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Inhibition of Adjuvant Arthritis by Statolon.* (32487)

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The parenteral administration of mycobacterial adjuvant into rats produces a disease characterized by polyarthritis, tendinitis and periostitis(1,2). Less constant features of adjuvant-disease or adjuvant arthritis (AA) include inflammation of portions of the eye, genital tract and skin. While the etiology of this disease is unknown, it is postulated that cellular or delayed hypersensitivity may play an important role in pathogenesis since: (a) the induction of tolerance to mycobacterial antigen in the neonatal period can inhibit subsequent production of "adjuvant-disease"(3), (b) there is a characteristic ten day latent period prior to the onset of arthritis(1), (c) the disease can be passively transferred between highly inbred rats by intact lymphoid cells(4), and (d) anti-rat lymphocyte serum inhibits the appearance of arthritis(5).

While it is possible that adjuvant activates a latent infection, against this is the fact that AA may be induced in gnotobiotic animals(6), and its onset is not inhibited by a variety of antibiotics(1). This evidence does not exclude the possibility of a virus or virus-like organism playing a role in the pathogenesis of AA. The effect of statolon (a broad spectrum anti-viral agent) was studied in this experimental model of arthritis in order to provide indirect evidence for virus or virus-like participation in the pathogenesis of AA.

Methods and material. Male, inbred, albino

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Fischer strain 344 rats† weighing 170-190 g were used in all experiments.

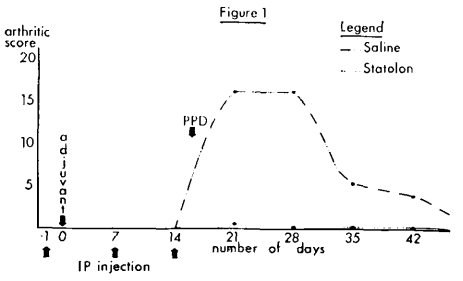
Production and evaluation of arthritis. Desiccated mycobacterium butyricum 2.5 mg (Difco) was suspended in 1 ml N.F. light paraffin oil and autoclaved. A 21 gauge needle was used to inject 0.1 ml of this adjuvant into the skin of the distal third of the tail. Twenty-one days later, groups of rats were coded and the severity of joint involvement evaluated by two independent observers. A single point was assigned for each involved wrist or ankle area, and an additional point was given for each involved phalangeal joint up to a maximum of 5 points per extremity. Inflammation of the tail was not scored since it was the site of injection.

Study groups in adjuvant-arthritis. Three groups of animals were studied as follows: 1) saline control. 2) rats which received 1% statolon‡ in saline and 3) rats which received denatured statolon, prepared by boiling the 1% statolon solution for one hour.

The injection schedule for all groups consisted of 1 ml of the appropriate solution given intraperitoneally 24 hours prior to, then 7 and 14 days following the administration of adjuvant. (Fig. 1).

Other studies. 1) H. & E. stained histological sections of decalcified metatarsalphalangeal and proximal interphalangeal joints of the second and third hind toes of the saline control and statolon treated rats *(vide supra)* were studied to ascertain the presence or absence of microscopic evidence of inflammation. 2) to study the possible effects of statolon on an acute inflammatory process, rats were given an intraperitoneal injection of either saline or statolon and 4 hours later received increasing amounts of turpentine into each paw (0.02, 0.04, 0.06 and 0.08 ml respectively). The animals were coded and the intensity of paw inflammation scored from 0 — 4+ by two independent observers 24 hours later. 3) To study the effects of statolon on delayed hypersensitivity a number of the saline control and statolon treated animals were skin tested 16 days after the injection of adjuvant according to the method of Flax and Waksman(7) (Fig. 1). Five μg of P. P. D. (Parke, Davis) in 0.1 ml of saline was injected intradermally into the freshly shaved skin of the flank. Saline was injected intradermally into the opposite flank. The diameter of induration was measured 24 hours later. A positive reaction is defined as one in which there is at least 5 mm of induration.

Results. For the purposes of this study, AA



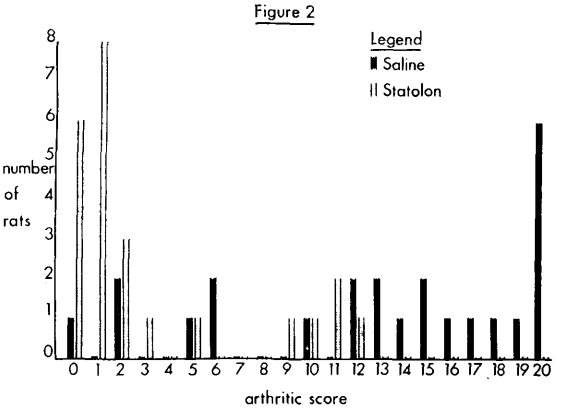


FIG. 1. Design and course of a typical experiment.
FIG. 2. Population data histogram of saline control and statolen treated rats.

† Charles River Breeding Laboratories, Wilmington, Mass.

‡ Statolon (#354-869B-139) was supplied as a powder through the courtesy of Dr. W. J. Kleinschmidt, Eli Lilly Co., Indianapolis, Ind.

is defined as the appearance of clinically apparent involvement of at least one joint. In the saline control rats which received adjuvant 92% developed arthritis. Fig. 1 shows the clinical course of a representative experiment. Saline treated rats achieved maximum disease by the 21st day. Joint involvement remained relatively constant for approximately one week beyond the 21st day, and then gradually diminished in intensity over the course of a month. Statolon treated rats had suppression of arthritis throughout the experiment. Since the largest consistent differences between groups appeared by the 21st day, this time was chosen for detailed presentation.

The data in Table I (representing results of 3 independent experiments) clearly demonstrated the beneficially modifying effect of statolon on AA in the rat. Statolon which had been denatured in such a way as to remove its interferon inducing capacity(8) was ineffective. Fig. 2 shows the distribution of data in saline and statolon treated animals. Two populations are noted. Histological sections of 44 clinically uninvolved digits from statolon treated animals confirmed the absence of joint pathology in these animals, but did reveal two instances of tendinitis, whereas identical digits of the saline-treated animals showed extensive inflammatory changes.

As noted in Table II, statolon had no modifying effect upon the inflammatory disease induced with turpentine when compared with their saline controls. The P.P. D. skin reactions in statolon and saline injected groups were compared. All tested animals developed positive skin tests and the intensity of the reactions were identical as seen in Table III.

Discussion. Statolon is an antiviral agent derived from the mold Penicillium stoloniferum. The antiviral activity is believed to be due to its capacity to induce interferon formation in the host(9). Interferon is a species

Table 1
Adjuvant Arthritis

	number of rats	mean arthritic score
Saline	24	13·1
Denatured Statolon	16	10·3
Statolon	24	2·9

Table 2
Turpentine Inflammation

	number of rats	mean inflammatory score
Saline	8	11·0
Statolon	8	12·0

Table 3
PPD Reactions

	number of rats	mean diameter of induration (mm)
Saline	15	10·6
Statolon	16	10·1

specific protein coded by the host cell genome which is induced by both viral and many non-viral agents(10). It inhibits the intracellular replication of "true viruses" as well as Bedsonia organisms(11). Although statolon in this study may have been effective by virtue of its antiviral action, the possibilities of an anti-inflammatory or an anti-immune effect of the drug were also considered.

The inability of statolon to modify turpentine inflammation strongly suggests that it did not function as an anti-inflammatory agent.

Cellular or delayed hypersensitivity is thought to play an important role in the pathogenesis of AA. The P.P. D. reaction is a prototype of cellular hypersensitivity and the intensity of the reaction is roughly proportional to the severity of arthritis seen in the animal(7). Since statolon did not inhibit the incidence or intensity of this reaction, it is unlikely that it has an important action on cellular hypersensitivity.

Statolon which is denatured by heating is ineffective as an inducer of interferon formation(8). Since denatured statolon did not protect the animals against the development of AA, it is probable that the effectiveness of unaltered statolon in these experiments was mediated through its interferon inducing capacity.

Preliminary studies in Sprague-Dawley rats which are reportedly poor interferon producers(12), demonstrated that this agent was not as effective as in the Fischer rats reported herein. This indicates the participation of other factors (genetic?) which will require elucidation.

The effectiveness of statolon in preventing AA suggests that a virus is involved in the pathogenesis of this model of disease. Statolon may be effective against Bedsonia organisms(9,11) as is tetracycline(13). Since tetracycline has been shown to be ineffective in preventing AA(1), it seems less likely that these organisms are of importance in the pathogenesis of AA.

The ultimate proof of an infectious etiology in AA will require the isolation of an infectious agent (virus) and demonstration of its ability to induce AA. AA can be passively transferred to syngeneic animals by means of viable lymphoid cells while anti-lymphocyte serum inhibits the production of AA. Together, these suggest a relationship between the postulated agent and lymphoid cells in the pathogenetic sequence.

Summary. 1. Statolon, a broad spectrum antiviral agent, effectively inhibits the development of adjuvant-arthritis in Fischer strain rats. 2. It is suggested that a virus is involved in the pathogenesis of the disease, and it probably has some relationship to lymphoid cells. 3. Evidence against an anti-inflammatory or anti-immunological effect of statolon in adjuvant-arthritis is presented. 4. The possible participation of other factors in the pathogenesis of this experimental model of arthritis requires further study.

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