Histamine in Full-Thickness Skin Autografts of Rat.* (29673)

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The processes of repair are characterized primarily by edema and secondarily by mucinous and fibrous organization of the fluid(1). Histamine induces increased vascular permeability and tissue edema. Mast cells contain and synthesize histamine, and histamine release has been associated with mast cell degranulation(2). The implication of histamine in the response of connective tissue to injury has been considered(3-8). Marckmann and Zachariae(8) studied skin histamine in autologous full-thickness skin grafts of the pig. During the first days after operation a release of histamine was observed. The histologic studies, however, revealed a superficial necrosis in the grafts. Although the necrosis was followed by development of new epidermis from the epithelial cells of the hair sheaths further studies on histamine in skin grafts were considered desirable.

The present paper reports results of analyses for histamine in autologous full-thickness skin grafts of the rat. Determinations were made by the spectrofluorometric assay method(9).

Material and methods. Fifty-four young male rats of an inbred Wistar strain, (State Serum Inst., Copenhagen), weighing 191 ± 3.8 g (standard error of mean) were maintained on an adequate diet with free access to water. The rats were kept in the animals' quarters at least 3 days before operation. Forty-seven rats had full thickness skin autografts, while 7 rats served as controls.

Anesthesia. The animals were anesthetized with ether during shaving, operation, and changes or removals of dressings.

Grafting-technic. The skin of the back and the sides of the abdomen was carefully shaved with an electrical clipper and a sharp knife. On the right side, midway between the costal arch and iliac crest, and midway between the spine and the umbilicus a circular skin area, 2 cm in diameter, was removed, excised down to the muscular fascia. The panniculus carnosus and adhering fat were carefully dissected off. The skin graft was turned 180 degrees, replaced, and sutured by means of surgical silk 000 in an atraumatic needle. The graft, its surroundings and the symmetrical side were treated with a thin layer of 3% tetracycline ointment and covered with a piece of filter paper. For compressive purpose a gauze pad was placed over the graft. Finally, the animal was dressed with 2 turns of adhesive tape around the trunk. Following operation the animals were individually caged. Dressings were changed at least 7 days after operation and definitely removed on the tenth or eleventh day after operation together with the sutures.

Skin sampling. After sacrificing the animals with ether from one to 28 days after operation, the grafts, their surroundings, and the symmetrical area were cleaned and shaved. The grafts were removed from the fascia. Until the fifth day this could be done by lifting off the graft from the wound bed. Later, it was necessary to dissect the graft free. This was done with great care to avoid the fascia. To exclude the marginal wound and the suture line a 2 mm broad brim was cut from the edge of the graft.

On the left side, symmetrical skin was removed and freed of panniculus carnosus and subcutaneous fat. 2 to 3 mm next to the graft, in front of this, a small skin sample was removed and treated in the same way. 4 mm punch biopsies were obtained from the center of the graft, from its symmetrical skin area, and from the skin next to the graft.

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TABLE I. Histamine Values in Back Skin of Control Animals.

Rat No.	μg Histamine/g dried defatted skin
1	49.2
2	60.7
3	53.3
4	42.0
5	51.4
6	61.2
7	61.9
Mean and S.D. of mean	54.2 ± 2.8

The non-wounded control animals were sacrificed and skin biopsies obtained in a similar way from the right side of the body.

Fixation and staining. For histological examination a skin specimen was excised from the punch hole to the margin of the graft, from symmetrical skin, and from normal skin of the control animals. The biopsies were fixed in a 4% aqueous lead subacetate solution for 24 hours, embedded in paraffin, sectioned and stained with a 0.5% aqueous solution of toluidine blue, which rather selectively stains acid mucopolysaccharides metachromatically.

Determination of water content. Skin samples were weighed immediately after removal. The minced samples were lyophilized for 48 hours over phosphopentoxide at room temperature under a pressure of 2 mm Hg. After weighing, the samples were defatted by shaking one hour in acetone, one hour in ether, and once more one hour in acetone. Finally the specimens were lyophilized for 48 hours. The weight now recorded is referred to as dry defatted weight. Values of the water content are given in per cent of dry defatted weight.

Histamine determinations. Histamine determinations were performed on approximately 4 mg dried defatted skin. The dried and defatted samples were homogenized in 5 ml of 0.4 N perchloric acid using a motor-driven glass homogenizer. The homogenate was allowed to stand for 10 minutes and then centrifuged. A 4 ml aliquot of the supernatant was analyzed for histamine by the spectrofluorometric method of assay according to a procedure previously described by one of the authors(10).

Results. The take of the grafts was assessed both grossly and microscopically. Only one graft showed signs of decreased viability. At the removal 8 days after operation it appeared yellowish and more or less necrotic. Microscopy revealed a marked infiltration of inflammatory cells. The rest of the grafts were all fully viable.

As summarized in Tables I and II, and Fig. 1, the data of the histamine determinations show that, within the first 24 hours following operation, the average histamine content of the skin graft was reduced to less than one-third of the average skin histamine level in the control animals. The reduction was highly significant ($p<0.0005$). This decrease continued, and on the third and fifth day after operation extremely low skin histamine values were demonstrated in the grafts. Thereafter, a gradual increase was observed.

TABLE II. Histamine Levels of Back Skin of Non-operated (Control Animals) and Operated Rats. Figures represent mean values and standard error of mean.

Days after transplantation	No. of animals	Skin histamine (μg/g dried defatted skin)		
		Uninjured skin		Graft
		Symmetrical area	Area next to graft	
Control	7	54.2 ± 2.8		
1	7*	46.7 ± 2.4	47.9 ± 5.6	16.0 ± 3.9
3	7	55.3 ± 4.8	50.7 ± 11.5	6.4 ± 2.5
5	7	43.4 ± 5.0	40.2 ± 6.2	7.7 ± 2.4
8	7	43.9 ± 2.8	45.1 ± 4.9	20.0 ± 3.9
12	7†	36.6 ± 3.6	43.5 ± 11.5	33.6 ± 5.1
21	6‡	32.8 ± 2.2	27.2 ± 4.7	51.7 ± 5.7
28	6	27.7 ± 3.3	not determined	57.0 ± 6.0

* Skin next to graft investigated in 6 animals. † Skin next to graft investigated in 5 animals. ‡ Skin of graft investigated in 5 animals.

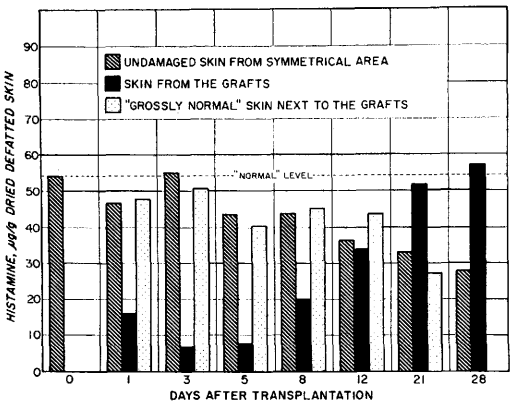

FIG. 1. Average amounts of histamine in full thickness skin autografts, uninjured skin from the corresponding area from the contralateral side, and grossly normal skin next to grafts followed after operation, together with average skin histamine value of control animals.

On the twenty-first day the histamine values of the grafts had exceeded the figures of the symmetrical uninjured areas and had reached the levels of the control animals. On the twenty-eighth day post operation histamine levels of the grafts were approximately twice as high as those of skin from symmetrical areas.

The skin histamine levels of uninjured symmetrical skin as well as of skin next to the grafts slowly decreased. On the twenty-first day after operation the reduction was significant for both areas ($p<0.0025$). On the twenty-eighth day only symmetrical skin was studied. Skin histamine levels were approximately half the values for non-operated rats. No traces of histamine could be demonstrated in the graft showing decreased viability on the eighth day after transplantation.

All values are given in μg histamine base per gram dried defatted skin.

Although no detailed countings of mast cells were performed, microscopy revealed a decrease in number of identifiable mast cells in the grafts early after operation. Later the mast cell population of the grafts increased to amounts at least not lower than in normal rat skin.

Determinations of water content (Table III) showed an immediate increase in the grafts. After being almost constant from the first to the fifth day after operation a secondary increase in water content was found from the eighth day reaching a maximum on the twenty-first day.

Discussion. Twenty-four hours after the operation edema was demonstrated in the grafts. The simultaneous reduction in histamine content indicates a considerable histamine release. This suggests that histamine may play a part in the early phase of repair and survival of the graft. Histamine probably increases vascular permeability in the graft and in the wound bed. Whether histamine release precedes and produces edema or the edema is primary and responsible for the release of histamine has not been established. Besides inducing edema, histamine may stimulate active transport processes across endothelial barriers(11). The decrease of the histamine values in the grafts is similar to the decrease previously found in full thickness skin grafts of pig(8). In linear wounds a decrease in histamine content has been demonstrated previously(5,6).

The measurements of water content showed a further increase. The edema of the graft was still pronounced 21 and 28 days after the operation, thus indicating that, although macroscopically normal, the grafts had not completed repair.

In the rat, the number of mast cells decreases in the region of a skin wound in the first 24 hours(12). Also, in the skin graft, early after operation, microscopic studies gave the impression of a reduction in number of mast cells. These findings suggest that there

TABLE III. Water Content in Skin of Non-operated (Control Animals) and Operated Rats in Per Cent of Dry Fat-Free Weight. Figures represent mean values and standard error of mean.

Days after transplantation	No. of animals	Water content (%)	
		Skin from symmetrical area	Graft
Control	7	193 ± 2.8	
1	7	193 ± 2.6	213 ± 4.0
3	7	192 ± 5.2	209 ± 6.6
5	7	188 ± 5.8	214 ± 5.5
8	6*	197 ± 8.8	238 ± 8.0
12	7	197 ± 7.5	239 ± 5.0
21	6	205 ± 1.7	273 ± 4.8
28	6	211 ± 3.5	258 ± 6.4

* One animal with decreased viability of graft was excluded.

is a connection between wound healing and repair on the one hand and mast cells and histamine on the other.

In studies on pig, microscopy revealed a superficial necrosis to take place regularly in full thickness skin grafts. Pig skin differs from rat skin by containing only one main concentration of mast cells and histamine in the subepidermal layer of the dermis(13), whereas the mast cells and histamine in the rat are mainly located in an inner layer(2) close to the base of the graft. This closer relation to the vessels of the wound bed may be of importance to survival and the early phase of repair.

The present study does not allow the conclusion that histamine is important during the continued repair processes in skin grafts. However, the increase in histamine forming capacity demonstrated in wound tissue by Kahlson and co-workers(6) may suggest such a further influence. The secondary reduction of skin histamine in uninjured areas indicates that regeneration of the graft histamine may take place at the expense of normal skin histamine.

Summary. Analyses of histamine and water content performed on full thickness autografts of rat skin showed a considerable histamine release during the first 24 hours after operation. Simultaneously an increase in water content of the graft was measured. It is suggested that histamine may play a role in the early phase of repair and to survival of the graft, presumably by increasing vascular permeability.

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Antigen Stimulated Proliferation in Lymphoid and Myeloid Tissues from Immunized Rabbits.*† (29674)

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Earlier work from this laboratory has established that the uptake of radioactive thymidine by spleen cell suspensions from previously immunized rabbits is stimulated by addition of antigen *in vitro*(1). This response represents an *in vitro* counterpart of the cellular proliferation seen in the *in vivo* secondary immune response.

The purpose of the experiments reported here was to determine if similar antigen-de-

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