RESEARCH PAPERS

THE MECHANISM OF THE ANTI-INFLAMMATORY ACTIVITY OF SALICYLATE

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Salicylate reduced the leakage of circulating dye in the reversed passive cutaneous anaphylaxis reaction in the guinea pig. 2,4-Dinitrophenol, although a more powerful uncoupling reagent than salicylate, had no effect compared to corresponding control animals. In this test in the guinea-pig, salicylate inhibits the increased capillary permeability due to cutaneous anaphylaxis and to histamine, but not to Miles-Wilhelm permeability factor. It is concluded that the effect of salicylate in reducing the increased capillary permeability produced in these reactions does not result from an uncoupling action on oxidative phosphorylation processes. The results suggest that salicylates act by preventing antigen-antibody combinations from exerting their effects on the capillary wall.

SALICYLATE uncouples oxidative phosphorylation reactions in respiring mitochondrial preparations (Brody, 1956) and many of its metabolic and toxic effects are explicable in terms of this action (Smith, 1959). Adams and Cobb (1958) studied the effects of a series of non-hormonal anti-inflammatory drugs, including salicylate, on erythema induced in the guinea pig by ultra-violet light. They observed a general parallelism between uncoupling and anti-inflammatory activity. It was noted however, that 2,4-dinitrophenol, which is a more powerful uncoupling reagent than salicylate, failed to affect the erythema test. In the present work we have compared the actions of salicylate and 2,4-dinitrophenol on the increased capillary permeability in passive cutaneous anaphylaxis in the guinea-pig. In addition, the effects of salicylate on the cutaneous lesions induced in the guinea-pig by the permeability factor of Miles and Wilhelm (1955) and by histamine, have been studied. A preliminary account of part of the work has already been published (Marks and Smith, 1960).

EXPERIMENTAL

Animals. Albino guinea-pigs (wt. 450–500 g.) all of which were bred from the same stock, were depilated by applying barium sulphide paste to the previously clipped hair of the back and flanks.

Materials. Specific pneumococcal polysaccharide type III (SIII) and antibodies to SIII prepared in rabbits (anti-SIII) were obtained from the National Institute for Medical Research. Guinea-pig permeability

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factor (PF) was obtained from the Lister Institute of Preventive Medicine. The histamine was used as a solution of its acid phosphate in 0·9 per cent (w/v) saline. Pontamine Sky Blue 6 BX (G. T. Gurr) was prepared as a 5 per cent solution in sterile 0·9 per cent (w/v) saline and given by intravenous injection in a dose of 6 mg./kg. body weight. The salicylate solution contained 125 mg. of salicylate ion per ml. and was prepared by dissolving sodium salicylate B.P. in 0·9 per cent (w/v) saline and adjusting to pH 7·5 with 0·1 N HCl. It was given by intraperitoneal or intravenous injection in a dose of either 500 or 100 mg./kg. body weight. 2,4-Dinitrophenol (DNP) solution, containing 5 mg./ml. in 0·9 per cent (w/v) saline adjusted to pH 7·5 with 0·1 N KOH, was administered by intraperitoneal injection in a dose of 20 mg./kg. body weight.

Passive cutaneous anaphylaxis. The following two methods involving either intradermal sensitisation with antiserum followed by a systemic challenge with the antigen after 24 hr. or systemic sensitisation with antiserum and an intradermal challenge with antigen after 48 hr. were used.

Method 1. The animals were given intradermal injections of 0·1 ml. saline containing amounts of anti-SIII ranging from 0·5 to 50 μg. of antibody N per ml. at random sites on the back and flanks. After 24 hr. the control animals received intraperitoneal injections of 2 ml. of saline and the test animals received either salicylate or DNP solution by the same route. 2 hr. later each animal received 200 μg. of SIII in 0·5 ml. saline, together with Pontamine Blue solution by intravenous injection. The diameters of the cutaneous lesions produced were measured after 20 min. and the degree of "blueing" of the lesions assessed visually in terms of arbitary units. Additional experiments were made in which the time interval between the intraperitoneal injection of 500 mg. of salicylate per kg. and the intravenous injection of the antigen plus dye was reduced from 2 hr. to between 15 and 30 min. Salicylate in a dose of 100 mg./kg. was also injected intravenously immediately before challenging with the antigen in a further group of 4 animals.

Method 2. The guinea-pigs received an intraperitoneal injection of anti-SIII in a dose of 0·8 mg./kg. contained in 1 ml. of saline, followed after 48 hr. by the intravenous injection of the pontamine blue solution. Each animal then received a total of 17 intradermal injections distributed at random. These injections, each of 0·1 ml., comprised one of saline, four of saline containing 30 μg. of SIII, four of a saline solution of PF, 20 μg./ml., and the remainder consisting of saline solutions of histamine, four containing 6·7 μg./ml., four containing 20 μg./ml. The diameters and intensity of "blueing" of the lesions were measured after 20 min. Each animal then received an intraperitoneal injection of either saline or salicylate 500 or 100 mg./kg. Intradermal injections of each of the four test solutions were then repeated at intervals of 15, 30 and 60 min. after the saline or salicylate had been given and their size and intensity of colour assessed after a further 20 min. The intraperitoneal injections of saline were given to determine the effects of the "blueing" of the cutaneous lesion due to the time intervals which had elapsed since the intravenous administration of the dye. Blood glucose determinations were made by the

glucose oxidase method on venous samples obtained before and at one and two hours after the intraperitoneal injections.

RESULTS

The results given in Table I show that the intraperitoneal injection of 500 mg./kg. of salicylate given 2 hr. before the administration of antigen and dye caused a significant reduction in the colour intensity of the cutaneous lesions produced by the intradermal injections of 10 to 50 μg./ml. concentrations of the antibody. The effects of the similar administration of 20 mg./kg. of DNP did not differ from that produced in corresponding control animals and neither of the uncoupling reagents altered the size of the areas in which extravasation of circulating dye had occurred.

In the control and DNP-treated animals, the cutaneous lesions were sharply circumscribed and the "blueing" was uniform throughout. However, in the animals injected with salicylate, the lesions were irregular in outline and also showed a mottled appearance. This "mottling" was the

TABLE I

EFFECT OF SALICYLATE (500 MG./KG. BODY WT.) AND DNP (20 MG./KG. BODY WT.) ON THE SIZE AND INTENSITY OF STAINING OF SKIN LESIONS PRODUCED IN THE REVERSED PCA TEST IN THE GUINEA-PIG

Antibody concentration (μg. antibody N./ml.)	No. of lesions per animal	Control (10)		Salicylate (10)		DNP (10)	
		Size (mm.)	Intensity	Size (mm.)	Intensity	Size (mm.)	Intensity
50	2	17·7 ± 2·6	2·8 ± 0·52	18·4 ± 2·0	2·0 ± 0·58	18·2 ± 2·5	2·5 ± 1·05
25	4	16·1 ± 2·3	2·6 ± 0·59	17·0 ± 2·0	1·7 ± 0·67	16·9 ± 2·5	2·7 ± 0·63
10	4	14·1 ± 2·2	2·4 ± 0·71	15·3 ± 2·7	1·7 ± 0·65	14·7 ± 2·4	2·4 ± 0·52
5	2	10·8 ± 2·4	1·4 ± 0·26	12·2 ± 2·9	1·0 ± 0·75	12·1 ± 1·8	1·8 ± 0·60
2·5	2	9·9 ± 1·8	0·8 ± 0·14	10·3 ± 2·6	0·8 ± 0·53	9·9 ± 3·2	1·1 ± 0·75
0·5	2	0	0	0	0	0	0

The salicylate and DNP were administered 2 hr. before the intradermal injection of the antigen. The number of animals in each group is given in parentheses and the intensity is expressed as arbitrary units. The results are given as means together with the standard deviations. Comparison of the results by the *t*-test showed a significant difference ($P < 0·05$) between the colour intensities of the skin lesions produced by 10 to 50 μg./ml. of antibody in the control and salicylate groups only.

most characteristic change produced by salicylate and was observed to occur in every animal tested. The application of intermittently weak negative pressure to the lesions, 5 to 10 mm. of Hg., failed to produce a uniform distribution of colour in the mottled areas showing that a reduced arteriolar pressure was not responsible for the mottling.

When the time interval between the intraperitoneal injection of salicylate and the intravenous injection of the antigen was reduced to between 15 and 30 min., these changes were less evident. There was no significant reduction in the intensity of blueing of the lesions and only one of the four salicylate-treated animals showed mottling. A similar result was obtained in 4 guinea-pigs which received 100 mg./kg. body weight of salicylate by intravenous injection immediately before challenging with the antigen. Mottling of the cutaneous lesions occurred in one animal but the extravasation of the circulating dye, as assessed by the colour intensity of the lesions, was not significantly affected.

The results, represented graphically in Fig. 1, show that both 500 and 100 mg./kg. of salicylate caused a marked reduction in the colour intensity of the lesions produced by passive cutaneous anaphylaxis to SIII. Mottling was observed in about three-quarters of the lesions in the animals given the larger dose of salicylate and in about half of those receiving the smaller dose. 500 mg./kg. of salicylate caused a slight reduction in the colour intensity of the lesions produced by PF but the smaller dose of salicylate was without effect. Mottling was not seen in any of the lesions produced by PF in salicylate-treated animals. 100 mg. salicylate caused reductions of more than 50 per cent in the colour intensities of the lesions

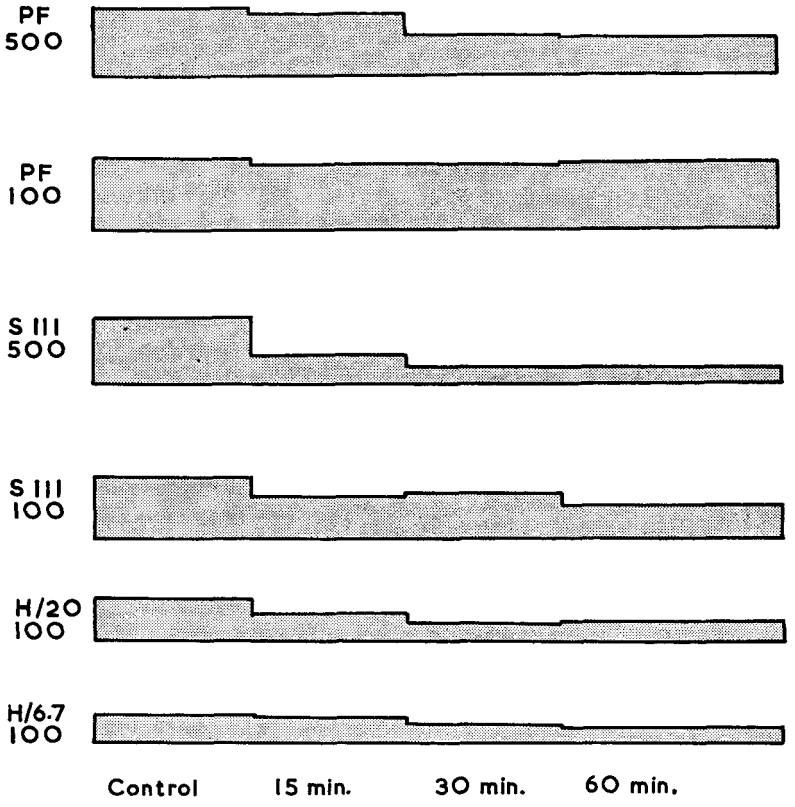

FIG. 1. The effects on the colour intensity of lesions produced by PF, SIII, and histamine (H) before, and 15, 30, and 60 min. after the intraperitoneal injection of salicylate in doses of 500 or 100 mg./kg. The thickness of the columns indicates the intensity of blueing in arbitary units. The sizes of the lesions were unaffected and are not shown.

produced by both doses of histamine. However, mottling occurred in about half of the lesions produced by histamine, both in control animals receiving saline and in test animals before the salicylate had been given. The administration of salicylate did not increase the frequency of mottling in the histamine-induced lesions.

Discussion

The results show that salicylate, but not DNP, affects anaphylactic capillary permeability in the guinea-pig. The inhibitory action of salicylate on these allergic lesions as well as in the erythema reaction (Adams and Cobb, 1958) must therefore be mediated by a mechanism other than an uncoupling action on oxidative phosphorylation processes. It was evident (Fig. 1) that an interval of 15 min. between the salicylate administration and the challenging dose of antigen sufficed to produce a reduced leakage of the circulating dye. The sizes of the lesions were not altered by the salicylate showing that the drug did not affect the sensitisation of the tissue by the antibody.

The present work has attempted to define the mode of action of salicylate more closely by using guinea-pig permeability factor and histamine in conjunction with the reversed passive cutaneous anaphylaxis reaction. The essential lesion in the cutaneous anaphylaxis reactions is an increased capillary permeability. Salicylate could reduce increased capillary permeability by a number of mechanisms. A suppression of the formation of antibody to the antigen is an unacceptable explanation because salicylate is effective both in the passive Arthus reaction (Smith and Humphrey, 1949) and in the present work, where preformed antibodies are injected in the animal. A dissociation of the antigen-antibody combination is unlikely since, although this has been reported to occur under *in vitro* conditions (Friend, 1953), it necessitated relatively large concentrations of salicylate and may have involved denaturation of the proteins. A further possibility is that salicylate stimulates the adrenal cortex and that adrenal corticosteroids are the effective agents in reducing capillary permeability but the balance of the evidence is against this interpretation (Spector, 1958). Adrenal medullary stimulation by salicylate leading to an increased secretion of adrenaline must also be considered as a possible mechanism. However it is not certain that adrenaline would diminish increased capillary permeability (Spector, 1958) and a determination of blood glucose levels of the salicylate-treated animals in the present work did not detect a hyperglycaemic response characteristic of adrenal medullary stimulation. There remain the possibilities that salicylate either prevents the antigen-antibody combination from exerting its effects on the capillary wall, whether this be direct or indirect, as by releasing chemical mediators, or that salicylate depresses the reactivity of the capillary wall to stimuli which increase permeability.

The present results would appear to support the penultimate hypothesis in that the effects of the antigen (SIII) and histamine were reduced by salicylate whereas that of guinea-pig permeability factor was not. If salicylate caused a general depression of reactivity of the capillary wall to stimuli capable of increasing permeability, then it would also be expected to inhibit the action of PF. However, as suggested by Spector (1958) for corticoids, it may be that salicylate may affect the reactivity of the capillary wall to some but not all stimuli which increase permeability. Mill and others (1958) have also reported that the effectiveness of human PF factor was reduced by previous admixture with salicylate. However, a direct

in vitro combination between the two materials cannot be excluded and their conditions and salicylate concentrations are not comparable with those used in the present *in vivo* experiments.

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