

CUTANEOUS RESORPTION OF HEPARIN AND HEPARINOIDS INVESTIGATED BY AUTORADIOGRAPHY*

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The question whether heparin and heparinoids are absorbed after cutaneous application still remains controversial. Jorpes in his extensive review (1) on heparin does not even mention this way of application. Heparin ointments are not mentioned at the "Symposium on Heparin" related in the American Journal of Cardiology 1964 (2). Finne denies any effectiveness of a heparinoid ointment (3). So does Konrad in a study with cattle (4).

On the other hand there are numerous papers mainly of clinical origin which claim some effectiveness of such ointments. Histochemical attempts to demonstrate absorbed heparin (5, 6) are not convincing in our view for reasons dealt with below.

Clear cut proofs for a systemic effectiveness in humans of epicutaneously applied heparinoids are lacking. The absorption, if any, must be very small. Therefore, we attempted to show, at least locally, absorbed heparin in the tissue by means of autoradiography which seemed especially suitable due to its high sensitivity.

MATERIAL AND METHODS

We used S^{35} labelled heparin, the heparinoid Ro 1-8307 and several other S^{35} labelled heparin derivatives with a radioactivity of 0.5 to 1.0 milliecurie per mg in the form of an ointment containing 5% of heparin or heparinoid. The S^{35} label was introduced in the N-sulfo-groups.

To test each substance, 6 rats with a body weight of approximately 200 gm were depilated on the left side with a depilatory ointment and electrically shaved on their right side. 0.5 gm of the radioactive ointment was rubbed in during 10 minutes in an area measuring about 2 cm in diameter. After 30 minutes, 2 and 8 hours, two animals were killed. The treated skin was carefully washed with water and soap, excised and the pieces cut in two halves. One half was fixed in Carnoy, embedded in paraffin and cut into 5 μ thick sections. The other half was cut on a cryostat without fixation.

Cryostat and paraffin sections were covered with stripping film Kodak AR 10 and exposed for 4 weeks. After development microscopical evalua-

tion was carried out, partly on unstained slides, partly on slides stained with toluidine blue. The method is open to two objections:

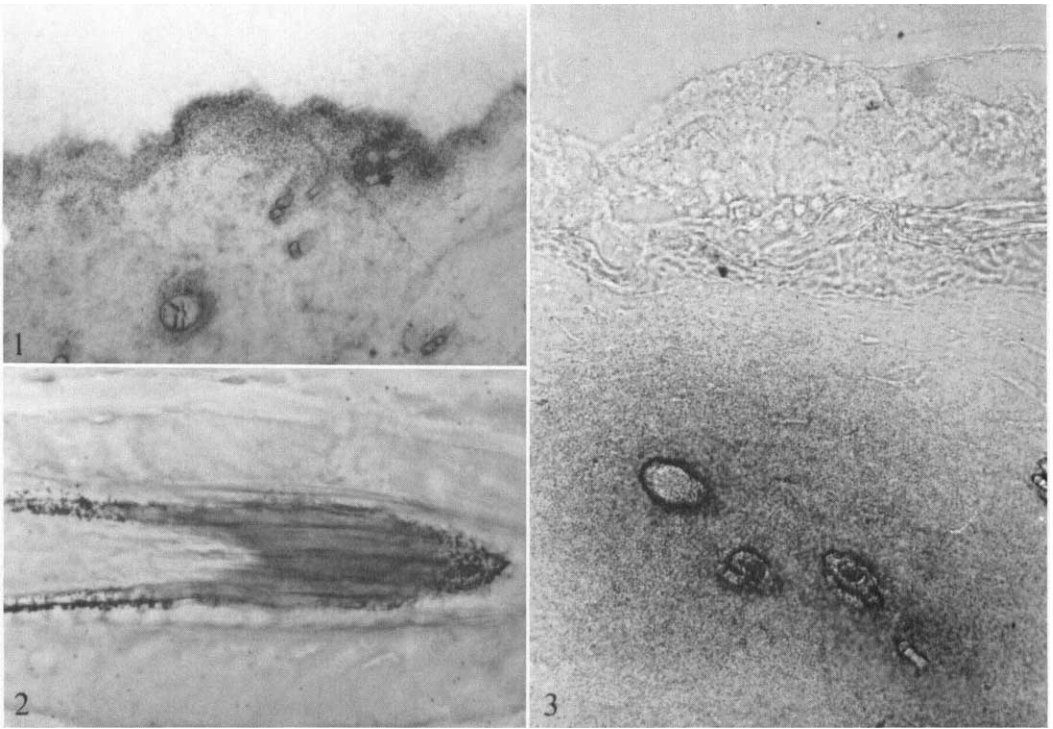
1. There is the risk that the label is enzymatically split off from the compound. The autoradiography shows, strictly speaking, only the localization of S^{35} , and not that of the biologically active compound. Positive findings would only indicate therefore that S^{35} (or sulfate) had penetrated into the skin but this would not prove the presence of the biologically active substance itself. This possibility exists, though we estimate it to be extremely low, since intracutaneously or subcutaneously injected heparin displays its undiminished biological activity, whereas desulfated heparin remains inactive.

2. The labeled substance could diffuse into the tissue during the preparation of the histological sections, for instance during fixation of the tissue or during thawing of the cryostat sections or during other phases of preparation, simulating absorption. It is difficult to exclude these possibilities by adequate controls. However, this bias is extremely unlikely, considering our results (see below).

RESULTS AND DISCUSSION

No difference could be found between electrically depilated and electrically shaved skin, nor between the various heparinoids used, nor with regard to the time at which the skin was taken after application of the ointment, nor between cryostat and paraffin sections. Radioactivity was limited to the epidermis and to the surface of the hair, penetrating into the depth of the hair follicles. In a few cases a diffuse radioactivity of a limited degree could be shown in the most superficial layers of the corium, especially in the immediate vicinity of the hair follicles or adjoining glands (Figs. 1-3). The identical findings in Carnoy fixed tissue and in cryostat sections as well as the sharp localization of the label usually found (for instance on the surface of hairs) speak against the possibility of the radioactivity being an artefact produced by diffusion during the histological preparation. Control slides from untreated animals never gave rise to silver grain formation. In conclusion, we estimate it, therefore, as probable that the small amounts of radioactivity found beneath the epidermis

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Autoradiographies from unstained sections. Skin inuncted with labelled heparin ointment. All sections cut perpendicular to the skin surface.

FIG. 1. Radioactivity limited mainly to the epidermis, penetrating only scarcely into the deeper layers of the cutis. $\times 120$.

FIG. 2. Longitudinal section of a hair. Radioactivity limited strictly to its surface. $\times 240$.

FIG. 3. Radioactivity in the immediate vicinity of hair follicles and adjoining glands. $\times 187$.

indicate penetration of labeled heparin(oid) through the epidermis *in vivo*.

Preparations stained with toluidine blue showed that there is no parallelism between radioactivity and metachromasia. On the other hand, the numerous and strongly metachromatically colored mast cells as well as other metachromatically colored structures showed no radioactivity. The offhand explanation appears to be that toluidine blue staining is much less sensitive compared to the autoradiographic method and that therefore the absorbed quantities of heparin or heparinoids must be very small, as compared to the quantities of heparin already present in mast cells and other structures.

In interpretation of our findings, one must bear in mind that these preparations like all histological preparations are "snapshots". They may provide a picture of the momentary

concentration of a label in a tissue but they cannot supply information on the movements of this label. Though we can only find small amounts of label in all preparations, it is possible that significant amounts have been absorbed.

In fact, this assumption seems probable, if we consider our knowledge on the effectiveness and intensity of microcirculation within the dermis. Scheuplein (7) states "...the rich vascularity of the superficial layers of the dermis should drastically limit the number of molecules free to diffuse deeper into the dermis..." and "...most molecules reaching the vascularized region... would be swept into systemic circulation. The stratum corneum limits the accumulation of substances within the viable epidermis by preventing their entrance except at rates so slow that the relatively rapid diffusion from the aqueous viable

cells to the cutaneous microcirculation is adequate to maintain their concentration at very low levels”.

The assumption that considerable amounts of heparin may cross the epidermal barrier is corroborated by the observation that even systemic effects may be observed in rats with chemically depilated skin (8).

Our findings are in good agreement with those of MacKee *et al.* (9) who studied the problem of percutaneous penetration in a general manner as early as in 1945. Their conclusion: “the most likely route of maximum penetration of the inuncted materials was as follows: From the outside into the horny layer; follicle openings; down the follicle, out of the follicle and into the cutis...” is corroborated by our observations, with the limitation that only very small quantities of material have been found in our experiments.

The histological findings of Wegmann and Garraud (6) and Lindner (5) who claim an increase of metachromatic substance in the tissue after application of heparin ointments could not be reproduced in our work. The variations in the distribution pattern of the metachromatic substances from one section to the other, even from one part of the same section to the other, are remarkable. Therefore, the increase in content of metachromatic substance would have to be very large indeed to reach the level of significance. Pictures as published in the above mentioned papers can easily be obtained from the same single preparation; thus, they carry no conviction.

SUMMARY

Autoradiography after epicutaneous application of S^{35} labeled heparin and heparinoid ointments shows small amounts of radioactivity beneath the epidermal barrier. Despite possible technical errors, this finding is interpreted as indicating penetration of the labeled substances *in vivo*. The concentration of radioactivity found in the tissue is very small because the absorbed substances are immediately transported away. Accumulation in defined structures beneath the epidermal barrier could not be demonstrated.

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