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# EFFECTS OF ANTI-RHEUMATIC COMPOUNDS AND PYRIDINE DERIVATIVES ON THE CUTANEOUS RESPONSE TO THURFYL NICOTINATE IN THE GUINEA-PIG

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The effects of certain anti-rheumatic drugs and compounds related to nicotinic acid on the cutaneous response to locally applied thurfyl nicotinate in guinea-pigs were investigated. Sodium salicylate, aspirin or phenylbutazone administered orally each reduced the response to thurfyl nicotinate at about half the dose needed for equivalent protection against the cutaneous effects of exposure to ultra-violet light. Nicotinic acid or thurfyl nicotinate given orally flushed the skin and reduced the intensity of the local reaction to thurfyl nicotinate without affecting the response to ultra-violet light. Nicotinamide, isonicotinic acid, picolinic acid and quinolinic acid were less effective in producing flushes and in reducing the effect of locally applied thurfyl nicotinate. The flush induced in guinea-pigs by oral nicotinic acid was reduced by the previous administration of phenylbutazone. Possible mechanisms for these effects are discussed.

Screening tests for potential anti-inflammatory agents are based on the ability of compounds to reduce the intensity of local reactions in experimental animals. Inflammation following the injection of an irritant may be so intense that the protective action of a drug can be demonstrated only with doses near the toxic level whereas against less severe responses, such as those evoked by exposure of the skin to ultra-violet light, smaller doses suffice. Miles (1961) has stressed the advantages of using minimally effective stimuli in examining the responses to local injury and this principle could be equally valuable in the study of anti-inflammatory compounds to allow the relative effectiveness of compounds to be determined in man and animals by the same means.

When a cream containing thurfyl nicotinate (5% w/w) is applied to the skin of human subjects it produces some of the signs of inflammation, but the responses of animal skin to the ester are not well marked (Gross & Merz, 1948). The observation of Truelove & Duthie (1959), that the erythema produced by thurfyl nicotinate cream in humans was reduced and its onset delayed after oral administration of aspirin 600 mg, was of interest since the dose needed to delay the development of erythema due to ultra-violet light in guinea-pigs by the same route is 100 to 200 mg/kg (Winder, Wax, Burr, Been & Rosiere, 1958 ; Adams, 1960). It seemed worthwhile to determine whether local reactions could be produced by the application

of thurfyl nicotinate to the depilated skin of experimental animals and, if so, whether protection was conferred by aspirin and other anti-rheumatic compounds at doses equivalent to those effective in man.

## METHODS

White guinea-pigs weighing about 500 g were deprived of food overnight. The hair on their backs was first clipped short and then removed with a depilatory cream on the morning of the test. Female animals were found to be more suitable since they fought less than males and the skin of their backs remained relatively free from scratches. The depilated area was marked to give four longitudinal parallel rows of three sites each. On each side of the mid-line the sites of one row were treated with thurfyl nicotinate and those of the other row were exposed to ultra-violet light. The left side was used in the morning, the animal was given the drug under test, and the right side was used in the afternoon. The following procedures were used to produce local reactions: (1) irradiation with ultra-violet light by placing the applicator attachment (no. 01203) of a Kromayer lamp, Type SK220, which has an aperture of 12 mm, in contact with the skin for 45 sec; (2) application of a disc of filter paper (6 mm diameter) soaked in an aqueous solution of thurfyl nicotinate (5% v/v) to the skin for 30 sec. Reactions were recorded at the following times: for ultra-violet light, 90 min after irradiation and 120 min after administration of a drug; for thurfyl nicotinate, 15 min after application of the irritant and 105 min after giving a drug.

The degrees of erythema were estimated by the same observer, who was unaware of the treatment employed in experiments reported in Tables 1 and 3, and in most of those reported in Table 2 where more than one dose level was employed. Scores were allotted according to the intensity of erythema on the following basis: just-visible faint pink=1, pink=2, dark pink=3. During tests animals were kept in an environmental temperature above 20° C. Compounds were suspended in water with compound tragacanth powder B.P. (20 mg/ml., the "vehicle") using an all-glass hand homogenizer, and were administered by stomach tube in a volume of 10 ml./kg of body weight.

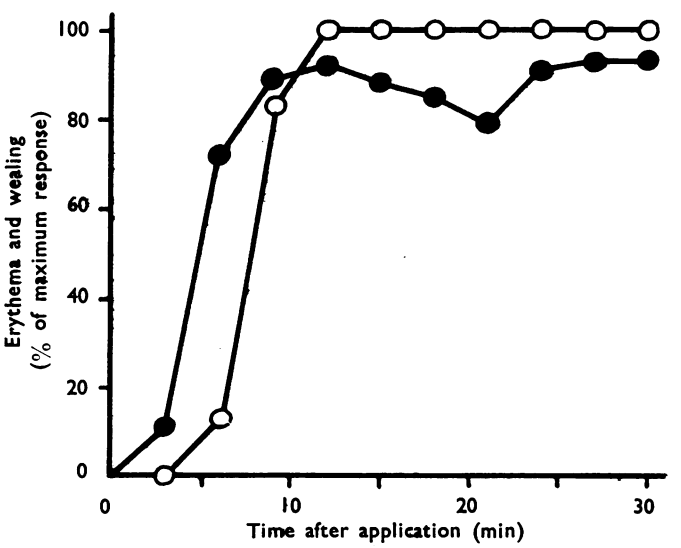


Fig. 1. Development of erythema and wealing in the depilated skin of guinea-pigs. Thurfyl nicotinate (5% v/v) was applied to the skin at six sites on each of five animals. The degree of erythema was assessed at regular intervals; just visible faint pink=1, pink=2, dark pink=3. ○ Percentage of sites showing wealing. ● Erythema score as a percentage of maximum possible response.

## RESULTS

Application of a solution of thurfyl nicotinate (5% v/v) to the depilated skin on the back of a guinea-pig produced an erythematous reaction confined to the area of contact and accompanied by wealing. The local reaction was less intense than that resulting from exposure to ultra-violet light and was more readily visible under good illumination from a tungsten light source than under daylight. Fig. 1 shows the rates at which erythema and weals developed. Wealing was confined to the area of erythema, which reached a maximum within 15 min and began to fade about 1 hr after application of thurfyl nicotinate. No reactions were seen with rabbits, rats or mice, even when undiluted thurfyl nicotinate was used.

### *Anti-rheumatic drugs*

The protective effects of anti-rheumatic drugs were determined against the erythema produced by application of thurfyl nicotinate solution and by exposure to ultra-violet light, in the same animals. The degree of protection was assessed by comparing the scores allotted to skin sites in the afternoon after administration of a drug with the control scores obtained in the morning. Administration of vehicle alone failed to produce any significant change in response; the total score in thirty animals was 99% of the control score at sites treated with thurfyl nicotinate, and 102% of the control score at sites exposed to ultra-violet light. Aspirin, sodium salicylate and phenylbutazone reduced the responses to both stimuli; the dose of each drug required for a given reduction in the response to thurfyl nicotinate was about half that needed to provide the same degree of protection against ultra-violet

TABLE 1

PROTECTIVE EFFECT OF ANTI-RHEUMATIC COMPOUNDS AGAINST THE SKIN RESPONSES TO THURFYL NICOTINATE AND TO ULTRA-VIOLET LIGHT IN GUINEA-PIGS

Erythema of guinea-pig skin was produced by the application of thurfyl nicotinate (5% v/v) or by exposure to ultra-violet light. The responses on one side of the animal were compared with those on the other side in the same way after oral administration of a drug or of the vehicle. Responses to treatment are expressed as percentages of the responses recorded before administration of drug or vehicle. * Significant difference ($P<0.05$) in response following treatment

| Treatment | Oral dose (mg/kg) | No. of expts. | Response (%) after Thurfyl nicotinate | Response (%) after Ultra-violet light |
|---|---|---|---|---|
| Vehicle only | | 30 | 99 | 102 |
| Sodium salicylate | 40 | 11 | 95 | 117* |
| | 80 | 12 | 88 | 91 |
| | 160 | 12 | 41* | 77* |
| | 320 | 12 | 33* | 57* |
| Phenylbutazone | 1·25 | 12 | 96* | 118 |
| | 2·5 | 12 | 74* | 86* |
| | 5 | 12 | 49* | 75* |
| | 10 | 18 | 48* | 70* |
| | 20 | 10 | 17* | 62* |
| | 40 | 21 | 40* | 37* |
| Aspirin | 25 | 11 | 112 | 102 |
| | 50 | 10 | 69* | 94 |
| | 100 | 12 | 17* | 61* |
| | 200 | 12 | 11* | 37* |

light. With these compounds the only effect observed was reduction of the intensity of erythema ; when aspirin was employed against thurfyl nicotinate there was no obvious blanching of the treated area as is seen in man (Truelove & Duthie, 1959). Doses needed to reduce by 30% the intensity of erythema following an application of thurfyl nicotinate were estimated to be 5 mg/kg of phenylbutazone, 50 mg/kg of aspirin and 100 mg/kg of sodium salicylate. After ultra-violet light corresponding doses were 10 mg/kg of phenylbutazone, 100 mg/kg of aspirin and 200 mg/kg of sodium salicylate (Table 1). Under the same conditions salicylamide (320 mg/kg) failed to confer protection ; this agrees with the findings of Adams (1960). The dose/response curve for phenylbutazone appeared less steep than those for aspirin or sodium salicylate against both thurfyl nicotinate and ultra-violet light.

### *Nicotinic acid and related compounds*

The effect of nicotinic acid on the cutaneous responses to thurfyl nicotinate and to exposure to ultra-violet light was investigated because of the reported interference

TABLE 2
EFFECTS OF NICOTINIC ACID AND RELATED COMPOUNDS ON SKIN RESPONSES TO THURFYL NICOTINATE AND TO ULTRA-VIOLET LIGHT IN GUINEA-PIGS

Erythema of guinea-pig skin was produced by the application of thurfyl nicotinate (5% v/v) or by exposure to ultra-violet light. The responses on one side of the animal were compared with those on the other side in the same way after oral administration of a compound. Responses to treatment are expressed as percentages of the responses recorded before administration of a drug. Local blanching refers to that at sites of application of thurfyl nicotinate. * Significant difference ($P<0{\cdot}05$) in response following treatment

| Treatment | Oral dose (mg/kg) | No. of expts. | Response (%) after Ultra-violet light | Response (%) after Topical thurfyl nicotinate | Proportion of guinea-pigs showing General flush | Proportion of guinea-pigs showing Local blanching |
|---|---|---|---|---|---|---|
| Thurfyl nicotinate | 10 | 5 | 100 | 103 | 5/5 | 0/5 |
| | 40 | 5 | 95 | 76 | 5/5 | 5/5 |
| | 100 | 5 | 100 | 31* | 5/5 | 5/5 |
| Nicotinic acid | 5 | 4 | 116 | 68 | 2/4 | 1/4 |
| | 10 | 5 | 98 | 69 | 3/5 | 2/5 |
| | 20 | 6 | 116 | 76 | 4/6 | 1/6 |
| | 40 | 6 | 102 | 26* | 6/6 | 4/6 |
| | 100 | 6 | 96 | 23* | 5/6 | 5/6 |
| | 400 | 6 | 100 | 22* | 6/6 | 6/6 |
| Isonicotinic acid | 100 | 5 | 108 | 106 | 3/5 | 0/5 |
| | 400 | 5 | 100 | 103 | 1/5 | 2/5 |
| Picolinic acid | 400 | 5 | 83 | 64* | 0/5 | 0/5 |
| Quinolinic acid | 100 | 4 | 110 | 121 | 0/4 | 0/4 |
| 2-Hydroxy-nicotinic acid | 160 | 5 | 105 | 110 | 0/5 | 0/5 |
| 2-Amino-nicotinic acid | 100 | 5 | 102 | 102 | 0/5 | 0/5 |
| Nicotinamide | 100 | 5 | 103 | 72 | 0/5 | 0/5 |
| | 200 | 5 | 108 | 76 | 0/5 | 0/5 |
| | 400 | 8 | 118* | 52* | 0/8 | 0/8 |
| | 1,000 | 5 | 96 | 16* | 3/5 | 0/5 |
| Iproniazid | 25 | 4 | 97 | 80 | 0/4 | 0/4 |
| | 100 | 2 | 94 | 83 | 0/2 | 2/2 |
| Pyridoxine hydrochloride | 200 | 4 | 100 | 110 | 0/4 | 2/4 |

of this drug with the *in vitro* release of histamine and with mast cell damage in guinea-pigs due to compound 48/80 and to anaphylaxis (Mota, Da Silva & Fernandes, 1960). Large oral doses of nicotinic acid produced a flush first observed in the ears, then in the neck and finally spreading over the back. The onset of flush occurred within 3 to 7 min, fading began at about 40 min and the skin appeared almost normal at 90 min. The results given in Table 2 show that after oral nicotinic acid responses to topically applied thurfyl nicotinate were considerably reduced but those to ultra-violet light were not affected. After the administration of nicotinic acid (40 to 400 mg/kg), during the period when the flush was most marked sites which had previously reacted with erythema to topically applied thurfyl nicotinate now appeared blanched. Of other compounds given orally, only thurfyl nicotinate produced effects similar in size to those with nicotinic acid. Significant reduction of the response to topical thurfyl nicotinate was obtained after an oral dose of 100 mg/kg of thurfyl nicotinate, but a flush was observed at 10 mg/kg and blanching at sites to which thurfyl nicotinate had been applied topically at 40 mg/kg. The following compounds were relatively ineffective in producing either a general flush or local blanching: isonicotinic acid, picolinic acid, quinolinic acid, 2-hydroxy-nicotinic acid and 2-aminonicotinic acid. Nicotinamide (400 mg/kg) reduced the local response to thurfyl nicotinate, but this effect was not associated with a flush or blanching. However, the response to ultra-violet light was unaffected even at a dose of 1 g/kg.

To determine whether sites treated with thurfyl nicotinate responded in the same way as those which had received tetrahydrofurfuryl alcohol, thurfyl nicotinate (5% w/v) was applied to six sites on the left side of four guinea-pigs and tetrahydrofurfuryl alcohol (5% w/v) to six sites on the right side. Another four guinea-pigs were treated similarly with thurfyl nicotinate on the right side and tetrahydrofurfuryl alcohol on the left and all sites were read 15 min later. The erythema score was 100 for sites treated with thurfyl nicotinate and 43 for those treated with tetrahydrofurfuryl alcohol. Wealing was observed at thirty-nine thurfyl nicotinate sites but at only one which had been treated with tetrahydrofurfuryl alcohol. When these animals were given nicotinic acid (400 mg/kg orally) blanching was observed only at the sites where thurfyl nicotinate had been applied.

*Effect of phenylbutazone on the responses to nicotinic acid*

Since phenylbutazone prevented the erythema produced by an application of thurfyl nicotinate to the skin, it was of interest to determine whether it would also prevent the flush induced by nicotinic acid administered orally. To test this possibility twelve male white guinea-pigs were starved overnight and depilated on the following morning. Six local reactions to thurfyl nicotinate and six to ultra-violet light were produced on the back of each animal and about 4 hr later half the animals were given phenylbutazone (40 mg/kg orally), the remainder receiving vehicle only. After 1 hr all animals were given nicotinic acid (100 mg/kg orally). A flush appeared in animals which had received vehicle only and blanching occurred at most of the sites of earlier reactions to thurfyl nicotinate but not at those which had received ultra-violet light (Table 3). The flush was less intense in

TABLE 3

EFFECT OF PHENYLBUTAZONE ON GUINEA-PIG CUTANEOUS RESPONSES TO NICOTINIC ACID ADMINISTERED ORALLY, THURFYL NICOTINATE APPLIED LOCALLY AND ULTRA-VIOLET LIGHT

Six local reactions to thurfyl nicotinate and six to ultra-violet light were produced on the depilated back of each guinea-pig. About 4 hr later control animals were given vehicle and the remainder a suspension of phenylbutazone (40 mg/kg orally). After 1 hr all animals were given nicotinic acid in solution (100 mg/kg orally). After 24 hr from the first dose of nicotinic acid all animals received a further dose of 100 mg/kg

| | Controls | | Animals treated with phenylbutazone on day 1 | |
|---|---|---|---|---|
| | Day 1 | Day 2 | Day 1 | Day 2 |
| Responses of six guinea-pigs to nicotinic acid: | | | | |
| Flush just visible | | | 4/6 | 1/6 |
| Pale pink flush | | | 2/6 | 2/6 |
| Deep pink flush | 1/6 | 2/6 | | 3/6 |
| Intense pink flush | 5/6 | 4/6 | | |
| Proportion of skin sites showing blanching after nicotinic acid: | | | | |
| Thurfyl nicotinate sites | 34/36 | 33/36 | 0/36 | 14/36 |
| Ultra-violet light sites | 0/36 | | | |

animals which had received phenylbutazone and little or no blanching occurred. After 24 hr from the first dose of nicotinic acid all animals received a further dose of 100 mg/kg. Flushing was again more intense in control animals, but the response in those treated with phenylbutazone appeared to be greater than on the previous day. Again blanching occurred at most thurfyl nicotinate sites in control animals, but on this occasion almost half the thurfyl nicotinate sites of the guinea-pigs treated with phenylbutazone responded in the same way.

## DISCUSSION

The doses of anti-rheumatic drugs needed to reduce the erythema resulting from irradiation of guinea-pig skin with ultra-violet light agree reasonably well with those reported by Adams (1960) and Winder *et al.* (1958), although the methods differ in detail. The ratios of effective doses (compared with phenylbutazone) obtained in these three studies were aspirin 5 to 11 and sodium salicylate 11 to 20. Winder, Wax, Scotti, Scherrer, Jones & Short (1962) estimated the potency of aspirin given orally to be 1/10th of that of phenylbutazone against the effect of ultra-violet light in guinea-pigs. In the present work, 30% reduction of the response to thurfyl nicotinate was obtained with oral doses of phenylbutazone (5 mg/kg), aspirin (50 mg/kg) or sodium salicylate (100 mg/kg), but twice the dose was needed for an equivalent degree of protection against ultra-violet light by each drug. It is not possible to conclude from these results that the reaction to thurfyl nicotinate is more susceptible to the action of drugs than is the reaction to ultra-violet light. Erythema produced by exposure to ultra-violet light developed between 30 and 120 min after drug administration and that produced by thurfyl nicotinate between 90 and 105 min, so that the effective concentrations of drug were not necessarily comparable in the two conditions.

The effectiveness of phenylbutazone as well as salicylates in reducing the response to thurfyl nicotinate and to ultra-violet light in guinea-pigs gives some support to the suggestion of Truelove & Duthie (1959) that the ability of a drug to protect against the response to thurfyl nicotinate may be related to its anti-rheumatic properties. Results of recent studies tend to confirm the predictive value of the guinea-pig ultra-violet erythema test in this respect (Winder *et al.*, 1962; Winder, Wax, Serrano, Jones & McPhee, 1963). As a screening procedure for potential anti-rheumatic compounds the thurfyl nicotinate test has the advantage that methods in guinea-pigs may also be used in human subjects, and it is possible that this would offer a rapid and simple means of assessing new compounds. A disadvantage of the test is that the local reaction produced by thurfyl nicotinate in guinea-pigs as described here is fainter and more difficult to read than that produced by ultra-violet light.

As the relative effectiveness of aspirin, sodium salicylate and phenylbutazone was similar whether the erythema resulted from exposure to ultra-violet light or the application of thurfyl nicotinate, it seems possible that these protective effects result from interference with mechanisms common to both reactions. Results with orally administered nicotinic acid indicate that there are differences in the biochemical reactions resulting from exposure to ultra-violet light and from application of thurfyl nicotinate, and that the mode of action of nicotinic acid in reducing the response to topically applied thurfyl nicotinate differs from that of either salicylates or phenylbutazone. Nicotinic acid, at oral doses as high as 400 mg/kg, failed to reduce the intensity of erythema due to exposure to ultra-violet light whereas a significant reduction of the response to locally applied thurfyl nicotinate was obtained after a dose of 40 mg/kg (Table 2). Under the same conditions salicylates and phenylbutazone would have produced a given degree of protection against ultra-violet light at only twice the dose needed to reduce the response to thurfyl nicotinate to the same extent.

Reduction of the cutaneous reaction to thurfyl nicotinate by previous oral administration of either nicotinic acid or thurfyl nicotinate itself was associated with development of a generalized flush and with blanching at sites where thurfyl nicotinate erythema had occurred earlier on the same day. These effects can be explained on the assumption that both the local reaction to thurfyl nicotinate and the flush resulting from oral administration of nicotinic acid are mediated by the same mechanism and that depletion of an agent responsible for the vasodilatation takes place.

It might be argued that the apparent intensity of erythema induced by locally applied thurfyl nicotinate would be reduced after oral administration of nicotinic acid or thurfyl nicotinate if there was any residual general flush at the time when readings were carried out. It is, however, unlikely that the readings were affected in this way as the flush induced by nicotinic acid in a dose sufficient to abolish the response to thurfyl nicotinate had almost entirely disappeared about 1.5 hr after its administration and no reduction was observed in the responses to ultra-violet light when assessed only 15 min after the responses to thurfyl nicotinate.

It is uncertain whether the vasodilating action of thurfyl nicotinate is due to the whole molecule. Tetrahydrofurfuryl alcohol produces erythema when applied to human skin (Brunner & Finkelstein, 1960), but a solution of tetrahydrofurfuryl alcohol in water (5% w/v) was less effective in this respect than a solution of thurfyl nicotinate at the same concentration in guinea-pigs. Oral or parenteral administration of nicotinic acid also produces vasodilatation in guinea-pigs and man (Bean & Spies, 1940; Chevillard, Charonnat & Giono, 1949; Giono & Chevillard, 1959; Chevillard & Laury, 1960), and the present results suggest that the mechanism is similar to or identical with that occurring after application of thurfyl nicotinate to the skin. The implication of this conclusion, if true, is that knowledge of the mechanism by which nicotinic acid induces vascular changes might throw light on some of the factors involved in inflammation. It is interesting in this respect that the development of erythema following application of thurfyl nicotinate to human skin appears to depend upon the innervation of cutaneous vessels (Crockford, Hellon & Heyman, 1962).

Structural requirements in compounds related to nicotinic acid for vasodilatation are fairly specific. In the present experiments, only nicotinic acid and thurfyl nicotinate gave both a general flush and also blanching at the sites of earlier reactions to topically applied thurfyl nicotinate at doses between 10 and 40 mg/kg; nicotinamide, isonicotinic acid, picolinic acid and quinolinic acid were ineffective or much less effective in both these respects. If, as has been suggested, orally administered nicotinic acid or thurfyl nicotinate depletes the skin of some active material, the degree to which this depletion takes place may vary with the vasodilating agent.

Results shown in Table 3 indicate that vasodilatation produced by oral administration of nicotinic acid can be reproduced 24 hr later but that the influence of thurfyl nicotinate on the skin persists for 2 days at least. In this experiment failure of animals treated with phenylbutazone to develop the same intensity of flush after nicotinic acid as did untreated animals on the first day could be related to the protective effect of the drug, and the improvement observed on the second day could be related to the lower level of phenylbutazone present at that time. The blanching at sites treated with thurfyl nicotinate in control animals on both the first and second days would indicate persistent depletion of active material by the locally applied thurfyl nicotinate. One possible explanation for the absence of blanching on the first day at thurfyl nicotinate sites in animals treated with phenylbutazone would be that the sites did not become visible because the flush due to nicotinic acid was so faint. The partial appearance of blanching on the second day could be a reflection of the deeper flush produced by nicotinic acid with less phenylbutazone present, indicating that phenylbutazone had not suppressed the reaction normally responsible for blanching and that its protective action was exerted at a later stage.

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