Evaluation of Immunosuppressive Potential of Cryogenine Using Developing and Established Adjuvant Arthritis in Rats

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Abstract □ Both developing (Days −1–+12 relative to inoculation) and established (Days +18–+29) stages of *Mycobacterium butyricum* adjuvant-induced polyarthritis in rats were treated orally with cryogenine (100 mg/kg/day), a prototype anti-inflammatory (phenylbutazone, 100 mg/kg/day), or a prototype immunosuppressive (cyclophosphamide, 6 mg/kg/day). During developing adjuvant arthritis, cryogenine and phenylbutazone significantly reduced the nonimmune-mediated inflammation in the inoculated hindpaw. Neither cryogenine nor phenylbutazone provided protection against the development of the delayed-onset, immune-mediated inflammation and the reduction in growth rate seen after Day +12 in unmedicated rats. Cyclophosphamide failed to reduce the nonimmune-mediated inflammation, but it provided significant protection against both the delayed-onset, immune-mediated inflammation and the reduced growth rate. During established adjuvant arthritis, cryogenine and phenylbutazone were effective against the established inflammation, while cyclophosphamide was ineffective. These results confirm the known anti-inflammatory and immunosuppressive activities of phenylbutazone and cyclophosphamide, respectively, and indicate that cryogenine lacks immunosuppressive capability at the effective anti-inflammatory dosage level used.

Keyphrases □ Cryogenine—immunosuppressive activity evaluated in developing and established adjuvant arthritis, rats □ Immunosuppressive activity—cryogenine evaluated in developing and established adjuvant arthritis, rats □ Anti-inflammatory activity—cryogenine evaluated in developing and established adjuvant arthritis, rats

Cryogenine[1] (I), an isoquinolizidine lactone, mol. wt. 435.53, has been shown to be effective against adjuvant-induced polyarthritis in the rat (1). Protection was provided against the disseminated lesions during therapy, and a continued anti-inflammatory effect was noted after discontinuance of therapy. Because the disseminated lesions are thought to be immune mediated (2), it was considered possible that part of the pharmacological activity of I might be attributable to immunosuppression. Earlier investigations documented the anti-inflammatory effectiveness of I in experimental models of nonimmune-mediated inflammation: yeast-induced foot edema (3), cotton-pellet granuloma (4), carrageenan-induced foot edema (4–6), and UV-induced erythema (7).

The immune basis of various animal and human inflammatory conditions and the efficacy of immunotherapy in such conditions have been established (8). Perper *et al.* (9) were able to discriminate between clinically effective immunosuppressive agents and anti-inflammatory drugs by selectively treating animals during either the developing (Days 0–+12 relative to inoculation) or established (Days +18–+29) stages of adjuvant-induced arthritis. The present investigation attempted to determine the immunosuppressive capability of I in the intact rat using the experimental design employed by Perper *et al.* (9).

[1] For structure, see *Tetrahedron Lett.*, **30**, 3641 (1966). This drug is not to be confused with the trade name product Cryogénine (phenylsemicarbazide, mol. wt. 151.2) distributed by Laboratoires Sarbach, Châtillon, France.

EXPERIMENTAL

Animals—Approximately 7-week-old, male, Sprague–Dawley albino rats, 120–170 g, were allowed to acclimate for at least 6 days prior to experimentation. Body weights were checked for normal growth patterns. The animals were supplied with block lab chow[2] and water *ad libitum*.

Adjuvant Preparation and Inoculation—Heat-killed, desiccated *Mycobacterium butyricum*[3] was triturated initially with light mineral oil and then homogenized in a tissue homogenizer equipped with an electrically driven pestle (about 10 complete strokes with the pestle spinning at approximately 1500 rpm). The finely divided suspension was autoclaved at 49° at 20 psi for 20 min. The final adjuvant preparation represented 5 mg of *M. butyricum*/ml of oil (10).

On Day 0, animals were lightly anesthetized with pentobarbital sodium (30 mg/5 ml/kg ip), and adjuvant then was injected intradermally (0.05 ml) under the subplantar surface of the right hindpaw in the metatarsal region. Positive controls and drug-treated animals received the adjuvant inoculation, while negative controls received only a subplantar injection of light mineral oil.

Drugs—The following drugs were used: I base[4] (100 mg/kg/day), phenylbutazone[5] (100 mg/kg/day), and cyclophosphamide[6] (6 mg/kg/day). Each was suspended or dissolved in aqueous 0.25% agar[7] and orally administered in a dosage volume of 10 ml/kg. Negative and positive controls received 10 ml/kg of the agar vehicle only.

Developing Adjuvant Arthritis—Animals were randomly divided into the various treatment groups, 10 animals/group (except the negative control group which consisted of 12 animals). On Day −1, rats were dosed with either the agar vehicle or one drug treatment. Dosing continued once daily through Day +12.

Established Adjuvant Arthritis—Ten animals were randomly selected and designated as negative controls. The remaining animal population was inoculated with adjuvant. On Day +18, animals with left hindpaw volumes between 2.09 and 4.35 ml were randomly assigned (block randomization) to a treatment group, 10 animals/group. Animals were dosed with either the agar vehicle or a test drug, beginning on Day +18 and continuing daily through Day +29.

Evaluation of Adjuvant Arthritis—Experimental measurements began just prior to the inoculation and were repeated every 3rd day thereafter. Drug effects were assessed according to changes in body weight, generalized inflammatory symptoms, and hindpaw volume. Generalized inflammatory symptoms were evaluated according to a subjective scoring system similar to systems used previously (1, 11–13). Each of the three anatomical regions of both the forepaws (carpal, metacarpal, and phalanx) and the hindpaws (tarsal, metatarsal, and phalanx) was graded with respect to the severity of swelling (range 0–2); each forepaw or hindpaw was separately graded with respect to the severity of erythema (range 0–2); the tibiotarsal joints of both hindpaws were individually graded with respect to the degree of dorsiflexion (0 = 150° of movement, 1 = >60–<150°, 2 = 30–60°, and 3 = <30°); the tail was graded with respect to both the severity of swelling (range 0–2) and the number of nodules (range 0–2); and the auricles of both ears were separately graded with respect to both the severity of erythema (range 0–2) and the number of nodules (range 0–2).

With this scoring system, an animal on a given day could develop an inflammogram score ranging from 0 to 50. Volumes for both the inoculated and the contralateral hindpaws were determined to the top edge of the lateral malleolus using a mercury-displacement technique similar

[2] Purina Laboratory Chow.
[3] No. 0640-33, Difco Laboratories, Detroit, Mich.
[4] Isolated from the Mexican plant, *Heimia salicifolia* Link & Otto, in this laboratory.
[5] Ciba Pharmaceutical Co., Summit, N.J.
[6] Mead Johnson Laboratories, Evansville, Ind.
[7] No. 0140-01, Difco Laboratories, Detroit, Mich.

Table I—Drug Effects on Inflammogram Scores in Developing Adjuvant Arthritis [a]

Day	Positive Control, Mean Score ± *SE*	Cryogenine, 100 mg/kg		Phenylbutazone, 100 mg/kg		Cyclophosphamide, 6 mg/kg	
		Mean Score ± *SE*	Inhibition, %	Mean Score ± *SE*	Inhibition, %	Mean Score ± *SE*	Inhibition, %
+3	5.2 ± 0.3	4.6 ± 0.3	−12	4.2 ± 0.2	−19[b]	6.1 ± 0.3	—
+6	6.1 ± 0.6	4.8 ± 0.4	−21	3.7 ± 0.4	−39[c]	6.6 ± 0.4	—
+9	6.3 ± 0.6	4.5 ± 0.4	−28[b]	4.1 ± 0.5	−35[b]	6.7 ± 0.4	—
+12	10.2 ± 2.0	8.3 ± 1.7	−19	6.5 ± 1.1	−36	6.1 ± 0.4	−40
+15	14.8 ± 2.7	13.2 ± 2.7	−11	15.3 ± 1.9	—	6.4 ± 0.5	−57[b]
+18	17.6 ± 3.0	16.7 ± 2.7	−5	17.6 ± 2.4	—	9.0 ± 2.0	−49[b]
+21	18.0 ± 2.4	18.0 ± 2.4	—	15.6 ± 2.1	−13	10.4 ± 2.4	−42[b]
+24	16.4 ± 3.0	17.1 ± 2.8	—	14.2 ± 1.9	−13	4.8 ± 0.7	−71[c]
+27	15.3 ± 2.9	14.9 ± 2.0	−3	13.0 ± 1.8	−15	5.0 ± 0.7	−67[c]
+30	14.6 ± 3.3	14.3 ± 2.2	−2	12.1 ± 2.1	−17	6.4 ± 1.8	−56[b]

[a] Drugs administered orally daily from Day −1 through and including Day +12. The negative control mean score was zero at all times. [b] Significantly different from positive control values at $p = 0.05$. [c] Very significantly different from positive controls at $p = 0.01$.

to the one described by Winter and Nuss (14). A dynograph[8] connected to a strain-gauge pressure transducer[9] was employed for recording hindpaw volumes. Drug effects were translated in terms of percentage change of increased hindpaw volume as described by Newbould (15):

$$\text{percentage change} = 100\{1 - [(a - x)/(b - y)]\} \qquad \text{(Eq. 1)}$$

where y is the mean hindpaw volume of positive control rats immediately prior to adjuvant inoculation, b is the mean hindpaw volume of positive control rats on a particular day, x is the mean hindpaw volume of drug-treated rats immediately prior to adjuvant inoculation, and a is the mean hindpaw volume of drug-treated rats on a particular day. Statistical analyses were generated using the Student t test.

RESULTS AND DISCUSSION

Treatment of Developing Adjuvant Arthritis—The inoculation of the rat hindpaw with adjuvant initially produces a nonimmune-mediated acute inflammatory reaction (16, 17). This reaction, characterized by swelling and erythema of the inoculated hindpaw, usually reaches maximum intensity between 6 and 9 days after injection. The course of this acute reaction in the positive control animals can be seen in Fig. 1 (inoculated hindpaw volume) and Table I (inflammogram). Because the swelling and erythema represent symptoms of nonimmune-mediated inflammation, any drug reducing these symptoms may be considered to have anti-inflammatory activity (9).

Phenylbutazone significantly reduced inoculated hindpaw volume on Days +3 (53% inhibition), +6 (46%), and +9 (38%), and a significant reduction of the inflammogram score was noted on these days. Cyclophosphamide, a clinically effective immunosuppressive agent, was inactive against the nonimmune-mediated inflammatory symptoms. Compound I was significantly effective against inoculated hindpaw swelling on Days +3 (26% inhibition), +6 (32%), +9 (36%), and +12 (31%) and significantly reduced the inflammogram score on Day +9.

The secondary increase in hindpaw volume seen beginning on Day +15 (Fig. 1, positive controls) represents the development of immune-mediated inflammation. The increases in both the contralateral hindpaw volume (Fig. 2, positive controls) and the inflammogram score (Table I, positive controls) beginning around Day +12 represent the dissemination of the immune response (18, 19). Collectively, these delayed-onset symptoms in the rat represent the adjuvant arthritis syndrome (20). This syndrome has been linked to a delayed hypersensitivity reaction specifically triggered by sensitized lymph node cells released into the circulation between Days +8 and +14 (19).

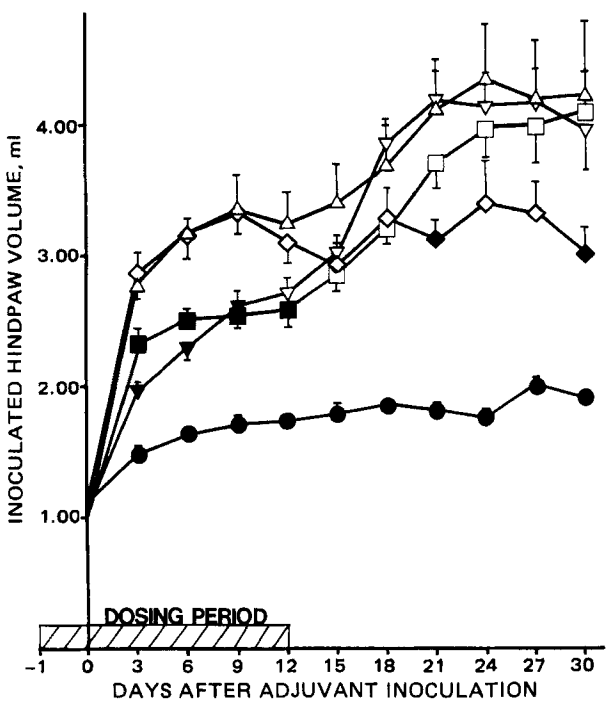

Figure 1—*Drug effects on inoculated hindpaw volume during developing adjuvant arthritis. Key:* ○, *negative control (10 ml/kg/day agar vehicle);* △, *positive control (10 ml/kg/day agar vehicle);* □, *cryogenine (100 mg/kg/day);* ▽, *phenylbutazone (100 mg/kg/day); and* ◇, *cyclophosphamide (6 mg/kg/day). Vertical bars indicate* ±SE. *Solid symbols indicate values significantly different from positive control values at* $p \leq 0.05$.

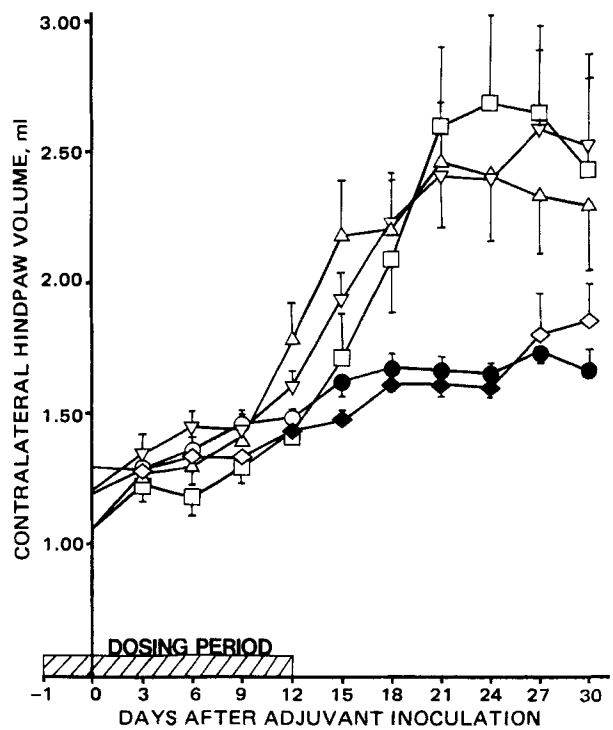

Figure 2—*Drug effects on contralateral hindpaw volume during developing adjuvant arthritis. Key:* ○, *negative control (10 ml/kg/day agar vehicle);* △, *positive control (10 ml/kg/day agar vehicle);* □, *cryogenine (100 mg/kg/day);* ▽, *phenylbutazone (100 mg/kg/day); and* ◇, *cyclophosphamide (6 mg/kg/day). Vertical bars indicate* ±SE. *Solid symbols indicate values significantly different from positive control values at* $p \leq 0.05$.

[8] Beckman RS.
[9] Statham.

Table II—Drug Effects on Body Weight in Developing Adjuvant Arthritis [a]

Day	Mean Body Weight, g ± *SE*				
	Negative Control	Positive Control	Cryogenine, 100 mg/kg	Phenylbutazone, 100 mg/kg	Cyclophosphamide, 6 mg/kg
0	152 ± 3	142 ± 4	140 ± 5	148 ± 5	155 ± 2
+3	166 ± 4[b]	148 ± 4	140 ± 5	153 ± 5	154 ± 4
+6	189 ± 5[b]	163 ± 5	158 ± 4	174 ± 6	171 ± 4
+9	213 ± 6[c]	179 ± 5	175 ± 5	194 ± 7	187 ± 5
+12	235 ± 7[c]	188 ± 7	182 ± 5	210 ± 8	203 ± 6
+15	256 ± 8[c]	198 ± 9	191 ± 5	220 ± 8	219 ± 6
+18	280 ± 7[c]	199 ± 11	195 ± 8	218 ± 9	229 ± 8[d]
+21	291 ± 10[c]	204 ± 14	200 ± 11	225 ± 11	243 ± 9[d]
+24	308 ± 11[c]	207 ± 17	194 ± 12	236 ± 10	256 ± 10[d]
+27	331 ± 10[c]	214 ± 18	204 ± 14	247 ± 11	272 ± 12[d]
+30	344 ± 10[c]	236 ± 19	217 ± 18	260 ± 12	295 ± 13[d]

[a] Drugs administered orally daily from Day −1 through and including Day +12. [b] Very significantly different from positive control values at $p = 0.01$. [c] Very highly significantly different from positive control values at $p = 0.001$. [d] Significantly different from positive control values at $p = 0.05$.

A drug that prevents the development of this syndrome when administered just up to the expected appearance of the associated inflammatory symptoms may be considered to have immunosuppressive activity (9). In the present study, cyclophosphamide was significantly effective in preventing the development of the delayed-onset, immune-mediated inflammatory syndrome. With respect to inoculated hindpaw volume (Fig. 1), cyclophosphamide pretreatment prevented the secondary increase. This reduction reached statistical significance on Day +21 (37% inhibition).

The total volume increase for the inoculated hindpaw seen after Day +12 (Fig. 1, positive controls) represents the sum of the residual nonimmune inflammatory effects resulting from the trauma of inoculation plus the immune-mediated inflammatory effects resulting from the delayed hypersensitivity reaction. In contrast, the increased volume of the contralateral hindpaw seen after Day +12 (Fig. 2, positive controls) can only represent immune-mediated inflammation (9). Cyclophosphamide significantly prevented the development of contralateral hindpaw edema (Fig. 2) on Days +12 (80% inhibition), +15 (85%), +18 (73%), +21 (77%), and +24 (78%). In addition, dissemination of immune-mediated inflammatory lesions to the forepaws, ears, and tail was significantly reduced beginning on Day +15 (Table I, inflammogram).

Body weights for animals during developing adjuvant arthritis are recorded in Table II. Growth rates for positive controls were significantly less than those for uninoculated negative control animals. These results are similar to those reported by others (1, 20). An observable reduction in growth rate is consistent with an ongoing, chronic, inflammatory disease. A drug treatment that prevents the development of the immune-mediated inflammatory syndrome, therefore, should prevent this reduced growth rate. Cyclophosphamide-pretreated animals grew at a significantly greater rate relative to the positive controls beginning on Day +18 (Table II). This effect on growth rate remained statistically significant through Day +30.

Phenylbutazone and I were ineffective in preventing the development of the immune-mediated inflammatory syndrome (Fig. 1, inoculated hindpaw volume; Fig. 2, contralateral hindpaw volume; and Table I, inflammogram) and in preventing the reduced growth rate (Table II).

Phenylbutazone treatment reduced the nonimmune-mediated acute inflammatory reaction in the inoculated hindpaw but failed to prevent the development of the immune-mediated inflammatory syndrome. Cyclophosphamide treatment failed to reduce the nonimmune-mediated

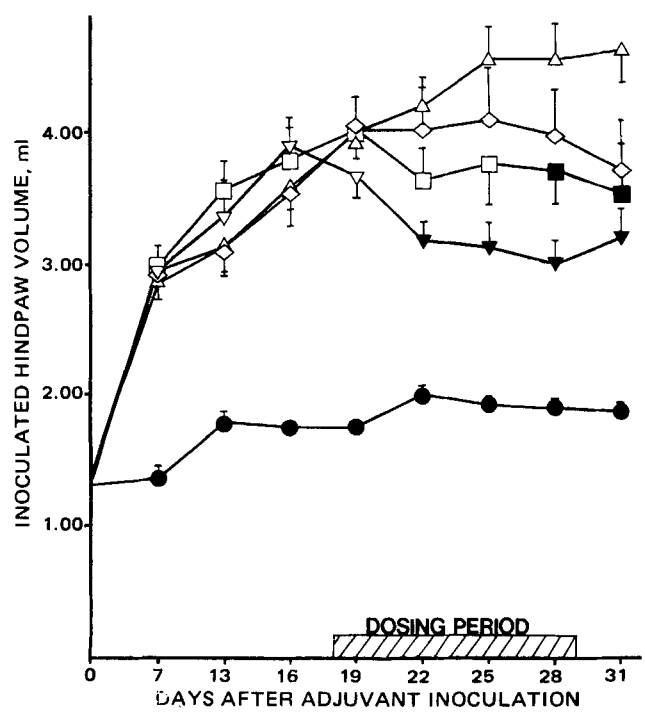

Figure 3—*Drug effects on inoculated hindpaw volume during established adjuvant arthritis. Key:* ○, *negative control (10 ml/kg/day agar vehicle);* △, *positive control (10 ml/kg/day agar vehicle);* □, *cryogenine (100 mg/kg/day);* ▽, *phenylbutazone (100 mg/kg/day); and* ◇, *cyclophosphamide (6 mg/kg/day). Vertical bars indicate* ±SE. *Solid symbols indicate values significantly different from positive control values at* $p \leq 0.05$.

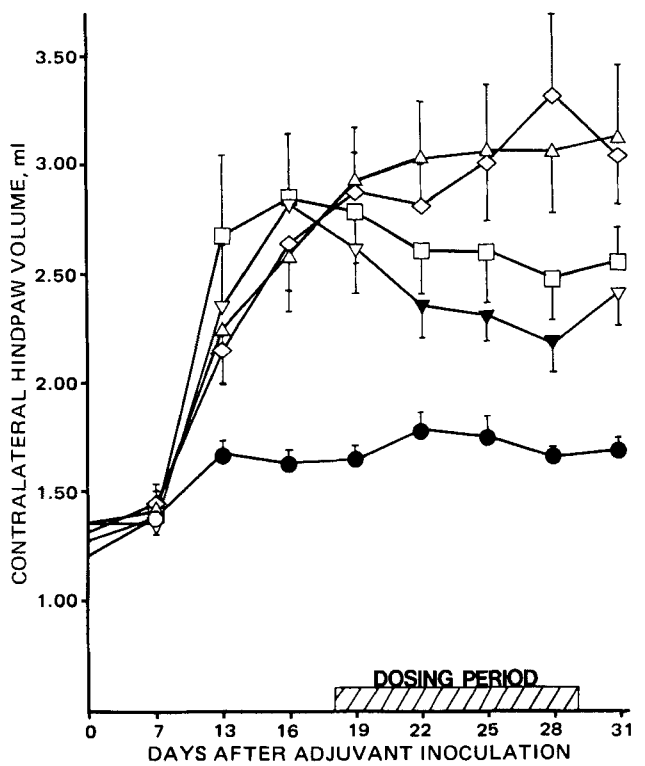

Figure 4—*Drug effects on contralateral hindpaw volume during established adjuvant arthritis. Key:* ○, *negative control (10 ml/kg/day agar vehicle);* △, *positive control (10 ml/kg/day agar vehicle);* □, *cryogenine (100 mg/kg/day);* ▽, *phenylbutazone (100 mg/kg/day); and* ◇, *cyclophosphamide (6 mg/kg/day). Vertical bars indicate* ±SE. *Solid symbols indicate values significantly different from positive control values at* $p \leq 0.05$.

Table III—Drug Effects on Inflammogram Scores in Established Adjuvant Arthritis [a]

Day	Positive Control, Mean Score ± *SE*	Cryogenine, 100 mg/kg		Phenylbutazone, 100 mg/kg		Cyclophosphamide, 6 mg/kg	
		Mean Score ± *SE*	Inhibition, %	Mean Score ± *SE*	Inhibition, %	Mean Score ± *SE*	Inhibition, %
+7	4.1 ± 0.4	5.7 ± 0.4[a]	—	4.6 ± 0.7	—	4.7 ± 0.8	—
+13	11.1 ± 2.2	16.8 ± 2.4	—	13.6 ± 2.2	—	13.3 ± 1.9	—
+16	18.3 ± 2.6	21.9 ± 2.7	—	20.7 ± 3.0	—	18.1 ± 2.3	—
+19	20.3 ± 2.0	21.9 ± 2.7	—	19.6 ± 2.5	−3	19.4 ± 2.0	−4
+22	19.4 ± 1.5	20.1 ± 2.3	—	18.4 ± 2.0	−5	19.3 ± 1.9	—
+25	20.1 ± 1.5	18.3 ± 2.5	−9	16.6 ± 2.0	−17	16.5 ± 2.6	−18
+28	19.9 ± 1.6	18.5 ± 2.7	−7	14.4 ± 2.2	−28	18.0 ± 2.9	−10
+31	20.1 ± 1.7	16.1 ± 3.0	−20	13.6 ± 1.8	−32[b]	16.8 ± 3.0	−16

[a] Drugs administered orally daily from Day +18 through and including Day +29. The negative control mean score was zero at all times. [b] Significantly different from positive control values at $p = 0.05$.

Table IV—Drug Effects on Body Weight in Established Adjuvant Arthritis [a]

Day	Mean Body Weight, g ± *SE*				
	Negative Control	Positive Control	Cryogenine, 100 mg/kg	Phenylbutazone, 100 mg/kg	Cyclophosphamide, 6 mg/kg
0	137 ± 4	143 ± 3	143 ± 4	145 ± 4	143 ± 5
+7	191 ± 7	180 ± 5	173 ± 4	175 ± 5	177 ± 5
+13	237 ± 9	205 ± 6	183 ± 5[b]	189 ± 6	194 ± 6
+16	261 ± 10[c]	203 ± 6	177 ± 7[b]	196 ± 10	186 ± 6
+19	281 ± 10[c]	198 ± 6	171 ± 8[b]	187 ± 9	180 ± 7
+22	298 ± 11[c]	194 ± 7	164 ± 9[b]	192 ± 12	178 ± 9
+25	310 ± 12[c]	200 ± 8	169 ± 10[b]	208 ± 12	181 ± 11
+28	323 ± 11[c]	208 ± 10	174 ± 13[b]	209 ± 14	184 ± 13
+31	329 ± 12[c]	212 ± 12	174 ± 16	231 ± 15	204 ± 14

[a] Drugs administered orally daily from Day +18 through and including Day +29. [b] Significantly different from positive control values at $p = 0.05$. [c] Very highly significantly different from positive control values at $p = 0.001$.

acute inflammatory reaction but prevented the development of the secondary immune-mediated syndrome. These results are in agreement with those observed previously (9). Results for I tended to parallel those of phenylbutazone. The activity observed against the nonimmune-mediated inflammatory reaction is consistent with the results recorded by Kosersky *et al.* (1).

Treatment of Established Adjuvant Arthritis—Sensitized lymph node cells are released into the circulation up to Day +14 (19). Pharmacological treatment during Days +18–+30 (*i.e.*, after the immunologically related induction period) should then detect only drugs having anti-inflammatory activity (9). However, this interpretation should be made cautiously, since (*a*) established adjuvant arthritis may be partially sustained by an autoimmune mechanism similar to the one proposed for rheumatoid arthritis (21–23), and (*b*) the hepatic microsomal mixed-function oxidase system is impaired in adjuvant arthritic rats during the established period of the disease (24).

In the present study, phenylbutazone treatment during Days +18 through +29 was significantly effective in reducing the already established chronic inflammation. Inoculated hindpaw volume (Fig. 3) was significantly reduced on Days +22 (37% inhibition), +25 (46%), +28 (50%), and +31 (45%). Contralateral hindpaw volume (Fig. 4) was significantly reduced on Days +22 (40% inhibition), +25 (44%), and +28 (52%). In addition, the inflammogram score (Table III) was significantly reduced on Day +31 (32% inhibition). Cyclophosphamide was ineffective against all of these experimental parameters. These results are in essential agreement with those of Perper *et al.* (9).

Treatment with I significantly reduced inoculated hindpaw volume (Fig. 3) on Days +28 (25% inhibition) and +31 (33%), but the apparent effects on contralateral hindpaw swelling (Fig. 4) and on the inflammogram score (Table III) were statistically not significant. Animals designated for treatment with I weighed significantly less than positive controls prior to the dosing period (Days +13 and +16) (Table IV). These animals continued to weigh significantly less through Day +28. The inflammogram score (Table III) was significantly greater prior to the dosing period (Day +7) for this same group of animals. On Day +18, the population of positive controls was randomly divided into the various drug treatment groups on the basis of an equivalent range of contralateral hindpaw swelling (*i.e.*, 2.09–4.35 ml). By chance, animals designated for I treatment may have been stronger reactors to the adjuvant inoculation. This possibility seems to explain the weight loss observed during drug treatment and accounts for why the drug's anti-inflammatory activity was less than expected. Previous investigators documented a weight gain for adjuvant-arthritic animals treated daily for 20 days with 100 mg/kg po of I (1). Moreover, in a preliminary study using a small group of rats with established adjuvant arthritis, I at 100 mg/kg po daily for 1 week appeared to reduce the already established inflammation (25).

The overall results using both developing and established treatment models tend to substantiate the known anti-inflammatory and immunosuppressive roles of phenylbutazone and cyclophosphamide, respectively. Assessment of the overall activity of I during both developing and established treatment periods suggests the lack of an immunosuppressive-type capability and supports previous investigations that indicated a nonimmune-type anti-inflammatory action mechanism for I (3–7).

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Calcium–Prostaglandin Aggregation and Its Effect on Prostaglandin Uptake by Isolated Rabbit Intestine

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Abstract □ A quantitative estimate of the role of calcium ions on the lipid–water partition coefficients of prostaglandin E_1 and dinoprost suggested the possibility of prostaglandin molecules and calcium ions aggregating in a 14:1 ratio to produce a lipid-soluble aggregate. The aggregation is postulated to be a characteristic of prostaglandin molecules as compared to simple fatty acids, *e.g.*, 1-octanoic acid, which, in the presence of calcium, behave differently than the prostaglandins. The uptake of prostaglandins by the mucosal surface of the rabbit intestine increased in the presence of calcium. For example, at 25 m*M* calcium, prostaglandin E_1 was transported at approximately twice the rate as in the system containing no calcium. The uptake rate of dinoprost was estimated to be three times faster with 10 m*M* calcium than in the absence of calcium. Therefore, it is proposed that a carrier-mediated diffusion process, for both the prostaglandin molecules and calcium ions, takes place in the uptake mechanism. Diffusion coefficients ranging from 0.48×10^{-5} to 7.19×10^{-5} cm²/sec and permeability coefficients ranging from 1.04×10^{-2} to 15.6×10^{-2} cm/sec were estimated for all systems studied.

Keyphrases □ Prostaglandin E_1—aggregation with calcium ions, effect on lipid–water partition coefficients and uptake by isolated rabbit intestine □ Dinoprost—aggregation with calcium ions, effect on lipid–water partition coefficients and uptake by isolated rabbit intestine □ Calcium ions—aggregation with prostaglandin E_1 and dinoprost, effect on lipid–water partition coefficients and uptake by isolated rabbit intestine □ Partition coefficients, lipid–water—prostaglandin E_1 and dinoprost, effect of aggregation with calcium ions □ Intestinal uptake, isolated—prostaglandin E_1 and dinoprost, effect of aggregation with calcium ions

Recent studies on the physical, physiological, and biochemical relationships between prostaglandin and calcium dealt with the cellular distribution of prostaglandins (1), the prostaglandin and cellular calcium interaction (2–5), and the effect of prostaglandin on the GI tract, including its influence on glucose, water, and electrolyte absorption (6–8). Very few studies attempted to explain the physical interaction between calcium and prostaglandins (9, 10).

The present study attempts to explain the nature of this interaction and to correlate the data to prostaglandin uptake by the intestinal mucosa of the rabbit.

EXPERIMENTAL

Partition Coefficient—The *n*-octane[1]–water, 1-octanol[2]–water, and 1-octanoic acid[3]–water partition coefficients of 5,6(n)-^{3}H-prostaglandin E_1[4] (I) and 5,6,8,11,12,14,15(n)-^{3}H-dinoprost (II) were measured as follows. In 10-ml screw-capped test tubes, 2.0 ml of the oil phase and 2.0 ml of a pH 5 aqueous phase containing the desired amount of I (24.4×10^{-9} m*M*), II (2.88×10^{-9} m*M*), and calcium ions (2.5, 5.0, 10.0, 25.0, 40.0, 50.0, 75.0, 100.0, 150.0, or 200.0 m*M*) were added. Prior to equilibration, nitrogen gas was cautiously bubbled into each test tube to ensure the displacement of air from the upper void spaces. The test tubes were then tightly capped and attached with rubber bands to the shaft of a rotating-bottle disintegration apparatus[5].

The apparatus with the test tubes was put in a stainless steel tank filled with water thermostatically controlled at 25°. The motor was allowed to rotate, mixing the two phases in the test tubes for 30 min. After equilibration, each test tube was detached from the apparatus and allowed to stand for 15 min at 25° for complete phase separation. Then 1.0 ml of each oil and aqueous phase was individually pipetted and assayed for I and II in a three-channel liquid scintillation spectrometer[6]. The calculated partition coefficients were the averages of four samples for each oil–water system.

The same procedure was followed for the estimation of the partition coefficient while varying the concentration of I and II and keeping the concentration of calcium ions constant (5 or 50 m*M*).

Partition Coefficient of 1-Octanoic Acid—Since prostaglandins are considered to be fatty acids, it was essential to compare their partition behavior with that of a fatty acid such as 1-octanoic acid in the presence of calcium ions. A potentiometric method was used for the quantitative analysis of 1-octanoic acid in the aqueous phase. A standard aqueous 1-octanoic acid solution (32.0 m*M*) was prepared and potentiometrically[7] titrated with 0.01 *N* sodium hydroxide. The standard solution was employed as the aqueous phase in the partition experiments after the addition of the desired amount of calcium ions (0, 100, or 200 m*M*). The oil phase chosen was 1-octanol.

Thus, for a single calcium-ion concentration system, two sets of 250-ml separators were prepared. For the 0 m*M* calcium system (A and A′),

[1] Eastman Kodak Co., Rochester, N.Y.
[2] Riedel-De Haen AG, Seelze-Hannover, West Germany.
[3] Hopkin and Williams Ltd., Chadwell Heath, Essex, England.
[4] Radiochemical Center, Amersham, Buckinghamshire, England.
[5] Ernest D. Menold, Lester, Pa.
[6] Packard Tri-Carb model 3320, Packard Instrument Co., Downers Grove, Ill.
[7] Radiometer, pH meter model 22, Copenhagen, Denmark.