Experimental Arthritis in the Rabbit Induced by Group A Streptococcal Products

Acute and chronic synovitis has been produced in rabbits following the intraarticular injection of streptolysin S (SLS); but none of the other extracellular products tested (streptolysin O, desoxyribonuclease, proteinase, streptokinase, erythrogenic toxin) had such an effect[1]. More recently it has been reported that severe arthritis developed in rabbits following intraarticular injections of group A streptococcal sonicates. The active fraction in the sonicates responsible for the initiation of the articular lesions was reported to be the cell-wall of the streptococci[2]. The results presented herein demonstrate the development of acute and chronic synovitis in rabbits following the intraarticular administration of group A streptococcal extracellular products (SEP); the SEP employed did not contain SLS.

SEP was kindly supplied by Dr. T. N. HARRIS from the Children's Hospital of Philadelphia. It was derived from a type 4 streptococcus grown in a chemostat, and contained 14 antigens as determined immunoelectrophoretically[3,4]. Eighteen rabbits were injected intraarticularly with 5–20 mg SEP. Seven rabbits dying within the first 2 days of injection had acute synovitis characterized by a heavy inflammatory infiltration mainly composed of neutrophilic granulocytes with a varying admixture of histiocytes and lymphocytes. The synovial cells were swollen or necrotic. Focally a moderate to heavy fibrinous deposits overlied the denuded connective tissue (Figure 1).

Seven animals sacrificed 7 days following the injection had subacute synovitis, the infiltration being composed of an admixture of mono- and polymorphonuclears with a moderate number of plasma cells. The synovial lining cells were low to high cuboidal, occasionally multilayered (Figure 2) with mild mitotic activity.

Four animals sacrificed on the twenty-fourth day had similar lesions but granulocytes were scarce within the infiltrate (Figure 3). Culture taken from knee joints of all animals were sterile. A fraction of positively charged antigens derived from SEP by chromatography on DEAE-sephadex also induced arthritis, this fraction contained only 5 antigens and was found to possess all the enzymatic activity of the whole SEP preparation[5]. Cardiac, hepatic and paraarticular inflammatory lesions identical with those described previously[4] were seen in animals injected with SEP or its DEAE-sephadex fraction. Sera of all rabbits showed a steep rise of serum glutamic oxaloacetic transaminase and sorbitol dehydrogenase 18–24 h following administration as shown previously[4].

Of 4 rabbits injected intraarticularly with 10 mg of SEP heated to 100 °C for 30 min, 3 showed normal synovia on the seventh day, 1 animal had minimal subacute inflammation. Animals injected with 25 mg of human serum albumin had normal synovia 10 days following injection (Figure 4). Rabbits injected intraarticularly with a type 12 streptococcal sonicate containing 10 mg of protein and 100 μg of rhamnose but devoid of SLS, and sacrificed on the tenth day following the injection, developed severe acute purulent necrotizing synovitis.

The results indicate that heat-labile group A streptococcal exo-products (SEP) and streptococcal sonicates which both did not contain any SLS, induced severe arthritis in the rabbit. Our findings differ from those described by WEISSMANN et al.[1] who found that SLS was the only streptococcal exo-products capable of initiating arthritis in the rabbit. This apparent discrepancy cannot be explained unless one assumes that the SEP used contained toxic components which were not present in any of the preparations used by WEISSMANN et al.[1]. The nature of the toxic component(s) present in SEP is not known. Since the intraarticular injection of SEP also caused cardiac, diaphragmatic and hepatic lesions, it is possible that the same toxic fraction(s) also act on the synovial membrane.

It remains to be elucidated whether SEP activity on the synovial cells involves damage to lysosomes as has been shown for SLS[1]. Since synovial tissue is known to be highly reactive to a variety of noxious agents, e.g. xylene, turpentine, formaldehyde[6] and to bacterial muco-

1 G. WEISSMANN, B. BECHER, G. WIEDERMANN and A. W. BERNHEIMER, Am. J. Path. *46*, 129 (1965).

2 W. J. CROMARTIE, in *Present State of Research on Group A Streptococci* (Excerpta med. Publications 1967), in press.

3 C. A. OGBURN, T. N. HARRIS and S. HARRIS, J. Bact. *76*, 142 (1958).

4 N. ZEIRI, Z. BENTWICH, J. H. BOSS, I. GINSBURG and T. N. HARRIS, Am. J. Path. *51*, 351 (1967).

5 G. SPIRA, Z. SILBERSTEIN, J. H. BOSS, T. N. HARRIS and I. GINSBURG, to be published.

6 J. A. KEY, J. Bone Jt Surg. *15*, 67 (1933).

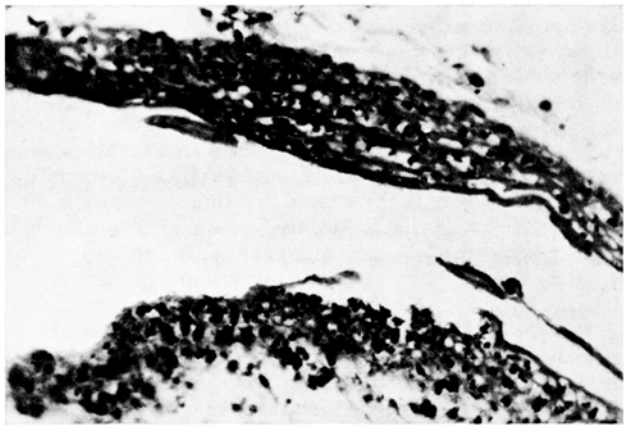

Fig. 1. Acute fibrinous synovitis in a rabbit succumbing within the first day of an intraarticular injection of 5 mg of SEP. × 270.

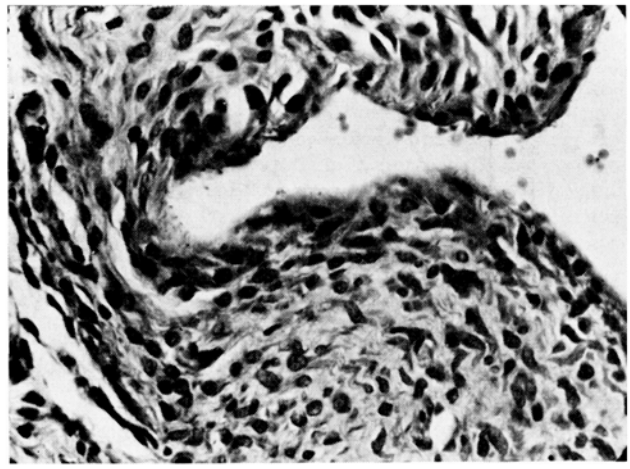

Fig. 2. Subacute synovitis in a rabbit killed 7 days following the intraarticular injection of 5 mg of SEP. Note the prominence of the synovial lining cells. × 450.

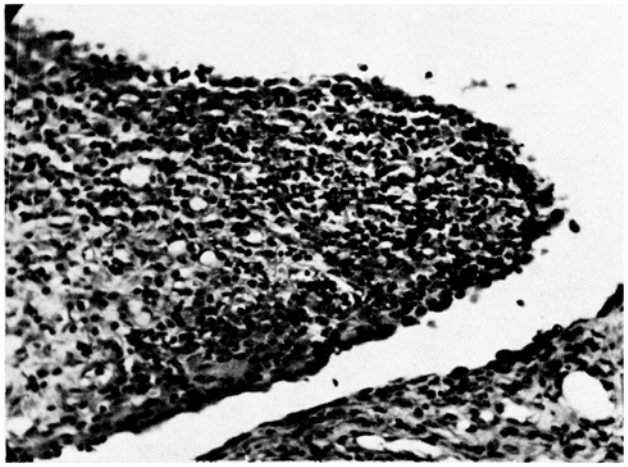

Fig. 3. Swollen synovial villous of a rabbit joint killed 20 days following intraarticular injection of 5 mg SEP. The villous is infiltrated by predominantly mononuclear inflammatory cells. The synovial lining cells are prominent. × 270.

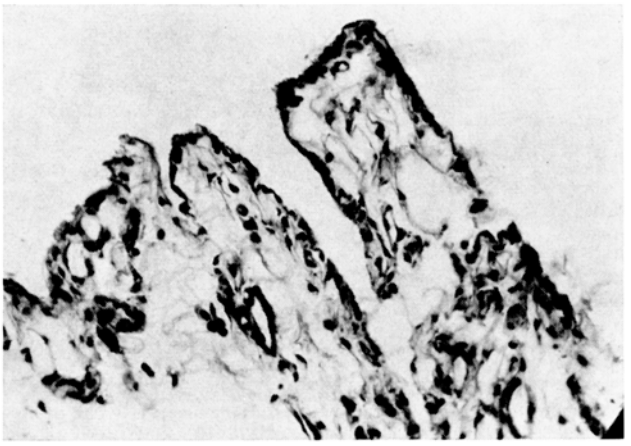

Fig. 4. Normal appearing synovia in a rabbit killed 7 days following an intraarticular injection of SEP heated to 100 °C. Note the delicate subsynovial connective tissue. × 270.

polysaccharides[7], the effect of the various streptococcal products (SLS, sonicates and SEP) on the synovial membrane are but 1 example of agents capable of injuring the joints. The sera of the rabbits used did not contain any detectable antibodies reactive with SEP. Thus, since the first synovial lesions become manifest as early as 16 h following injection, the possibility that the synovial alterations are due to an immune response should be considered only to be excluded[8].

Zusammenfassung. Intraartikuläre Injektion von Streptolysin-S-freien extrazellulären Produkten der Streptokokken Gruppe A verursacht eine zunächst akute, in der Folge aber subkutane Synovitis. Die Veränderungen gleichen denjenigen nach Injektion von Streptololensonikaten, so dass angenommen wird, ausser Streptolysin S bedingen auch andere streptokokkale Faktoren eine Arthritis.

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19 October 1967.

[7] R. S. Jones and Y. Carter, A.M.A. Archs Path. *58*, 613 (1954).
[8] Supported by Research Grant BSS-CD-IS-2 from the U.S. Public Health Service.

Effect of Alloxan Diabetes and Insulin Administration on the Incidence of Pituitary Necrosis Caused by Hexadimethrine Bromide in Rats

According to obduction data necrosis of anterior pituitary occurs more frequently in diabetic patients[1]. As administration of hexadimethrine bromide to rats causes necrosis in the anterior lobe of the pituitary gland[2–4], the possibility arose of studying whether the incidence and extent of adenohypophysial necrosis due to hexadimethrine bromide (HB) is modified in rats with alloxan diabetes and in those injected with insulin.

The experiments were carried out on female albino rats of the same strain weighing ca. 200 g and kept on a standard diet. One group of rats received HB (Polybrene®, Abbott) alone in a dose of 5 mg/rat i.v. Three groups of rats were given a single i.v. injection of 40 mg/kg body weight alloxan monohydrate. Two, 6 and 30 days later they were i.v. injected with 5 mg/rat of HB. Blood sugar levels were determined before the administration of HB and only the rats having hyperglycaemia were used. Other groups fasting for 12 h were given 1.0 IU/100 g body weight aqueous insulin (Richter, Budapest) i.p. simultaneously with HB.

The results are shown in the Table. It can be seen that, contrary to our expectation, the rats with alloxan diabetes did not become more sensitive to the effect of HB; their survival time was not shorter, mortality and incidence of adenohypophysial necrosis did not increase. On the other hand, insulin administration strikingly sensitized the rats to the effect of HB; most of the rats died within 48 h, survival time was shortened and adenohypophysial necrosis more often appeared.

[1] C. F. Brennan, R. G. S. Malone and J. A. Weaver, Lancet *2*, 12 (1956).
[2] K. Kovács, R. Carroll and E. Tapp, Lancet *2*, 919 (1964).
[3] K. Kovács, Schweiz. med. Wschr. *97*, 1047 (1967).
[4] J. Nichols, Lab. Invest. *15*, 412 (1966).