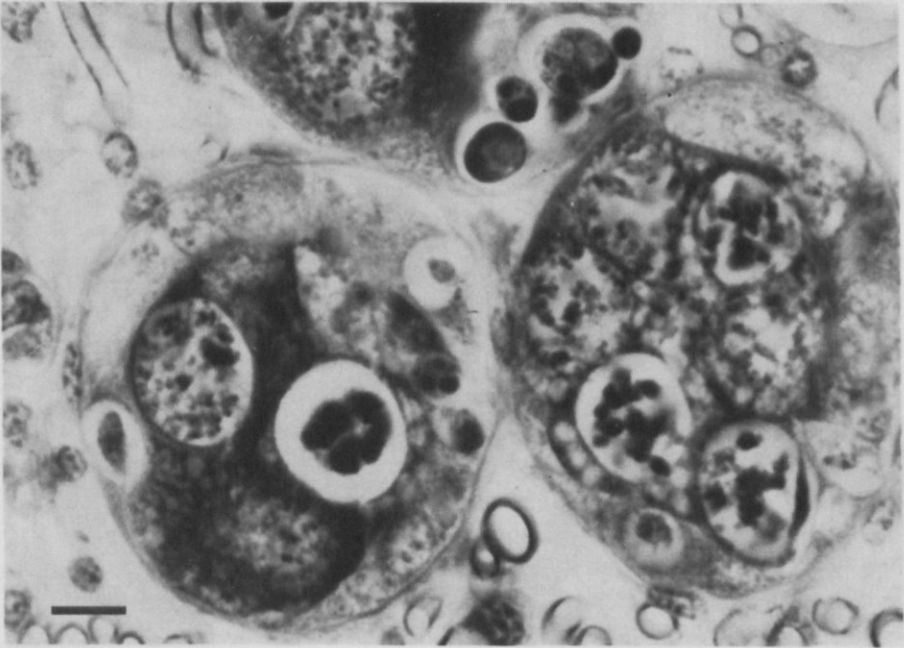
FIG. 41. S1/S2 egg chambers in ovary irradiated with 2 kR. Abnormal mixture of morphologically nearly normal chromatin structures in trophocyte nuclei (cf. Theunissen 1976, fig. 83). Swelling and cytolysis of follicle epithelium are most prominent symptoms here. Bar = 10 μ.

peared due to cytolysis. Boundaries between trophocytes and follicle epithelium are often indistinct and irregular. The epithelium which covers the basal part of the germarium is often swollen, chromatokinetic and irregular of size and shape.

In the germarium usually a population of 'diffuse' oogonia is found. The situation in the apical part is very variable. The local populations can be seriously depleted and show a large variation of pathological symptoms and total desorganization (fig. 42). In other ovaria only some chromatokinetic degeneration can be seen in these populations (fig. 43).

0 days, 3.0 kR

The reactions of the ovary of the newly emerged fly to the sterilizing dose have already been described (4.3.1.) (figs. 40, 3 and 35).

Summary 0 days

In newly emerged females the ovary reacts in various ways on irradiation 3 days earlier. A clear tendency is the decreasing rate of development of the first and second egg chambers at increasing dose (table 6) (cf. THEUNISSEN, 1974, and page 63).
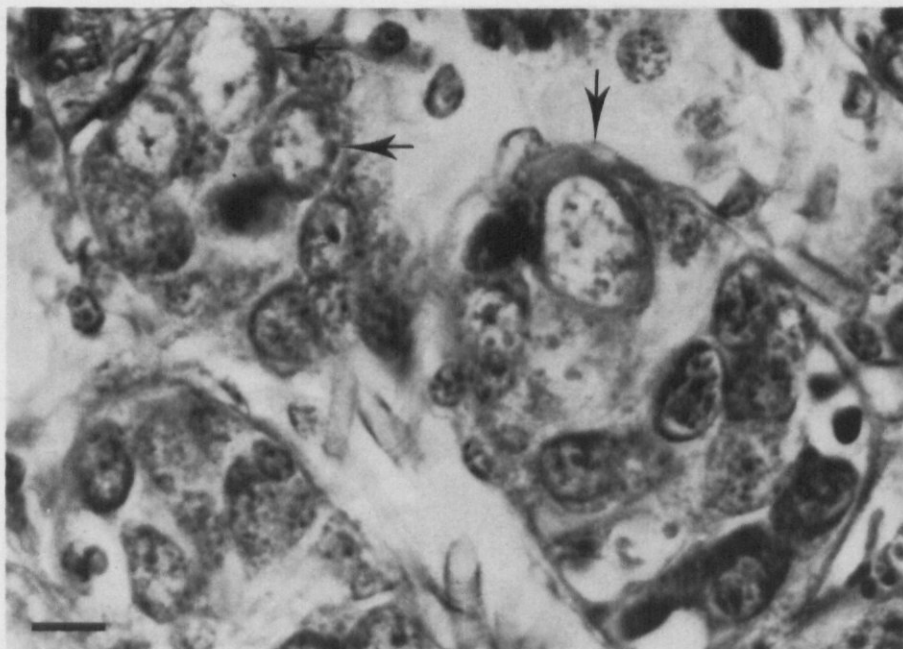
FIG. 42. Swelling of individual germinal cells (arrows) in 2 kR irradiated germaria. Cell depletion by pycnosis and cytolosis is also observed. Bar = 10 μ.

FIG. 43. Chromatokinetic degeneration in germarium (arrow) and metaplasia of follicle epithelium of first, S2/S3 egg chamber. In germaria 'diffuse' oogonia are still observed. Newly emerged adult, dose 2 kR. Bar = 10 μ.

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TABLE 6. Average egg chamber development in newly emerged females 3 days after irradiation with various doses.
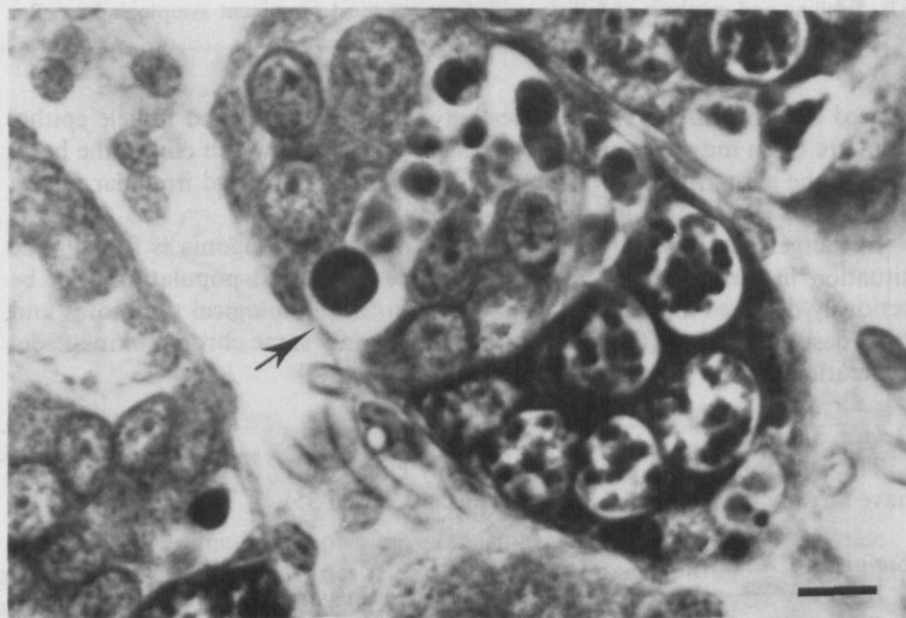
dose[1]	first egg chamber[2]	second egg chamber[3]	ovary[4]
0	3.0	1.0	4.0
0.5	3.0	1.0	4.0
1.0	2.7	0.6	3.3
1.5	2.5	0.4	2.9
2.0	2.5	0	2.5
3.0	1.9	0	1.9

[1] dose in kR
[2] first egg chamber denotes most advanced one
[3] second egg chamber is egg chamber following first one in the ovariole
[4] sum of average development of all consecutive egg chambers in the ovariole

The radiation effect on the development is also reflected by the size of the first egg chambers in the various dose groups. In order to obtain some quantitative data on this effect volumes of these egg chambers have been measured In just emerged flies the most advanced egg chamber is ovoid. When the diameters of the egg chamber, including the follicle epithelium, are measured along the longitudinal and maximum transversal axes of the egg chamber, the long and short axes of the rotation body of an oval have been determined. When the long and short axes are 2a and 2b respectively, the volume of the rotation body when rotated along the long axis is: $I = 4/3 \pi ab^2$. According to this method the volumes of first egg chambers have been determined. Per dose group 5 flies were taken. From the ovaries of each fly 10 first egg chambers were chosen at random and the long and short axes measured. The results are summarized in table 7.

These figures represent the order of magnitude of the egg chamber volumes as samples were small and the individual variability was large. First egg chambers of irradiated flies can be somewhat irregular of shape at emergence al-

TABLE 7. Volume of most advanced egg chamber of newly emerged flies 3 days following irradiation with various doses.

dose[1]	average volume[2]
0	69.7
0.5	69.6
1.0	59.4
1.5	58.0
2.0	39.0
3.0	30.7

[1] dose in kR
[2] volume in $10^3 \mu^3$

ready (figs. 37 and 40), which depends on the dose received. This aberration from the ovoid shape is not considered to impair an approximate determination of these egg chamber volumes. These data confirm the picture of general dystrophy of the ovary at increasing doses.

With regard to histopathological symptoms the follicle epithelium shows the strongest reaction, which increases in severity at increasing dose. The germarium is clearly split in a relatively radioresistant population of 'diffuse' oogonia and a population of 'concentrated' oogonial types. At low doses they show a shift towards a 'diffuse' type (0.5 kR). After 1.0 kR irradiation the cells seem to be unable to make the shift due to interfering degeneration. At higher doses a variable pattern of pathological reactions is seen, which generally increases in severity and degree of desorganization of local cell populations with increasing dose.

4.3.2.3. 5 days post-emergence

5 days, 0.5 kR

Egg chamber and trophocyte development are almost normal, except for a somewhat irregular shape. The second egg chamber is normally developed as S3 or S2. S1 egg chambers differentiate when the preceding one is in the S3 stage.

The follicle epithelium occasionally shows some swelling although incidentally more serious symptoms can be observed: chromatokinesis, pycnosis, dysplasia in individual abnormal egg chambers which are perhaps degenerating independently of the irradiation effect. The covering epithelium of S1 chambers can be swollen or shows locally metaplasia or beginning desorganization.

The germinal cell population in the germarium is very variable. Sometimes relatively large numbers of 'concentrated' oogonia are found but usually they are scarce or absent. The 'diffuse' type is generally present. A number of pathological symptoms is found incidentally and dispersed: coarse chromatin structure, cytolysis, and local chromatokinetic degeneration leading to pycnosis. These symptoms are, however, frequently absent. Oocytes seem to be morphologically normal. Cell divisions are found.

5 days, 1.0 kR

The pathological reactions are considerably more severe. The ovary as a whole shows moderate-serious dystrophy. The first egg chamber often has not yet separated fully from the germarium in which the second one is still enclosed. Locally abnormal development may take place. For instance, an S4 egg chamber may develop from an S2 which is preceded by an S4 egg chamber already present (fig. 44). Trophocyte nuclei do not show specific pathological symptoms but their cytoplasm often stains irregularly indicating a progressing cytolysis.

The follicle epithelium is often metaplastic, swollen, chromatokinetic, pycnotic and showing karyolysis. The boundary with the trophocytes is ir-
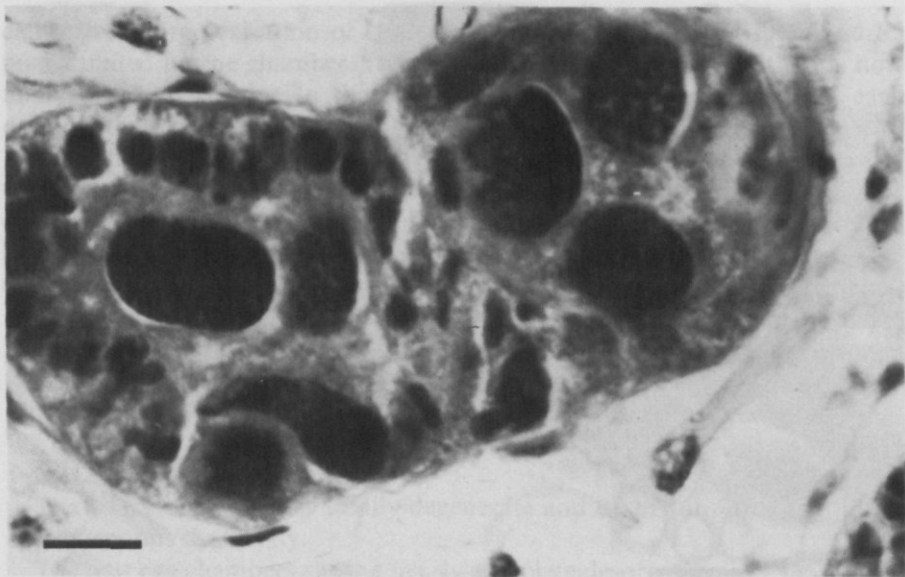
FIG. 44. Abnormal egg chamber development. Two subsequent egg chambers in an ovariole have developed to the same stage. Adult 5 days, dose 1.0 kR, 8 days p.i. Bar = 10 μ.

regular and indistinct which later results in desorganization of the entire egg chamber. The boundary between trophocytes and oocyte, which does not show any abnormalities, can also fade. The b-type nuclei in the epithelium become gradually pycnotic.

The germarium contains few normal cells. Extensive cytolysis and swelling of cells is taking place. The normal spatial arrangement of the cells seems to be distorted, for instance resulting in egg chamber development in the germarium. These widespread acute dysplastic symptoms can dominate the general picture of abnormal and abortive development.

5 days, 1.5 kR

The severity of all pathological reactions has increased even more. The ovary as a whole is seriously dystrophic. The first egg chamber can be heavily deformed by severe cytolysis of the trophocytes. Their cytoplasm as well as that of the oocyte, whose nucleus appears to be normal, is vacuolated. The oocyte may show an abnormal orientation relative to the trophocytes and can be found laterally or even apically of them. The trophocytes occasionally show extrusion into the germarium by rupture of the degenerating follicle epithelium (fig. 46). Generally, the boundary between epithelium and trophocytes is irregular and indistinct. Often infiltration of epithelium cells between the degenerating trophocytes is observed. Trophocytes of the second egg chamber

FIG. 45. Cell debris (arrows) in pedicel. Left: an egg chamber, right: the lateral oviduct. Adult 5 days, dose 1.0 kR, 8 days p.i. Bar = 10 μ.

FIG. 46. Extrusion of trophocyte (arrow) from S4 egg chamber into adjacent S2 egg chamber. Note desorganized and metaplastic follicle epithelium. Adult 5 days, dose 1.5 kR, 8 days p.i. Bar = 10 μ.
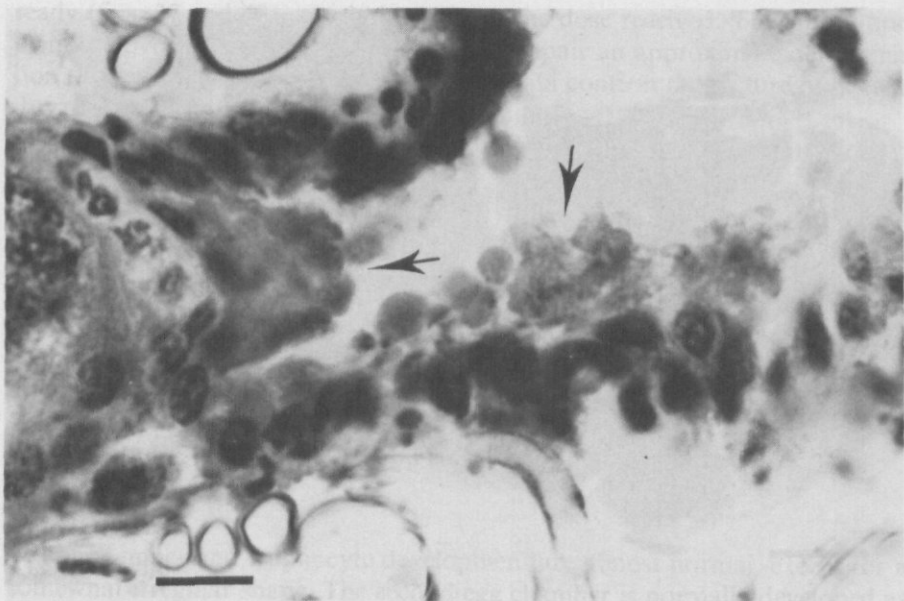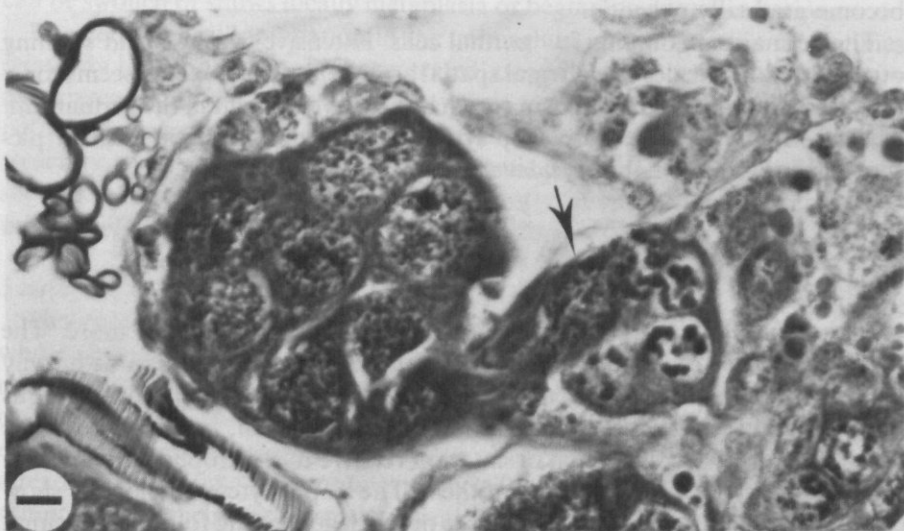
can show a large variation of chromatin patterns typical for S1, S2 and S3 nuclei united in one chamber. Its development proceeds further than is normally observed relative to the development of the first chamber. In each ovariole the second egg chamber can differ in developmental stage.

The follicle epithelium shows a large variety of serious pathological symptoms: heavy swelling, widespread acute metaplasia and dysplasia, extensive cytolysis, karyolysis and chromatokinetic degeneration. These symptoms are less generally found and less severe in the follicle epithelium of the second egg chamber.

The germarium shows a considerable desorganization. It may contain trophocytes. The normal mutual relations between the cells are distorted. Sometimes relatively many 'diffuse' type oogonia and cell divisions are present. All symptoms of chromatokinetic degeneration and swelling can be observed.

5 days, 2.0 kR

The ovary as a whole is totally degenerate and desorganized, presenting a case of serious dystrophy.

The first egg chambers show a nearly complete desorganization. Only a few can still be classified according to their stage of development. The trophocyte cytoplasm has disappeared or is reduced by cytolysis. The remaining nuclei show chromatokinetic degeneration as structureless lumps in infiltrated egg chambers.

The follicle epithelium is extremely abnormal. It infiltrates the egg chambers between the remnants of the trophocytes but it hardly covers the egg chamber anymore because of extensive acute metaplasia and in particular cytolysis of epithelium cells. The egg chamber as a whole presents a chaotic picture of total desorganization. The boundaries between egg chamber and germarium are indistinct.

The germarium also shows a total desorganization. It can contain a rudimentary second egg chamber in any given place. This egg chamber usually shows cytolysis. Locally 'diffuse' oogonia can still be present, but extensive cell depletion by cytolysis is evident. Many cells show chromatokinetic degeneration, karyolysis, swelling, pycnosis and abnormal chromatin structures. The pathological picture is extremely variable. Often squamous epithelium seems to be the only structure which holds the entire degenerated mass of cells together.

5 days, 3.0 kR

Only remnants of egg chambers are left which can not be recognized as having developed into a certain stage. These remnants stain very variable and show many differents shapes and abnormal structures.

Former follicle epithelium, usually devoid of cells, partially surrounds the remnants of trophocytes as a light, thin layer. Sometimes cellular membranes are still intact.

The germarium contains a large variety of cells with different chromatin

patterns: from finely granular to coarse, from dispersed to concentrated, from concretions to filaments in all possible combinations. Large cavities are formed due to extensive cytolysis (fig. 53, 2). Cells in chromatokinetic degeneration are present at a large scale. The somatic elements often show swelling, chromatokinetic degeneration, karyolysis and chromatolysis.

Summary 5 days

At an age of 5 days the differences in pathological reactions between the ovaries in the various dose groups have become considerably larger. While the ovaries hardly show radiation effects after 0.5 kR irradiation, the highest dose results in a total desorganization, degeneration and serious dystrophic condition of the entire ovary and its constituing parts. The follicle epithelium and germarial cells react most sensitively to the radiation injury by conspicuous and dose dependent pathological symptoms. At higher doses the trophocyte cytoplasm and germarial somatic cells become visibly involved in the histopathological pattern. Both a large individual variability and a gradual increase in severity of most symptoms prevent the formulation of stages in the pathological processes, thus obstructing a more quantitative approach in the assessment of these radiation effects.

As for average egg chamber and total ovary development, table 8 summarizes the data obtained.

4.3.2.4. 10 days post-emergence

10 days, 0.5 kR

The first egg chambers look quite normal but are slightly retarded in development when compared to untreated ovaries, in spite of their similar size. Regularly radiation induced interference with synchronization of egg chamber development in the ovariole is observed. The second egg chamber also shows shrinkage of the trophocyte cytoplasm.

TABLE 8. Average egg chamber development in 5 days old females 8 days after irradiation with various doses.

dose	first egg chamber	second egg chamber	ovary
0	4.2	2.7	7.6
0.5	3.9	2.7	7.4
1.0	4.0	1.6	5.6
1.5	3.8	1.8	5.6
2.0	3.0	2.0	5.0
3.0	—[1]	—[1]	—[1]

[1] – denotes the inability to determine the stage of development due to pathological effects. For legend see table 6.

The follicle epithelium occasionally shows some shrinkage and chromato-kinesis but looks otherwise normal.

The germarium contains many dividing cells and 'concentrated' and 'diffuse' oogonia and their intermediate types. Nevertheless, locally cytolysis and chromatokinesis can be found as well as swelling of individual germinal cells.

10 days, 1.0 kR

The ovary as a whole gives an abnormal and dystrophic impression. All egg chambers are clearly pathological and retarded in development. The trophocyte cytoplasm is shrunken and often chromatokinetic. The chromatin pattern is nearly normal except for a slight condensation of the chromatin structures which are denser and coarser. Irregularities in synchronization of egg chamber development extend also to the third egg chamber. An abnormal orientation of the oocyte relative to the trophocytes, trophocyte resorption (fig. 47) in some cases and a retarded migration of the border cells between the trophocytes are observed.

The follicle epithelium is abnormal: local hyperchromatosis, chromatokinetic degeneration, swelling, local acute dysplasia, sometimes heavy acute metaplasia and widespread cytolysis are observed.

The germarium mostly contains S1 trophocytes in chromatokinetic degeneration or already in cytolysis. Many germinal cell nuclei show coarse chromatin structures, part of which are presumably failed prophases. Karyolysis is relatively frequently seen.
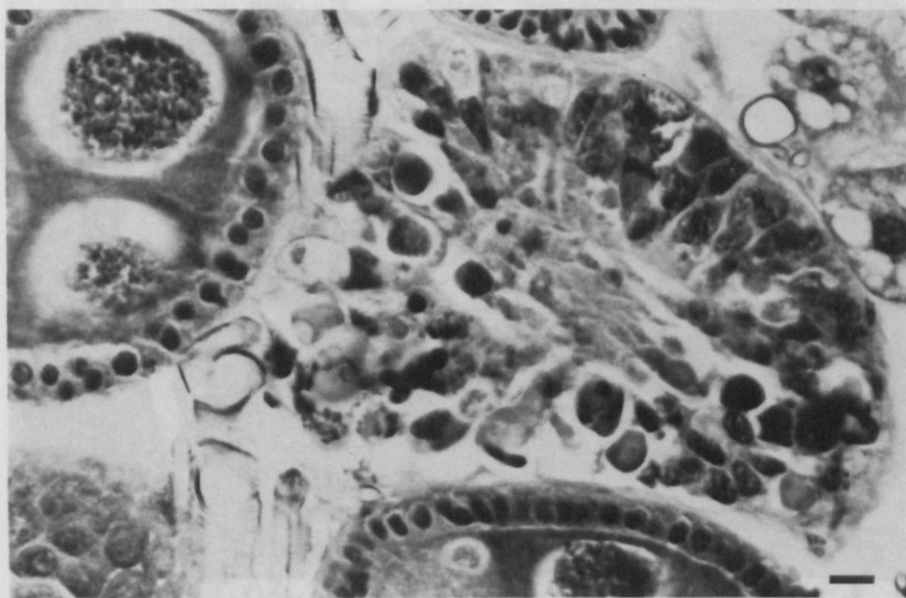
FIG. 47. Egg chamber in resorption. Apparent proliferation of the follicle epithelium without showing any mitotic divisions. Adult 10 days, dose 1.0 kR, 13 days p.i. Bar = 10 μ.
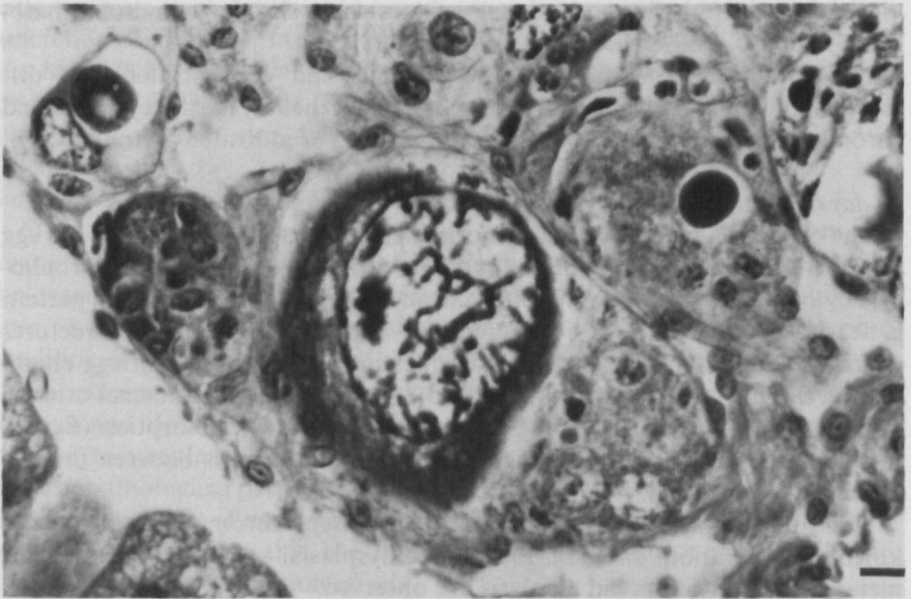
FIG. 48. Abnormal hypertrophic development of a single trophocyte. Note the polytene chromosomes in the nucleus. Adult 10 days, dose 1.5 kR, 13 days p.i. Bar = 10 μ.

FIG. 49. Lysis in both trophocyte cytoplasm and follicle epithelium of egg chamber. Trophocyte nuclei are morphologically relatively radioresistant. Adult 10 days, dose 1.5 kR. 13 days p.i. Bar = 10 μ.
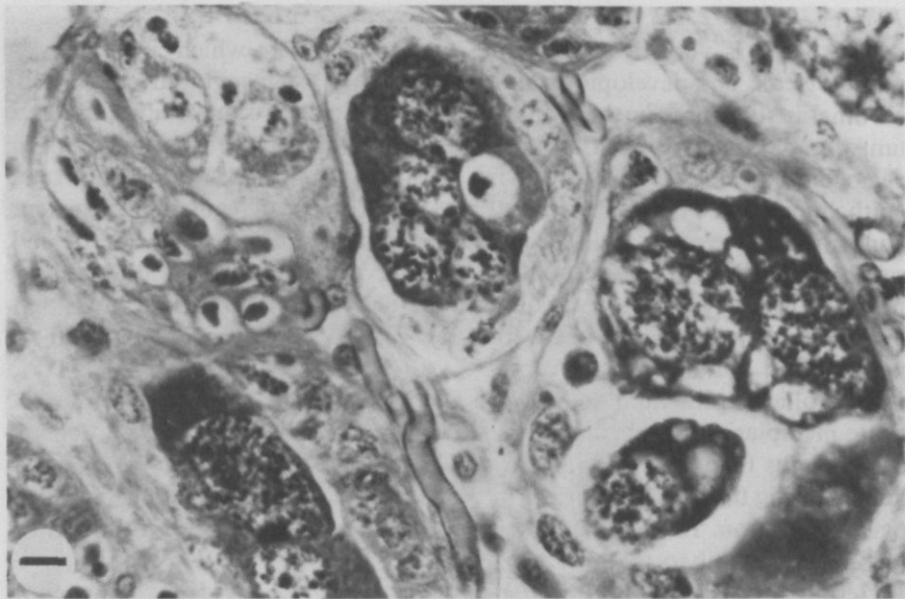
FIG. 50. Deformation of first egg chambers caused by lysis of trophocyte cytoplasm (vacuolization), swelling and metaplastic changes in the follicle epithelium and pressure of the surrounding tissues. Adult 10 days, dose 1.5. kR, 13 days p.i. Bar = 10 μ.
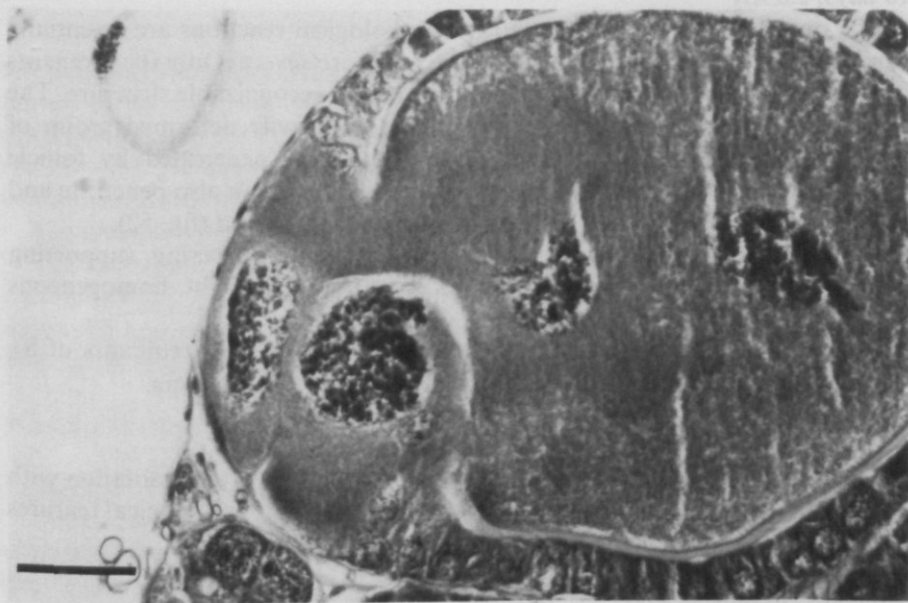
FIG. 51. Extrusion of trophocytes into oocyte. Adult 10 days, dose 1.5 kR, 13 days p.i. Bar = 50 μ.

10 days, 1.5 kR

The ovary is very dystrophic due to egg chambers which are extremely pathological. The developmental stage can often be recognized only by the chromatin structures in the trophocyte nuclei which are relatively radioresistant (fig. 48). Synchronization is seriously in disorder. The trophocytes of an egg chamber can be resorbed, leaving only the spongy 'skeleton' of infiltrated epithelium cells. Other pathological features are: very irregular development, abnormal oocyte orientation, vacuolization and lysis of the cytoplasm (fig. 49), swelling of cells in lysis, fusion of egg chambers in an ovariole, variable shape because of the pressure of surrounding tissues (fig. 50), extrusion of trophocytes into germarium or oocyte (fig. 51). These symptoms contribute to the pathological picture of the ovary.

The follicle epithelium adds to this picture by severe cytolysis, widespread acute metaplasia, local acute dysplasia and swelling of the remaining cells. A conspicuous feature is the breaking through the normal limits of the epithelium, causing the cells to infiltrate into the egg chamber in spite of their pathological condition. In untreated ovaria this kind of activity is seen only in resorbing egg chambers (DAVIES and KING, 1972).

Germinal cell populations in the germarium are severely depleted by cytolysis. Remnants of S1 egg chambers are sometimes left as darker stained structures. Chromatokinetic structures, cell debris and some cell divisions are observed.

10 days, 2.0 kR

The ovary is very dystrophic and the pathological reactions are essentially similar to those described above but they are more severe. Only the remnants of the first egg chamber and the germarium have a recognizable structure. The egg chamber consists of an irregularly shaped, heavily deformed group of trophocytes with vacuolated and lysing cytoplasm, penetrated by follicle epithelium cells. Trophocytes of younger egg chambers can also penetrate and even fusion of adjacent egg chambers is frequently observed (fig. 52).

The follicle epithelium has virtually disappeared as a covering, supporting and trophic tissue and it has changed its structure into a light, homogeneous zone, if present at all. It has completely degenerated.

The germaria are almost empty, except for somatic cells, remnants of S1 egg chambers and sometimes germinal cells in very small numbers.

10 days, 3.0 kR

The condition of the ovaries is essentially the same as after irradiation with 2.0 kR. The higher dose has not resulted in more distinct pathological features (fig. 53, 3).

Summary 10 days

The still increasing differences in pathological radiation effects in ovaries treated with various doses are accentuated. At the lowest dose the effects seem

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FIG. 52. Result of fusion of two adjacent egg chambers as indicated by mixture of trophocyte nuclei of two different stages: S2 (arrows) and S4. Adult 10 days, dose 2.0 kR, 13 days p.i. Bar = 10 μ.

to be limited when compared to untreated ovaries. After higher doses the intensity of the pathological reactions increases with dose till an added 1 kR does not seem to exert an additional negative influence. When compared to the respective situations in 5 days old flies the severity of the pathological symptoms has increased.

Egg chamber development at this age is summarized in table 9.

The apparently more developed first egg chamber in the 500 R irradiated ovaries reflects the retarded development since the untreated females already deposited eggs at this age, thus lowering the recorded average developmental stage of the first egg chamber. At this age the 500 R group had not yet oviposited.

4.3.2.5. 15 days post-emergence

15 days, 0.5 kR

The ovaries do not show differences in size or degree of development when compared to untreated ones. A conspicuous change in the appearance of the entire ovary, including the trophocytes, is the widespread chromato-kinesis. The cytoplasm of the trophocytes of first and second egg chamber is vacuolated and shrunken. Eggs have been deposited as is also shown by the presence of 'follicle resorption bodies' (PHIPPS, 1966), formerly called 'corpora
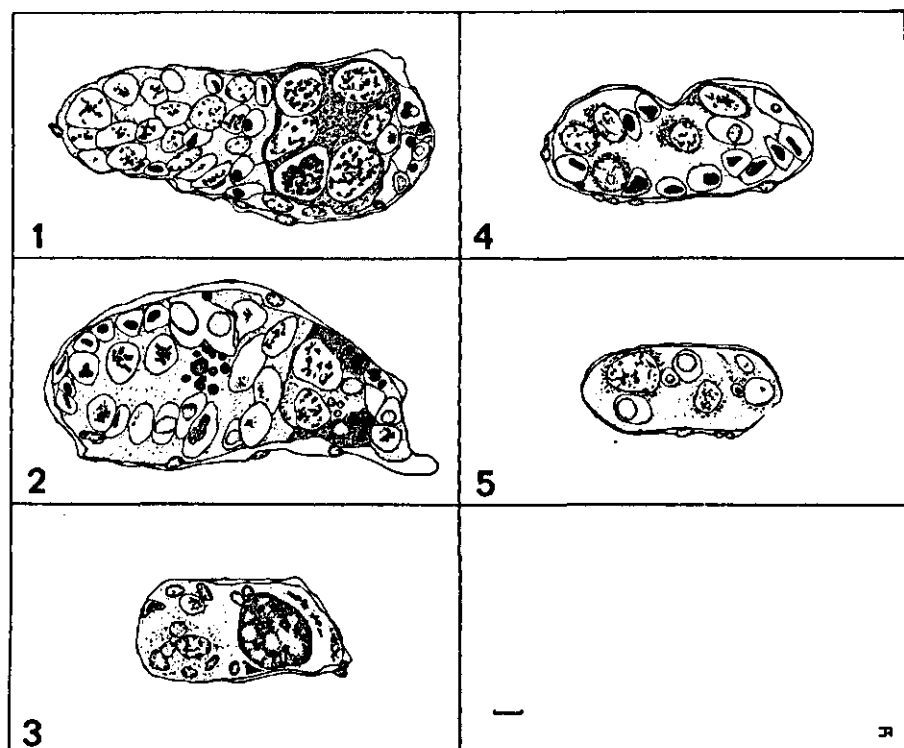
FIG. 53. Ovarioles of females irradiated with 3 kR, the sterilizing dose, 3 days prior to emergence, at various ages: at emergence (1), 5 (2), 10 (3), 15 (4) and 20 days (5). Bar = 10 μ.

TABLE 9. Average egg chamber development in 10 days old flies 13 days after irradiation with various doses.

dose	first egg chamber	second egg chamber	ovary
0	8.5	5.2	17.9
0.5	9.3	5.3	19.4
1.0	7.8	4.0	14.8
1.5	3.8	1.1	4.9
2.0	3.7	0.2	4.0
3.0	—	—	—

For legend see table 6.

lutea' which term has been critisized by LUSIS (1963) and others as is summarized by PHIPPS (1966) (fig. 54). The younger egg chambers look normal.

The germarium contains large populations of 'diffuse' and 'concentrated' oogonia and their intermediate types.
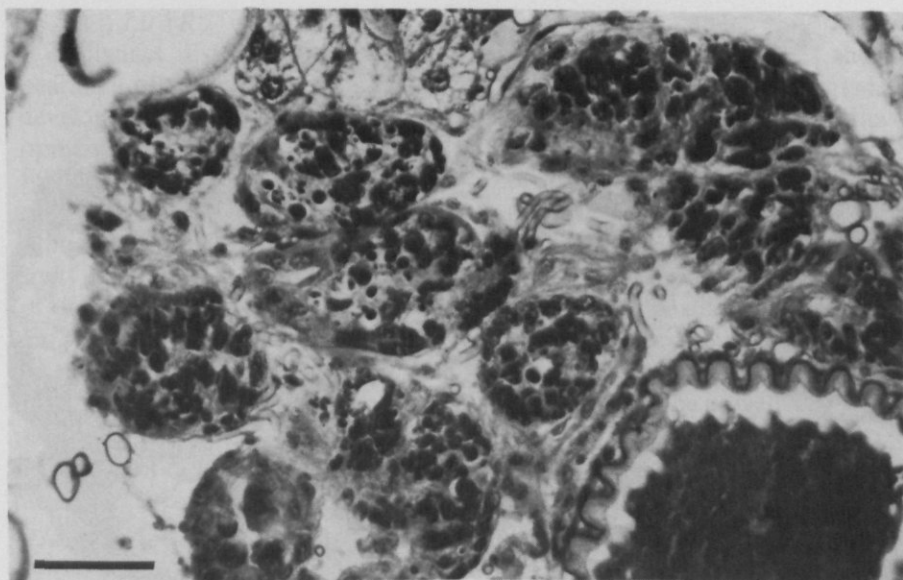
FIG. 54. Follicle resorption bodies in ovary of 20 days old untreated adult. Bar = 50 μ.
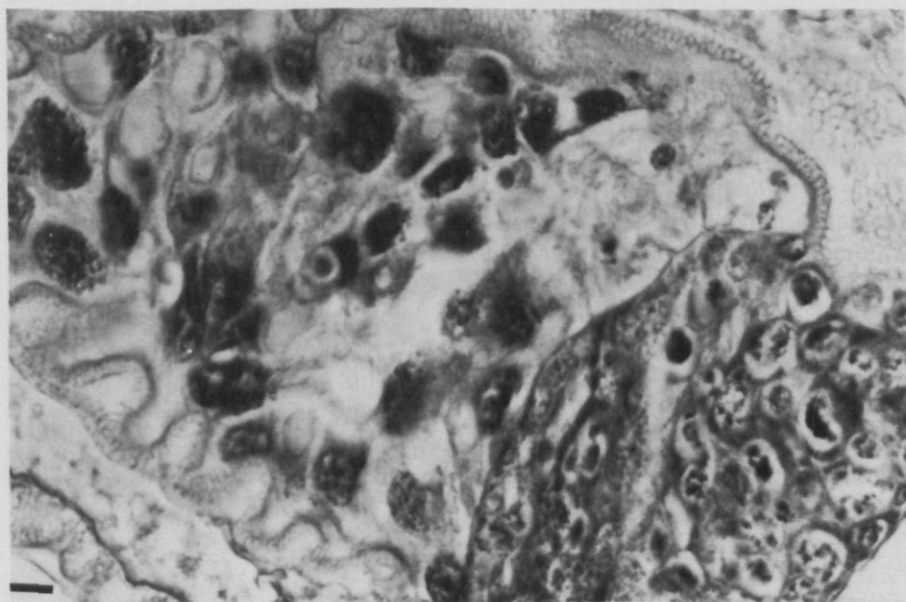
FIG. 55. Dissolution of S10 egg chamber. Note absence of differentiation of younger egg chambers. Adult 15 days, dose 1.0 kR, 18 days p.i. Bar = 10 μ.
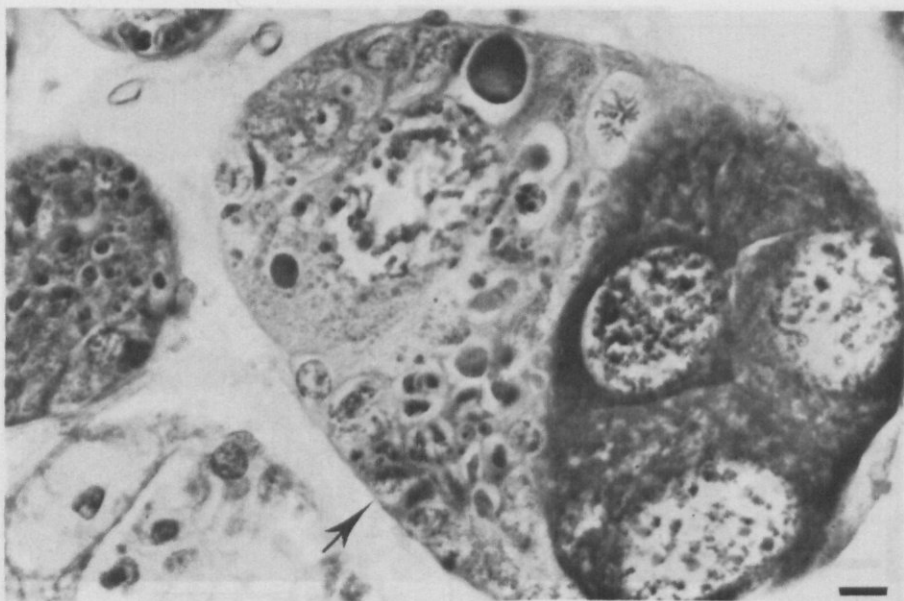
FIG. 56. Abnormal development of successive egg chambers. Lysis of trophocytes in first egg chamber, local hyperplastic proliferation (arrow) and meaplasia of follicle epithelium, and hypertrophic growth of a single trophocyte in second egg chamber. Adult 15 days, dose 1.0 kR, 18 days p.i. Bar = 10 μ.
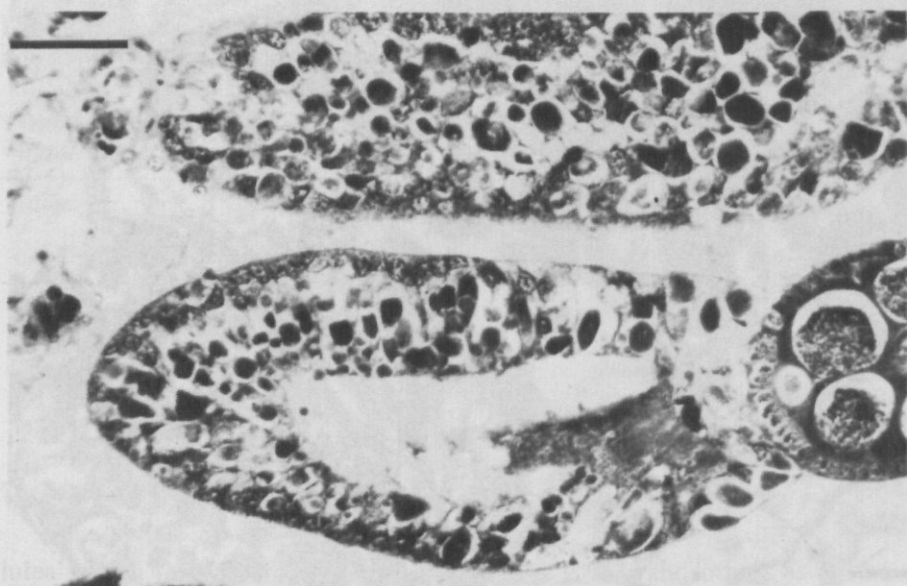
FIG. 57. Egg chambers in resorption in 15 days old, untreated female. Note the proliferation of the follicle epithelium. Bar = 50 μ.

15 days, 1.0 kR

A distinct distortion of egg chamber development characterizes the appearance of the ovary. In some ovarioles S10 chambers, mature eggs, have developed which are partly in a state of dissolution (fig. 55). In others development has stopped and degeneration is initiated in an earlier stage of egg formation (fig. 56). The degeneration process of the first egg chamber is not called: 'resorption', because this term implies an active participation in this process of the follicle cells (fig. 57) (DAVIES and KING, 1972). As such an activity has not been observed here and the nuclear and cytoplasmic structures of the trophocytes and oocyte, including yolk, seem to be dissolved by internal enzymatic action, the term 'dissolution' seems to be more appropriate. Whether or not eggs have been deposited can not be established from the histological data because suspected 'follicle resorption bodies' might be as well 'egg resorption bodies' (PHIPPS, 1966).

The second egg chamber shows severe histopathological reactions: vacuolated and chromatokinetic cytoplasm of trophocytes and oocyte, infiltration by follicle cells, deformed shape by pressure of surrounding tissues, chromatokinetic and metaplastic follicle epithelium which locally may proliferate to large hyperplastic masses of cells which sometimes show signs of beginning anaplasia.

Younger egg chambers often have not developed. The germarium often contains swollen remains of S1 trophocytes or egg chambers, in addition to surprisingly many 'diffuse' oogonia and intermediate types.

15 days, 1.5 kR

The ovaries are characterized by an almost total absence of recognizable egg chambers and a very dystrophic condition. Younger egg chambers are not formed anymore. The stage of development of the first egg chambers usually can not be determined anymore because of heavy distortion, vacuolization, chromatokinetic degeneration including pycnosis of the trophocyte nuclei, cytolysis, acute metaplasia and local hyperplasia of the epithelium. Masses of hyperplastic epithelium cells may develop into spheroidal cell concretions which contain dividing cells and a-type epithelium cells. These cells often show a variable appearance by irregular chromatin patterns, swelling of individual cells and variable stainability. Sometimes abnormally large and light cells are found resembling swollen 'diffuse' oogonia. In some cases germaria are included in this mass. The symptoms of active proliferation and variable appearance of the cells suggest a clear case of anaplasia of the follicle epithelium.

The germaria still contain germinal cells with a coarsely granular chromatin pattern.

15 days, 2.0 kR

The ovaries are very dystrophic. The first egg chambers can not be recognized any more relative to their stage of development. They show heavy distortion of shape, chromatokinesis, vacuolization and shrinkage of the cytoplasm.

Oocytes are barely recognizable.

The germaria can still be distinguished but contain swollen somatic cells with a-type nuclei in which chromatokinetic and shrunken chromatin is observed. These nuclei show a large variation in stainability.

15 days, 3.0 kR

Except for a progressing process of general degeneration and desorganization no essential differences are observed relative to the situation at 10 days of age. The same pathological symptoms in the germarium and remnants of egg chambers are still present. Some swollen and somewhat hyperchromatic germinal cells are found, resembling S1 trophocytes (fig. 53, 4).

Summary 15 days

The development of the patterns of histopathological reactions per dose group after increasing age is also observed here. The ·differences have again been enhanced. The 0.5 kR irradiated females show very moderate symptoms, especially in view of the occurrence at this age of resorption of egg chambers also in untreated ovaries. In these ovaries the frequency of abnormal egg chambers and cells increases with age. Differences between 'spontaneous' and radiation induced abnormalities are, however, distinct. The former are phenomena which are clearly isolated in time and location in individual structures whereas the latter are a part of a whole pattern of more or less abnormal structures and phenomena interwoven in a radiation syndrome.

At low doses eggs may be deposited although the number is small in the 1.0 kR group. After irradiation with 1.5 kR or more egg chamber formation is totally inhibited, the trophocytes are degenerating and the follicle epithelium shows a late proliferating activity leading to anaplastic phenomena or total disappearance. The differences between ovaries irradiated with 2.0 kR and 3.0 kR are small, reflecting a stabilization of the overall pathological picture at these doses with increasing age.

The average egg chamber development is summarized in table 10.

TABLE 10. Average egg chamber development in 15 days old flies 18 days after irradiation with various doses.

dose	first egg chamber	second egg chamber	ovary
0	7.8	3.8	14.3
0.5	8.8	4.7	17.1
1.0	8.3	4.0	14.0
1.5	0.7	—	0.7
2.0	—	—	—
3.0	—	—	—

For legend see table 6.

4.3.2.6. 20 days post-emergence

20 days, 0.5 kR

The egg chambers are typical although the incidence of egg chamber resorption and degeneration clearly increases. The synchronization of their development is failing, resulting in second and third egg chambers of many different stages. In some cases an obviously retarded growth of the egg chamber (S5) in a small number of ovarioles is accompanied by the development of a second egg chamber of nearly the same stage (S4). The not inhibited ovarioles produced S10 chambers (eggs) of which some were deposited. Probably the development of the second chambers in all ovarioles is determined by the combined feedback of the S10 and S5 first egg chambers (cf. THEUNISSEN, 1974). Follicle resorption bodies are frequently observed (fig. 54).

Follicle epithelium of a-type cells is sometimes hyperchromatic but otherwise normal.

The germarium contains 'concentrated' and 'diffuse' oogonia in the apical and basal parts respectively, dividing cells, and differentiating S1 trophocytes.

20 days, 1.0 kR

In most ovaria the S10 egg chambers dominate. Follicle resorption bodies are frequently found, indicating oviposition which actually has been observed in the cages. A number of S10 first egg chambers is degenerated and dissolved, others have not yet developed this far. These retarded egg chambers show a variable pathological pattern: distortion of shape, abnormal orientation of the oocyte, vacuolized and cytolytic cytoplasm of trophocytes and oocytes, follicle epithelium: chromatokinetic degeneration, karyolysis, karyorrhexis, local metaplasia, dysplasia or absence, infiltration of egg chamber. Locally masses of hyperplastic follicle cells are found between or against the germarium and S10 egg chamber (fig. 58).

The germarium looks rather normal with the presence of 'concentrated' and 'diffuse' oogonia, but it can in some cases have been transformed into a large, irregularly shaped mass of anaplastic cells which shows cytolysis in its centre. These cells are extremely variable in size, shape, orientation and staining properties. Their nuclei vary from 'diffuse' oogonia to abnormally large or small pycnotic ones. Some resemble irregular, enlarged follicle epithelium cell nuclei with occasional hyperchromatosis. Extremely lightly stained cells of unknown origin and some large trophocyte-like cells are seen. As dividing cells are scarce and thus proliferation is limited and since no invasive action of the cells in neighbouring tissues has been observed a case of beginning neoplasia is excluded.

20 days, 1.5 kR

Very small and dystrophic ovaries. Occasionally some S10 egg chambers have been formed. Younger ones are degenerated and often dissolved. They are distorted (figs. 59 and 60), showing chromatokinetic degeneration of tro-
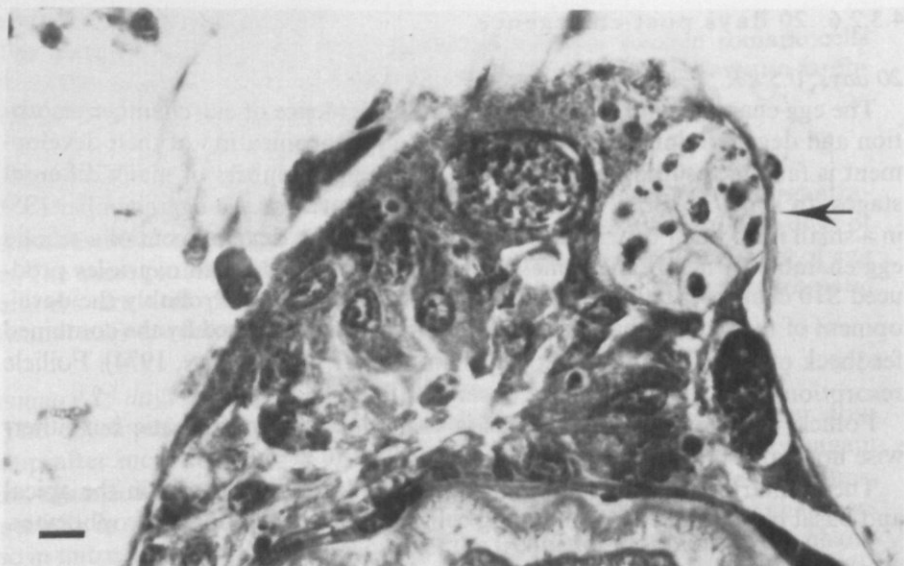
FIG. 58. Follicle epithelium at apical part of an S10 egg chamber, one trophocyte of second egg chamber and remnants of germarium (arrow). Adult 20 days, dose 1.0 kR, 23 days p.i. Bar = 10 μ.
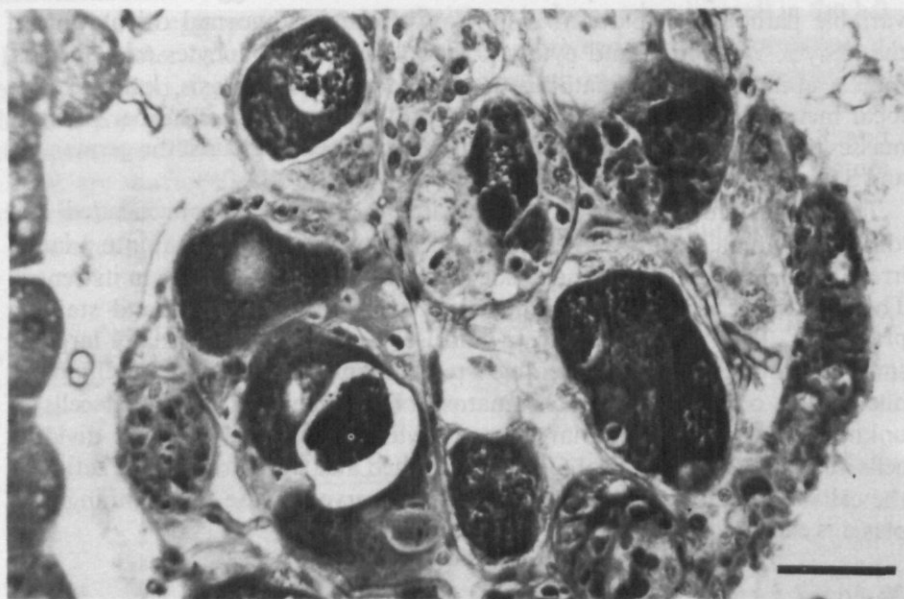
FIG. 59. Deformed egg chambers due to cytolysis of trophocytes and pathological reactions of the epithelium: swelling, necrosis by continuing chromatokinetic degeneration, metaplasia and dysplasia. Adult 20 days, dose 1.5 kR, 23 days p.i. Bar = 50 μ.
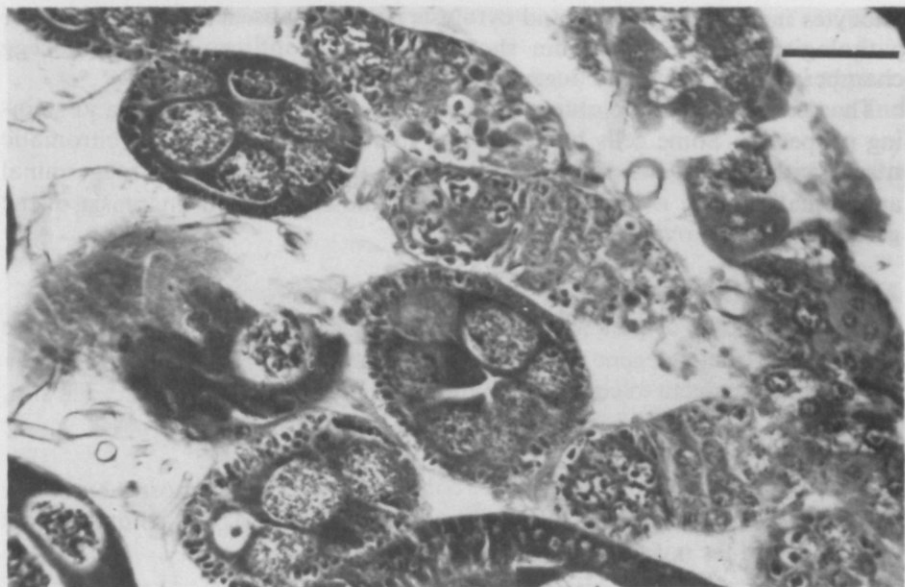
FIG. 60. Germaria, S2 and S4 egg chambers in ovary of a 20 days old, untreated adult. Bar = 50 μ.
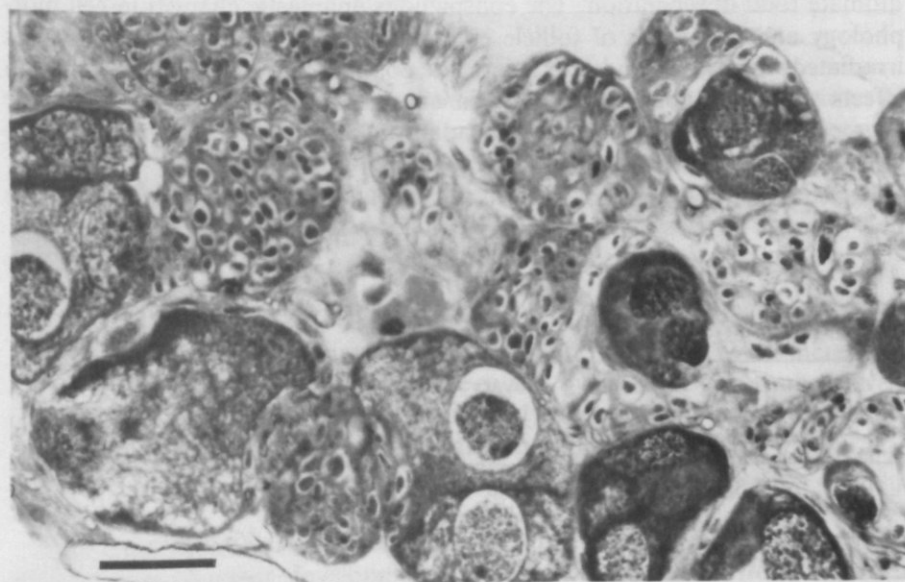
FIG. 61. Deformed egg chambers, cytolysis and individual hypertrophic growth of trophocytes mainly represent here the radiation syndrome. Adult 20 days, dose 1.5 kR, 23 days p.i. Bar = 50 μ.

phocytes including pycnosis and cytolysis (fig. 61), absence of epithelium or pathological follicle epithelium showing swelling, infiltration into the egg chamber, chromatokinetic degeneration, local acute metaplasia.

The germarial cells are abnormally variable in size, morphology and staining properties. Some cells have swollen, light nuclei, others hyperchromatic nuclei with concentrated chromatin. Distinction between cells of germinal and somatic origin is lost. These symptoms indicate a clear anaplasia of the germaria in these ovaries.

20 days, 2.0 kR

Very dystrophic ovaries with remnants of egg chambers vaguely present are in chromatokinetic degeneration. They are heavily distorted.

The germaria seem to contain somatic cells only and are very reduced in size.

20 days, 3.0 kR

The ovaries strongly reduced in size contain somatic cells almost exclusively, leaving a very small number of germinal ones (fig. 53, 5). The somatic tissue which is left does not seem to degenerate quickly.

Summary 20 days

In view of the increasing frequency of degeneration of egg chambers in untreated ovaries the 0.5 kR group shows relatively little effect of irradiation. The pathological pattern in the other groups shows a continued trend to the ultimate total degeneration. The conspicuous anaplastic changes in cell morphology and behaviour of follicle epithelium and germarial cells in ovaries irradiated with 1.0 kR and 1.5 kR indicate possible cancer inducing irradiation effects which are commonly found in animals with a longer life span. The degree of degeneration following irradiation with 2.0 kR and 3.0 kR may prevent such types of radiation damage.

Egg chamber development as far as it is still taking place is shown in table 11.

TABLE 11. Average egg chamber development in 20 days old flies 23 days after irradiation with various doses.

dose	first egg chamber	second egg chamber	ovary	
0	6.3	3.7	12.8	
0.5	7.4	4.0	15.5	
1.0	8.9	0.2	9.2	
1.5	0.8	–	0.8	
2.0	–	–	–	
3.0	–	–	–	

For legend see table 6.

4.3.3. *General pathological reactions of ovary*

After irradiation with relatively low doses ($\leqslant$ 3.0 kR) the external appearance of the ovaries changes considerably according to the received dose and post-irradiation period.

The overall impression of the character of the radiation damage in the various dose groups can be summarized as follows:

0 kR

Normal development as has been described earlier (THEUNISSEN, 1976). Average first egg chamber development increasing till oviposition takes place at about 10 days of adult life. In 20 days old females young egg chamber degeneration in frequency and the speed of egg formation seems to be decreasing. Occurrence of pathological symptoms in untreated ovaries is normal but limited to a few incidental cases of resorbed egg chambers, chromatokinetic somatic and germinal cells or entire ovaries in degeneration. In very old females degeneration of ovaries is commonly found.

0.5 kR

The ultimate result of the irradiation is limited in histopathological sense. The ovary is capable to compensate for the treatment by a larger but somewhat retarded production of eggs. Apart from the chromatin transformation of oogonia, most pathological symptoms appear occasionally and only an increased incidence of abnormalities indicates the preceding irradiation.

1.0 kR

Irradiation effects are clearly visible, especially because of the inhibition of young egg chamber formation and a wide variety of pathological reactions. The total effect, however, is moderate because still eggs can be produced and deposited. After an initial pathological reaction of germarial cells which eliminates many of them, germinal cell populations are capable of recovery and survival of the irradiation.

1.5 kR

Distinct and irreversible irradiation effects are observed. With increasing age the symptoms differ more from those caused by 1.0 kR. Egg chamber development stagnates. Cell functioning is not only impaired but cells are killed at a large scale. Degeneration and desorganization prevail.

2.0 kR

At emergence the pathological effects are already clear, including seriously inhibited egg chamber development. The severity of these effects increase sharply with age till between 5 and 10 days a stabilization of the pathological picture slows down the overall degeneration process which results in a totally dystrophic ovary.

3.0 kR

During the first days of adult life a serious pathological situation gradually becomes stabilized into a slowly proceding degeneration process of the entire ovary.

4.3.4. *Development of radiation syndrome in ovaries following irradiation with relatively high doses*

A conspicuous element in the histopathological reaction of ovaries to irradiation with relatively high doses (6–9 kR) is the continuity with pathological patterns of ovarial cell populations already described.

Egg chamber and ovary development is regressive and contributes to serious dystrophy of the ovaries. Determination of egg chamber development is hardly possible because of profound changes in chromatin appearance of trophocyte nuclei. Already more or less developed egg chambers which were irradiated 1–3 days prior to emergence show an abnormal trophocyte chromatin structure of coarse granules connected with filaments which sometimes are locally broadened. This chromatin pattern is classified as S1, sometimes S2/S1, based on the definitions given earlier (THEUNISSEN, 1973a, 1976). Behind the first egg chamber a second one is sometimes present, mostly an S1 chamber. In table 12 the results are summarized of egg chamber development assessed in ovaries irradiated with 6, 7, 8 and 9 kR 1, 2 and 3 days prior to emergence.

As dose effects on the general histopathological pattern are relatively small (fig. 62) it seems to be appropriate to describe the reaction of important elements briefly, according to their post-irradiation period.

1 day p.i.

In ovaries of 1 day p.i. the first egg chambers show stages of S2/S1 after 6 kR treatment (fig. 63). With increasing dose irregulatities in shape and size as well as stagnation in the separation of egg chamber and germarium become more distinct. Trophocyte cytoplasm is vacuolated in egg chambers of the highest dose group. The follicle epithelium is one of the first reacting tissues, as has

TABLE 12. Average egg chamber development in newly emerged females irradiated with various doses of hard X-rays 1–3 days prior to emergence.

	first egg chamber[2]			second egg chamber[2]			ovary		
dose[1]	1[3]	2	3	1	2	3	1	2	3
6	1.5	1.0	1.0	0.3	—	—	1.8	1.0	1.0
7	1.0	1.0	1.0	0.7	—	—	1.7	1.0	1.0
8	1.0	1.0	1.0	—	—	—	1.0	1.0	1.0
9	1.0	1.0	1.0	0.7	—	—	1.7	1.0	1.0

[1] dose in kR
[2] see legend table 6 (page 75)
[3] days between irradiation and emergence
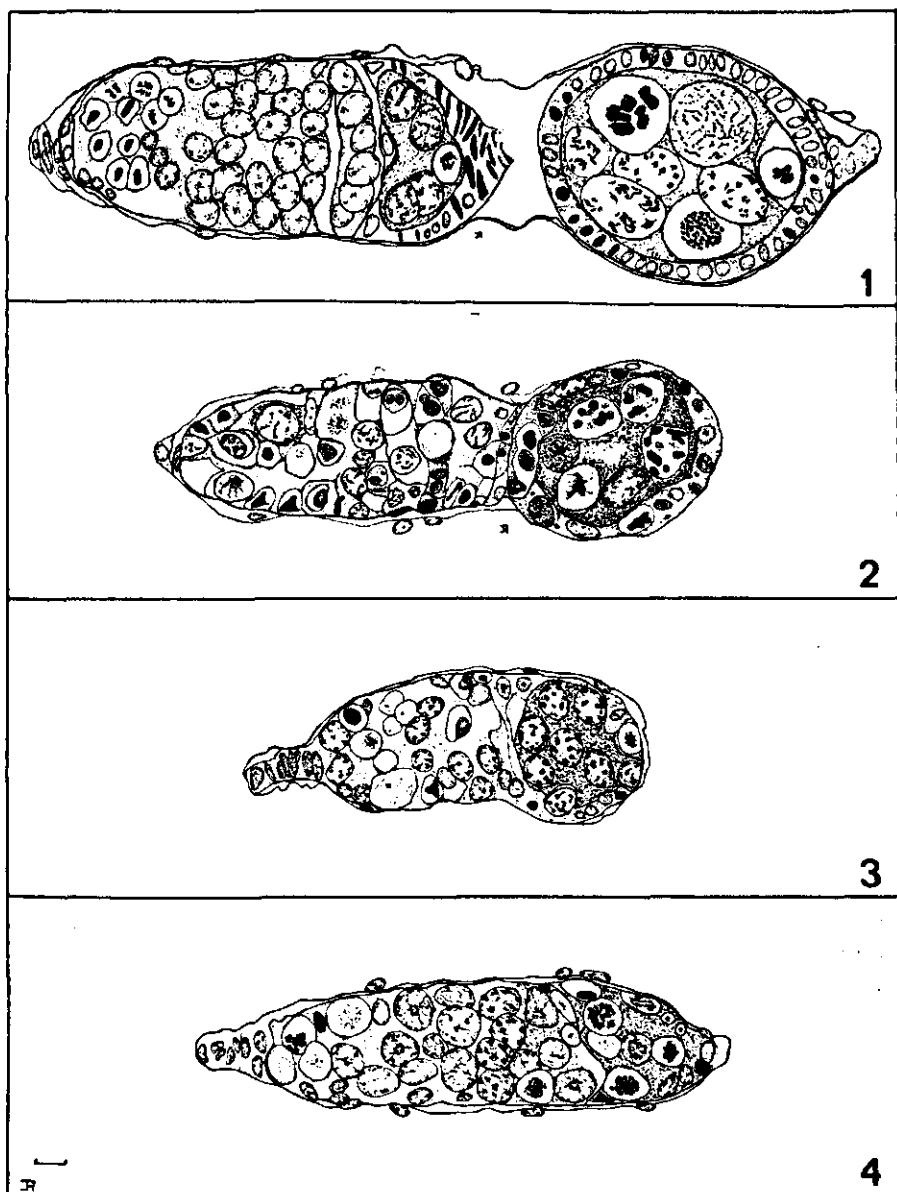
FIG. 62. Ovarioles of just emerged flies irradiated with various doses of hard X-rays 3 days earlier.
1. 0 kR   2. 3.0 kR   3. 6.0 kR   4. 9.0 kR
Note the differences in pathological appearance caused by each added dose of 3 kR. Compare with figs 35, 37 and 40. Bar = 10 μ.
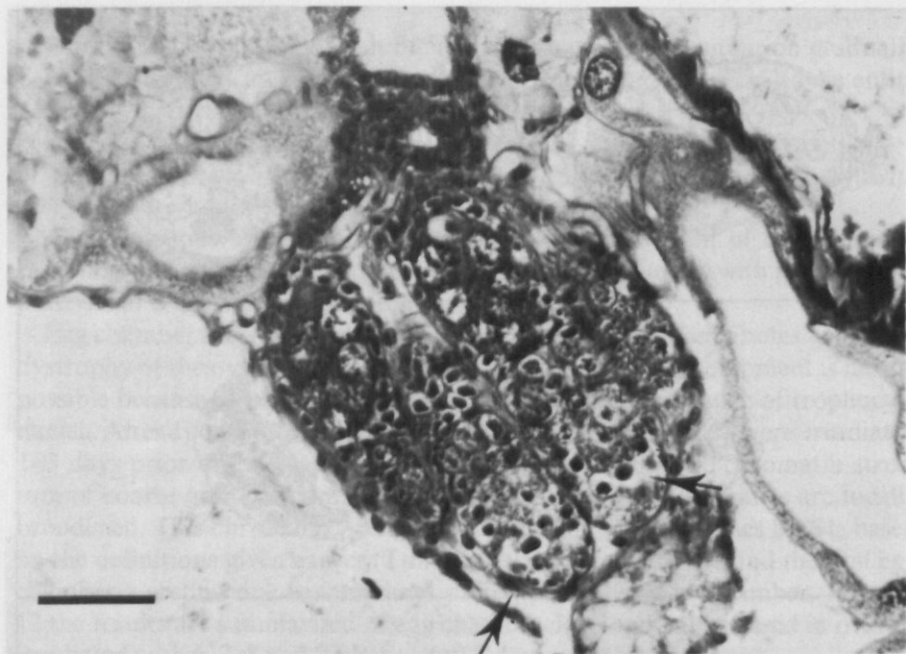
FIG. 63–66. Series of low magnification photographs of ovaries irradiated with relatively high doses (6–9 kR) of hard X-rays. All same magnification, see fig. 67.
FIG. 63. Ovary of newly emerged adult 1 day after irradiation with 6 kR. Swollen follicle epithelia of first egg chambers. Chromatokinetic degeneration in germaria (arrows). Bar = 50 μ.

been established earlier. These cells often show swelling and chromatokinetic degeneration at 6 kR. At higher doses these symptoms are more severe including karyorrhexis, local cytolysis, acute dysplasia and metaplasia and other abnormalities.

In the germaria of 6 kR irradiated ovaries a rather normal looking population of 'concentrated' and 'diffuse' oogonia is present with localized degeneration symptoms like chromatolysis and cytolysis. At increasing dose these symptoms become more severe and also hyperchromatosis, chromatokinetic degeneration, swelling and karyorrhexis occur. After treatment with 8 kR and more the 'concentrated' oogonia are absent, contrary to the 'diffuse' ones which are situated in the basal part of the germarium which shows remarkably few symptoms (fig. 64).

2 days p.i.

At 2 days p.i. degeneration symptoms in first egg chambers are more distinct, especially in the follicle epithelium. The latter shows a more severe pathological picture with increasing dose, involving general desorganization, varying thickness, chromatokinetic degeneration, widespread cytolysis, an indistinct

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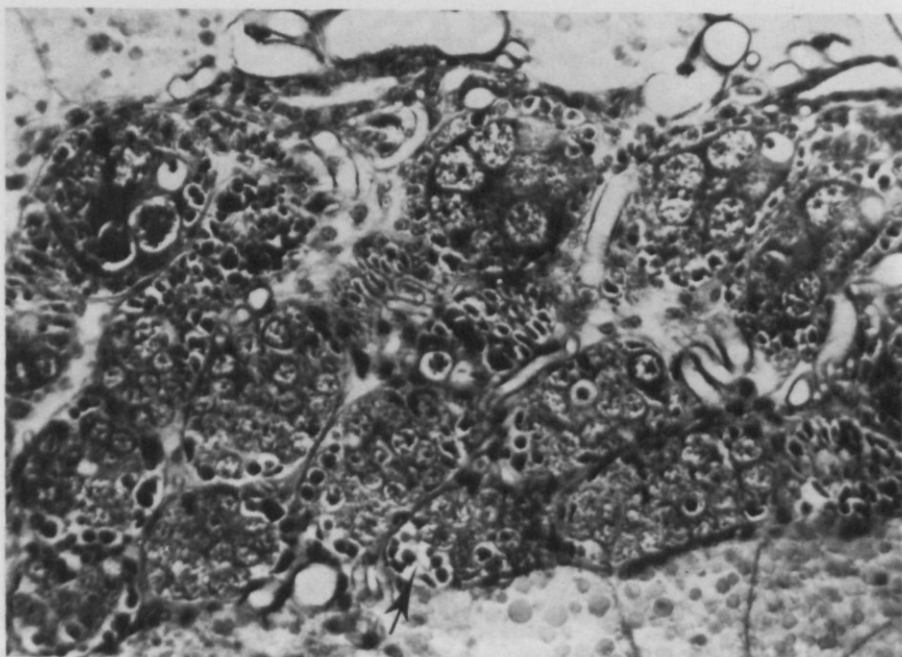
FIG. 64. Newly emerged adult, treated 1 day earlier with 9 kR. Similar but more severe pathological reactions as in fig. 63. Shape of first egg chambers is sometimes already distorted. Swelling and some signs of acute dysplasia of the follicle epithelium. In the germaria chromatokinetic degeneration (arrow). The size of the ovary is individually determined and not yet influenced significantly by the treatment at this post-irradiated time. Comparison with fig. 63 illustrates the individual variability in this respect.

border with the trophocytes, acute metaplasia or clear disappearance of epithelium and acute dysplasia.

Cytolysis in the germaria increases, leaving cavities which contain hyperchromatic, pycnotic and chromatokinetic cells and structures. Chromatolysis and karyorrhexis become increasingly rare following 6 kR irradiation. At increasing doses 'concentrated' oogonia are not observed anymore, only 'diffuse' ones which often contain filamentous chromatin structures, possibly indicating attempts to resume division activity. The cavities become larger at higher doses.

3 days p.i.

Ovaries of 3 days p.i. show first egg chambers which are still a part of their germaria (fig. 62, 3 and 4), which may contain two egg chambers. The trophocyte cytoplasm and chromatin reveal signs of dissolution. The egg chambers can be slightly angular and variable in shape as if they give way to the pressure of the surrounding tissues (fig. 65). The follicle epithelium changes in structure by the disappearance of radial cell membranes, into a light, structureless
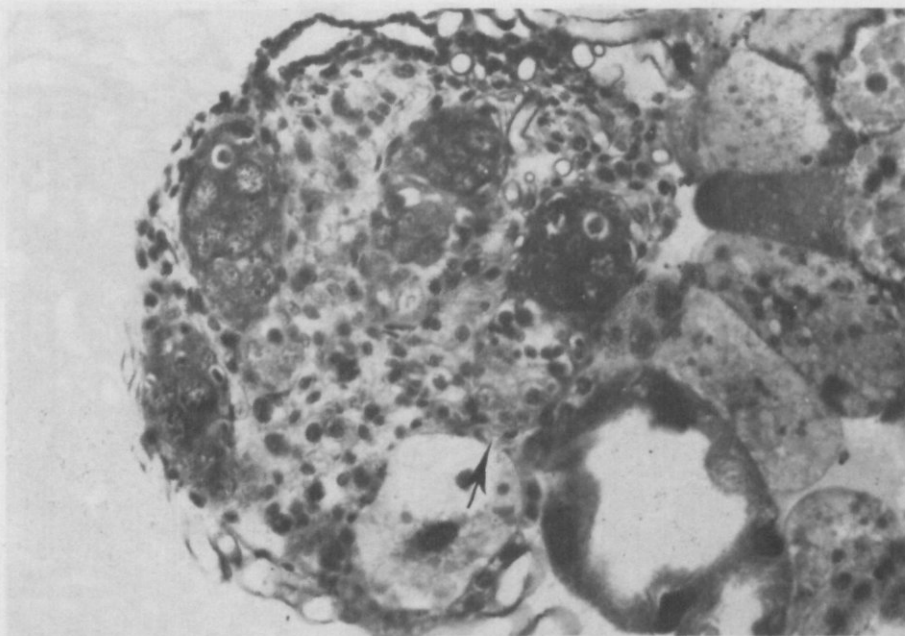
FIG. 65. Ovary of newly emerged adult, 3 days after treatment with 6 kR. The first egg chambers are conspicuous partly because of disappearance of oogonia from the germaria (arrow). Chromatokinetic degeneration, pycnosis and cytolysis are common signs of general necrotic changes in the germarium. Metaplastic changes in the follicle epithelium of the first egg chambers are evident.

layer which also may disappear entirely. After 9 kR irradiation the egg chambers have almost disappeared (fig. 62, 4). The trophocyte cytoplasm is dissolved, the chromatin shows loss of structure and the remnants of these chambers lie close to the germarium. The follicle epithelium still shows cells in division after irradiation with 6 kR in spite of its pathological condition.

In the germaria of flies of all dose groups the severity of the symptoms mentioned gradually increases with dose. 'Diffuse' oogonia are numerous (fig. 66), 'concentrated' ones are absent. Prophase stages of mitotically active cells are observed.

6 days p.i.

The ovaries show egg chambers which cannot be classified anymore regarding their development. Trophocyte cytoplasm stains irregularly, contains vacuoles and shows lytic processes. Their nuclei are indistincly bordered and the chromatin seems to coagulate and to lose structure prior to pycnosis. The egg chambers are highly variable in shape. They are often absent in ovaries treated with 8 kR and totally absent in those of the 9 kR group.

Cytolysis of both somatic and germinal cells has very much increased. The number of both normal and pathological cells seems to decrease quickly and
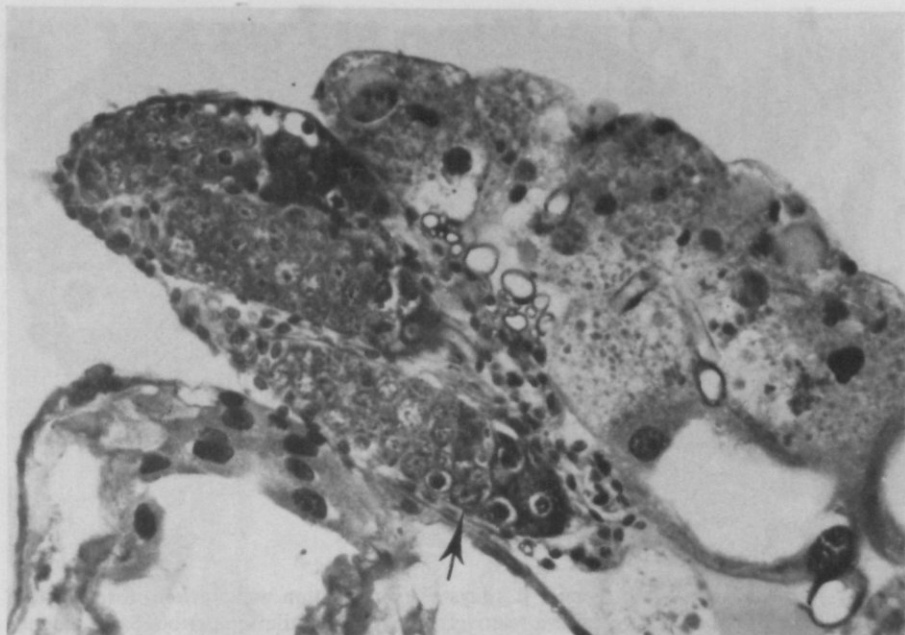
FIG. 66. Ovary of newly emerged adult, 3 days after treatment with 9 kR. The first egg chambers are hardly developed and remain within the germaria. The cyst which was due to become the second egg chamber (arrow) is sometimes visible between the first egg chamber and the germarium.

without excessive display of pathological abnormalities. All types of pathological reactions mentioned can be found but never comprising entire cell populations. Except for the 'diffuse' oogonia, the germinal cells die individually and each in its own way. The 'diffuse' type also decreases in numbers but does hardly show any symptoms. These cells are still present in germaria of flies of all dose groups.

15 days p.i.

In ovaries of the 6 kR group some remnants of the first egg chamber are still observed (fig. 67). In those of other dose groups egg chambers are absent (figs. 68–70).

In all dose groups germaria have disappeared from the ovaries in a different degree according to the dose received. In the remaining germaria 'diffuse' oogonia are still present. In cavities pathological cells or cell debris show the symptoms already mentioned. General desorganization is very far advanced.

4.3.5. Discussion

Because of the gradual development of histopathological symptoms with increasing age in irradiated ovaries and the individual variability in their ap-

FIG. 67–70. Ovaries of adult females, 15 days after irradiation with various doses of hard X-rays, illustrating dose effects after a relatively long post-irradiation period. Same magnification as fig. 63.

FIG. 67. Dose: 6 kR. Clearly visible but highly abnormal first egg chambers and germaria.

FIG. 68. Dose: 7 kR. Ovary strongly dystrophic. Some highly abnormal germinal structures left. Often it is impossible to distinguish between a former egg chamber and a germarium.

FIG. 69. Dose: 8 kR. Similar situation as in fig. 68.

FIG. 70. Dose: 9 kR. Hardly any remnant left of egg chambers or germaria. Picture of nearly total dystrophy and castration.

pearance, these symptoms cannot be fitted into a quantitative system for radiation damage assessment. Hence, the 'degree of pathology' cannot be superimposed on the system which is used to determine egg chamber development.
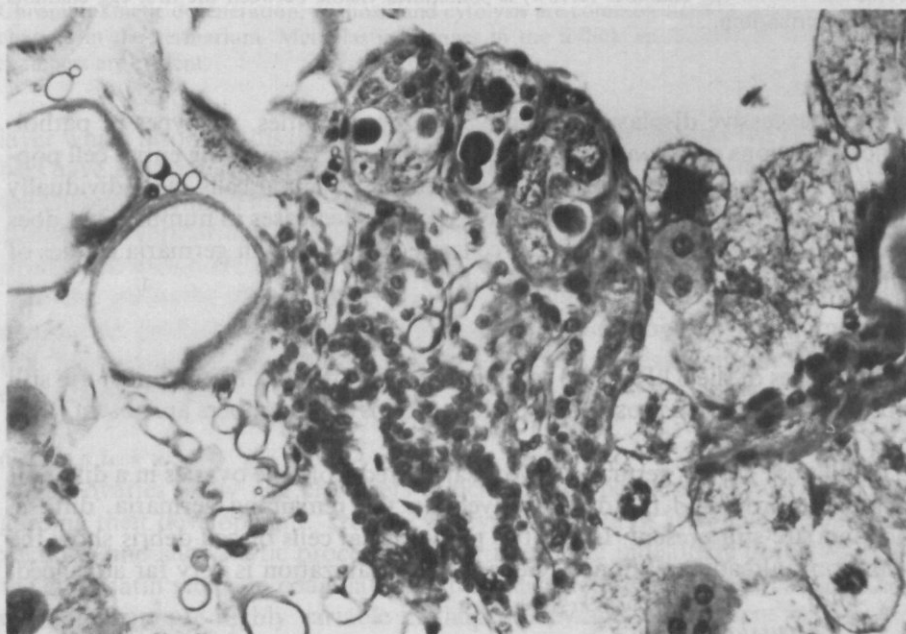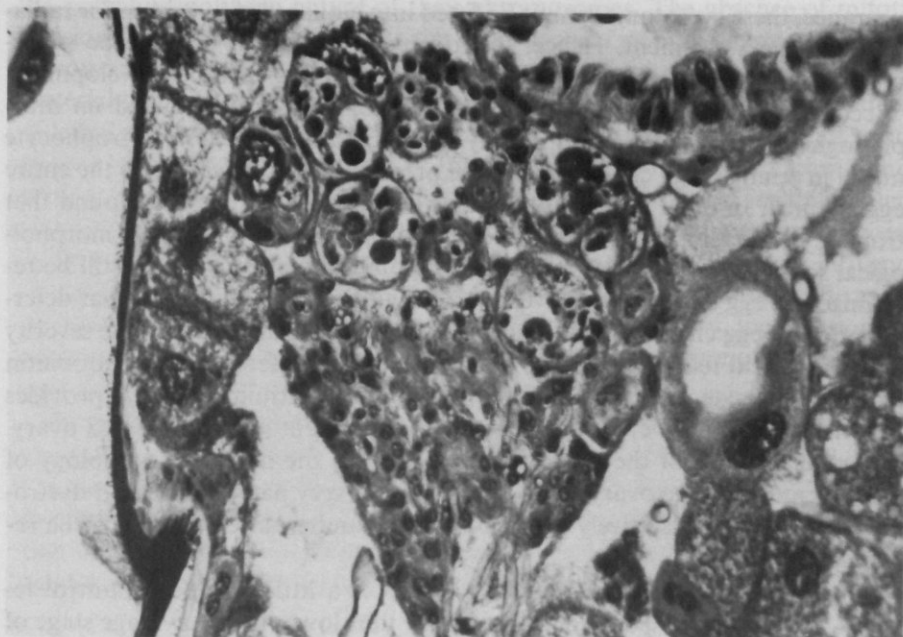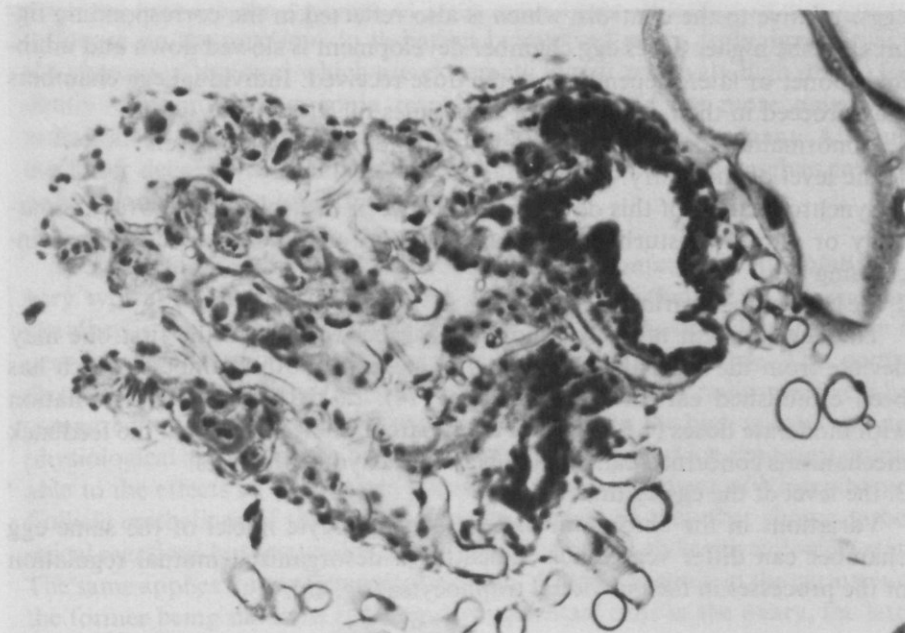
According to this system the stage of development is determined on morphological criteria which refer mainly to the chromatin pattern of trophocyte nuclei in younger egg chambers and the proportion of oocyte versus the entire egg chamber in older ones (THEUNISSEN, 1973a, b). As it has been found that trophocyte nuclei are the most radioresistant part of the ovary in a morphological sense, the stage of development of young egg chambers can still be recognized in egg chambers in a very pathological state. This means that determination of egg chamber development does not take into account the severity of pathological reactions as long as these do not interfere with the chromatin of the trophocyte nuclei. Therefore, this way of determination only provides information on the average egg chamber development in the irradiated ovary. The independence of the trophocyte nuclei from the degree of pathology of other elements of the ovary is the reason that in very pathological and dystrophic ovaries still a relatively 'advanced' egg chamber development can be recorded.

At lower doses egg chamber development is a little retarded. Control females younger than 10 days deposited eggs, thus lowering the average stage of first egg chamber development found in 10 days old females. In the 0.5 kR group the eggs were not yet deposited, which accounts for the higher average stage of development (table 9). Females of the 1.0 kR group deposited fewer eggs, relative to the controls, which is also reflected in the corresponding figures. At the higher doses egg chamber development is slowed down and inhibited sooner or later, depending on the dose received. Individual egg chambers may proceed in their development sometimes till S10.

Abnormalities in egg chamber development are found at 3 levels:
1. the level of the ovary.

Synchronization of this development between ovarioles in the ovary is partially or entirely disturbed, especially at relatively moderate doses and increasing age.
2. the level of the ovariole.

The development of the second egg chamber relative to the first one may deviate from the normal combination of stages of both chambers, which has been established earlier (THEUNISSEN, 1974). In particular after irradiation with moderate doses (1.5 kR) such an apparent interference with the feedback mechanisms concerned can already be found in young females.
3. the level of the egg chamber.

Variations in the chromatin pattern of trophocyte nuclei of the same egg chamber can differ very much indicating a desorganized mutual regulation of the processes in the individual trophocytes (fig. 48).

The radioresistance of the trophocyte nuclei can be due to a combination of factors: their mitotic inactivity, the syncytial nature of their population and

the polyploid/polytene nature of their chromosomes. The absence of mitotic divisions in trophocytes prevents the expression of possible damage to chromosomes in metaphase-anaphase stages as breakage or uneven distribution of material to daughter cells. Hence, no selection takes place by way of the effects of lethal mutations or structural chromosomal lesions. Whether or not endomitotic activities still take place in trophocyte nuclei after irradiation is not always clear. Where no development is observed they are obviously inhibited. At the lower doses they seem to be able to repair possible damage and proceed in their activity. This process of repair may originate partly from the influence of the syncytium, as has been discussed earlier for the radiation effects on primary and secondary spermatogonia. The correction of local lesions in chromosomes may also be facilitated by the multiple presence of any damaged part whether in polyploid or in polytene chromosomes.

As polytene chromosomes have been observed in germarial germinal cells (0 days, 1.5 kR) endomitotic activity must take place in pupal germaria, which has also been reported earlier (THEUNISSEN, 1973b). Relative to both normal development and repair of chromosomal damage and subsequent continuation of egg chamber development a more detailed investigation of early endomitotic activities in the pupal ovary is needed and warranted.

The apical part of the germarium shows pathological symptoms considerably earlier after irradiation than the basal part where 'diffuse' oogonia are situated. This difference in reaction is probably based on the nature of the intercellular relations between the oogonia of both main types. Whether or not synctial influence on the reactions to radiation is involved seems to be imperative to the chances of survival which are extremely low at relatively high doses. Evidently 'concentrated' oogonia react individually and are more sensitive to radiation whereas 'diffuse' oogonia are much more radioresistant. Although the latter decrease in numbers they are able to maintain themselves in small populations during a long time.

The extremely pathological reaction of some somatic cell populations is very typical of the irradiated ovary. The follicle epithelium of the first egg chamber is the strongest reacting tissue of the entire ovary. It appears to be very sensitive to radiation, at least in a histopathological sense. This contradicts the general tendency of somatic cells being far less radiosensitive when compared to germinal ones. An explanation might be the high mitotic rate and physiological activity of the normal follicle epithelium which renders it vulnerable to the effects of irradiation, according to the statement of Vintemberger. Follicle epithelium of the second egg chamber or S1 chamber shows pathological reactions but less severe, probably according to their physiological state. The same applies for the 'concentrated' and 'diffuse' oogonia in the germarium, the former being the most radiosensitive germinal cells in the ovary, the latter being relatively little radiosensitive.

In spite of the strong pathological reaction of the follicle epithelium it

remains capable to perform certain functions when its most common ones i.e. covering, protecting, supporting and feeding have ceased to operate. The infiltration of a population of degenerating trophocytes by cells from a very pathological follicle epithelium to perform an obviously physiological function is quite an achievement under these circumstances. This function of oosorption and destruction is possibly triggered when the degeneration of the trophocytes has reached a certain level. Obviously the internal communication and organization remains at least partly intact even when the ovary as a whole is very dystrophic and its constituing elements are severely degenerating. Distortion of synchronization in egg chamber formation or of the mutual relations between tissues only reflects a part of the picture of the internal rellations between cell populations as influenced by irradiation. Some signals seem to be powerful enough to activate very pathological cells to perform a duty which mostly remains hidden in normal situations. This process needs more detailed investigation as it is highly important to the general knowledge on intercellular relations and communications.

After irradiation with relatively high doses (between 4.0 and 9.0 kR) general dystrophy is found in the ovaries of all ages and dose groups. The degree of dystrophy depends on the dose received. An irreversibly regressive and pathological development leads to a total elimination of all germinal elements from the ovaries, leaving shrunken and distorted covering tissues: a packing without contents.

The quickly spreading desorganization of egg chambers and germaria is somewhat camouflaged by the widespread and fast proceeding cytolysis which removes cell debris etc within a short time (figs. 71–74) leaving only cavities which disappear by the reduction in size of the entire ovary and its constituing parts. Direct cytolysis of cells and cell debris without preceding chromatokinesis and pycnosis seems to take place at higher rate and more generally at increasing doses and post-irradiation period. This results in a 'clean' degeneration in spite of the variety of pathological symptoms observed. This conspicuous feature of the general pathological pattern has also been established and consecutively discussed in testicular cell populations following irradiation at higher doses (page 62).

4.4. QUANTITATIVE APPROACH TO EVALUATION OF RADIATION EFFECTS

Study of the histopathological effects of irradiation of gonads reveals patterns which present a large number of symptoms in varying degree of severity relative to the dose received. Examination of a sufficiently large number of comparable preparations permits general statements to be made on these radiation effects. This qualitative approach is suitable to investigate pathological processes and their expression in the cell populations concerned. For the purpose of a quick comparative assessment of radiation damage quantitative methods would be useful, provided the appropriate criteria would be
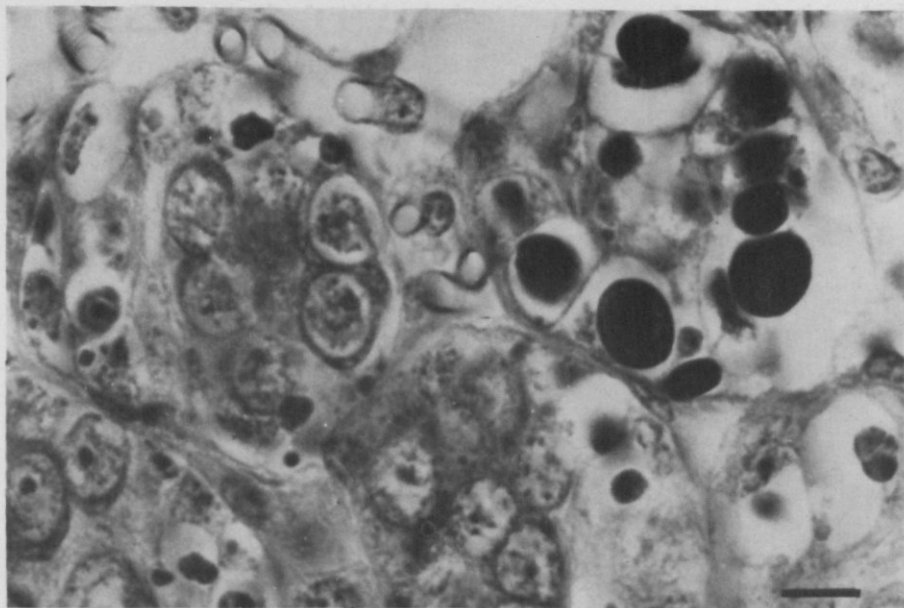
FIG. 71–74. Histopathological effects of treatment with higher doses on egg chambers and germaria in newly emerged adults. Bar = 10 μ.
FIG. 71. Extensive chromatokinetic degeneration in germaria. Dose 5 kR.
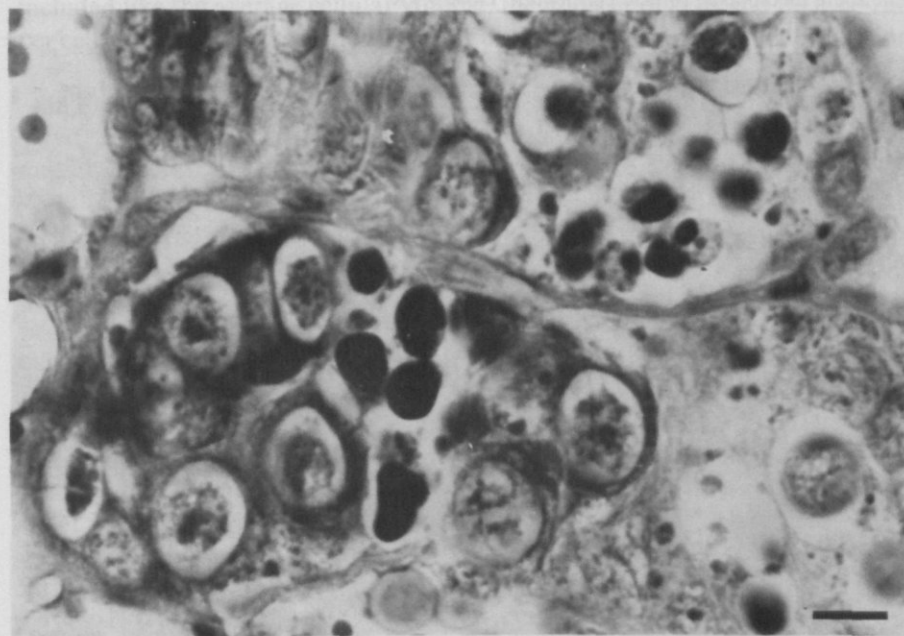
FIG. 72. Considerable swelling of oogonıa which also show increased cytoplasmic staining. Chromatokinetic degeneration, cytolysis, pycnosis and desorganization are also observed. Dose 5 kR.
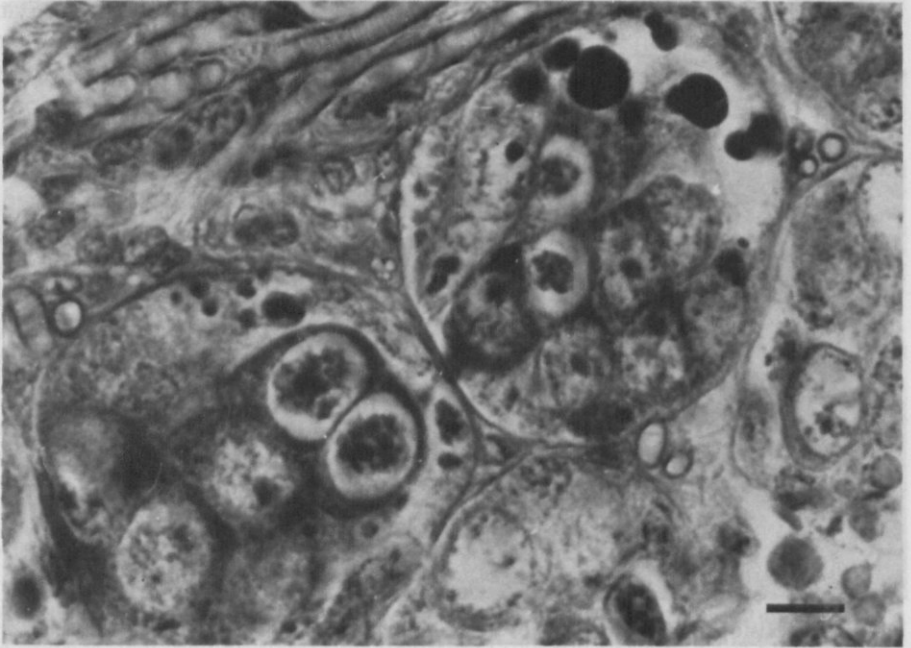
FIG. 73. Young egg chambers with swollen trophocytes and degenerating, swollen, dysplastic and acute metaplastic follicle epithelium. Dose 5 kR.

FIG. 74. Remnants of young egg chamber (arrow) with nearly disappeared follicle epithelium. Dose 5 kR.

available. These criteria should be derived from measurable properties which change because of irradiation.

A conspicuous feature in both irradiated and untreated testes is the decrease in germinal cell populations at increasing age. Irradiation contributes to this decrease at a rate which roughly depends on the dose received. This property could provide a clue to a quantitative approach of radiation effects. A short description of such a method will be given.

Histological research of untreated testes provides criteria to identify the various germinal cell types (THEUNISSEN, 1976) and their pathological forms in irradiated testes. Therefore, in a given testis the location of each cell population can be indicated. They are situated in zones which, though irregular and sometimes indistinct, can be outlined. In such a way cysts of cells in meiotic division, the apical cell and the central cavity can also be outlined. Using a good microscope with drawing tube, a kind of map of each section of the testis, its zones of germinal cell types and other structures can be drawn at a fixed magnification. This map shows the borders of the various cell populations within the limits of the internal testicular space, excluding the testicular sheath. The surface of the various zones in each section can be measured by means of a planimeter and totalled per cell type or structure. Given a certain thickness of the sections an approximate figure for the volume per cell type is obtained. In this way changes in the relative volume of the different cell populations are recorded. Besides the volume of these populations, other parameters can be included like the average cellular volume, the number of cells and their density per cell type. These factors, however, are excluded here from further discussion.

In exploring this method testes of 0, 10, 20 and 30 days old flies were measured. Suitable serial sections of testes were selected, drawn section by section, measured and the results calculated. These results are summarized in table 13.

The small number of testes measured excludes a representative and statistically reliable picture. The point is to demonstrate here a possibility to acquire quantitative data on normal and experimentally treated testes with a fair reproducibility. When drawn maps were repeatedly measured and their results calculated independently the deviations from the figures obtained first were less than 1%. Although the figures are not representative they do not contradict the existing histological data and they could well represent real situations. They show a stable population of primary spermatogonia which increases relatively in volume as does the apical cell in testes which become smaller at older age. The population of secondary spermatogonia and most other cell populations decrease gradually with increasing age. The central cavity becomes larger in accordance with its changing function as a vesicula seminalis in the basal part of the testis. In the irradiated testis the swelling of the primary spermatogonia and apical cavities is reflected in a higher relative volume. The stagnation in meiosis and the morphological identity of 'diffuse' secondary spermatogonia and interphase primary spermatocytes in irradiated testes is dem-

TABLE 13. Volumes of zones of cell types and structures as a percentage of the internal testicular space.

cell type[1]	age[2]				
	0		10	20	30
	i[3]	n[4]			
p.spg	11.4	2.2	3.3	4.2	9.8
s.spg	48.8	23.8	11.8	10.2	2.9
p.spc.	—[5]	8.2	5.2	3.4	—
s.spc.	0.9	1.0	1.9	0.9	—
e.spt	3.1	5.5	2.1	1.0	—
i/t.spt	8.0	22.5	14.5	3.2	4.8
apical cell	0.09	0.02	0.05	0.06	0.20
central cavity	24.2	35.3	59.8	77.1	77.5

[1] including apical cell and central cavity
[2] age in days of adult life
[3] testis irradiated with 3 kR of hard X-rays 3 days earlier
[4] not irradiated testis of newly emerged adult
[5] — = not present or identifiable

onstrated by the large population of secondary spermatogonia. The trapped secondary spermatocytes do not change much when compared to the untreated testis. The quick pathological reaction of the spermatids is also evident in the figures.

The usefulness of this essentially attractive method is seriously reduced by the time consuming and elaborate collecting of the data. Especially drawing and measuring the surfaces takes a long time (about 2–3 days/testis). Calculations can be carried out quickly by using standardized formulae. When proper facilities would be available, however, the time consuming steps of drawing and measuring could be shortened considerably. These facilities are computerized instruments designed to project maps, blue prints etc on a screen. The investigator can modify the projected item by adding a drawing or changing parts on the screen followed by a calculation of the consequences of the modification by the computer. When sections of testes are projected, zones of cell types could be drawn, integrated and used for the calculations of the data immediately after the last section has been drawn. Large series of comparable preparations could be studied this way, thus making the method practical for routine evaluations of radiation damage or effects of other pathogenic factors acting on the testicular cell populations.

4.5. GENERAL DISCUSSION

Relatively few studies have been entirely devoted to histopathological radiation effects in insects. Most accounts on irradiation are based on genetical

or physiological criteria and a number of them report also histopathological data. As it is hardly possible to compare in detail all relevant papers to the findings with *H. antiqua* only papers concerning radiation histopathology are mentioned and those referring to genetical or physiological research are omitted, save for some exceptions. Histopathological radiation effects on testes, ovaries and other tissues and organs will be briefly considered.

In early radiation studies genetical criteria or a mixture of genetical, physiological and occasionally histopathological criteria were used; for instance by WHITING, 1940; FRITZ-NIGGLI, 1952, 1958; GROSCH and SULLIVAN, 1954; AMY, 1955; ANNAN, 1955; KAUFMAN and WASSERMAN, 1957; WELSHONS and RUSSELL, 1957 and ALEXANDER, 1958.

Testes

After irradiation of Drosophila pupae with doses varying from 6–80 kR no histopathological reactions of the germinal cells were observed by FRITZ-NIGGLI (1952). Irradiation of Drosophila larvae 72 hours old with 3 kR resulted in degeneration of late spermatogonia and the subsequent formation of a layer of necrotic cells between spermatogonia and primary spermatocytes (BOURGIN et al., 1956), which is very similar to the picture of apical cavities filled with degenerating spermatogonia in *H. antiqua*. Recovery of the spermatogonial population became apparent at about 3 days p.i. Mitotic activity showed a similar delay as in *H. antiqua*. BOURGIN et al. (1956) recognized two categories of effects: the immediately and the later appearing ones. The examples given illustrate the use of different criteria for both categories; cyto- and histopathological and physiological respectively. These different effects are probably only parts of a continuous series of developing pathological processes.

Irradiation of pupae of *Ceratitis capitata* prior to emergence with various doses of gamma-rays (2–9 kR) results within 4 days in the complete disappearance of spermatids and in desorganized premeiotic cell populations (CAUSSE et al., 1968). These workers concluded that a quick differentiation of spermatids to sperm cells accounted for the rapid spermatid disappearance. This conclusion, however, is not substantiated by their data, nor their conclusion that at doses of about 2–3 kR the spermatocytes are partly capable of a normal development. As far as can be concluded from their brief descriptions of the reactions of *C. capitata* to gamma irradiation these are in fact very similar to those found in *H. antiqua*, in spite of a possibly different, histological processing, which was unfortunately not mentioned.

A dose of 10 kR of gamma-rays administered 2 days prior to or 2 days after emergence of *Ceratitis capitata* adults (ANWAR at al., 1971) heavily affected spermatogenic processes. Due to different histological techniques a direct comparison is out of the question. Very striking, however, is the quick disappearance of spermatogonia at this dose, which has also been found in the higher dose range (6–9 kR) applied to *H. antiqua*. This fast cytolysis has been discussed earlier (cf. pages 62 and 106) for both male and female germinal cells

of *H. antiqua*. As in *H. antiqua*, the spermatids in *C. capitata* seem to be the most radiosensitive cell types in a histopathological sense. TAEGE (1963) found vacuolization, pycnosis and cytolysis of spermatogonia of *Calliphora erythrocephala* after irradiation with doses of 1–8 kR of X-rays. He also observed amitotic spermatogonial divisions. He presumed a certain independence of differentiation of irradiated spermatocytes towards spermatids from division difficulties of the spermatocyte chromosomes. A hypothesis explaining this phenomenon, which probably also was observed by CAUSSE et al. (1968) in *Ceratitis capitata*, is given by LINDSLEY and GRELL (1969) (cf. page 62).

RIEMANN and THORSON (1969) discussed the radiosensitivity of spermatogonia to X-rays in a comparative study of *Musca domestica, Phormia regina* and *Cochliomyia macellaria*. They used as a criterium the rate of recovery, partial or complete, of the spermatogonial cell populations after 2 weeks as determined hy histological examination. Their descriptions of partially recovering spermatogonial populations present a picture largely similar to what has been found in *H. antiqua*. They refer to the formation of syncytia in absence of typical cysts. Increased coarseness of chromatin following irradiation is a commonly observed feature, in particular in germinal cells. Therefore, the proliferation of somatic cells as assumed by RIEMANN and THORSON (1969) might well represent recovering spermatogonia showing a less organized population as compared to untreated populations.

Despite the different histological techniques used by RIEMANN (1967) in his study óf radiation effects on *Cochliomyia hominivorax* similarities and differences with *H. antiqua* are found. The general pathological picture described is rather similar to that in *H. antiqua* with a number of clear exceptions, for instance:

– contrary to mitotic divisions meiotic activity in *C. hominivorax* seems to be hardly inhibited by treatment with the sterilizing dose of 6.2 kR, or lower doses.

– spermatids do not seem to be affected morphologically by the treatment when irradiated as such, nor functionally relative to their capacity to differentiate into sperm cells.

– sperm cells derived from irradiated preceding germinal cell types show morphological abnormalities.

– irradiation with their respective sterilizing doses seems to cause severe histopathological reactions quicker in *C. hominivorax* than in *H. antiqua*.

Radiosensitivity of spermatogonia of *Anthonomus grandis* is less when compared to the regenerating cells in nidi of the midgut epithelium (RIEMANN and FLINT, 1967). Recovery of the spermatogonial populations occurred up to a dose of 4 kR.

AMERESEKERE et al. (1971) described briefly irradiation effects on testes of *Circulifer tenellus* after treatment with 15 and 20 kR. They found cell depletion, pycnosis of spermatogonia and spermatocytes and sometimes abnormal sperm cells.

High doses were also applied to *Earias insulana* by ASHRAF et al. (1974). An increasing dose range of 5, 15, 30 and 45 kR caused a number of pathological

reactions which were essentially similar but differing in severity according to the received dose. Spermatogonia were found to be most radiosensitive.

Lower doses were used to irradiate larvae of *Plodia interpunctella* (ASHRAFI et al., 1972). Spermatogonia, spermatocytes and spermatids were increasingly radioresistant in this order at the various doses employed.

A most important study of radiation effects on Lepidopteran spermatogenesis has been made by SADO (1963) using *Bombys mori*. He described the normal spermatogenesis, the various germinal cell types and their reactions to irradiation. His results on differential radiosensitivity of various germinal cell types confirm the general trend in similar papers of other authors on *Lepidoptera*. The distinct difference between early and late spermatogonia which are respectively resistant and highly sensitive to radiation is tempting to suspect possible similarities with the reaction of 'diffuse' and 'concentrated' spermatogonia in *H. antiqua* to irradiation. The clear radioresistance of spermatids in Lepidoptera is contrary to spermatid radiosensitivity in many Diptera, which has been discussed earlier (cf. page 63), although exceptions are reported (RIEMANN, 1967).

Ovaries

In the ovary of *Drosophila melanogaster* irradiated with 4 kR KING (1957) found 12 days p.i. pycnosis in trophocyte nuclei, extrusion of trophcytes into oocyte or ovariole, degeneration of entire ovarioles following lysis of egg chambers, abnormalities in egg chamber formation relative to developmental stages and number of trophocytes, fusion of egg chambers, abnormal oocyte orientation and stagnation of chromatin pattern development in trophocyte nuclei. These phenomena have also been observed in *H. antiqua*. KING (1957) does not mention a very sensitive reaction of the follicle epithelium, infiltration of the egg chamber by epithelium cells and hyperplasia as have been observed in *H. antiqua*.

BACCETTI and DE DOMINICIS (1963) concluded that the thickening of chromatin is a symptom of radiation damage, which has been confirmed by the increasing coarseness of chromatin elements at increasing doses in *H. antiqua*. They also found in *Dacus oleae* the sensitivity of the germarial cell populations, abnormalities in egg chamber formation and other features which have also been established in irradiated ovaries of *H. antiqua*, except the reported accumulation of collagen fibrillae around the ovarioles of *D. oleae*. The continuity of histopathological patterns in irradiated testes and ovaries of *H. antiqua* is consistent with their conclusion that irradiation damage in the ovaries of *D. oleae* is quantitatively different according to the received dose but qualitatively similar.

In 26 days old females of *Ceratitis capitata* CAUSSE et al. (1968) observed pathological and abberrantly developed egg chambers following irradiation with 3 kR of gamma-rays 17 days earlier. Irradiation with 2 kR resulted in smaller sized ovaries, apparently without major histopathological symptoms, whereas after 4 kR complete inhibition of pupal ovary development and severe

desorganization and degeneration of the germaria was found.

ERDMAN (1961) noted in irradiated larvae of *Habrobracon juglandis* retarded and inhibited oocyte development, mainly due to failure of trophocyte function which is induced in oogonia a number of cell generations earlier. He also found that the presence of killed undifferentiated germaria stimulates the development of ovarian somatic tissue. This is not observed in *H. antiqua*. Differentiation of young egg chambers from oogonia was inhibited when pupae were irradiated.

Irradiation of 1 day old females of *Circulifer tenellus* with 10 kR of gamma-rays resulted in a retarded development of the ovaries (AMERESEKERE et al., 1971).

Other tissues

Histopathological effects after irradiation of other insect tissues have been studied incidentically. Especially the radiation effects on the midgut of some insects received considerable attention: *Blabera fusca* (MONTREUIL-LANGLOIS, 1960, 1963), *Dacus olae* (BACCETTI et al., 1961), *Ceratitis capitata* and *Chelisoches morio* (LITTLE, 1967), *Anthonomus grandis* (RIEMANN and FLINT, 1967), *Heliothis virescens* (VINSON et al., 1969), *Plodia interpunctella* (ASHRAF et al., 1971) and *Tenebrio* (BROWER and ASHRAF, 1972). Nervous tissue has been little studied. BOURGIN et al. (1956) noted the radiosensitivity of larval brain cells of *Drosophila melanogaster* which became clear at mitosis. Retarded and hypoplastic development of the brain resulted from 3 kR irradiation. They also observed a beginning dystrophic reaction of the ring gland 4–5 days p.i. with 3 kR. BHAKTHAN and NAIR (1972) investigated irradiated flight muscles of *Musca domestica*. GRÉGOIRE (1955) examined the reactions of hemocytes to X-rays of *Locusta migratoria, Carausius morosus* and *Periplaneta americana*.

No distinct histopathological reactions in *H. antiqua* were found, in accessory glands, hemocytes, nervous and muscular tissues, adipose tissue, and oenocytes. Irradiated flies, especially females, clearly had more fat cells with a high content. As far as could be detected no identifiable physiological functions in *H. antiqua* are disturbed by irradiation, except those connected with reproduction.

Physiological irradiation effects have been studied more frequently and only some examples can be mentioned here. Radiation induced effects have been investigated on pheromone production in *Tenebrio molitor* (MENON and NAIR, 1972), on neurosecretion in *Gryllus domesticus* (THEODORESCU and NOVAK, 1970), on ecdysis (JOLY and BIELLMANN, 1958) and wing development, integument pigmentation and growth (BIELLMANN, 1963) in *Locusta migratoria*, on physical and chemical properties of the hemolymph in *Galleria mellonella* (MESTRES and LAVIOLETTE, 1968), *Apis mellifera* (RICHARDSON and MYSER, 1975) and *Haematobia irritans* (MAYER et al., 1975), and on excretion in *Periplaneta americana* (WHARTON and WHARTON, 1961).

When commenting on histopathological studies of radiation effects in insects some points seem to be important because they influence efficient scien-

tific information and maximum quality of the presentation.

– Some authors do not define or describe adequately the germinal or other cell types they are referring to in their papers. This matter is important because it decreases their scientific value and reliability considerably and prevents a proper understanding. Identification of the cell types and populations concerned is left to the imagination of the reader.

– Histopathological terms are usually not defined, resulting in perhaps colourful but unscientific denotations of pathological situations which require interpretation by the reader. Examples are the use of the term 'cellular atrophy' (AMERESEKERE et al., 1971) which presumably refers to cellular necrosis; 'liquefaction' (ASHRAFI et al., 1972) perhaps denoting cytolysis; 'contraction of secondary spermatocytes' (ASHRAFI et al., 1974) which term is quite obscure beyond guess.

– A good quality of the illustrations contributes largely to the understanding of the treated subject.

Effects of dose

Dose effects assessed by comparing ovaries of the same age and post-irradiation period and subjected to different doses are recognized by different degrees of severity of pathological symptoms or disturbances of egg chamber structure. A marked difference between the development of pathological reactions of ovaries and testes is found in the effects of the doses. These are most distinct in ovaries of older females. With increasing age the differences due to the dose received become increasingly clear resulting in ovaries with separate pathological conditions in relation to the dose group. Conversely, in testes dose effects are most clear in young flies because of the differential radiosensitivity of their germinal cell types. Depending on the dose received the pathological condition of the germinal testicular cell populations reach the same point sooner or later. Although in testes the morphological dose effects are trivial at relatively high doses, they are distinct at lower doses, including the sterilizing one, especially in younger adults.

The large individual variability of the histopathological reactions of cells and cell populations to irradiation (figs. 75–77) illustrates the probability character of the kind and severity of the inflicted damage. At increasing doses the probability of receiving an essential or acute lethal lesion also increases. Properties of the cell population or the individual cell which influence this lesion in either a protecting or a sensibilizing sense further complicate this reaction pattern. These properties are genetically determined but expressed physiologically by internal and external factors acting in and upon the individual organism.
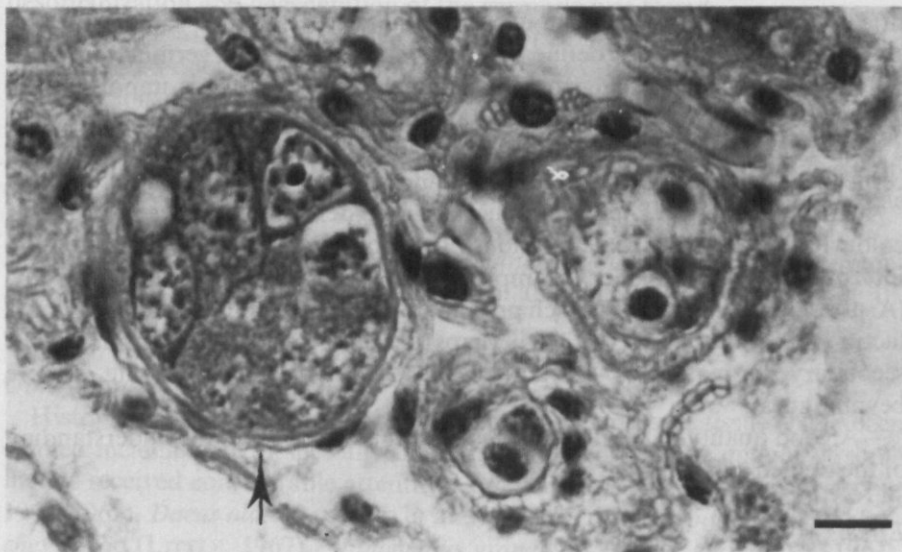
FIG. 75–77. Illustration of effect of individual variability on pathological reaction to irradiation. Stabilization of the pathological condition of the ovary, as reflected by symptoms, obviously prevented their continued quick lytic breakdown. Bar $= 10\ \mu$.

FIG. 75. Remnants of young egg chamber (arrow) in ovary of 30 days old female. In most comparable females such structures are already absent at 15 days. Dose 3 kR, 33 days p.i.
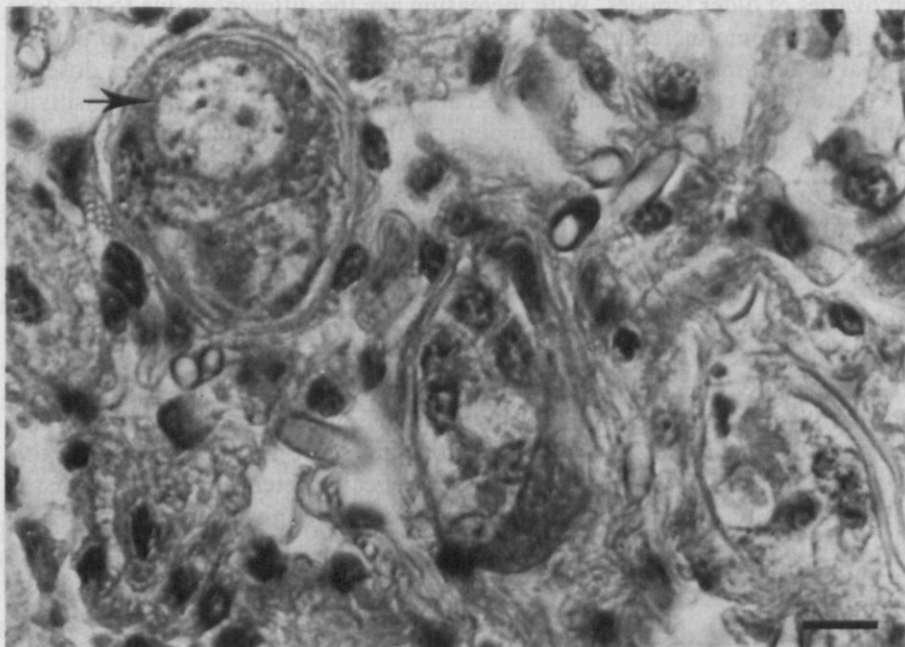
FIG. 76. Swollen trophocyte (arrow) in ovary of 30 days old female, 33 days after irradiation with 3 kR.

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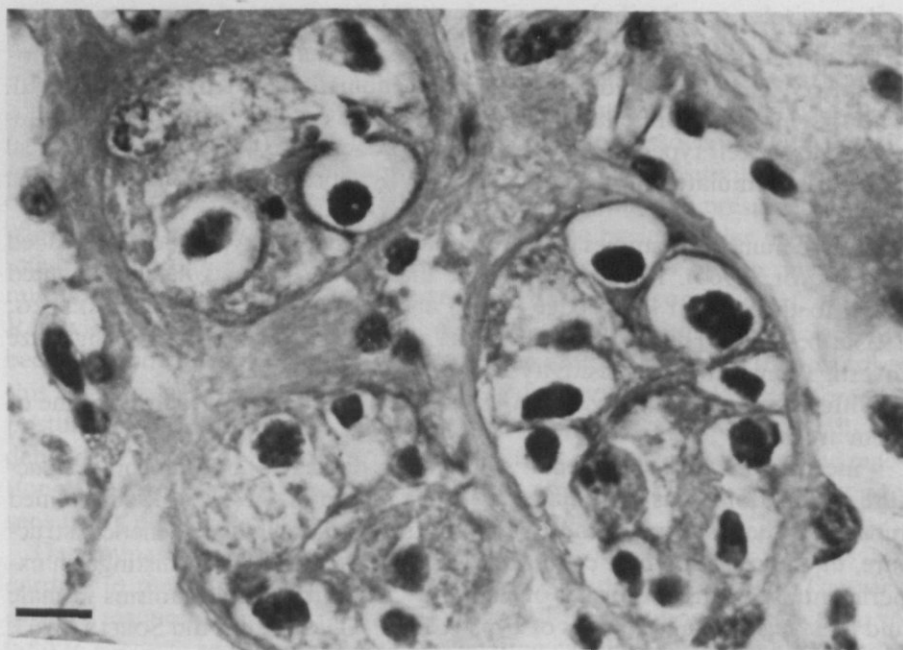
FIG. 77. Pycnotic remnants of egg chambers and germaria in 30 days old female, 33 days after irradiation with 3 kR.

Dose effects are reflected by:

1. the speed of development of pathological reactions. For instance the change of structure of the follicle epithelium takes place earlier at increasing dose:

dose (kR):	2	3	7
adult age (days):	10	5	0
post-irradiation (days):	13	8	3

2. the absence of symptoms which is observed in ovaries which are less heavily irradiated. For instance infiltration of egg chambers by follicle epithelium cells and hyperplasia and anaplasia of follicle epithelium. These processes probably require time in addition to a certain minimum state of health of the cells involved.

3. the presence of symptoms at a certain dose which are absent in ovaries treated with lower doses. For instance the direct cytolysis which already has been discussed and the quick reaction of the chromatin of trophocyte nuclei at higher doses, as reflected by their coarse chromatin granules, are commonly observed. Once in this condition the trophocyte nucleus is able to maintain its existence unchanged during a relatively long period, often surviving by far the cytoplasm.

Radiosensitivity

An intrigueing problem in radiobiology is the matter of differential radio-sensitivity. The nature and causes of this phenomenon are still largely ignored. The first problem presenting itself is by which criteria the range of radio-sensitivity of cell types is measured. An approach based on histopathological criteria is formulated in this study. Usually genetical or physiological criteria are used.

A large number of studies on differential radiosensitivity has been published (cf. MANDL, 1964). Research on some mammalian species has contributed much to the present knowledge (FOGG and COWING, 1951, 1952; OAKBERG, 1955a, b, 1968). The reviews of DUCOFF (1972) and GROSCH (1974) signalize investigations on radiosensitivity in insects.

Differential radiosensitivity can be caused by a number of factors. Some of them are:

– mechanisms leading to repair of radiation damage of chromosomal structure or functioning as determined by genetical criteria. TRAUT (1966) defined repair as: 'the restoring of the normal condition of a damaged genetical structure, or the restoring of a condition which we are not able to distinguish experimentally from the normal one'. He discussed repair mechanisms in male and female germinal cell types of *Drosophila melanogaster* as did SOBELS (1963, 1966). KOCH et al. (1970) discussed repair of chromatid breaks on *Drosophila melanogaster* oocytes, thus influencing their radiosensitivity. Repair of DNA as a determining factor in radiosensitivity has been described by DEAN (1968).

– length of stages in the cell cycle or changes in the rate of cellular proliferation influencing the manifestation of radiation damage (PATT, 1968). In this context a connection could be made with the concept of blocking transformation of non-cycling cells to cycling ones and vice versa (GELFANT and SMITH, 1972) as a factor contributing to ageing.

– the degree of radiation induced inhibition of DNA synthesis (TUNG et al., 1971), which in *Musca autumnalis* testes seems to take place mainly in spermatogonia.

Other factors may be added here, although they have been mentioned by several authors and elsewhere in the present study:

– the degree of polyploidy and polyteny of the nuclei of the irradiated cells, providing multiple presence of possibly damaged parts of chromosomes.

– the influence of syncytia on their individual elements as a protecting factor, probably also because of compensation of chromosomal damage (PONTECORVO, 1944).

– the physiological state of the cell at the time of irradiation, which already has been discussed (cf. page 60).

The 'law' of Bergonié and Tribondeau thus seems to become increasingly debatable (HABER and ROTHSTEIN, 1969). Spermatogonia, for example, which are supposed to be relatively undifferentiated and mitotically active are quite radioresistant in *H. antiqua* in their 'diffuse' form but sensitive in their 'concentrated' types. TRAUT (1966) found low mutation frequencies in sperma-

togonia and oogonia of *Drosophila melanogaster* indicating a relative radio-resistance which could be based on an low intrinsic radiosensitivity and repair mechanisms. In *H. antiqua* 'diffuse' spermatogonia could possess a low intrinsic radiosensitivity, although according to HABER and ROTHSTEIN (1969) the presence or absence of a physiological condition associated with mitotic activity does not alter significantly the intrinsic radiosensitivity of the cells concerned. If this is true for *H. antiqua* spermatogonia, the presumed low intrinsic radiosensitivity depends on the level of the basic cellular metabolism, independent of mitotic activity. Such an independence of interphase and mitotic activity has indeed been observed in irradiated gonads of *H. antiqua*. Regardless of the severity of pathological cellular conditions mitotic activity becomes evident following the post-irradiation inhibition period. In most cases this activity leads to death by failing cell divisions. It could, therefore, be postulated that interphase physiological and mitotic activity are partially independent factors as far as their influence on the radiosensitivity and the reaction of the whole cell to ionizing radiation is concerned, both contributing to sensitizing or protecting physiological circumstances.

Determination of differential radiosensitivity of testicular cell types according to the described method (page 58) is easier at lower doses. The very distinct reaction of the testicular germinal cell populations of *H. antiqua* to 0.5 kR suggests an increasingly discriminating capacity of this method at decreasing doses. At very low doses the pathological reaction might become apparent only later in the adult stage. Therefore, observations should be made up to 20 days. Not only radiation effects can be assessed by this method. In fact all pathogenic factors which act on testicular cell populations can be evaluated in this way, for instance effects of hormonally active substances, chemosterilants and light flashes (STÜBEN, 1973). The combination of this method with the possibility to use the standardized histological methods for insect species of various orders (THEUNISSEN, 1976) provides a tool for extensive comparative research on radiation and other pathogenic effects on testicular cell populations of insects.

Terminology

In chapter 3.1. the terms 'dysplasia' and 'metaplasia' have been discussed. In medical pathology these expression refer to processes which develop gradually during a relatively long period, by far exceeding the life span of *H. antiqua*, by cellular proliferation and replacement of aged cells by newly formed ones. Since cell proliferation is reduced or absent following irradiation of *H. antiqua* and dysplastic and metaplastic symptoms appear within a relatively short time, they lack the aspects of a progressive change of a cell population by active proliferation. These terms, therefore, should be preceded in this case by the indication 'acute' denoting their recessive and short term character, though the conditions and appearance of the cell populations concerned agree with the definitions of the terms 'dysplasia' and 'metaplasia'.

FIG. 78. Resorption of S3 egg chamber in newly emerged untreated female. Left: part of comparable normal S3 egg chamber (cf. Theunissen, 1976, fig. 83). Bar = 10 μ.

Cell proliferation

Cell proliferation in the absence of normal mitoses has been observed when studying recovery symptoms of the spermatogonial populations after irradiation with 0.5 and 1.0 kR (cf. page 57). This phenomenon has also been observed in physiologically (fig. 78) and pathologically degenerating egg chambers. The latter were infiltrated by cells of the severely pathological follicle epithelium which did not show any mitotic activity at all. This infiltration could be realized because of:

– migration of still viable cells into the egg chamber.

– cell proliferation by means of very fast mitotic divisions which are not likely to be observed.

– cell proliferation by means of inconspicuous amitotic divisions. The first possibility could be valid but seems to be remote because of the preceding serious cell depletion of the epithelium by cytolysis. In view of the number of cells in the egg chamber some form of proliferation is more probable. The second way seems to be unlikely because of the already inhibited normal mitotic activity at the prevailing conditions. The third possibility, can not be ruled out (cf. fig. 33). TAEGE (1963) observed amitotic divisions of irradiated spermatogonia of *Calliphora erythrocephala*. More information, however, is necessary to confirm the presence of amitotic divisions.

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Ageing

The 'ageing effect' of ionizing radiation comprises a number of symptoms and phenomena which cause prematurely a situation which is found in old untreated adults. The ageing effect of irradiation in *H. antiqua* only bears upon the testis because of the general cell depletion and increasing thickening and pigmentation of the testicular sheath. In normally ageing ovaries still far advanced egg chambers develop, contrary to irradiated ovaries. Other symptoms of premature ageing are absent in other tissues which sometimes can give an even younger impression, for instance the adipose tissue in irradiated females which presents a far less 'worn out' appearance as compared to untreated females of the same age. Ageing may also refer to premature mortality which is often used as a criterium for ageing and which has been attributed to chromosomal loss or deficiency in *Drosophila melanogaster* (OSTER, 1959, c), to transformation of cycling to non-cycling cells in which G1 or G2 are blocked (GELFANT and SMITH, 1972), or to somatic mutations caused by radiation which gradually impair proper functioning of cell populations, tissues and organs (BELLAMY, 1973). In insects a shortened life span because of irradiation is usually supposed to be caused mainly by genetic damage.

Differences and similarities of the histopathological reaction of testes and ovaries

Differences in reaction to irradiation between testes and ovaries have to be mentioned briefly. The external morphological effects which are limited in testes, are very conspicuous in ovaries because of the general dystrophy of the entire ovary and the in most cases widespread degenerative changes in its parts. In irradiated ovaries no ghosts are formed, in contrast to testes where they are very conspicuous. Differences in the physiological state of somatic and germinal cell populations in testes and ovaries can be reflected in the pathological reaction of both categories. In testes only germinal cells show symptoms. In ovaries large populations of follicle cells are involved in the total pathological pattern. Recovery following irradiation with low doses takes place in testes after an interval of about 10 days. Recovery in ovaries means a continuing egg chamber and ovary development after receiving low doses, perhaps at a lower rate but without interrupting the production of germinal cells. A symptom of temporarily reduced germinal cell activity is the scarcity of 'concentrated' oogonia in 5 days old females after irradiation with 0.5 kR. Later on they are present again in the apical part of the germarium.

The continuity of the histopathological patterns in gonads of *H. antiqua* at increasing age and dose is very clear. An example is the disappearance of 'concentrated' oogonia from the germarium which takes place earlier at increasing doses:

dose (kR)	:	0.5	1.0	3.0	6.0	8.0
adult age (days)	:	20	5	5	0	0
post-irradiation (days)	:	23	8	8	2	1

Except a possible effect on the repair of radiation damage by syncytia of trophocytes and encysted male germinal cells a few other similatities in pathological patterns and reactions between ovaries and testes are found. A very conspicuous and intrigueing similarity is the shift in oogonial and spermatogonial cell types from 'concentrated' towards 'diffuse' types after irradiation. This phenomenon has already been discussed in relation to the differential radiosensitivity of primary and secondary spermatogonia. The same arguments apply to the 'concentrated' oogonia which behave as individual cells in the apical part of the germarium and the 'diffuse' oogonia which are mainly found in morphologically uniform groups in the basal part of the germarium and which are considerably more radioresistant. Single 'diffuse' oogonia are usually found to be the last recognizable oogonia to survive in an irradiated germarium, which also supports the similarity of oogonia and spermatogonia in reaction to ionizing radiation.

A feature common to ovary and testis is the relatively decreasing pathological reaction at increasing dose and age. The first 0.5 kR during the first days post-irradiation up to about 5 days of adult life seems to cause most damage per added 500 R as compared to higher doses and ages. The higher the dose received the earlier the damage caused seems to have reached a level at which a kind of stabilization of the pathological pattern takes place. This stabilization is observed as a distinct slowing down of the degeneration process and other pathological developments which continue to proceed but on a seemingly increasing lower speed at increasing dose and age, of course within certain limits. In females for instance this stabilization begins later than 20 days of adult age in the 0.5 kR group but between emergence and 5 days in the 3.0 kR group. Table 3 shows the quick and profound changes up till about 5 days in male gonads and the further development of the pathological pattern taking a relatively long and short time after irradiation with low and higher doses respectively before stabilization begins. During this process both in ovaries and in testes the somatic cell populations, as far as they are not directly involved in the pathological changes themselves, adjust to the size of the remaining germinal cell populations.

Concluding remarks

In order to summarize the main results of this study, a few short concluding remarks are made:

– In spite of a considerable individual variability it is possible to establish a general trend in the histopathological reaction of the gonads and their cell populations to irradiation.

– A reproducible histopathological pattern, the radiation syndrome, has been observed.

– In testes this pattern can be analyzed per germinal cell type based on the possibility to identify both 'normal' and pathological forms of these cell types.

– This analysis can be used to assess radiation effects on the various cell types at any moment. It permits determination of their differential radio-

sensitivity and provides information about effects on spermatogenic processes.

– Using histopathological criteria to evaluate irradiation effects, a new group of standards has been described in addition to the customary groups of genetical and physiological criteria.

– These histopathological standards can be applied to other pathogenic factors which influence male germinal cell populations, for instance chemosterilants.

– The data on egg chamber development reflect irradiation effects on ovaries as far as egg formation is concerned. They do not take into account the severity of the histopathological radiation symptoms.

– The study of radiation effects on chromosome morphology does not provide, qualitatively nor quantitatively, a suitable system for reliable assessment of these effects after irradiation with doses around the sterilizing dose and higher.

– There are few histopathological effects and symptoms in *H. antiqua* which are specific for irradiation.

– The appearance of a particular symptom is determined by a combination of factors. Important ones are: dose, age, post-irradiation period, the nature of the organ or tissue and the physiological activity of target cells.

– The development of the pattern of pathological symptoms following irradiation is generally a gradual and continuous process.

– The effect of X-rays on the combined testicular germinal cell populations as it is expressed by the radiation syndrome is qualitative in nature. Whatever the administered dose, within certain limits, the histopathological pattern does not change essentially.

– The general dose effect on the radiation syndrome is quantitative in nature. With few exceptions a higher dose results in a higher degree of severity of the symptoms.

– The lower the dose applied the more prominent is the effect of every added unit of radiation. The pathological reaction of a unit of 500 R added at a low dose level is much more distinct than when added at high dose levels.

– At relatively high doses the histopathological reactions are more determined by the length of the post-irradiation period than by the received dose.

– 'Concentrated' and 'b-type' cells are more radiosensitive than 'diffuse' and 'a-type' cells.

– Sperm cells are radioresistent from a histopathological point of view.

– In *H. antiqua* male germinal cell types show an increasing radiosensitivity in the following order: sperm cells- primary spermatogonia- secondary spermatogonia/primary spermatocytes- spermatids.

– Available information on differential radiosensitivity of male germinal cell types of Diptera and Lepidoptera indicates a reversed ranking order.

– Most effective irradiation of reproductive organs, permitting the use of the lowest possible dose, is achieved at a moment at which the most sensitive cell type is the most advanced one, and is relatively predominant.

– The causes and nature of the phenomenon of differential radiosensitivity constitute the most important problem in radiobiology.

5. SUMMARY

This paper is the second volume of a publication on gametogenesis and radiation pathology in the onion fly, *Hylemya antiqua* (Meigen).

Histopathological effects of irradiation of advanced pupae on the adult gonads have been described. Irradiation with hard X-rays usually took place 3 days before emergence. The dose range used was 0.5–9.0 kR, with 0 kR as a control. A dose of 3.0 kR was used to 'sterilize' the males, the females being more sensitive to irradiation.

In an introductory chapter (radiation pathology) a survey is given of the terminology we applied. As much as possible histopathological terms are used as in medecine. To avoid repeated lengthy descriptions an original terminology was designed for some pathological phenomena.

Observed histopathological changes after irradiation are described in the next chapter (results). The pathogenesis of the radiation syndrome has been followed in young testes at various post-irradiation times. The sensitivity and the quick reaction of the testicular germinal cell populations to irradiation are very suitable to study the development of this pattern of histopathological symptoms.

The account of the radiohistopathology of the testis begins with a description of the reaction of the various germinal cell types to irradiation with the sterilizing dose. The dose-effect relationship of these pathological reactions in a number of adult age-groups is described for the full range of doses. Recovery of germinal cell populations starts in a limited scale at about 5–10 days after irradiation with 0.5 and 1.0 kR and lasts for another 10 days. After that period the newly formed germinal cells degenerate quickly, so they do not contribute to the sperm supply. The differential disappearance and the possibility to identify both morphologically normal and pathological types of germinal cells provide a system to establish the presence or absence of the morphologically normal cells of various germinal cell types per testis. By means of this system the patterns of pathological reactions of the germinal cell populations are demonstrated, reflecting the radiation damage for each age- and dose-group. It may also serve to designate effects of other pathogenic agents which influence testicular germinal cell populations.

Pathological changes in the ovary and radiation-induced delay and inhibition of egg chamber development have been described and evaluated. Observations on dose-effect relationships with respect to the ovaries in various adult age-groups are discussed. The effects of relatively high doses, between 6.0 and 9.0 kR, on the pattern of pathological symptoms have been described.

A brief outline is given of a possible approach to a quantitative evaluation of radiation damage in testes. In principle this sensitive method can be used to assess also other pathogenic effects on germinal testicular cell populations.

Concluding the general discussion, the main results of this study are summarized.

6. ACKNOWLEDGEMENTS

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