competitively reverses the inhibitory action of cycloserine on *S. faecalis* cells that are synthesizing cell wall substance. Both cycloserine and penicillin can inhibit cells that are not growing but that are making cell wall substance. However, they differ in mode of action.

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1. Bondi, A., Kornblum, J., Forte, C., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 270.

2. Harris, D. A., Ruger, M., Reagan, M. A., Wolf, F. S., Peck, R. L., Wallick, H., Woodruff, H. B., *Antibiot. & Chemother.,* 1955, v5, 183.

3. Kuehl, F. A., Wolf, F. S., Trenner, N. R., Peck, R. L., Howe, E. E., Hunnewell, B. D., Downing, G., Newstead, E., Buhs, R. P., Putter, I., Ormond, R., Lyons, J. E., Chaiet, L., Folkers, K., *J. Am. Chem. Soc.,* 1955, v77, 2344.

4. Ciak, J., Hahn, F. E., *Antibiot. & Chemother.,* 1959, v9, 47.

5. Park, J. T., *Symp. Soc. Gen. Microbiol.,* 1958, v8, 49.

6. Shockman, G. D., *Bact. Proc.,* 1959, 128.

7. Shockman, G. D., Kolb, J. J., Toennies, G., *J. Biol. Chem.,* 1958, v230, 961.

8. ———, *Bact. Proc.,* 1957, 131.

9. Woolley, D. W., *A Study of Antimetabolites,* John Wiley & Sons, N. Y., 1952.

10. Snell, E. E., *J. Biol. Chem.,* 1945, v158, 497.

11. Lark, C., Lark, K. G., *Bact. Proc.,* 1958, 108.

12. Park, J. T., Strominger, J. L., *Science,* 1957, v125, 99.

13. McQuillen, K., *Proc. 4th Int. Congr. Biochem., Vienna,* 1958, v13, 406.

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Factors Responsible for Decline of Inflammation in Arthus Hypersensitivity Vasculitis.*† (25065)

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In recent studies of Arthus-type vascular inflammatory reactions(1,2), a rough correlation was found to exist between disappearance of antigen from the vessel and a decrease of inflammation in the reaction. This was noted both macroscopically, and in the vessels, microscopically. These results suggested that removal or perhaps some form of isolation of the antigen-antibody complex eliminated the phlogogenic stimulus and allowed healing of the damaged vessels to take place. However, such factors as a possible exhaustion of cellular or humoral components essential to development of the reaction, and/or production of inhibitors locally might account for the decrease in inflammatory reaction also. In order to find if these latter factors played an essential role in the decline of inflammation, experiments were performed in which antigen was reintroduced into healing Arthus sites at various times. This was performed under the supposition that any inflammation secondary to the presence of the reinjected antigen and its corresponding antibody would be prevented or at least decreased in intensity, if an exhaustion of essential materials had occurred, or if inhibitors played an important role.

Materials and methods. Antigen: Bovine serum albumin (BSA), Armour Laboratories, Lot #T-68204 was used to elicit the Arthus reactions. Aside from BSA, other antigens used for control purposes or to obtain antibodies for the fluorescent antibody studies included rabbit serum albumin (RSA) and rabbit serum globulin (RSG), (Fraction II), Pentex, Inc., Lot #6901 and human gamma globulin (HGG), (Fraction II), Squibb Laboratories, Lot #1615. *Antisera*: Antibodies to the antigens mentioned above were produced, characterized and absorbed to remove con-

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TABLE I. Macroscopic Reaction to Reinjection of Antigen into Healing Arthus Sites in Rabbits.*

Time of reinj. (after 1st inj.) (hr)	No. of rabbits	Reinj. antigen	Reactions†	
			Non-reinj. site	Reinj. site
48	3	75 μg N BSA	2+, 1+, 2+	4+, 4+, 4+
72	3	*Idem*	±, ±, ±	3+, 4+, 4+
	2	75 μg N HGG	±, ±	+, +
	2	27 μg N BSA	±, ±	3+, 3+
96	3	75 μg "	±, ±, ±	4+, 4+, 4+
216	3	*Idem*	±, ±, ±	4+, 4+, 4+

* Original Arthus reactions produced by I.D. inj. of 75 μg BSA N.
† Reactions read 7 hr after reinjection. Injections into virgin sites closely resembled reinjection sites in each case.

taminating antibodies as described previously (2). *Production of reactions*: Adult male albino rabbits were used for this study. The hair at the reaction site was carefully removed with electric clippers. (a) Active or classical Arthus reactions were produced by injecting 75 μg BSA nitrogen (N) intradermally (I.D.) into rabbits previously sensitized and containing between 150 and 1100 μg anti BSA N/ml serum. Reinjections of the antigen into the healing reactions were performed by injecting 27 or 75 μg BSA N into the identical site of the first injection at various times after the first as will be indicated later. Volumes of injected antigen were 0.15 ml or less. Reactions were graded as previously(1,2), and biopsies were taken either at 7, 16, or 24 hours after the second, superimposed injection. In each case half the biopsy was fixed in 10% formalin for hematoxylin and eosin (H&E) staining, while the other half was rapidly frozen at −70°C for fluorescent antibody studies. *Fluorescent antibody technic*: Frozen tissue sections were studied using the fluorescent antibody technic of Coons and Kaplan(3) with certain modifications(1).

Results. Four Arthus reactions were produced on the flanks of each of 15 sensitized rabbits. Typical severe hemorrhagic, edematous reactions appeared within 4 hours, reaching a maximum between 7 and 10 hours, declining somewhat in intensity at 48 hours and to a much greater extent at 72 hours. Microscopically, the classically described acute vasculitis was present in sections taken at 24 hours and fluorescent antibody studies at this time revealed the presence of antigen localized in the walls of diseased vessels. However, by 48 hours microscopic evidence for healing of the lesions was apparent with loss of most of the polymorphonuclear leukocytes (polymorphs, or rabbit heterophiles) in and around damaged vessels and with the presence of many mononuclear cells and a scattering of eosinophiles. Necrotic material and thromboses were still present at 48 hours although to a lesser extent than 24 hours. By 72 hours further evidence of resolution was present. Fluorescent antibody studies at 64 hours revealed that little or no antigen remained in vessel walls similar to that previously described for 48 hour reactions(2). The occasional small flecks of antigen remaining appeared to be surrounded by mononuclear cells and might well have been isolated from the surrounding milieu by this means.

Reinjection of 75 μg BSA N intradermally into the site of the healing lesions was performed at 48, 72, 96 or 216 hours after the original antigen injection. Three rabbits were used at each time of reinjection. To study the effect of small amounts of antigen, 2 rabbits were reinjected 72 hours after the first injection with 27 μg BSA N. In each rabbit a virgin site was also injected for purposes of comparison. As control, one of the healing sites on each of 2 rabbits was given a similar superimposed injection of 75 μg N HGG at 72 hours. The results are shown in Table I. In each case in which BSA was reinjected, a severe hemorrhagic edematous reaction rapidly ensued identical to not only the original Arthus reactions, but also to the virgin sites made at the time of reinjection (Fig. 1). The reactions to the reinjected antigen also developed at the same time as the virgin sites,

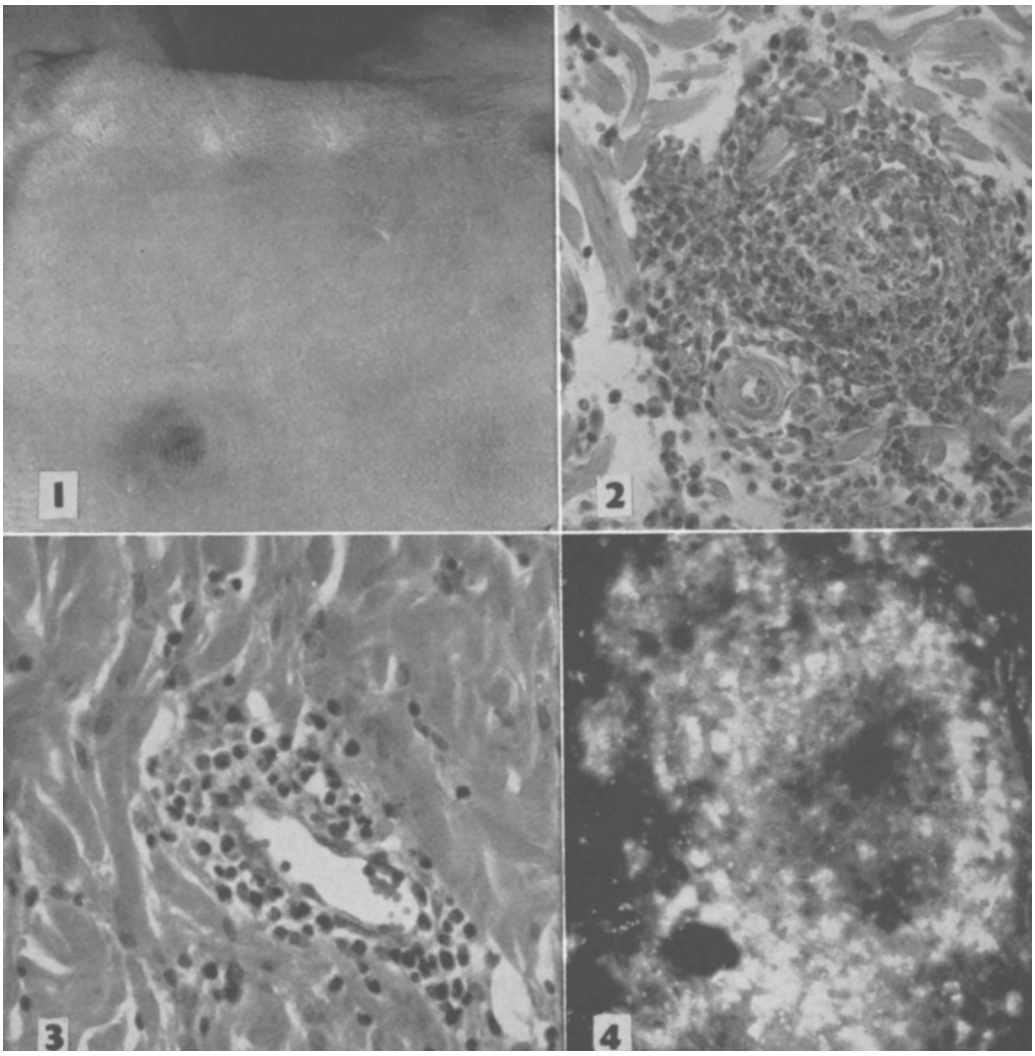

FIG. 1. Photograph of rabbit showing an Arthus reaction produced by a superimposed inj. of 75 μg BSA N into a healing 72-hr-old Arthus site. A 72-hr healing site is seen at right for comparison. Photo taken 6 hr after reinjection. × 0.6.

FIG. 2. Photomicrograph showing acute vascular inflammation 6 hr after reinjection of 75 μg BSA N into a 72 hr Arthus site. Section was taken from a reaction similar to that seen in Fig. 1. Hematoxylin and eosin. × 300.

FIG. 3. Photomicrograph showing a vessel in a section of healing Arthus site at 72 hr. Note that leukocytes are mononuclear in type and little evidence of inflammation persists in contrast to vessel seen in Fig. 2. Hematoxylin and eosin. × 300.

FIG. 4. Fluorescence photomicrograph showing presence of BSA in wall of an inflamed vessel similar to that seen in Fig. 2. Section was taken from a reinjected Arthus reaction 6 hr after second inj. This indicates that after reinjection of antigen in skin, antigen and antibody again concentrate in vessel walls. × 280.

showing distinct evidence of reaction by 2 hours. The sites not reinjected continued the normal resolution in each case showing the normal course of events of a healing Arthus reaction. The control superimposed injections of HGG failed to cause more than a mild edema and erythema that largely disappeared by 6 hours.

Microscopic sections of these lesions taken either at 7, 16, or 24 hours after reinjection of

BSA revealed severe vascular inflammatory changes (Fig. 2). These acutely damaged vessels were found throughout the section. In particular, acutely inflamed vessels were found in areas where mononuclear cells were present in considerable numbers. These sections were similar to the virgin injection sites except that an abundance of mononuclear cells was also present. The healing sites that were not reinjected showed the usual resolution seen at that particular time after antigen injection (Fig. 3).

Fluorescent antibody studies of the reinjected sites again revealed localization of the antigen and RSG in the vessel walls throughout the section (Fig. 4). This suggested that the specific etiologic agents, *i.e.*, antigen and antibody had again combined in the vessel walls.

Controls for the fluorescent antibody studies included the use of unrelated fluorescent sera, *i.e.*, fluorescent anti HGG and fluorescent normal rabbit globulin which yielded negative fluorescence in each case. Fluorescent anti RSA failed to show concentrations of RSA in the vessel wall although albumin was found diffusely scattered in the dermis. As further control, sections were flooded first with *non*-fluorescent anti BSA for 20 minutes and then, after washing, with fluorescent anti-BSA for 10 minutes and the intensity of these slides was compared with slides flooded first with non-fluorescent anti HGG for 20 minutes and then fluorescent anti BSA for 10 minutes. In each case the latter section revealed far greater intensity of fluorescence indicating that the fluorescence marking of the BSA could be blocked by prior application of unlabelled specific antibody.

Discussion. Previous studies have indicated that loss of antigen from a hypersensitivity vasculitis of the Arthus type corresponded in time with a diminution of the vascular inflammatory reaction. The studies reported here would indicate that it is the loss of the offending antigen that is necessary before diminution of reaction and healing of the vessels take place. This is in keeping with the hypothesis that the antigen and antibody are the phlogogenic agents responsible not only for initiation, but also for continuation of the Arthus vascular reaction. This conclusion is supported by the finding that reactivation of vascular inflammation in healing vessels followed the union of fresh antigen and antibody in the vessel walls.

Two other conclusions seem possible: 1). That the various cellular and humoral factors essential to development of the inflammation are present in quantities capable of supporting an inflammatory reaction at a time when the Arthus reaction is subsiding. It would seem, therefore, that any depletion of these essential elements does not play an important role in diminution of the vascular reaction. It was found that reunion of antigen and antibody in the healing vessels after reinjection was again accompanied by an influx of polymorphs, which from previous studies(2,4,5) have been shown to provide an essential key to the reaction. This stresses the important role of polymorphs in development of the vascular inflammation. Rosenberg, Chandler and Fischel(6) have recently found that passive cutaneous anaphylaxis reactions can be elicited in the same skin site in guinea pigs on 3 consecutive days suggesting that in these reactions also the materials necessary to support the inflammation were present in sufficient quantity. Stone(7) has shown that during protracted systemic anaphylaxis in mice and guinea pigs that development of Arthus reactions was inhibited. Bier *et al.*(8) and Osler *et al.*(9) have demonstrated an inhibition of passive cutaneous anaphylaxis in rats following protracted anaphylaxis, and have indicated that depletion of complement is responsible for the inhibition. The lack of inhibition of the reactions in our studies in contrast to that noted by these authors might be explained in several ways: First, in the experiments quoted above, a relatively severe systemic reaction took place, perhaps capable of removing considerably more of the factors essential for development of inflammation than are removed in the local Arthus reactions studied here. Second, the reactions that were apparently inhibited were produced at a relatively short time after or even during the process of exhaustion, while our testing reactions were performed at a much later time. Furthermore, the importance of complement is

apparently not as great in development of Arthus reactions as in passive cutaneous anaphylaxis, and also the ease of depleting an animal of factors essential for inflammation might well vary among different species.

2). The finding reported here would also suggest that an inhibitor, such as reported by Hayashi(10,11), does not play a substantial role in cessation of vascular inflammation, at least under the experimental conditions in our study. It should be noted that a fresh, acute reaction developed in vessels about which mononuclear cells, without doubt left over from the previous reaction, were abundant. This occurred even when small amounts (27 μg NBSA) of antigen were used. It is very possible that the inhibitor reported by Hayashi, which he indicates is related to the mononuclear cells, is overwhelmed by the inflammatory reaction that occurs following fresh antigen-antibody combination in vessel walls and with the subsequent influx of polymorphs.

Thrombosis of vessels in the Arthus reactions unquestionably plays an important role in development of the macroscopic lesion, and resolution of these thromboses is most certainly a factor in termination of the reaction as a whole. However, from previous studies in which the Arthus vascular inflammation was prevented by depletion of polymorphs(2), few cellular thromboses occurred indicating that thrombosis comes about secondary to the inflammatory reaction of the vessels. Moreover, that the thromboses resolve secondary to decreasing vascular inflammation seems most likely.

Summary. 1) Our studies suggest that neither exhaustion of humoral or cellular factors necessary for inflammation, nor presence of possible inhibitors of the reaction play a dominant role in diminution of inflammation and healing of a hypersensitivity vasculitis of the Arthus type. 2) This study supports the importance of ridding the diseased vessel of antigen and antibody in bringing about a decrease in reaction and healing.

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1. Cochrane, C. G., Weigle, W. O., *J. Exp. Med.*, 1958, v108, 591.

2. Cochrane, C. G., Weigle, W. O., Dixon, F. J., *ibid.*, 1959, in press.

3. Coons, A. H., Kaplan, M. H., *ibid.*, 1950, v91, 1.

4. Stetson, C. A., *ibid.*, 1951, v94, 347.

5. Humphrey, J. H., *Brit. J. Exp. Path.*, 1955, v36, 268.

6. Rosenberg, L. T., Chandler, M. H., Fischel, E. E., *Fed. Proc.*, 1959, v18, 594.

7. Stone, S. H., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 269.

8. Bier, O. G., Siqueira, M., Asler, A. G., *Intern. Arch. Allergy Appl. Immunol.*, 1955, v7, 1.

9. Osler, A. G., Hawrisiak, M. M., Ovary, Z., *J. Exp. Med.*, 1957, v106, 811.

10. Ungar, G., Hayashi, H., *Ann. Allergy*, 1958, v16, 542.

11. Hayashi, H., Tokuda, A., Matsuba, K., Inoue, T., Udaka, K., Kuze, T., Ito, K., *Mie Med. J.*, 1958, v8, 329.

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Lactogenic Hormone Requirements for Milk Secretion in Intact Lactating Rats.* (25066)

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Early studies indicated that lactogenic hormone released from the adenohypophysis in response to nursing stimuli plays an important role in maintenance of lactation(1,2,5). Recent experiments have indicated that lactogenic hormone is rather ineffective in maintaining milk secretion in hypophysectomized

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