Summary. Manganous ion, 0.0001 M, hastened activation of crude prothrombokinase in a 3-stage analysis of blood coagulation. It did not prevent the subsequent steep decline of thrombokinase activity. Rapid quenching of thrombokinase activity might help in the physiologic control of blood clotting.

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Relation of Hair Proliferation to Damage Induced in the Mouse Skin. (18381)

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Physiological conditions prevailing in the skin at the time of experimental treatment with chemical or physical agents may be expected to influence the nature and degree of the response. A condition that may be readily observed and controlled is the stage of development of the hair. In the developmental cycle of a hair follicle, the anagen stage is the phase of active growth and development (17 days). In a more detailed analysis of the anagen phase, Chase *et al.*(1) have observed 6 clearly defined substages. The subsequent catagen stage is the phase of growth cessation, club hair formation, and setting aside of the dormant hair germ for the next hair generation (2 days). The telogen stage is the resting phase(2). The degree of injury which can be inflicted upon skin appears to depend not only upon the types of treatment but also upon the stage of development of the existing hair follicles. X-radiation with 300 r to 1000 r during anagen stages results in cessation of development in the follicle and the loss of any growing hair. During catagen and telogen stages, such x-ray doses cause no loss of hair, the club hairs remaining apparently undisturbed(3). In addition, x-radiation has also a profound effect upon hair pigment. Pigment loss for the ensuing hair generations is greatest in follicles x-rayed in catagen or telogen(3-5). In contrast to the effects of x-ray on the hair, single or repeated applications by painting of irritants such as K_2PO_4, ether, benzene(6), or methylcholanthrene-in-benzene have no effect on hairs in anagen but cause loss of hairs in the telogen stage. Whereas such doses of x-radiation have only a slight effect upon the pigment cells in the epidermis, upon the integrity of sebaceous glands, and upon hyperplasia of the epidermis—a single painting of 0.6% methylcholanthrene-in-benzene results in increased melanization in dendritic cells of the epidermis, loss of sebaceous glands(7-9), and hyperplasia of the epidermis. The painting, however, has no effect upon the growing hairs. After the loss of sebaceous glands by treatment with methylcholanthrene, a complete redifferentiation of the glands from external sheath cells occurs if and when there is active hair development in the follicles(9).

Methods. Experiments were carried out to

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determine the extent of injury in the epidermis and in the cutaneous appendages following application of methylcholanthrene alone and in combination with exposure to 1000 r of x-rays. In order to ascertain whether the stage of the hair follicles had any significant influence on the extent or type of injury induced by these agents, the right half of the dorsum of mice whose hair follicles were in the telogen (resting) stage, was plucked, while the left side was merely clipped. Plucking of club hairs initiates the growth of the next hair generation in that area whereas careful clipping or shaving does not(3). Eighteen mice (13 males and 5 females, 3 to 6 months of age) of the inbred C57 Black strain were used. In the first experiment the carcinogenic agent was applied after a period of 6 days when the clipped area was still in the telogen stage, and the plucked area was in substage anagen IV (an active phase of hair development in which the bulb is at maximal mitotic activity although the tip of the hair shaft has not yet emerged through the skin surface)(1). At this time, then, the whole dorsum was painted with 0.6% methylcholanthrene-in-benzene while only the posterior one-half of the dorsum received in addition an x-ray dose of 1000 r (100 kv, 10 ma, 17.4 cm, 389 r/min, air dose). Thus, in the same animal 4 skin areas were obtained in which conditions were different. To simplify matters these areas will be referred to as A, B, C and D (A, anterior left half with club hairs clipped, and treated with methylcholanthrene; B, anterior right half with club hairs previously plucked, and now in substage anagen IV, treated with methylcholanthrene; C, posterior left half, like A but, in addition, irradiated with 1000 r; D, posterior right half, like B, but also irradiated).

In a second experiment all of the skin areas treated were in the telogen stage. The left half of the dorsum was simply clipped while the right half was plucked at the time of treatment with methylcholanthrene and x-ray. The manipulations of this experiment were similar to the first experiment, with the exception of the 6-day interval between the time of plucking and the treatments.

Observations. Daily gross observations in the first experiment show in area A, a progressive keratosis and epilation of the stumps of clipped hairs, which persist a variable period of time up to 17 days following treatment when the experiment was terminated. In many instances, by the end of the experiment, the hair follicles are reactivated and growth proceeds apparently normally. In area B there is a moderate keratosis but no inhibition of hair growth. In area C there is keratosis, epilation, and subsequent ulceration which in some cases continues to the termination of the experiment. Area D shows some keratosis, a retardation of hair growth, and occasionally some ulceration.

In the second experiment (treated at the time of plucking and clipping) areas A and B are comparable to A of the first experiment, *i.e.* telogen follicles. Area B shows a more progressive keratosis and occasional ulcerations. In some cases, A as well as B has entered the anagen stage by the time of termination of the experiment. Areas C and D become keratotic and ulcerated. Although these two regions are comparable (they are also comparable to C of the first experiment), D, which has been plucked rather than clipped, in most cases shows a more advanced state of injury.

Histological sections of biopsies taken at 1, 4, 7, 10, 13 and 17 days after treatment substantiate the gross observations. A more extensive description of the cytological effects will be published in a separate paper. Area A in the first and in the second experiment, shows hyperkeratosis and hyperplasia of the epidermis accompanied by hypertrophy of the nucleus and cytoplasm in both surface epidermis and in cells of the hair follicles. These conditions are progressive for about 10 days and then recede. In some cases the condition is alleviated by the end of the experiment. Amelioration is always accompanied by reactivation of the hair follicles.

Area B in the first experiment shows only a moderate hyperplasia and keratosis up to the 4th day. A normal condition, including a restoration of the sebaceous glands is observed by the 10th day. In the second ex-

periment, the epidermis of area B shows massive amounts of hyperplasia with hyperkeratosis and parakeratosis. There are numerous giant cells with nuclear aberrations. Moderate acanthosis is often found. These changes are progressive until the 10th or 13th day at which time there is some repair of the injury which is accompanied by a restoration of hair growth activity.

Areas C, in the first and second experiments, are alike. Massive progressive hyperplasia and hyperkeratosis of the epidermis and hair follicles occurs at first. Gigantic epidermal cells having either giant nuclei or clusters of nuclei are numerous throughout the duration of the observations. In sites of ulcerations single flattened giant cells may often be seen extending over the granular tissue of the dermis. In other places large areas of hyperplastic epidermis are underlain by invading epidermis. In still other areas, nothing remains of the epidermis except a few cords of giant cells extending into the dermis. Damage here in area C is severe and at the termination of the experiments only rarely did we encounter even slight amelioration or repair.

Area D in the first experiment, with follicles in anagen IV at the time of treatment, shows some hyperkeratosis and hyperplasia. This, however, is not severe. There is, in addition a cessation of growth of the hair follicles which lasts a variable interval of time. In all cases, however, the skin appears to be normal by the end of the experiment. In the second experiment, however, area D, with follicles in telogen at the time of treatment but the club hairs plucked instead of clipped, shows the most severe damage. It is essentially like area C of both experiments, but the degree of injury and other induced changes are even more severe. At the end of 17 days the epidermis in area D of the second experiment is still hyperplastic and acanthosis and utricular cysts are commonly found.

Conclusions. The combined treatment of methylcholanthrene-in-benzene painting plus 1000 r irradiation results in changes in the epidermis which are more severe than those occurring after either treatment alone. Sebaceous glands are destroyed by methylcholanthrene and redifferentiate only when hair growth is active. Where hair development is arrested or delayed by the irradiation, there is a failure of new sebaceous glands to redifferentiate until hair development again occurs. Furthermore, the initial damage to the epidermis in terms of hyperplasias and hyperkeratoses is less in areas where hairs were in anagen IV at the time of the single or combined treatment. Maximal damage occurs in telogen areas. Not only, then, is active hair proliferation associated with restoration, as in the case of sebaceous glands, but it also protects the epidermis from damage and it is associated with the repair of epidermal damage.

The explanation of such phenomena is as yet incompletely known. Cells of the external sheath of the follicle are directly involved in redifferentiation of sebaceous glands and to a considerable extent in repair of the epidermis. This layer of external sheath cells forms the morphological continuity between the hair germ or bulb and the basal layer of the epidermis. The resistance of epidermis to damage, as well as the capacity for repair, which are associated with hair proliferation may well indicate a comparable functional continuity of the external sheath. In any case, the importance of considering and controlling conditions in the skin at the time of and after treatments is obvious.

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