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BIOLOGY SECTION OF RESEARCH DIVISION

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THE GENERAL PICTURE OF IRRADIATION DAMAGE TO TISSUES

Part I. Superficial Radiation (Beta rays)
Part II. Penetrating Radiations (Neutrons, Gamma rays)

By

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Part I. Superficial Radiation (Beta rays)

Introduction

The skin is the most readily accessible organ of the body. It has a comparatively simple structure, a simple pattern of differentiation and a functional purpose that is easy to understand. For these reasons it is an important organ in which to study the carcinogenic process. Since beta rays from external sources are absorbed almost exclusively in the skin and since beta rays have recently been discovered to have remarkable cancer producing power in the skin(1), this paper is devoted to an analysis of the skin response to superficial radiations in the light of present information.

Features of the Skin

Composition: Normal skin consists of two general parts, the epidermis and the dermis. The epidermis is made up exclusively of epithelial cells which are usually arranged so as to show four recognizable layers of cells. The first is the stratum germinativum, the germinal layer which gives rise to all the other cell types in the epithelium; the second is the stratum mucosum, consisting of larger and more rounded or cuboidal cells

2172

(sometimes called stratum malpighii); the third is the stratum granulosum, comprised of flattened, sometimes granular, cells; and the fourth is the stratum corneum, the horny surface layer. For purposes of simplicity we may omit the stratum granulosum since it is usually absent and refer to the other layers as the germinal (or basal) layer, the intermediate layer, and the corneal layer. These layers vary in degree of accentuation in different species and merge more or less smoothly into an orderly pattern of differentiation.

Immediately beneath the epithelium is the derma, which is composed mainly of connective tissue and blood vessels. The derma then is a sustaining base for the epithelium and its general condition has an important bearing on the function of the epithelium.

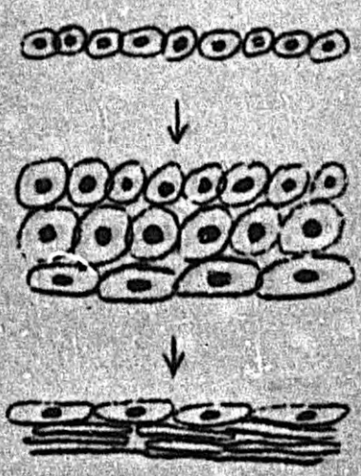
Normal differentiation of epithelium: Diagrammatically the steps in differentiation of epithelium may be represented as shown in figure 1.

Figure 1. Diagrammatic arrangement of cell types in the epidermis, showing progressive stages of differentiation.

Differentiation of the skin takes place by a process of maturation to death - that is, by the germinal layer proliferating a flow of cells which become modified as they move toward and finally comprise the surface layer of the skin. Since the outer layer is made up of non-viable horny cells, it is appropriate to regard the progressive change as one of differentiation which culminates in cell death. In such a process, the germinal layer must form two types of cells: (1) cells which become differentiated as just indicated, and (2) new germinal cells like themselves which in turn produce more germinal and more corneal cells. There must continually be a residuum of germinal cells.

Figure I.

SKIN



4172

In addition to formation of the horny integument, the epithelial layer gives rise to special organs such as hair, teeth, nails, scales, feathers, beaks, claws, sebacious and mammary glands, etc. In all these instances, likewise, the stratum germinativum is the source of cells; it gives rise to the particular cells that become modified to form the special parts. Damage to either the germinal or to the intermediate layer may lead to abnormal structures.

Kinds of abnormalities: The skin may show three general types of abnormalities: failures, anomalies, and excesses.

Failures of the skin are manifested as drying, scaling, fissuring, lessened resiliency, reduced regenerative capacity and inability to grow appendages such as hair or nails. Changes of this type can result from functional defects in the derma or stratum germinativum, or both.

Anomalies, such as badly formed nails, poor quality of hair, poorly developed teeth, and the like, result from improper differentiation of the epithelium; hence, these changes are due to the inherent condition of the cells of the basal layer.

Excesses result from overgrowth or overfunction. All the cell components capable of mitosis can manifest excess growth. Let us consider the consequences of excessive activity in certain of the cell layers in simple skin. Excessive activity of the intermediate layer would lead to thickening of this layer and in some cases to unusual formation of squamous. When this is confined to the surface there may be piling up of horny material with consequent keratoses. Then there is a folding of the surface tissues and the process of keratinization continues, the squamous materials are thrown into circular "pearls" embedded within the tissue masses and recognized as acanthoma. If this condition is accompanied by down growth with extensions into

other tissue parts, squamous carcinoma is obtained. The middle layer may also undergo excessive growth with a tendency toward glandular manifestations, hence the growths that result are designated as adenomas. The histological picture in these tumors may resemble mammary glands, sebacious glands, sweat glands, or even hair follicles. It is important to note that in these instances the power of proliferative growth is retained by the differentiated glandular structures and that little or no dependence is placed on the stratum germinativum as the source of cells.

Occasionally the stratum germinativum itself may undergo excessive growth with the cells showing little tendency to differentiate, and resulting in a tumor type known as reticulated epithelioma. The histologic picture here is one of primitive cells showing thickening and extensions of downgrowth. The clinical picture in this type of tumor is interesting in that the behavior is essentially opposite to that of epithelization in wound repair. In human beings, the early epithelioma lesions appear as broader flattened elevations or as multiple small thickenings which coalesce. After a period, which may cover several years, the lesion may ulcerate displaying a characteristic rodent ulcer with a remarkable tendency to remain superficial. This neoplasm destroys the skin down to superficial fascia where its progress is long restrained. Extensive fibrosis of the base may limit the downward growth and at the same time obstruct any natural or artificial effort at cure.

In those forms that have pigment cells in the skin, excessive growths in the stratum granulosum may also occur, giving rise to clinical forms known as xeroderma pigmentosum (malignant freckles) and malignant lentigo.

Excessive growth in general is detrimental to the well being of a host organism. The degree of interference with life however may vary all the way from inconvenience to lethal. Death from excesses usually result from malignant manifestations characterized by the power of tumor cells to invade other tissues and the ability of the tumor to metastasize. Death eventually results either from the involvement of vital organs or a generalized toxemia.

Kinds of Damaging Agents

Skin is continually affected by its external environment. Excessive dryness or excessive moisture are less favorable for the best skin function; likewise repeated traumatic injury varying from the slightest irritation to complex wounds alter its function. Thermal burns and sunlight reach exposed parts, but except for excessive exposures alters only the surface layers. Other types of agents such as chemical compounds and penetrating radiations traverse deeply and may or may not affect the superficial layers depending upon the properties of the agents used and the quality of skin acted upon. Hence some correlation can be made between the type of agent employed and the kind of damage obtained.

Effects of Damage on the Separate Cell Layers

It is advantageous at this point to consider in hypothetical manner the results of damage to each of the cell layers

separately. Beginning at the surface, the corneal layer is little affected by doses of agents which will seriously affect the tissues beneath. This is because the corneal layer is made up of cells that are already non-viable. The intermediate layer and the stratum granulosum (when present) are made up of living cells. When any appreciable number of these elements are destroyed at a given time, a break occurs in the chain of differentiation. If the squamous wears away, as it does in normal life, there is an insufficient quantity of maturing cells to replace those that disappear, and for a period there is an area of immature skin exposed. Where the stratum germinativum is not destroyed, the damaged layer is soon replaced by new cells and a new layer of squamous is established. The stratum germinativum is likewise made up of living cells and, as already pointed out, is the fountain head of cells for the whole epithelium. Complete destruction of this layer leads in time to an open ulcer as there are no cells remaining in the area for replacement. Healing in such a case must take place from the margin by lateral extension of epithelial sheets from the periphery of the wound so as to lay down a new epithelial field.

Chemical Carcinogens

In order to better understand the carcinogenic action of radiation, let us consider also the action of chemical compounds. It is now well known that repeated application of certain coal tar and other products to the skin will elicit different kinds of skin cancer. When these products are painted on the skin surface a larger proportion of epithelial growths are obtained, whereas when they are planted or injected subcutaneously a greater proportion of sarcomas are derived. This suggests that the cells are acted upon directly by the agents.

It is of interest also that precancerous conditions appear to be developed when the various carcinogenic hydrocarbons are applied as a forerunner of the full blown cancerous conditions. Usually after the agents have been applied once or twice a week for several weeks, the skin shows evidence of injury in the form of mild ulceration. If treatment is discontinued at this stage, the lesion will subside and disappear; whereas if it is continued skin carcinoma will develop. Moreover, skin carcinoma may be induced in the second phase of treatment when treatment consists of nothing more than mild heat application (hot water in a test tube laid across the wounds)[2].

A most illuminating experiment has been carried out by Orr[3]. Small paraffin pellets were prepared, some of which were plain, others of which contained non-carcinogenic hydrocarbons, and still others of which contained certain carcinogenic hydrocarbons (methylcholanthrene). These pellets were implanted subcutaneously in animals, and tissue sections were prepared at different times to determine the course of the reactions. The plain pellets and those containing non-carcinogenic hydrocarbons were found to elicit the usual foreign-body reaction leading to enclosure of the pellet within fibrous connective tissue in two to three months. After this, the reaction was complete and there were no further changes. Pellets containing carcinogenic agents on the other hand prevented satisfactory encapsulation. There was an inadequate formation of fibrous material and the collagen formed was small in amount and loose in texture. The connective tissue beyond the point where a capsule was expected to form, showed evidence of irritation and remained in a mild state of inflammation. After some months, foci of secondary cellular proliferation appeared beyond the primary lesion at a distance from the pellet and it was from these areas that sarcomas developed. It was suggested that the presence of carcinogen tended to restrict the usual tissue reaction to a foreign body, thereby preventing an effective

and satisfactory end-point in the healing process from being reached. The surrounding connective tissues, being held under a constant stimulus for repair, eventually gave way to neoplastic growth.

Thus, one sees in chemical carcinogenesis the features of mild injury together with incomplete healing over a long period of time.

Physical Carcinogens (Irradiation)

Under the general heading of superficial irradiation may be listed sunlight, ultraviolet, beta rays, cathode rays and soft x-rays. We shall deal with the carcinogenic effect of the latter first.

Cancer of the skin resulting from exposure to x-rays was observed as early as 1902[4] which was within six years after the discovery of these rays was announced.

Actually, the x-ray exposures of the patient observed were received in the year 1896 and lasted for several hours each day at a short distance from a low vacuum tube. After some weeks of such exposure the hands became red and dry and soon were painful and greatly swollen, despite the cessation of exposure. The nails became dry and striated, then softened and finally sloughed off. Within a few months blisters formed and sloughing of skin areas began. Treatment of this case, like that in many others since run a tragic course of excision, grafting and amputation.

Helbich[5] in 1909 used excised tissues from persons with late x-ray dermatitus and made a careful study of histological changes therein. Comparing the tissues of normal skin with those of lesions approaching the cancerous state, he

10 l 72

described four types of modification: (1) a thickening and downgrowth of epithelium: (2) a rarification of the loose connective tissue immediately beneath the dermis, characterized as being in a state of imperfect repair; (3) vascular changes, consisting mainly of telangiectasia, loss of elasticity, and obliterative endarteritis; and (4) atrophy of the smooth muscle, mostly in blood vessels.

Kolbach stated that in general the increased thickening of the epidermis and the production of wart-like growths with keratoses and downgrowth are best explained as the result of constant, active, proliferative repair called forth by the continuous occurrence of small defects in the underlining corium. In a later paper Kolbach(6) stated that "the outstanding factor of importance is that conditions are continually occurring that will call forth proliferative activity of the epidermis greatly in excess of the rate of normal proliferation", and inferred that the acquisition of malignant properties is not sudden but is gradual during the course of years.

Histologic changes in the skin and underlining tissues following exposure to ionizing radiations have been worked out in some detail by Montgomery(7).

Soft x-rays penetrate well beyond the surface epithelium and affect the dermis as well as the epidermis. Hence changes in epithelium may be secondary to changes in blood vessels and connective tissues beneath. It is of particular interest then to consider the effects of radiations which are absorbed mainly by the epithelial layers.

Sunlight as a possible causal agent in skin cancer has long been recognized. The precancerous condition called Seamen's Skin has been attributed to continued exposure to light. Blum(8) stated that, while few oppose the idea that sunlight may be an etiologic factor in cutaneous cancer, opinions vary regarding its relative importance.

11272

Blum summarized the conclusions of various workers as follows: "(1) cancer of the skin occurs principally on parts exposed to sunlight; (2) cancer of the skin is more prevalent in outdoor workers; (3) the incidence of cancer of the skin is greater in regions of the earth which receive the greatest insolation; and (4) cancer of the skin occurs more often in blondes than in brunetes".

MacKee and Cipollaro[9] give a description of "farmers and sailors' skin" on exposed areas of adults, mostly middle-aged and elderly persons who have been exposed to the sun for many years. The skin becomes wrinkled and dry, showing permanent lentigo, telangiectasia, and white sclerotic spots. The lips are apt to become dry and fissure easily. Keratoses of the senile type frequently develop in such skin. The condition bears some resemblance to chronic dermatitis. Epithelioma, often of the squamous cell type, develops commonly from farmers and sailors skin. Preceding the onset of active growth, degeneration and atrophy of the connective tissue occur in the upper layers of true skin. The epidermis is somewhat atrophic except in areas where keratoses are developing.

Experiments with ultraviolet light are still more instructive. Blum[10], giving repeated exposures five times weekly to mice, was able to induce tumors on the ears of mice in 100% of the animals. Both carcinomas and sarcomas were obtained. The interval between the beginning of treatment and the appearance of tumors was shortened (to 3 or 4 months) by increasing the daily dose up to a certain limit. Increasing the dose beyond this point produced no further change in the induction time. Other experiments showed that if the treatment schedule was interrupted or stopped, the induction time was longer and that it varied inversely with the number of treatments applied.

Blum and his associates[11] were able to obtain tumors with only slight evidence of precancerous lesions. They pointed out that with large daily doses the tissue destruction preceding tumor formation might be severe, whereas with small daily doses it might consist of no more than a slight hyperplasia with no skin abrasions or ulcers. They stated in fact "it is difficult to say that hyperplasia is an essential

1317

factor for tumor formation, or even that the two processes are directly related".

Without attempting to give figures, it may be said that the epithelium of most skins is, or at least very nearly totally absorbing for the active components of the visible and ultraviolet light. Blum made a series of measurements on the transmission of ultraviolet light in the skin of mice and decided that a significant proportion actually reached the derma. He offers this as an explanation for the fact that some sarcomas were obtained along with the carcinomas in the ultraviolet treated animals.

The interesting new findings by Raper with phosphorus (P^{32}) beta rays provide certain additional features[1]. The half value thickness of tissue for this particular radiation is approximately 0.8 mm., which is well within the derma, and in rat skin is near the base of the hair follicles. Raper obtained both sarcomas and carcinomas, the latter in much higher frequency, and after single applications of the radiation.

The beta ray induced tumors began to appear at about 4 months after treatment and arose from scattered discrete foci on intact skin. When single doses of 4000 rep were used, no gross changes were noted in the skin previous to tumor formation. When the doses were 5000 rep significant skin damage was noted. At three to four weeks there was epilation with some desquamation which was followed by a fairly normal healing and regrowth of hair within four to six weeks. The skin in these animals then remained fairly normal until the tumors began to appear.

In resume, the carcinogenic action of beta rays appears to be different from that of chemical compounds as no visible injury need necessarily precede tumor formation, and different from that of ultraviolet light in that a single application of the agent is sufficient to induce tumors. Since almost every known type of epithelial tumor was obtained with beta rays it appears that cells of the skin at practically every level of differentiation can be induced to malignant behavior. Various types of cells are wrenched from their organizational mooring and impelled to assume a different type of activity. It is pertinent that with daily treatments of 50 rep per day, tumors should arise in the face of exposure after several months.

1472

Summary

The pertinent facts associated at this time with induction of skin tumors with superficial radiations may be listed as follows:

1. It is important to remember that skin consists of layers of cells with the germinal layer at the base, and that the germinal layer proliferates other germinal cells and cells which become differentiated first to form an intermediate layer and finally the dead corneal layer.

2. In the skin there must continually be a residuum of active germinal cells and a process of maturation to death of cells in order to have normal skin function.

3. Skin abnormalities consist of three types: (1) failures manifested as drying, scaling, fissuring, lessened resiliency, and reduced regenerative capacity; (2) anomalies such as badly formed nails, poor quality of hair, and poorly developed teeth; and (3) excesses involving overgrowth and over-function as in neoplasia.

/PART I

4. Radiation absorbed in the skin causes damage primarily in the germinal layer. When the damage to this layer is partial, the effect is a skin failure; the germinal layer is unable to proliferate enough cells to maintain the integument with the result that there is drying, scaling, fissuring, possible loss of hair, etc. Repair takes place from the floor of the damaged area, however, from living germinal cells that remain. When damage to the germinal layer is complete the effect is skin sterilization: not only is there drying and scaling, but also there is exudation, sloughing and ulcer formation. In this case healing must be from the edge as no germinal elements remain. /END

5. Skin tumors often arise out of such wounds that have become refractory.

6. In the case of superficial radiations skin tumors (of practically every type) have been observed to appear in animals that display little or no gross evidence of skin break down.

7. Single applications of beta rays have been observed to be carcinogenic, as many as 50 to 100 foci of tumor growth being produced on rats that ordinarily show no skin lesions.

15172

Part II. Penetrating Radiations (Neutrons, Gamma rays)

Introduction

A description has been given(Part I.) of tumor formation and the accompanying changes that follow applications of superficial radiations to mammalian forms. A similar description is given here of changes which follow exposure to penetrating radiations and an attempt is made to indicate some of the salient features involved in the process of irradiation injury and in irradiation carcinogenesis.

Relative Vulnerability of Tissues

Experience has revealed that when penetrating radiations are used to affect mammalian organisms, essentially the same kinds of changes are produced irrespective of the type of radiation used - that is, x-rays gamma rays, fast neutrons or slow neutrons(2-6). When such agents are applied to the whole body, lymphoid organs are among the first to respond; as a rule, some damage in the form of cell disintegration can be found in lymph nodes as early as two hours after doses as low as 25 to 50 r.

1672

The germinal epithelium of the testis is almost as sensitive, although damage cannot be detected as soon after exposure. The erythroblast and myelocyte of the bone marrow are a little more resistant and the epithelial lining of the gastro-intestinal tract is more resistant still. The susceptibility of the ovary varies considerably from specie to specie, and accordingly, cannot be readily fitted into a scheme of vulnerability. Suffice it to say that the sensitivity of the ovary is intermediate among the other organs. Connective tissue and bone are comparatively quite resistant as are the mature elements of the blood stream and organs such as the pancreas, liver, and kidney. At the bottom of the list are organs such as nerves, brain and muscle. Since the fate of organisms following irradiation is determined by the more sensitive parts rather than the most resistant, the discussion here will pertain more to the former.

Significance of Cell Maturation

In the discussion on skin responses, it was pointed out that cells in this organ pass through various progressive levels of differentiation and that cells at certain levels are more susceptible to radiation damage than those at other levels. A similar picture appears in the germinal epithelium of the testis. Near the basement membrane of the seminiferous tubules there is a layer of spermatogonial cells which are parent types in the sense that they give rise to primary spermatocytes which in turn give rise to secondary spermatocytes, these to spermatids and finally the latter mature sperm (Fig. II.). In this series, the primary spermatocytes appear to be the most sensitive and spermatogonia only a little less so.

1272

Figure II. Schematic arrangement showing the order of maturation of the skin and testicual epithelium in mammalian forms.

Similarly, the mature granulocytes of the blood stream are derived through differentiation. The hemocytoblast develops from the primitive mesenchyme cell and in turn gives rise either to more hemocytoblasts or to myelocytes. The myelocytes change gradually and become the precursors of mature granulocytes (Fig. III.)

Figure III. Schematic arrangement showing the order of maturation of various germinal elements of the mammalian body.

In the same manner, the mature erythrocytes develop by passing through progressive levels of differentiation almost identical to the mature granulocytes - i.e. mesenchyme cells to hemocytoblasts to erythroblasts to mature erythrocytes (Fig. III.)

Mature erythrocytes and mature granulocytes are relatively very resistant to irradiation damage but as in the case of the skin and testis the younger cells such as hemocytoblasts, myelocytes, and erythroblasts, are relatively very sensitive. It is emphasized, however, that the most primitive cell of them all - the mesenchyme cell - is quite resistant to irradiation injury. It will remain and be functional following exposure when most of the other elements have disappeared.

1817

Figure II.

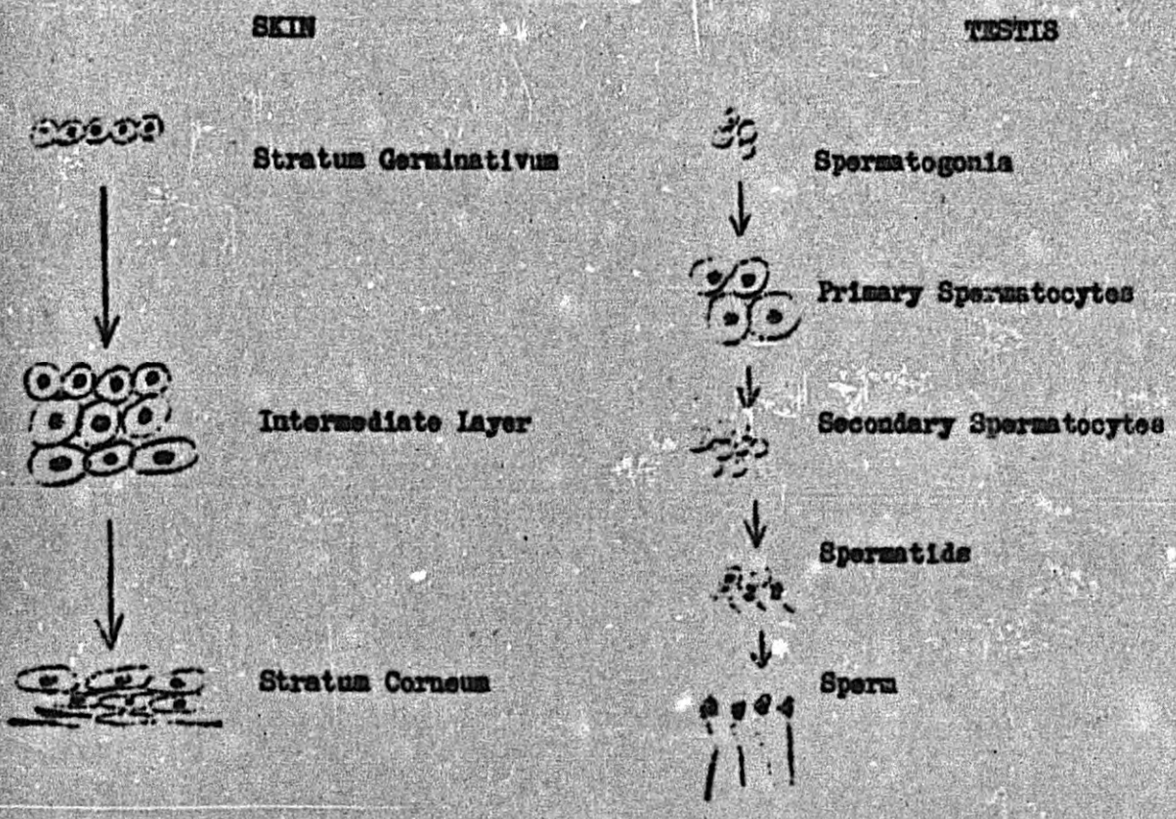
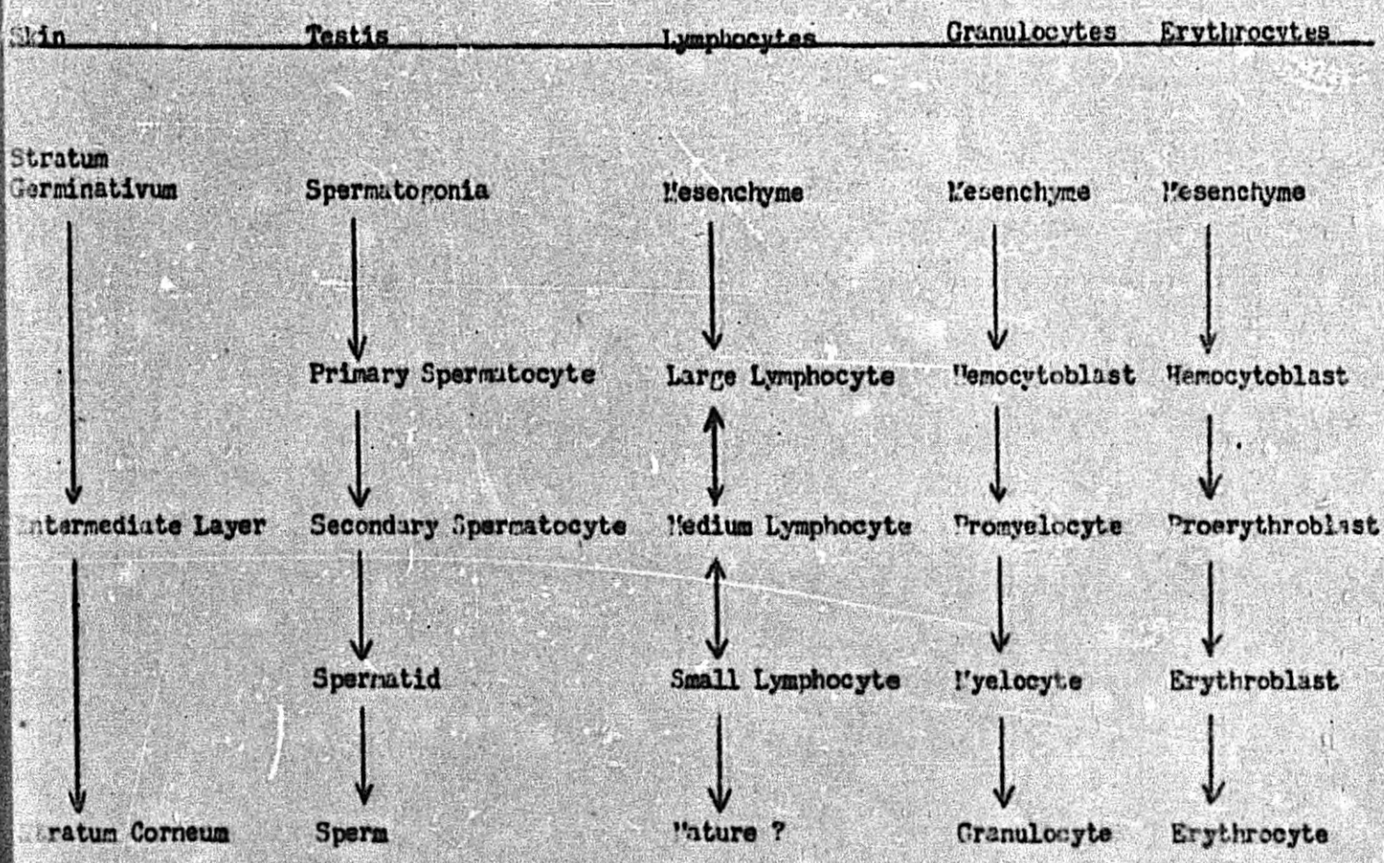

SKIN
TESTIS
Stratum Germinativum
Spermatogonia
Primary Spermatocytes
Intermediate Layer
Secondary Spermatocytes
Spermatids
Stratum Corneum
Sperm

Figure III.



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The lymphocyte series can, with appropriate consideration, be fitted into the same scheme. The primitive mesenchyme cell (parent to both the myelocyte and lymphocyte series) is resistant while the large lymphocyte (identical with hemocytoblast), the medium and small lymphocytes are very susceptible to irradiation injury. It is not entirely clear which is the mature cell in the development of the lymphocyte series; hence, one cannot correlate with certainty the stage in development with radiosensitivity. But, if as some believe, the lymphocyte can develop into mature granulocytes, monocytes, and plasma cells, which are resistant, then the lymphocyte picture is consistant with the others (Fig. III.)

This general picture can also be applied to the epithelial lining of certain parts of the gastro-intestinal tract because cells near the bottom of the crypts of Lieberkuhn in the doudenum, for example, are more sensitive to radiation damage than are those in the upper part of the crypt. It is interesting that regeneration of the single layer of epithelium takes place in or near the bottom of the crypt and new cells move upward along the neck of the crypt differentiating into goblet cells and into columnar cells with striated borders. These latter cells are resistant to radiation damage.

Figure III. is a diagrammatic arrangement showing the progressive changes of differentiation in the various types of cells. Whereas the types of tissues involved and the end products obtained are very different in each instance, there are certain features common to all. In each there is a source

2i l72

element and a process of maturation of cells to death. The source element functions both to maintain itself and to provide a continuous supply of cells for a particular pattern of differentiation and use. Stress must be laid on the fact that the most primitive cells (i.e., mesenchyme, etc.) are more resistant to radiation than are the intermediate in a differentiating series. As can be seen from Fig. III., the number of transitional stages in differentiation of the tissues mentioned varies, but the flow from source to a specialized cell type and finally death is much the same in all. It is significant from the standpoint of susceptibility to radiation that these tissues are all of the generative series and are at the same time the tissues designated as the most sensitive to radiation.

Nature of Irradiation Damage

It was pointed out for the skin that the cells of the basal layer are the most susceptible to radiation and that injury to the cells of this layer will result in poor skin function for a period of time or in a complete loss of the skin in the exposed regions, depending upon the amount of radiation applied. A similar reaction takes place in each of the tissues or organs under consideration here.

The testis furnishes an excellent example. As emphasized already, there is a progressive sequence of differentiation, starting with the spermatogonial cells which in turn give rise to spermatocytes, spermatids and sperm in this order. Breeding tests following the exposure of various laboratory animals to radiation reveal periods of temporary or permanent sterility, again depending upon the dose of radiation applied. Tests carried out to correlate the nature of testicular changes with dose of radiation applied have shown that the primary spermatocytes and spermatogonia are the first cells to show damage and disappear. As in the skin, when an intermediate or

12 l

source cell drops out, the more advanced cells complete their cycle of maturation and eventually disappear. Thus after a latent interval, the tissue or organ shows a period of injury or sterility. When an intermediate or source type cell is the most vulnerable, the susceptibility of the more mature forms is of little consequence, as the effect of a break in the supply has the appearance of destruction of mature cells also by irradiation. This is because the mature cells disappear in the usual manner and, for a period at least, are not replaced. The threshold of dosage for testicular injury which can be detected by histological means in laboratory animals is in the range of 100 to 200 r of x-rays or gamma rays. The sterilizing dose is 4 to 8 times this amount. From the size of the sterilizing dose, it is plain that complete sterility is difficult to obtain without at the same time producing other changes that will be lethal.

The power of regeneration in the testis is extremely great. After periods of sterility when the spermatic tubules are almost devoid of germinal elements, regrowth of the epithelium will take place and the production of functional sperm will occur apparently like normal.

Irradiation damage to the hemopoietic organs shows points of similarity and points of difference when compared with changes induced in the skin and testis. Small lymphocytes (as seen in mice) are the most radiosensitive and the first to show degenerative changes following exposure. Degenerating lymphocytes may be found in the lymph nodes and spleen within 2 to 4 hours after x-ray doses as low as 50 r or less. The threshold doses for most myeloid elements are in the range of 100 to 200 r and disintegration does not begin for several hours after exposure. The minimum leukocyte levels in the peripheral blood and the maximum aplasia of the hemopoietic organs are reached at 4 days to 1 week. If the organisms survive, recovery to a condition resembling normal takes place in most cases in 3 to 4 weeks. Erythrocytes, having no nuclei and being inanimate, so to speak, show no significant disintegration following doses of a few hundred r.

The overall picture so far as the hemopoietic organs are concerned appears to be as follows. The source cells, that is the hemocytoblasts, are secondary with respect to radiosensitivity, the more advanced cells (erythroblasts and myelocytes) being more sensitive. When the doses of radiation are increased or repeated, the hemocytoblast also disappears. Thus at 4 days to 1 week a stage is reached at which the blood is composed almost exclusively of erythrocytes, and the lymph nodes, spleen, and bone marrow are nearly devoid of cells. The erythrocyte levels for the most part remain in the normal range despite a regimen of treatment that will practically erase the leukocyte components. This appears to be true despite the fact that leukocytes and erythrocytes spring from a common ancestral type cell.

Although the erythrocyte is not damaged particularly by the direct action of radiation, the question arises as to why a fall in erythrocyte level does not occur when the supply is cut off by the destruction of the ancestral hemocytoblast. The explanation appears to lie in 2 facts: first, that the mature erythrocyte has a comparatively long life and second, that recovery of the ability to proliferate new erythrocytes is rapid. The life span of erythrocytes, according to recent data, appears to be 1 to 3 months. Hence, since the bone marrow even after extensive damage can return to apparently normal in 3 to 6 weeks, it is evident that a safe carry-over period exists for the erythrocytes when single treatments of radiation are administered.

When repeated treatments are given in such a manner as to shorten the life span significantly, yet not cause death within 6 to 8 months (a period well beyond the normal life of erythrocytes), one might expect to see a downward drift in erythrocyte level of the peripheral blood. Although such a drift does occur in a few species (i.e., guinea pigs [12],

uaually the normal erythrocyte leval is maintained until death or until an abrupt anemia occurs just before death. In these latter cases it would appear that a partial bone marrow is able to keep a fairly normal erythrocyte level, but eventually gives way in a state of exhaustion (aplasia anemia). The fact that some animals apparently die from irradiation injury with the normal erythrocyte level indicates that aplastic anemia is not the cause of irradiation death as a rule, despite the fact that it may be in some instances.

Turning now to the gastro-intestinal epithelium, the response of this tissue to radiation is also clear-cut. Since there is much mitotic activity near the base of the crypts and since the cells mature as they push their way to the surface, it is significant that those cells near the base are the sensitive ones whereas those near the surface are more resistant. When the damage to this layer is sufficiently great whole patches of epithelium are sloughed away and if the areas are denuded in such a manner that recovery cannot occur, nutrition is interfered with and death may result (i.e., from this and other commensurate injuries).

Tumor Induction with Radiation

As in the skin, abnormalities of the various tissues of internal organs may be characterized as failures, anomalies, and excesses, the most significant of which is the latter.

In the discussion thus far, it has been pointed out that, in general, the tissues most vulnerable to radiation are those that have a normal active proliferative function, and further that the first irradiation effects were of an injurious character to fragile and potentially active cells. It was also pointed out that malignant growths occurred in certain of the damaged tissues, although not all. In studies

2572

with mice and penetrating radiations as used, only the hemopoietic organs were found to give a really significant rise in incidence of malignant disease.

The cycle of changes in the blood and blood-forming organs, as indicated, consists of a fall in leukocyte levels which begins within 2 to 4 hours after exposure and the degeneration of lymphoid and myeloid elements beginning at about the same time. Both of these effects are progressive and the time of maximum damage is reached in 1 to 7 days, depending upon the dose of radiation and the type of cell considered. Regrowth of the marrow and lymphoid organs begins at about 1 week and the apparent normal condition is reached again at 3 to 6 weeks after exposure. Return to normal leukocyte levels is reached as a rule at about the same time. At 4 to 6 months, death begins to occur with the animal groups either in a state of generalized atrophy or neoplasia of hemopoietic organs. In certain strains of mice the latter consists mainly of mediastinal lymphomatosis with occasional general involvement of all lymph nodes and spleen; in most cases the peripheral blood shows no increased white count. In the various strains of mice thus far studied the predominant cell type in these tumors has been the small or medium-sized lymphocyte.

In brief, the sequence of events is as follows: (1) a period of degeneration and atrophy during the first week after (a single) exposure; (2) a period of recovery and regeneration during the second and third weeks; (3) a period of reasonably normal existence for several months; and (4) general atrophy or neoplasia.

Since the highest incidence of lymphoma is found in animals which suffered severe damage to the hemopoietic organs, the question is raised as to whether injury is a necessary forerunner of neoplasia, and in like manner whether the extensive repair process may be a contributing factor. While

the findings on hemopoietic organs would tend to bear out such an hypothesis, the results obtained with the other organs are opposed to it. In the testis, the damage produced was comparable in extent to that in the hemopoietic organs, yet no tumors of the testis were found. Furthermore, as indicated in Part I., tumors of the skin may be obtained with ultraviolet light and beta rays where it is doubtful that any tissue degeneration preceded the neoplasia. In chemical carcinogenesis of the skin, long periods of imperfect repair was a conspicuous factor but such is not readily apparent in irradiation carcinogenesis of the hemopoietic organs where extensive regeneration has occurred. It appears, therefore, that extensive tissue injury is not always a necessary prerequisite of tumor formation in irradiation carcinogenesis.

Discussion

When penetrating radiations from external sources, such as fast neutrons, slow neutrons, and gamma rays are applied to animals as small as mice the distribution through the animal body is reasonably uniform. Under these circumstances, as already made clear, the organs and tissues most readily affected are the blood-forming components, the germinal epithelium of the testis, and the gastro-intestinal mucosa. Skin and bone by comparison are little affected. It has been emphasized also that following damage to the four organs mentioned, malignant growths are confined mainly to only one of these, the hemocoietic (in mice).

When other kinds and other sources of radiation are used, the major damage can be shifted from the parts mentioned to other parts. With beta rays for example, damage and tumor induction can be confined largely to the skin, and with ingested radioactive materials having an affinity for particular organs, damage and tumor formation can be confined mainly to particular internal organs.

It has been shown already[1] that with beta rays, more radiation can be applied to the skin without killing the animals, and that skin tumors can be obtained in great profusion in this manner (in Sprague-Dawley rats). Similarly, as indicated elsewhere[13], the feeding of radiostrontium leads to a greatly increased incidence of bone tumors in animals that show mainly lymphomas after exposure to external sources of radiation.

It has been found furthermore that the tumor spectrum following irradiation will vary with different species of animals. As indicated above, lymphoma is the predominant tumor type seen in certain strains of mice following uniform whole-body irradiation. In other studies with mice, ovarian tumors are most frequently seen. In addition, certain organs, such as the hemopoietic, will respond readily to radiation in some strains or species whereas in others great resistance is shown.

The cycle of damage and repair in the case of single and repeat treatments is quite different. Following small repeated treatments, instead of sudden atrophy and fairly rapid repair, the evidence at any time for injury is slight. By intricate means, however, subliminal effects can be demonstrated. It would appear then that when subthreshold doses are given over a long period of time, damage is produced as usual by irradiation but due to the rate of repair when the damage is small, the latter is sufficient to compensate largely so that injury often is not apparent. By this means, an animal might be pressed near to the limit of its reserve capacities without showing extensive outward signs, yet under stress--as in combatting infection--be unable to muster sufficient reserve to do so. Although generalized atrophy was not observed in the animals receiving small daily treatments, the experiments thus far have revealed little information on subliminal effects which are so very important from the standpoint of low grade injury and late effects. There is need for much additional information concerning whether vigor is lowered, resistance to disease is reduced, or life span is shortened appreciably by small daily exposures in the accepted tolerance range.

Summary

1. It was pointed out that the most radiosensitive tissues are of the generative type - that is, those which in life are continually proliferating new cells which undergo maturation, serve a useful function for a limited period and then die.

2. The skin, testis, hemopoietic organs, gastro-intestinal epithelium and perhaps other tissue series show a parallel type of differentiation with these features in common.

3. In all cases (with the possible exception of lymphocytes), certain parent cells (but not necessarily the most primitive types), were found to be the most susceptible to radiation.

4. Partial damage to a particular tissue series results in a limited period during which mature cells are being formed, whereas complete damage results in sterilization.

5. Recovery to an apparently normal condition takes place even when the acute histologic destruction has been almost complete.

6. Subliminal damage in the case of daily exposure may hold an animal nearer to a state of exhaustion so that he is less able to cope with a crisis.

7. Animals receiving large doses of radiation (either single or repeat treatments) die prematurely usually either a state of atrophy or neoplasia.

8. When penetrating radiations are applied generally to the whole body, leukemia is the predominant tumor type obtained; when only slightly penetrating radiations are applied to the body surface, skin tumors are obtained; and when internal emitters that are bone seekers are applied, bone tumors are obtained.

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30l72