Effects of Salicylate on Prophylactic Counter-Irritation.* (26959)

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A pre-existing inflammation produced by mechanical (1) or chemical (2) irritation has been shown to inhibit partially the response elicited by a subsequent irritation. It has been found in rats that when $AgNO_3$ is used as the chemical irritant, the maximum inhibition of inflammation, as evaluated by the edematous response, occurs when the initial irritation precedes the second irritation by 12-15 hours (3). Because of the extensive use of salicylates in treatment of inflammation, this study was undertaken to evaluate their effects on this prophylactic counter-irritation mechanism.

Materials and methods. The test sites used in this study were the ankles of male albino rats (300-450 g). Chemical irritation was accomplished by injection of 0.10 ml of 1% silver nitrate into the joint under light ether anesthesia (4). Salicylate administration was accomplished by introducing 50 mg of sodium salicylate in 2 ml of water via stomach tube. The edematous response was evaluated by measuring to the nearest millimeter the circumference of the leg immediately superior to the ankle joint. An analysis of variance has shown that there is no significant difference between investigators or repeat measurements in this laboratory. For comparative purposes and ease in presentation, the percent increments in circumference were calculated.

During this study 2 series of experiments were performed. Since statistical analysis ("t" ratios) showed no significant differences between the 2 series, they have been combined for economy of presentation. The animals were divided into the following treatment groups:

A—only the test ankle was irritated (control group);

B—sodium salicylate was administered 1 hour prior to irritation of the test ankle;

C—the opposite ankle was irritated 15 hours prior to irritation of the test ankle;

D—sodium salicylate was administered 16 hours prior, and the opposite ankle was irritated 15 hours prior to irritation of the test ankle; and

E—the opposite ankle was irritated 15 hours prior, and sodium salicylate was administered 1 hour prior to irritation of the test ankle.

Results. In Table I are presented the mean percent increases in circumference of the test ankles following irritation with $AgNO_3$. For the control group (A) the peak response (ca. 55%) was reached within 12 hours and was maintained through the second day; thereafter the swelling subsided and the ankle regained its initial size in 2 weeks. Administration of sodium salicylate one hour prior to irritation of the test ankle resulted in a marked inhibition of edema. The amount of swelling for this treatment (B) was significantly less ($P < .01$) than that occurring in the control group at the 12, 24, and 48 hour determinations, which as noted above correspond to the period of maximum swelling.

In the prior-irritation group (treatment C), in which the opposite ankle was irritated 15 hours previous to injection of the test ankle, the ankle swelling was much less than in the control group. The differences were significant at the 6, 12, 24, 48, and 120-hour determinations. Also, this prior-irritation group exhibited less mean swelling than the salicylate-treated group, but the

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TABLE I. Effects of Prior Treatment with Sodium Salicylate and/or Prior Irritation on Percent Increments of Ankle Edema.

Treatments	n	Hours after irritation of test ankle with $AgNO_3$ 6	12	24	48	72	120	168	336
A. None	10	44 ± 5*	55 ± 5	55 ± 4	56 ± 7	48 ±10	42 ± 9	27 ± 9	2 ± 2
B. Na sal. (−1 hr)	10	37 ± 7	44 ± 6	45 ± 8	44 ± 8	40 ±15	34 ±15	22 ±13	3 ± 4
C. Prior irrit. (−15 hr)	21	26 ± 9	37 ±10	42 ± 8	40 ± 8	38 ± 9	26 ±11	16 ±10	1 ± 2
D. Na sal. (−16 hr); prior irrit. (−15 hr)	21	38 ±11	50 ± 8	56 ±10	53 ±11	46 ±10	37 ±15	25 ±14	4 ± 4
E. Prior irrit. (−15 hr; Na sal. (−1 hr)	21	28 ±11	38 ±11	41 ± 8	40 ±11	34 ±19	31 ±15	24 ±14	2 ± 5

Treatment comparisons showing significant differences ($P < .01$) †

A *vs* B	12, 24, 48 hr	B *vs* C	6 hr	C *vs* D	6, 12, 24, 48, 72, 336 hr
A *vs* C	6, 12, 24, 48, 72, 120, 168 hr	B *vs* D	24 hr	C *vs* E	—
A *vs* D	—	B *vs* E	—	D *vs* E	6, 12, 24, 48 hr
A *vs* E	6, 12, 24, 48 hr				

* Mean ± stand. dev. † "t" ratio.

difference was significant at the 6 hour reading only. When salicylate was administered prior to irritation of the opposite ankle (treatment D), thereby inhibiting the "inflammatory" response of the first ankle, the test ankles swelled nearly as much as those of the controls and differed as much from group C as did the controls. In those rats receiving salicylate one hour prior to irritation of the test ankle (treatment E), the opposite ankle having been irriated 15 hours previously, the amount of edema was less than that of the controls; the difference was significant at 6, 12, 24 and 48 hours. The amount of swelling, however, was not significantly less than that obtained with salicylate (treatment B) or prior-irritation (treatment C) alone.

Discussion. As we reported previously, the maximum inhibition of edema in the test ankle occurs when the prophylactic counter-irritation (PCI) precedes by 12 to 15 hours the irritation of the test ankle. In this study, PCI inhibited the edematous response of the test ankle to a slightly greater extent than did sodium salicylate. This, however, is not a significant finding since we have noted greater inhibition of swelling with higher doses of sodium salicylate than were used here. This substantiates the reports of others (4,5). On the other hand, a greater inhibition of the swelling might occur if the degree of inflammation of the first ankle was increased by injecting a larger quantity of irritant. The dose of 0.10 ml of 1% $AgNO_3$ was chosen because with larger quantities sloughing and visible necrosis often resulted.

Administration of salicylate shortly before irritation of the test ankle did not augment the inhibition produced by PCI. One explanation for the lack of summation is the possible interference of one mechanism with the other. An alternative explanation is that both PCI and salicylate utilize essentially the same terminal mechanisms with the result that under the conditions of this experiment either treatment alone leads to near exhaustion of the anti-edema mechanisms. Laden et al (1) have shown that the "protection" afforded by PCI is also present in adrenalectomized and hypophysectomized animals. Further, Kelemen (5), subscribing to the permissive theory for corticoid activity, reports that although corticoids are necessary for the anti-edema action of salicylates, the latter do not operate through the hypophyseal-cortical axis. These

observations, therefore, rule out the hypophyseal-cortical axis as a common mechanism.

Of even more interest is the effect seen when salicylate was given just prior to PCI. Here the inhibition of the edematous response usually produced by PCI was markedly reduced, if not lost all together. The interference by salicylate upon the PCI mechanism, as mentioned above, is one possibility for explaining this effect. An alternate possibility of this salicylate effect is that by reducing the inflammatory response to the first irritation the stimulus to the counter-irritation mechanism is sufficiently reduced as not to alter the second inflammation under the conditions of this study.

The existing evidence suggests that PCI leads to production of a generalized hyporeactivity to irritation. Local hyporeactivity at the site of irritation has been established (6,7,8,9). By altering or blocking the PCI mechanism, salicylates may allow a more rapid return to the initial reactivity. However, until the nature of this "protection" against inflammation afforded by PCI is established, it is difficult to evaluate the salicylate effect in this study.

Summary. The effects of sodium salicylate on prophylactic counter-irritation, accomplished by irritating the opposite ankle 15 hours prior to the test ankle, were studied in rats. It was found that: (1) The capacities to inhibit edema by prophylactic counter-irritation and sodium salicylate were similar; (2) Sodium salicylate administered 1 hour prior to irritation of the test ankle did not increase the degree of inhibition produced by prophylactic counter-irritation; and (3) Sodium salicylate given 1 hour prior to the prophylactic counter-irritation caused the latter's anti-edema effect to be lost.

1. Hamdy, M. K., Kunkel, L. E., Rheins, M. S., Deatherage, F. E., *J. Animal Sci.,* 1957, v16, 496.

2. Laden, C., Blackwell, R. Quentin, Fosdick, L. S., *Am. J. Physiol.,* 1958, v195, 712.

3. Bowman, D. C., Jr., Doemling, D. B., Harris, S. C., *Fed. Proc.,* 1960, v19, 87.

4. LaBelle, A., Tornaben, J. A., *Science,* 1951. v114, 187.

5. Kelemen, E., *Permeability in Acute Experimental Inflammatory Oedema in the Light of the Action of Salicylates,* Hungarian Acad. Sci., 1960.

6. Miles, A. A., Miles, E. M., *J. Physiol.,* 1952, v118, 228.

7. Hoene, R., Labbe, P., Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 65.

8. Arteta, J. L., *ibid.,* 1954, v85, 162.

9. Salgado, E., Hoene, R., *ibid.,* 1954, v86, 190.

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Species-Related Antigens of Mammalian Cell Strains as Determined by Immunofluorescence.*† (26960)

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In the development of characterizing procedures for cultured cell strains, identification of their species of origin is of primary importance, especially since it is commonly known that some cell strains have become contaminated with others of diverse parentage. This problem has been approached in several ways, including chromosomal analyses(1), virus susceptibility(2), and immunologic identification(3-4). In an effort to identify cell strains in a manner analogous to the systematic serology long employed in

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