Ann. rheum. Dis. (1974) 33, 413

Experimental herpes virus arthritis

Factors in chronicity

P. A. BACON, R. BLUESTONE, L. S. GOLDBERG, F. W. WEBB,*
C. M. PEARSON, M. COOKE, AND J. G. STEVENS
From the University of California at Los Angeles

A chronic erosive arthritis in rabbits can be produced by a single intra-articular injection of herpes simplex virus (HSV) (Webb, Bluestone, Goldberg, Douglas, and Pearson, 1973). The experiments described here were designed to further delineate some important aspects of this viral arthritis. In particular, we have examined the natural history of the lesion over 12 months and evaluated the role of persistence of virus together with immune response to HSV in the production of chronic arthritis. Finally, the capacity of HSV to induce arthritis in species other than the rabbit has been tested by using another laboratory animal, the guinea pig.

Materials and methods

ANIMALS

Twenty young outbred female New Zealand white rabbits weighing approximately 3 kg each were used together with 12 young outbred Hartley guinea pigs weighing approximately 300 g each.

Herpes simplex virus (HSV), H4 strain (Stevens and Cook, 1971a) was cultured on monolayers of rabbit kidney cells (RKC) grown and sonicated as previously described (Webb and others, 1973). In this study, primary cultures of RK 13 cells known to be free of contaminating mycoplasma were used. The infected sonicate was diluted to give $7{\cdot}5 \times 10^5$ plaque forming units (pfu) of virus/ml before use; noninfected RKC sonicates had protein concentrations of 8 mg/ml.

Knee joint injections of HSV or RKC were performed under thiopentone anaesthesia using an aseptic technique. The rabbits received a single injection of 1·0 ml volume in the left knee only, 10 receiving HSV and 10 RKC sonicates. The rabbits were sacrificed in pairs at 1- or 2-month intervals, up to 10 months after injection. The guinea pigs all received an injection in both knees of 0·12 ml. 6 animals received 0·10 ml HSV ($7{\cdot}5 \times 10^4$ pfu) mixed with 0·02 ml saline or stock latex suspension. The stock latex is a 20% suspension of particles of average size 0·8 μm diameter as used for the latex fixation test. 4 of the guinea pigs received the HSV/latex in one knee and HSV alone in the other knee. Latex particles are known to produce transient acute synovitis (Bacon, Jones, Radwanski, and Dumonde, 1969) which could effect the chronicity of the HSV arthritis. 2 weeks after the initial injection, 3 guinea pigs received a second unilateral injection of 0·1 ml containing HSV only, to determine the effect of a repeat injection. The 6 control guinea pigs received RKC with or without added latex particles according to a similar schedule. All guinea pigs were sacrificed at 1 month after injection.

Preparation of joints for examination. After sacrifice by exsanguination under thiopentone anaesthesia, the rabbit joints were opened under aseptic conditions. A portion of synovium from both the anterior and the posterior joint space was immediately removed for virological examination, together with a section of meniscus, a shaving of articular cartilage from the femoral condyle, and a portion of the cruciate ligament. The gross appearances of the joint were noted before preparation for histology, as described previously (Webb and others, 1973). 3 large sections of each joint, from femoral condyle, patella, and tibial articular surfaces, were examined independently by two observers and graded as follows:

Normal synovium (Grade 0)—1 to 2 layers of flattened synovial cells with subsynovial fat abutting onto membrane.
Mild synovitis (Grade 1)—2 to 3 layers flattened or somewhat rounded synovial cells, scanty mononuclear infiltrate.
Moderate synovitis (Grade 2)—3 to 4 or more layers of rounded cells; definite synovial hyperplasia, many mononuclear cells.
Moderately severe synovitis (Grade 3)—Moderate synovitis with lymphoid aggregation.
Severe synovitis (Grade 4)—Moderately severe synovitis with marginal bony erosions and/or periostitis.

Guinea pig knee joints were excised after killing, carefully cleared free of muscle, and fixed whole after reflection of the patella ligament. The joints were then decalcified and prepared for histology as for the rabbits. Examination of slightly oblique sagittal sections from anterior and posterior surfaces of the block allowed adequate examination of patellofemoral and tibiofemoral articulations together with menisci, ligaments, and synovium. These were similarly graded by the same two observers.

Accepted for publication March 17, 1974.
Reprint requests to: Dr. P. A. Bacon, Royal National Hospital for Rheumatic Diseases, Bath.
* Current address: Ipswich Hospital, Suffolk.

Table I *HSV arthritis in rabbits—histological grading**

Months after injection	*HSV challenge†*		*RKC challenge‡*	
	Injected knee	*Other knee*	*Injected knee*	*Other knee*
1	3–4	0–1	1–2	0
	3	1		
2	3	1–2	0–1	0–1
	2	1		
3	1–2	0	0–1	0
4, 5, and 6	0–1	0–1	0–1	0–1
8	1–2	1	0	0
10	1–2	1	0	0

* Grading 0–4 (see Materials and Methods) assessed by 2 independent observers.
† Rabbits injected with 1×10^6 plaque forming units of herpes simplex virus in 1 ml RKC medium.
‡ Rabbits injected with uninfected rabbit kidney cell sonicates.

Herpes virus identification in joint tissues was determined by culture, immunofluorescence, and electron microscopy by methods known to reveal herpes virus in other situations (Stevens and Cook, 1971b). Culture was performed by co-cultivation of small cut portions and homogenized joint tissue with RKB cells for 3 weeks. PPLO studies were also performed on representative samples of each tissue. Immunofluorescent studies were performed by an indirect technique, using a rabbit antibody specific for herpes simplex. Electron microscopical studies were performed after fixation in gluteraldehyde.

Skin tests were performed with 0·1 ml of killed (ultraviolet irradiated) HSV containing 1×10^4 pfu of virus, or with 0·1 ml RKC containing 12 μg protein. The rabbits were skin tested before injection, at 2 and 4 weeks, and again before sacrifice. The guinea pigs were tested once only, 48 hours before sacrifice.

Tests of cell-mediated immunity in vitro were performed on the rabbits using a leucocyte migration test (Soborg and Bendixen, 1967), modified for use with rabbit peripheral blood leucocytes. This test has been shown to measure reactivity to standard protein antigens such as bovine γ-globulin and PPD (Bacon, Goldberg, and Bluestone, 1973). Inhibition of leucocyte migration from capillary tubes was performed on each pair of rabbits 48 to 72 hours before sacrifice. The guinea pigs were tested by a macrophage migration inhibition test using peritoneal macrophages induced by intraperitoneal injection of 10 ml sterile liquid paraffin inserted 72 hours before sacrifice. Irradiated HSV or RKC preparations, 120 μg/ml, were added as antigen to each culture chamber and the tests were performed in quadruplicate for both species. The results were expressed as the migration index, which is the area of migration in the presence of antigen divided by the area of migration in the absence of antigen × 100.

Results

I RABBITS

Histology (Table I)

The rabbits injected with HSV showed a marked arthritis early on which later healed with focal, chronic arthritis. At 1 month, severe (grades 3 to 4) synovitis was seen in the injected knee of both rabbits sacrificed at this time (Fig. 1). The opposite knees showed mild (0–1) changes only. At 2 months the 2 injected knees showed a similar picture but more patchy changes, while the uninjected knees showed a variable synovitis (grade 1 to 2). At 3 months milder synovitis (grade 1 to 2) was seen in the injected knee (Fig. 2), with a normal uninjected knee. Thereafter, the knee joints appeared virtually normal at 4 to 6 months, though late sequelae (grade 1 to 2) were seen in the rabbits sacrificed at 8 and 10 months (Fig. 3).

The control animals injected with RKC showed no consistent synovitis apart from variable but definite changes (grade 1 to 2) seen in the injected knee in the rabbits sacrificed at 1 month. No synovitis was observed in the opposite uninjected knee.

Herpes virus isolation No evidence for persistence of live virus, or viral specific products, was found in any rabbit joints by explant culture, immunofluorescence, or electron microscopy. In addition, mycoplasma could not be cultured from the joints. This was true for the early joints with marked synovitis, as well as for the later more normal joints.

Immune response

(a) Skin tests to HSV and RKC were essentially negative at all times in the HSV injected animals. In the RKC controls, weak early reactions to RKC protein were seen at times after the joint challenge, but no sign of delayed reactivity developed.

(b) Leucocyte migration tests (Fig. 4) to HSV were positive at 1 month in the HSV challenged animals and remained persistently positive when followed up

FIG. 1 *Grade 3 arthritis induced by HSV in rabbits at 1 month. The injected knee showing (a) the marked synovial cell hyperplasia and hypertrophy, the normal lining layer being 1 to 2 cells thick.* ×183; *(b) perivascular infiltration of mononuclear cells in the subsynovial fatty tissue.* ×366

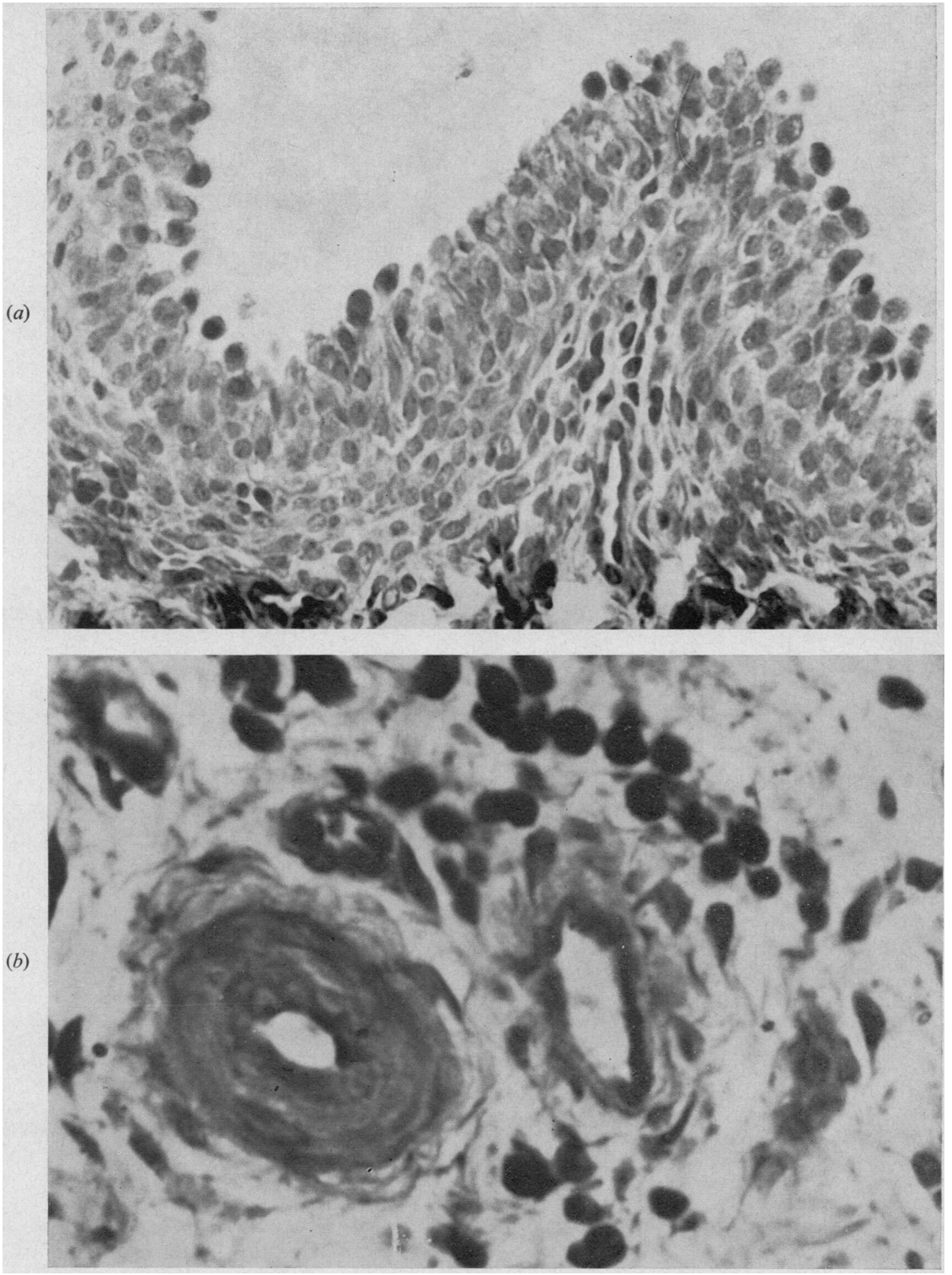


FIG. 1.

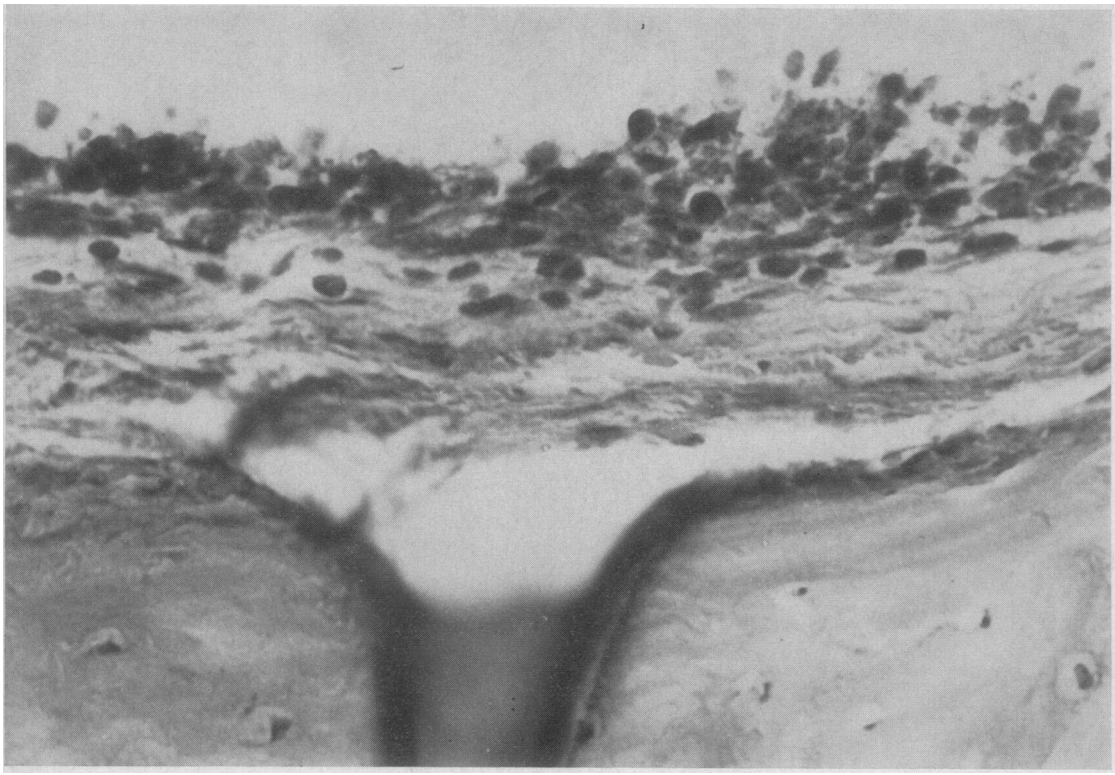


FIG. 2 *Grade 2 synovitis in rabbits at 3 months, showing persistent synovial cell hyperplasia with occasional small lymphocytes beneath this layer.* ×200

to 8 months, with a borderline result at 10 months. No positive responses to RKC were found. The control rabbits challenged with RKC had consistently negative responses to both RKC and HSV. The difference between the *in vitro* response to HSV in the HSV challenged animals and the controls is highly significant (P = 0·005).

II GUINEA PIGS

Histology

Moderate to severe (grade 2–4) arthritis was seen in 5 of the 6 animals in both knees, 1 guinea pig showing only mild (grade 1) changes in both knees. The synovitis appeared different in this species in that small lymphocyte accumulation and lymphoid follicles were not seen. This agrees with the previously described findings in immune arthritis in guinea pigs (Loewi, 1968). In contrast, the oedema of the synovitis was more pronounced and the frequency of cartilage or tendon erosion was greater (Fig. 5). The average score was 2·4 in the 8 joints injected with HSV alone, and 3·0 in the 4 joints receiving HSV plus latex. This difference was not significant, suggesting the acute latex synovitis had not influenced the chronic HSV arthritis. There was also no apparent effect of a second injection in the 3 animals that received this—indeed the animal with the least change was in this group.

Immune response

Skin tests to HSV and RKC were essentially negative in the HSV challenged guinea pigs. The RKC challenged controls showed a flare at 4 hours to both antigens with an absent delayed reaction (Table II).

Inhibition of macrophage migration was shown with HSV but not with RKC in all the HSV injected animals (Table II). None of the RKC challenged controls gave a positive response with either antigen. The mean migration index for the 6 animals challenged with HSV was 62% (40–88) when HSV was used as the antigen; the mean migration index was 94% (87–105) when RKC was used as the test antigen. The 6 animals injected with RKC showed mean migration indices of 96% (89–104) and 101% (98–106) using HSV and RKC respectively as antigens.

Discussion

Previous work from this laboratory showed that after a single intra-articular injection of HSV, a moderate

to severe arthritis was present at 3 months in the injected knees of all 10 animals studied; milder changes occurred in the majority of noninjected contralateral knees (Webb and others, 1973). The present study has revealed a different picture when the time course of the arthritis was examined in more detail. Severe arthritis was seen at 1 and 2 months, with only milder changes at 3 months and thereafter. There are at least two possible reasons for the differences between the two studies. First, there may

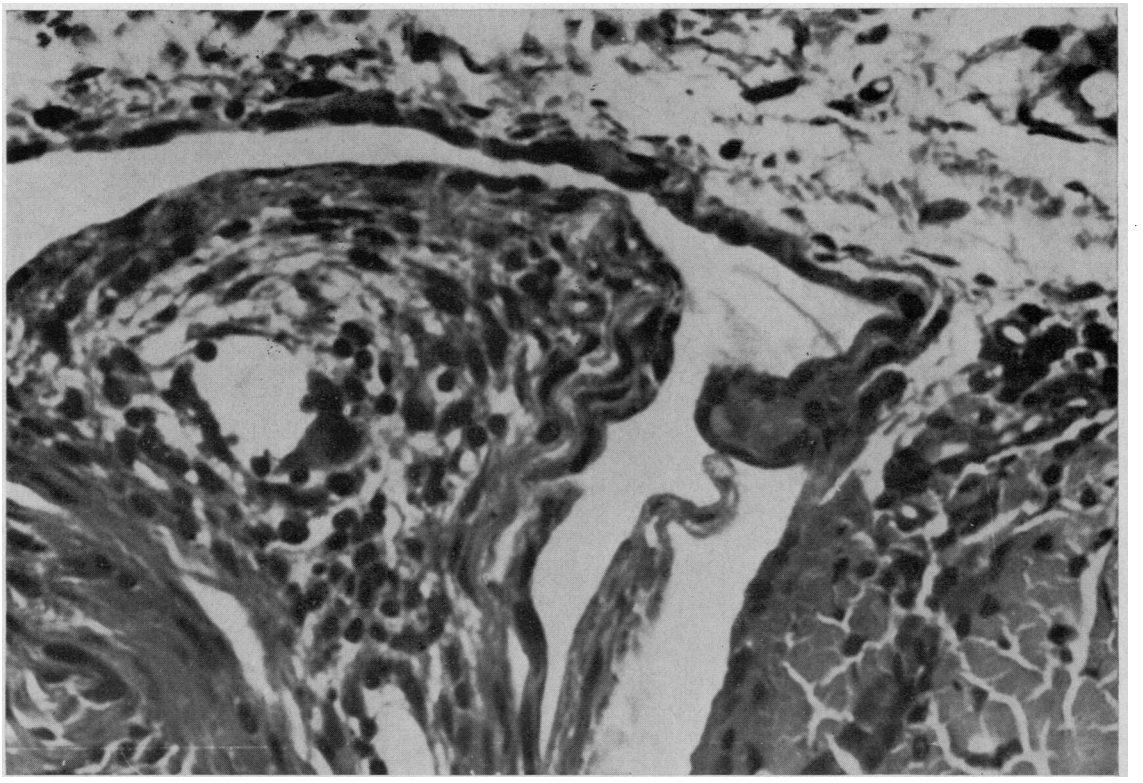


FIG. 3 *HSV synovitis in rabbits; injected knee at 8 months showing chronic granulomatous focus with occasional small multinucleate giant cells and many mononuclear cells.* ×100

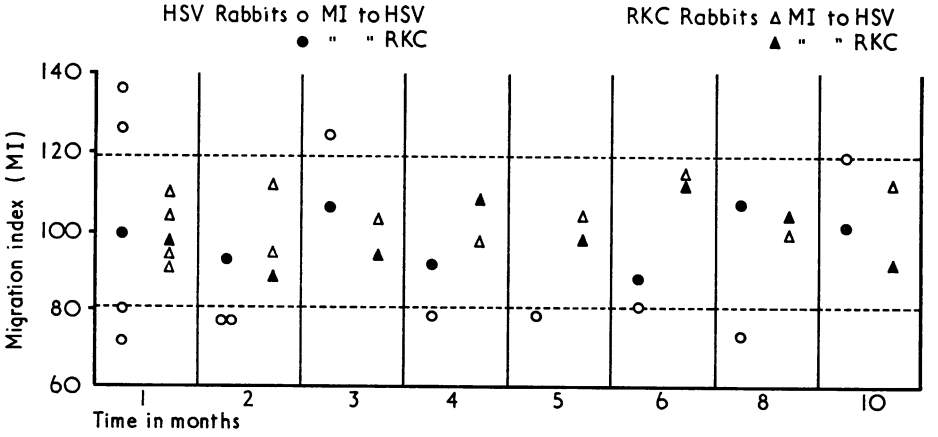


FIG. 4 *Results of the leucocyte migration-inhibition tests using ultraviolet irradiated HSV or RKC as antigens to test HSV and RKC challenged rabbits* in vitro. *The dotted lines indicate the normal range of the MI from 80 to 120. The responses of the herpes challenged rabbits to HSV antigen* (○) *are all outside this range*

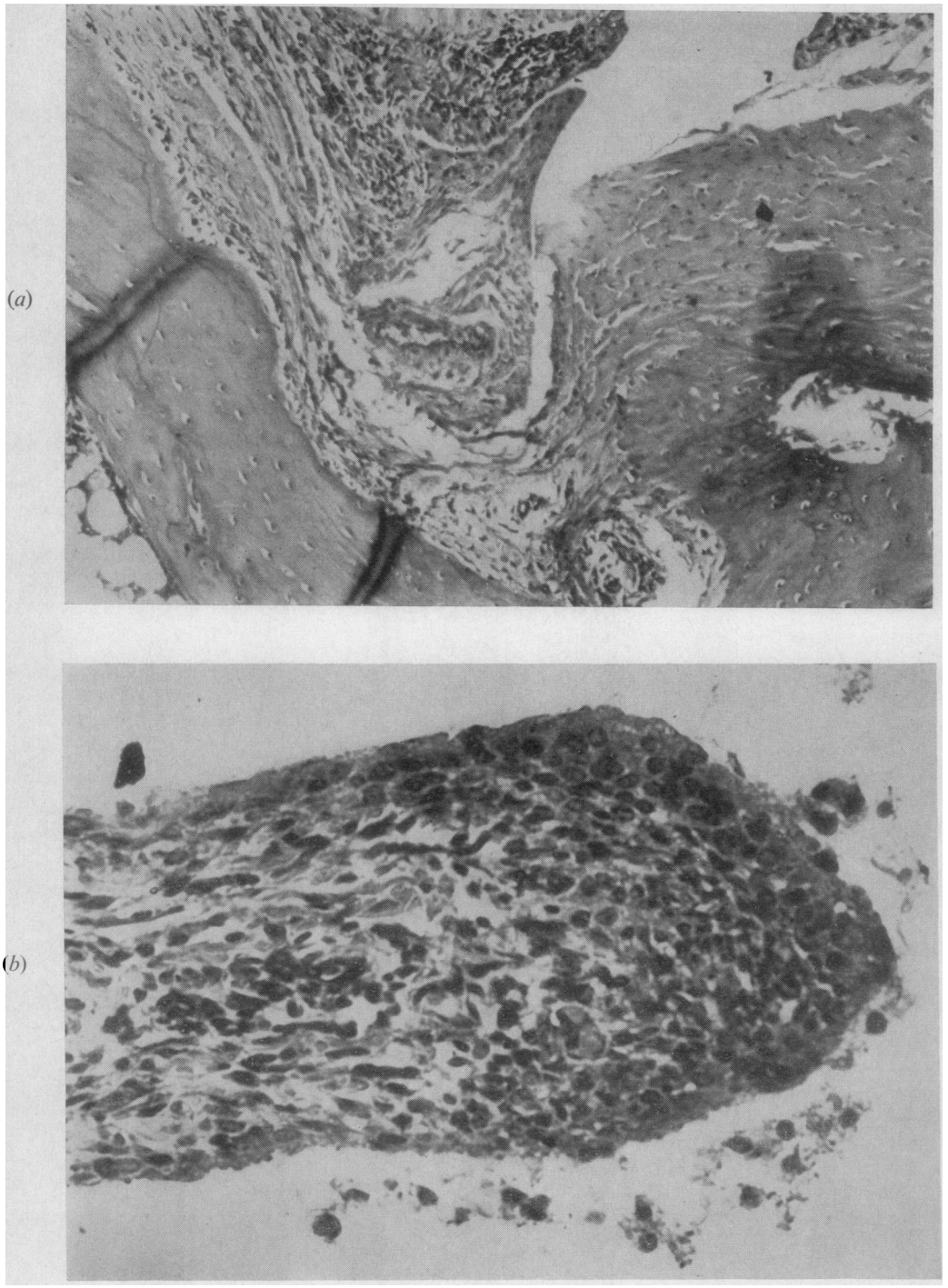
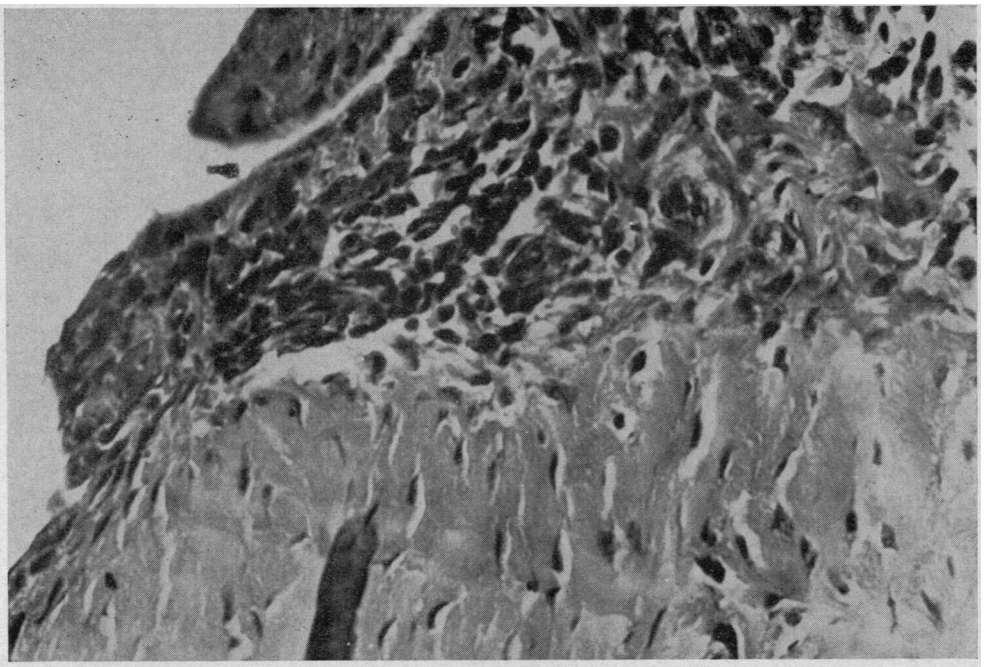
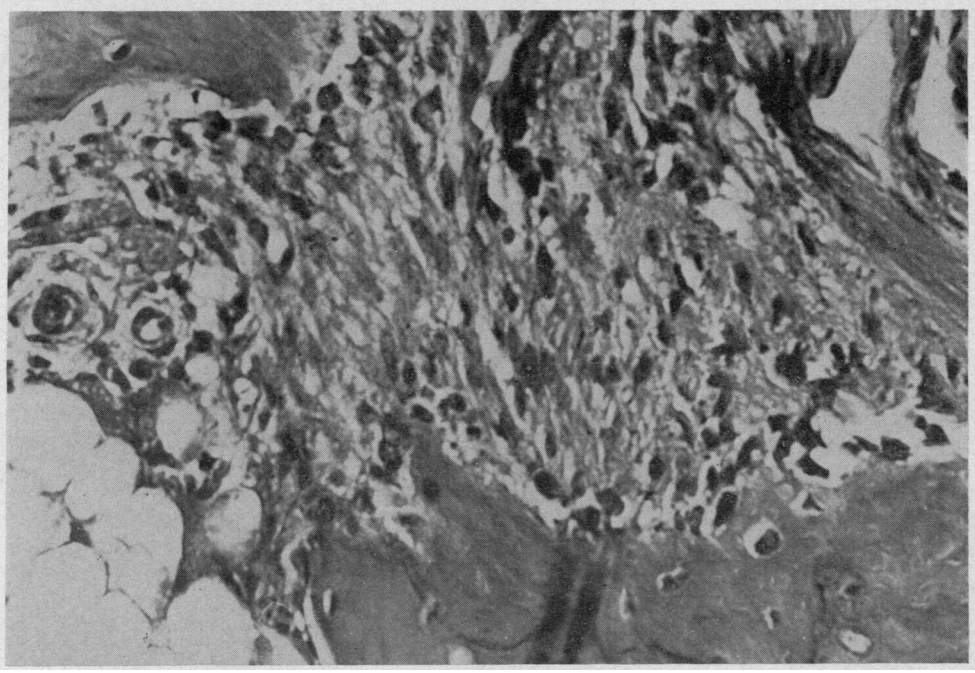


FIG. 5 *Arthritis induced by HSV in guinea pigs. (a) Intercondylar notch, showing marked infiltration of inflammatory tissue in an area normally occupied by bland loose fibro-fatty tissue.* ×52; *(b) synovial frond in the lateral joint angle with synovial cell hyperplasia and deeper inflammatory cell infiltration.* ×173; *(c) intercondylar area showing pannus invading the ligament.* ×173; *(d) intercondylar area showing pannus invading and eroding through subcortical bone.* ×173

(*c*)



(*d*)



have been a difference of host responsiveness between the two sets of rabbits used. It is possible that the rabbits studied by Webb and others would have shown considerable healing had they been examined a few weeks later. The second possibility is that the different preparations of HSV used for the two experiments accounted for the different results. The virus used in this study was not devoid of arthritogenicity, as shown by the changes induced in rabbits early after injection and the uniform changes seen in the guinea pigs. However, there was an important difference in that the virus used in this study had been carefully cleared of *Mycoplasma laidlawii*, which had been known to be a contaminant in the previous

Table II *HSV arthritis in guinea pigs; macrophage migration and skin tests*

	MI vs. HSV	*vs. RKC*	*Skin test vs. HSV*		*vs. RKC*
HSV challenged animals (6)	62% (40–88)	94% (87–105)	4 hrs	0	0
			24 hrs	7 (f)	5 (f)
			48 hrs	0	0
RKC challenged animals (6)	96% (89–104)	101% (98–106)	4 hrs	13	18
			24 hrs	7 (f)	5 (f)
			48 hrs	0	0

The mean migration index (MI), using inhibition of macrophage migration *in vitro* compared with skin tests in the HSV challenged guinea pigs and the controls.

study. The mycoplasma does not apparently cause an arthritis on its own, since sonicates of the contaminated kidney cell cultures were without effect (Webb and others, 1973). Mycoplasmas have recently been shown to possess mitogenic properties (Ginsburg and Nicolet, 1973) and conceivably mycoplasma may have had a synergistic effect on the arthrogenicity of the herpes virus. This adjuvant effect of mycoplasma may be more important in rabbits than in other species since the guinea pigs responded strongly to the mycoplasma-free HSV preparation. The ability of HSV to induce arthritis does not appear to be host specific; the factors involved in the chronicity of viral arthritis appear much more complex than in simple immunological models.

The natural regression of the arthritis in this study made it more difficult to define all the mechanisms involved in chronicity, but several points are clear. Persistence of either virus or recognizable virus induced antigens does not appear to be the cause of the synovial inflammation. Even at 1 month, when active synovitis was seen in all 3 rabbits studied, no evidence for persistence of the viral genome could be found even though both synovium and cartilage were examined in detail by methods known to reveal latent herpes infection in other situations (Stevens and Cook, 1971b). The synovitis was present for some weeks after this, so that neither the subacute nor the chronic phase of this arthritis depends upon the persistence of virus within the joint. This suggests that infection *per se* may be the trigger factor, but is not the actual cause of chronicity in this model.

The role of immunological factors is obviously of interest for several reasons. First, the simple models of immune inflammation in the synovial joint are known to cause chronic arthritis in both rabbits (Dumonde and Glynn, 1962) and guinea pigs (Loewi, 1968). Secondly, much of the pathology related to viral infections can be ascribed to the host immune response to the virus (Mims, 1973). An antibody response to herpes virus was reported in all rabbits injected with live virus (Webb and others, 1973), but the skin tests were predominantly negative, suggesting a degree of energy which can occur with viral infection (Salaman, 1970). Brisk and prolonged antibody responses to herpes virus in rabbits after inoculation in various sites has been described (Lam and Hsiung, 1973). In that study, persistence of virus was found only after intravenous inoculation, but not after intraperitoneal or subcutaneous injection, in accord with our findings after intra-articular injections.

The induction of chronicity in experimental immune arthritis appears to depend on cell-mediated reactivity rather than antibody (McCluskey, Gell, and Felix-Davies, 1961; Bacon and others, 1969). Also, current evidence implicates cell-mediated immunity in the host defence mechanism against herpes virus (Wilton, Ivanyi, and Lehner, 1972; Russell, 1974). It is therefore of interest that both the rabbits and the guinea pigs studied here had *in vitro* evidence of cell-mediated immunity to herpes virus. This response occurred in the absence of the Freund's complete adjuvant so frequently used to induce experimental allergic disease, and is in accord with the known ability of the virus infection to evoke cellular responses (Rosenberg, Farber, and Notkins, 1972).

The relationship between cell-mediated immunity to HSV and the arthritis induced by HSV is unknown. The former might be a factor in the initial synovitis, though this has not been examined here. However, it cannot explain the more chronic lesions in the absence of detectable persistent virus. In the experimental immune arthritis of rabbits, antigen can be detected in the joint for several months (Webb, Ford, and Glynn, 1971; Consden, Doble, Glynn, and Nind, 1971), yet doubts have been raised as to whether the very persistent arthritis is related to the retained antigen (Glynn, 1968). The difference between the immune model and the HSV arthritis may thus be one of degree only. A reasonable hypothesis is that the herpes virus initiates an immune response, while at the same time producing alterations in host tissue antigens. The continued arthritis in the absence of live virus would then be a consequence of the immune response to altered tissue antigens. This would be analogous to the situation in chronic viral keratitis where cellular immunity to corneal antigen, and not to the aetiological virus, appears to be responsible for the chronicity of keratitis (Henley, Shore, and Leopold, 1971).

Summary

A pronounced arthritis was produced in rabbits by a single intra-articular injection of herpes simplex virus (HSV). After 3 months considerable healing occurred, but local chronic changes were seen up to the end of the study at 10 months. Persistence of virus in the joints was not found even when the synovitis was most severe, although sought in detail using direct cultivation, immunofluorescence, and electron microscopy. An *in vitro* cellular immune response to HSV was shown by inhibition of leucocyte migration in all the HSV injected rabbits, but not in the controls.

The species specificity of this viral arthritis was tested by intra-articular injection of HSV in guinea pigs. This species also showed both arthritis and a cellular immune response to HSV. The role of virus in initiating arthritis and of the immune response in the induction of the chronic lesion is discussed.

P. A. B. was a Geigy Travelling Fellow of the A.R.C., and in receipt of a Peel Travel Scholarship. We gratefully acknowledge the expert technical assistance of Robin Yeaton and Barbara Van de Sande.

This work was supported by USPHS Grant GM15759 and research support from the Veterans Administration.

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