Reactivation of Arthus-Type Inflammation by Reduced Glutathione. (26825)

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Recent reviews have summarized the evidence for the activation of proteolytic enzyme systems as a common mechanism for various types of allergic reactions(1,2). We found that the activity of an SH-dependent skin protease increases simultaneously with development of the cutaneous Arthus phenomenon and decreases as the inflammation subsides. The latter phenomenon is due, at least in part, to local accumulation of a protease inhibitor of polypeptide nature(3-5). Both enzyme and inhibitor are released from mesenchymal connective tissue cells and can be extracted in the euglobulin fraction of the inflamed skin(5-7). The Arthus reaction was shown to be inhibited by the inhibitor-containing euglobulin(8) or the partially purified inhibitor.

It was observed in a preliminary study that the euglobulin extracted from healing Arthus sites shows little protease activity but becomes proteolytic on addition of reduced glutathione (GSH). The present paper shows that intradermal injection into completely healed Arthus sites results in rapid reactivation of the inflammatory process.

Materials and methods. Adult male albino rabbits were sensitized with 3 subcutaneous injections of 5 ml bovine serum every other day. On the 11th day after last injection the precipitin titer of the sera was measured by the ring test(5). Animals showing precipitin titers of 2^6 were given 4 intradermal injections of 0.2 ml antigen in the flanks. The reaction became visible 5 hours later, reached its peak in 24 hours and decreased thereafter. Inflammatory reactions were graded according to the method of Cochrane and Weigle(9). The present study was done 12 to 19 days after injection when the reaction had completely disappeared.

A commercial preparation of GSH was used after verification of its reduced state by amperometric titration(10). Amounts of 0.2 or 0.3 ml of 0.1 M GSH (in physiological saline) were injected intradermally into the center of 2 of the healed sites, shaved 24 hours prior to injection. The 2 opposite sites received the same volume of saline. The reactions produced were compared macroscopically and histologically after fixation in 10% formol and hematoxylin-eosin staining. An injection of GSH was also made into a virgin skin site of each animal.

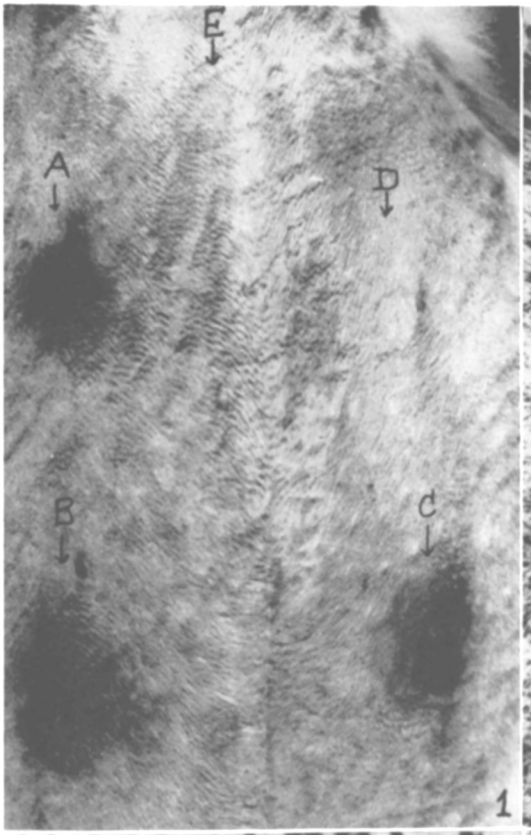
Results. Injection of GSH into healed Arthus sites was followed in all cases by the appearance of visible inflammatory reactions within 2 hours, which reached a peak in 9 to 11 hours and lasted 72 to 96 hours. These reactions developed and regressed more rapidly than the original Arthus phenomenon. At the height of the reaction, moderate to severe hemorrhage was observed with mild sloughing in the center of the lesion; there was no severe desquamation or necrosis. In 3 out of 8 cases the inflammation was almost as intense as in the original Arthus reaction; in the other 5 cases the reactions were moderate (Fig. 1). With 0.2 ml GSH the inflammatory reaction followed the same sequence as above but the reaction was less marked; mean value of the great diameter of the lesion was 22 mm. In Table I is a comparison between the original Arthus reaction

FIG. 1. Reactivated inflammation in rabbit skin. A, B and C, 18-day-old healed Arthus sites inj. with 0.3 ml 0.1 M GSH (3+ grade reactions); D, healed site inj. with 0.3 ml saline; E, virgin site inj. with 0.3 ml 0.1 M GSH. Picture taken 10 hr after inj.

FIG. 2. Photomicrograph of reactivated lesion. Hematoxylin-eosin staining; ×45. Skin specimen collected 10 hr after inj. of GSH. The reaction is almost indistinguishable from original Arthus phenomenon.

FIG. 3. Marked degree of perivascular infiltration of mononuclear cells in proximity of the muscular layer. Hematoxylin-eosin, × 400.

FIG. 4. Fibrinoid exudation and leucocytic infiltration in reticular layer; small blood vessel showing lesions of panarteritis nodosa type. Hematoxylin, ×150.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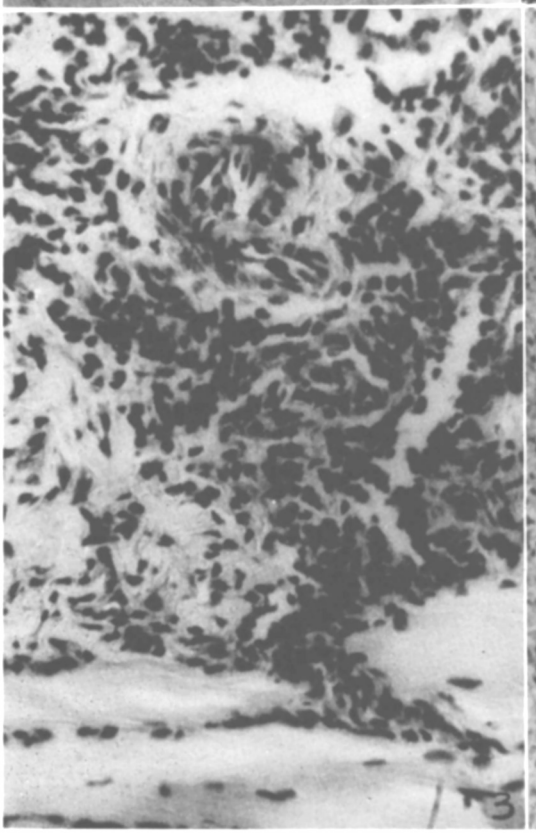
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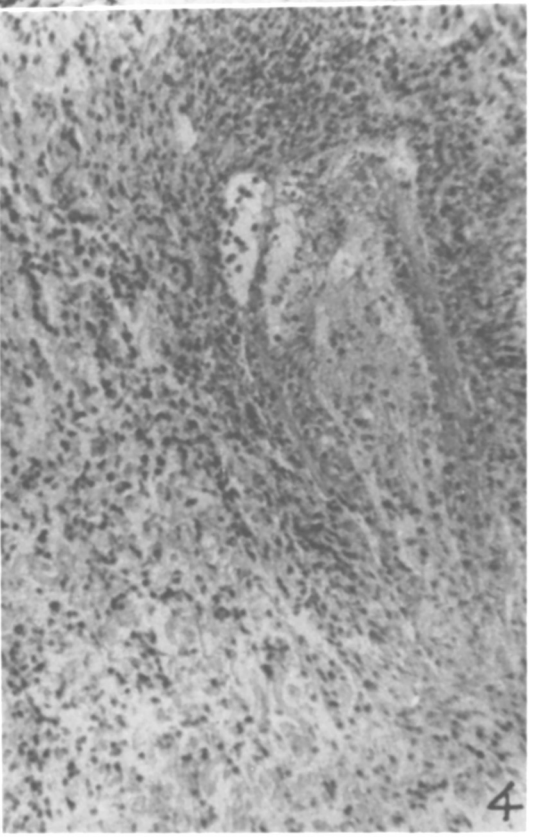
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TABLE I. Macroscopic Reactions to Injection of GSH into Healed Arthus Sites.

Animal groups No.	Inj. GSH, cc	Reactions* Original Arthus†	Healing day	GSH-inj. site‡
1, 2, 3	.3	r. 4+, 4+, 4+	12, 12, 14	4+, 4+, 3+
4, 5, 6§	"	r. 4+, 4+, 4+	15, 14, 18	2+, 2+, 3+
7, 8	"	r. 4+, 4+, 4+	19, 13	2+, 2+, 2+
9, 10, 11	.2	r. 4+, 4+, 4+	15, 18, 15	1+, 2+, 1+

* Mean grade of inflammation in each rabbit; there was little difference in reaction intensity in different sites of the same rabbit.
† Reactions at 24 hr.
‡ " " 10 hr.
§ Shown in Fig. 1.
Inj. of GSH into virgin site or of saline into healed site induced no reaction.

and the reactivated phenomenon.

No reactions were observed after injection of GSH into virgin skin sites of sensitized animals or after injection of saline into healed Arthus sites.

Histological studies on biopsy specimens taken 10 hours after injection of GSH showed varying degrees of cutaneous lesions similar to those of the original reaction. The lesions were characterized by: 1. diffuse and local infiltration by inflammatory cells; 2. fibrinoid exudation as shown by eosinophilic swelling of collagen bundles and interfibrillar deposition of an eosinophilic amorphous material; 3. vascular changes visible as eosinophilic swelling and necrosis of vessel walls; 4. hemorrhage or formation of hyaline and fibrinous thrombi in the small blood vessels. Varying degrees of these lesions were found in all sites. The most severe reactions (3 animals) were almost indistinguishable from the original Arthus phenomenon (Fig. 2). In 8 other cases the reactions were moderate and 2 among these were characterized mostly by extensive fibrinoid exudation.

The type of cells participating in the reaction is of interest. Mononuclear infiltration appeared either in a diffuse or a focal (perivascular) pattern with a preference for the papillary layer of the corium and the subcutaneous tissue close to the muscular layer (Fig. 3). Some of the cells were lymphocytes but most of them were monocytes and histiocytes. Large number of plasma cells were found in the same area, especially around small blood vessels. The dense mononuclear accumulation resembled the picture observed at a late stage of the Arthus phenomenon. Polymorphs and eosinophiles were also distributed in either a diffuse or a focal pattern but the greatest accumulation took place in the reticular layer of the corium. Fibrinoid exudation and vascular reaction also predominated in this area where varying degrees of lesions of the peri- or panarterites type were observed (Fig. 4). These lesions were similar to those observed in the early phase of the Arthus reaction.

Varying numbers of mitoses were observed in the epidermis of the reactivated area, especially in the 3 cases which showed the most intense reaction. Mitoses were also seen in the muscular layer of the walls of small and middle sized arteries.

No significant histological changes were observed in the saline injected sites or the virgin sites treated with GSH.

Discussion. Cochrane *et al.*(11) showed that reinjection of the antigen into a healed Arthus site induces a lesion similar to the original reaction. This reactivation is certainly different from the phenomenon described in this paper, and it was shown to be produced by an antigen-antibody reaction in the blood vessel walls. Our observations can probably be interpreted in terms of activation of proteolytic enzymes by GSH. It is also possible that GSH acts on the inhibitor polypeptide mentioned above since it has been shown that this substance loses its activity under the influence of reducing agents(12). GSH is a powerful endogenous hydrogen carrier and it is believed that one of its functions is to keep SH-dependent enzymes in the

reduced form. The observations presented here may have some bearing on the mechanism of the recurrence of chronic inflammatory processes in rheumatic myocarditis and rheumatoid arthritis.

Summary. Injection of GSH into recently healed sites of Arthus phenomenon produces a reactivation of the inflammatory process. The reactivated lesion has the same aspect and same histological characteristics as the original reaction.

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