# THE COMPARATIVE ANTIPYRETIC ACTIVITY OF ACETYLSALICYLIC ACID AND SALYCYLAMIDE IN FEVER-INDUCED RATS

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The literature reveals relatively few investigations of the antipyretic activity of salicylamide, although many have been conducted on its analgesic activity and ultimate fate. This investigation was initiated to determine the extent of antipyretic activity of salicylamide as compared to that of acetylsalicylic acid and a salicylate combination[1]. The combination (the P-tablet)† contains both the above ingredients in addition to caffeine, aluminium hydroxide, tartaric acid and thiamine hydrochloride. The antipyretic action was also compared with the plasma-salicylamide level. The antipyretic activity of salicylamide has been reported to range from none detectable to equal or greater than aspirin[2-9] in rats, rabbits and man. The fact that salicylamide demonstrates desirable qualities as an analgesic[2,4,6,7,10,11] but inferior qualities as an antipyretic is of interest. The possible cause has not been elucidated, but it is suggested that this drug may be effective as an antipyretic only in the free form, whereas protein-binding and conjugation which ultimately results apparently has no effect on its activity as an analgesic.

## Experimental

*Experimental Animal.* Adult albino Spragus-Dawley rats of mixed sex varying in weight from 200–350 g. were employed. They ate a standard proprietary laboratory diet, with water *ad libitum*. Drugs were administered to pyrexic animals after 12–16 hours fasting.

*Fevering Agent.* Animals were rendered febrile by subcutaneous injection of 0·6 ml. of a 5 per cent aqueous solution of Witte Peptone. When filtered and incubated for 12–24 hours at 98·6° F., this agent produced fevers averaging 103° F., four hours after injection. Storing under refrigeration preserved its activity indefinitely.

*Instruments.* A Tele-thermometer was used inserting the rectal electrode approximately two inches. Faecal matter was previously removed by gentle digital pressure and the rectum lubricated with liquid paraffin.

*Administration of Drugs.* Drugs were suspended in a 0·5 per cent solution of Kelcosol‡ in water and administered orally, four hours after peptone injection, by means of a stomach tube. All concentrations of the

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† An analgesic tablet formulated for the Purdue University Student Health Service containing approximately 20 per cent salicylamide and 32 per cent aspirin.

‡ A sodium alginate product.

drugs were suspended in this agent in such a way that the volume of solution administered to the animals remained constant.

*Method.* Four groups of 12 animals each were selected for the antipyretic studies, and subdivided into groups of four and identified. Twelve animals were used for each test. Of these, four served as non-fevered controls receiving no drug nor fevering agent, four as fevered controls receiving fevering agent only and the remaining four received fevering agent and the specific test dose of a drug four hours after the administration of peptone (fever peak).

The large groups of 12 animals were crossed-over, each group being used every fourth day. The smaller groups were also crossed-over so that each would serve at one time or another as non-fevered controls, fevered controls or receive fevering agent and the drug. This cross-over method permitted all animals to serve as their own controls, thus minimising individual variation, was more exemplary of a population and permitted sufficient rest and recovery from the drug and fevering agent. Fatalities were not observed and all animals appeared to thrive during the duration of the tests.

The fever responses to peptone from a group of eight to 20 rats, at the four hour (peak fever) and seven hour intervals upon three successive tests (two weeks apart) were statistically compared. At both time intervals no significant differences in fevered temperatures for the three successive tests were found (P range = 0·3 to 0·5). The errors and tests for significance were calculated by Students method of "*t*". Hence, neither the intensity nor the duration of the fever induced by peptone injections was significantly altered when the rats were repeatedly used.

In determining plasma-salicylamide levels the method of Keller[12], as modified by Cosmides[13] was used. Animals were again rendered febrile as in the antipyretic studies, drug introduced orally at peak fever, four hours after introduction of peptone, and blood withdrawn by cardiac puncture at specific time intervals after administration of the drug. Heparin 0·1 ml. served as the anticoagulant. Approximately 5 ml. of blood was withdrawn from each etherized animal and the animal subsequently killed.

## Results

At completion of the tests the recorded temperatures of fevered and non-fevered controls were averaged. Figure 1 shows the resultant temperature curves. The curves from 4–7 hours in Figure 1 are used as control curves in Figures 2–5.

Figure 2 shows the effect of 100, 200, 250 and 300 mg./kg. of acetylsalicylic acid. The three higher doses exert similar antipyretic effects and reduce peptone-induced fever to approximately the normal level. For this reason, the 200 mg./kg. dose was employed for subsequent comparisons. Salicylamide in doses ranging from 50–300 mg./kg. failed to demonstrate a sustained decrease in temperature. Maximum decrease was observed at approximately 30 minutes after drug administration and rapidly rose thereafter (Fig. 3). These results confirm those reported by

Bavin and colleagues[7]. The 300 mg./kg. dose was observed to maintain a somewhat longer duration of activity.

Fever reductions observed with combinations of salicylamide and aspirin failed to demonstrate a significant difference at peak activity from those seen with aspirin alone (Fig. 2, 4 and Table II). At the end of two hours the animals receiving 200 mg./kg. of aspirin had a significantly lower temperature than those receiving 100 mg./kg. each of aspirin and

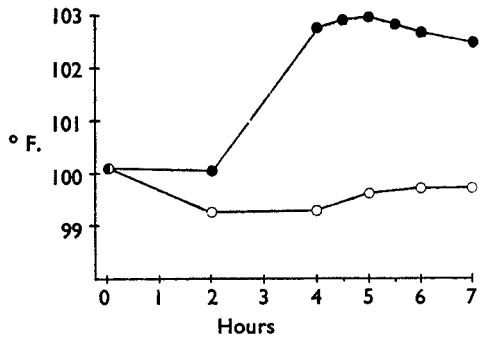


FIG. 1. Comparison of fevered and non-fevered controls.

● Fevered controls.
○ Nonfevered controls.

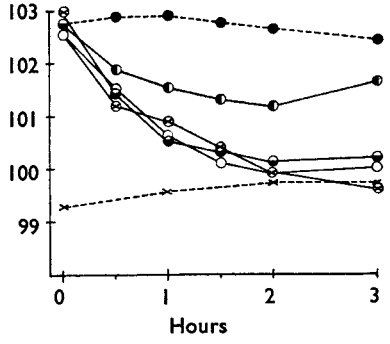


FIG. 2. Effects of acetylsalicylic acid upon oral introduction to peptone pyrexic rats. Fevered controls, ●; nonfevered controls ×; acetylsalicylic acid in mg./kg., ◐, 100; ◒, 200; ○, 250; ⊕, 300.

salicylamide (Table III). Comparison of the effects of the P-tablet with aspirin and salicylamide may be observed in Figure 5. It can be readily seen that the P-tablet has less than half the antipyretic activity of aspirin ($P < 0.01$, see Table II). Aspirin 200 mg./kg. was shown to be a more potent antipyretic than 100 and 200 mg./kg. of salicylamide and 200 mg./kg. of the P-tablet* (Table II). The antipyretic activity of 300 mg./kg. of salicylamide and aspirin-salicylamide, 100 mg./kg. of each,

TABLE I

COMPARISON OF THE PLASMA-SALICYLAMIDE LEVELS IN MG. PER CENT AFTER ORAL ADMINISTRATION OF 200 AND 300 MG./KG. TO PEPTONE PYREXIAL RATS*

| | | Minutes | | |
|---|---|---|---|---|
| Normal | 5 | 10 | 20 | 30 |
| Mg. per cent after 200 mg./kg. 6·54 | 16·00 | 12·54 | 9·37 | 9·36 |
| Standard error ±0·71 | ± 0·24 | ± 0·89 | ± 0·55 | ±1·24 |
| Mg. per cent after 300 mg./kg. 6·54 | 13·70 | 14·28 | 10·80 | 9·43 |
| Standard error ±0·71 | ± 1·40 | ± 2·22 | ± 0·81 | ±1·13 |

* Between 5 and 13 animals used at each time interval.

were not significantly different when compared to aspirin at peak activity (Table II), however, at the end of two hours they both exhibited significantly less effects than aspirin (Table III).

* Equivalent to approximately 39 mg./kg. of salicylamide and 64 mg./kg. of aspirin.

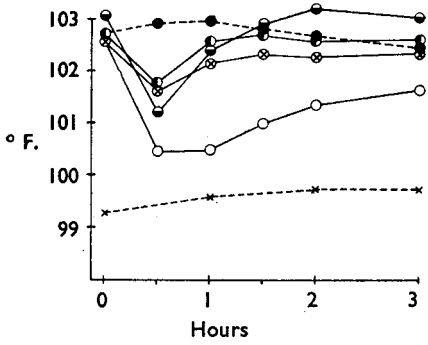


FIG. 3. Effects of salicylamide upon oral introduction to peptone pyrexic rats. Fevered controls, ●; nonfevered controls, ×; salicylamide in mg/kg., ⊕, 50; ◐, 100; ◒, 200; ○, 300.

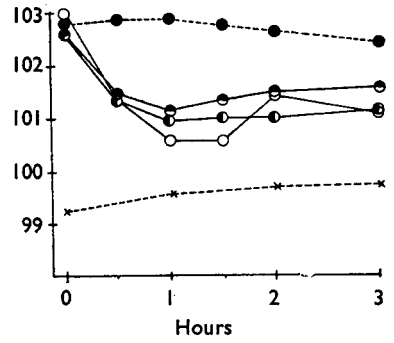


FIG. 4. Comparison of the effects of acetylsalicylic acid-salicylamide combinations upon oral introduction to peptone pyrexic rats. Fevered controls, ●; nonfevered controls, ×; acetylsalicylic acid-salicylamide combinations in mg./kg. of each, ◓, 25; ◐, 50; ○, 100.

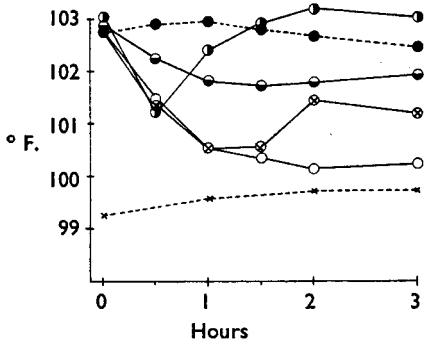


FIG. 5. Comparison of the effects of the P-tablet, acetylsalicylic acid and salicylamide upon oral introduction to peptone pyrexic rats. Nonfevered controls, ×; fevered controls, ●; acetylsalicylic acid 200 mgl/kg., ○; salicylamide 200 mg./kg., ◑; acetylsalicylic acid-salicylamide combination 100 mg./kg. of each, ⊕; P-tablet 200 mg./kg., ◒.

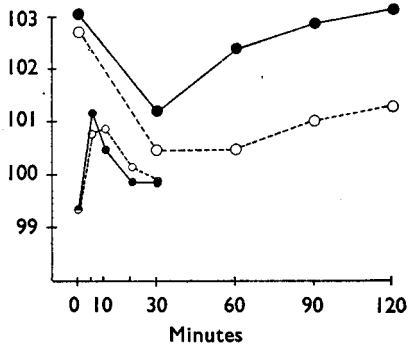


FIG. 6. Comparison of the plasma-salicylamide levels to salicylamide antipyresis after oral administration of 200 and 300 mg./kg. doses to peptone pyrexic rats. Salicylamide in mg./kg., ●, 200; ○, 300. The ° F. scale between 99 and 103° F. also corresponds to plasma levels of 5, 10, 15, 20 and 25 mg. per cent.

Plasma-salicylamide levels were determined with 200 and 300 mg./kg. doses (Table I). The peak concentration was observed five minutes after administration with 200 mg./kg. and at 10 minutes with 300 mg./kg. The differences in concentrations at 20 and 30 minutes are insignificant. Figure 6 shows a comparison between antipyretic values obtained with 200 and 300 mg./kg. doses and the plasma-salicylamide levels obtained with the same doses. The reflection of high plasma-salicylamide levels on pyrexia is not immediately manifested, and its short antipyretic quality appears to be related to the rapid disappearance of the free salicylamide from the blood.

TABLE II

A COMPARISON OF PEAK ANTIPYRETIC ACTIVITY OF ASPIRIN WITH SALICYLAMIDE AND ASPIRIN-SALICYLAMIDE COMBINATIONS

| Drug | Peak activity time* hours | No. of animals | Dose mg./kg. | Fever reduction | SE | P |
|---|---|---|---|---|---|---|
| Aspirin | 2 | 8 | 200 | 2·6 ± | 0·17 | — |
| Salicylamide | 0·5 | 8 | 100 | 1·1 ± | 0·33 | <0·01 |
| Salicylamide | 0·5 | 8 | 200 | 1·7 ± | 0·36 | <0·05 |
| Salicylamide | 0·5 | 8 | 300 | 2·4 ± | 0·32 | >0·5 |
| Aspirin/Amide† | 1·5 | 8 | 200 | 2·3 ± | 0·29 | >0·5 |
| P-Tablet‡ | 1·5 | 16 | 200 | 1·1 ± | 0·17 | <0·01 |

* Calculated from the time of introduction of drug at peak fever (4 hours after peptone injection.)
† 100 mg./kg. of each.
‡ An analgesic tablet formulated for the Purdue University Student Health Service.

TABLE III

A COMPARISON OF THE ANTIPYRETIC ACTIVITY OF ASPIRIN WITH SALICYLAMIDE AND ASPIRIN-SALICYLAMIDE COMBINATION AT 2 HOURS*

| Drug | No. of animals | Dose mg./kg. | Fever reduction | SE | P |
|---|---|---|---|---|---|
| Aspirin | 8 | 200 | 2·6 ± | 0·17 | — |
| Salicylamide | 8 | 300 | 1·4 ± | 0·41 | <0·05 |
| Aspirin/Amide† | 8 | 200 | 1·2 ± | 0·42 | <0·02 |

* Termination of test.
† 100 mg./kg. of each.

## DISCUSSION

Acetylsalicylic acid is shown to be superior to aspirin-salicylamide combinations, to salicylamide alone, and to the P-tablet.

The antipyretic effect of salicylamide is in conformity with that reported by Bavin and colleagues[7]. Initial onset is rapid, but duration is very short. Consideration of the blood levels obtained through colorimetric readings supports the belief that the antipyretic activity of this compound is due to the unconjugated form.

Seeberg and associates[14] report salicylate concentrations in the brain to be twice as high with salicylamide as with aspirin when administered orally to rabbits in 500 mg./kg. doses. Peak concentration was demonstrated at one half-hour after dosing. The concentration fell while that of aspirin gradually rose until the curves crossed three hours after introduction of the compounds. They report further that concentrations of salicylamide in other body tissues is equal to that found in the serum 10 minutes after intravenous introduction of 50 mg./kg. in rabbits. Protein-binding was found to occur much more rapidly with aspirin than with salicylamide. They conclude that the low serum concentrations elicited with salicylamide is not due to protein-binding, but to rapid diffusion throughout the body tissues. It appears, then, that antipyresis is dependent on the presence of free salicylamide in the blood.

Combinations of aspirin and salicylamide are shown to be less effective than those of aspirin alone. Therefore there is no apparent potentiation between the compounds. The P-tablet demonstrates an antipyretic curve similar to that seen with aspirin, but to a much lesser degree. This may be due to the presence of caffeine in the formula[11]. Comparisons of the two

active ingredients of the P-tablet indicates that the antipyretic activity is largely due to the presence of aspirin.

## Summary

1. Aspirin is shown to be superior to aspirin-salicylamide combinations, salicylamide alone, and to the P-tablet as an antipyretic.

2. The antipyretic effect of salicylamide is rapid in onset, but short in duration.

3. Comparison of plasma-salicylamide levels to antipyresis indicates that the activity of this compound as an antipyretic is probably due to the unconjugated, free amide.

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