**Interaction of aspirin with nonsteroidal anti-inflammatory drugs in rats**

SIR,—Clinical evaluation of new anti-arthritic drugs is difficult because of the unpredictable course of arthritis. It is even more difficult if an analgesic drug, such as a salicylate which also has anti-inflammatory activity (Smith & Smith, 1966), is administered at the same time. The difficulty in assessing the anti-inflammatory activity of a nonsteroidal drug, while a mild analgesic (usually a salicylate) is permitted *ad libitum*, is reflected in some of the reports on the anti-arthritic activity of indomethacin. While numerous investigators have reported indomethacin to be active against arthritis, Mainland (1967) and Donnelly, Lloyd & Campbell (1967) concluded that indomethacin was approximately as effective as placebo. Of the reports in which indomethacin was found to be active, only a few stated whether salicylates were permitted. In three of these, indomethacin was compared side-by-side with a salicylate (Pinals & Frank, 1967; Gaspardy, Gaspardy & others, 1966; Pitkeathly, Banerjee & others, 1966); in two other reports salicylates were gradually withdrawn in some cases (Rothermich, 1966; Englund, 1966); and in one study, free intake of aspirin was allowed (Smyth, 1965).

Does the intake of salicylates mask the anti-inflammatory activity of anti-inflammatory agents? In an attempt to answer this, two anti-inflammatory drugs, indomethacin and phenylbutazone, have been administered alone or in a combination with aspirin to rats in which oedema in the foot had been induced by carrageenan according to a slightly modified method of Winter, Risley & Nuss (1962).

Male Sprague-Dawley rats from A and E Farms, Altamont, N.Y., 125 g, were fasted overnight and indomethacin (Merck), 1·56 and 6·25 mg/kg, and phenylbutazone (Geigy), 25 and 100 mg/kg, were administered by stomach tube as suspensions in 1% gum tragacanth, 2 ml/100 g. Aspirin (Monsanto) was administered similarly at 100 mg/kg alone and at 100 mg/kg in combinations with each of the above doses of indomethacin or phenylbutazone. Control rats received only the gum tragacanth vehicle. Each single and combined dose was examined in duplicate experiments.

One hr after administration of the drugs, 0·05 ml of 0·75% carrageenan (Viscarin standard) suspension was injected into the plantar tissue of the right hind foot of each rat. Three hr later, all rats were killed with chloroform and both feet were cut off at the tibio-talar joint, and weighed. The oedema was determined from the difference between weights of injected and non-injected feet, and the percent inhibition of oedema was calculated for each group. The significance of difference between the medicated groups and the control groups, and between the groups that received combined drugs and single drugs were calculated according to the *t*-test.

The doses of all compounds were selected so that, in the event of additive inhibition of oedema, the maximal inhibition due to combined medication would not be expected to be greater than the limits of attainable inhibition in this test system.

Table 1 shows that aspirin, 100 mg/kg, or indomethacin or phenylbutazone at two doses each resulted in significant ($P \leq 0{\cdot}01$) inhibition of oedema in all experiments. The degree of inhibition by indomethacin or phenylbutazone was dose-related.

Combined administration of aspirin with indomethacin resulted in inhibition of oedema that was not significantly different from that obtained with the same doses of either compound alone.

Combined administration of aspirin with phenylbutazone resulted in slightly

TABLE 1. INHIBITION OF CARRAGEENAN OEDEMA BY COMBINED ADMINISTRATION OF ASPIRIN WITH INDOMETHACIN OR PHENYLBUTAZONE

| | Aspirin (mg/kg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | | 100 | | 0 | | 100 | |
| | Oedema (mg) | Inhibition (%) | Oedema (mg) | Inhibition (%) | Oedema (mg) | Inhibition (%) | Oedema (mg) | Inhibition (%) |
| Indomethacin (mg/kg) | Exp. 1 (5 rats/group) | | | | Exp. 2 (8 rats/group) | | | |
| 0 | 647 ± 25 | — | 349 ± 44 | 46 | 556 ± 27 | — | 343 ± 35 | 38 |
| 1·56 | 402 ± 26 | 38 | 526 ± 18 | 34 | 329 ± 20 | 41 | 394 ± 13 | 29 |
| 6·25 | 320 ± 65 | 50 | 294 ± 37 | 54 | 271 ± 26 | 51 | 290 ± 39 | 48 |
| Phenylbutazone (mg/kg) | Exp. 1 (10 rats/group) | | | | Exp. 2 (10 rats/group) | | | |
| 0 | 647 ± 25 | — | 349 ± 44 | 46 | 648 ± 30 | — | 381 ± 29 | 41 |
| 25 | 386 ± 29 | 40 | 273 ± 38 | 58* | 364 ± 22 | 44 | 247 ± 42 | 62*† |
| 100 | 302 ± 26 | 53 | 371 ± 55 | 43 | 279 ± 32 | 57 | 207 ± 42 | 68† |

* $P < 0.05$ for differences between inhibition due to combined drugs and aspirin alone.
† $P < 0.01$ for differences between inhibition due to combined drugs and phenylbutazone alone.

greater inhibition of oedema than that achieved with aspirin alone in one of the experiments at the lower dose of phenylbutazone and this increase was significant at the $P \leq 0.01$ level, but no significant differences were noted in the other experiment or at the higher dose.

In comparing the effects of administering aspirin with phenylbutazone with those of phenylbutazone alone, aspirin with 25 mg/kg of phenylbutazone resulted in slightly greater inhibition ($P \leq 0.05$) than that of 25 mg/kg of phenylbutazone alone in both experiments, but combined administration of aspirin with 100 mg/kg of phenylbutazone resulted in no significant differences compared to administration of 100 mg/kg of phenylbutazone alone.

All rats were normal in behaviour and appearance throughout.

The results of the combined medications of aspirin and indomethacin in rats indirectly support the conclusions of Mainland (1967) and Donnelly & others (1967) who reported that the effects of indomethacin (in patients receiving salicylates *ad libitum*) were indistinguishable from the effects of placebo treatment. The combined administration of aspirin with indomethacin to rats resulted in no additive inhibition of oedema which suggests drug interaction.

The results after combined administration of aspirin with phenylbutazone were not as consistent, but even here the anti-inflammatory responses were not strictly additive, and they again suggest drug interaction.

These data lend support to the claim by Boardman & Hart (1967) that high doses of salicylates are anti-inflammatory as well as analgesic, and that the intake of salicylates should be eliminated from comparative clinical trials of non-steroidal anti-inflammatory agents.

*Acknowledgements.* We are grateful to Dr. A. L. Beyler for his critical comments, to Mr. G. L. Brown, Jr. for his skilful technical assistance, and to Dr. Terry A. Terzakis (Geigy) for the supply of phenylbutazone and to Dr. Walter B. Gall (Merck) for the supply of indomethacin.

Section of Toxicology and Experimental Pathology,
Sterling-Winthrop Research Institute,
Rensselaer, New York,
U.S.A. 12144.

Z. E. MIELENS
H. P. DROBECK
J. ROZITIS, JR.
V. J. SANSONE, JR.

March 25, 1968

**References**

Boardman, P. L. & Hart, F. D. (1967). *Br. med. J.*, **4**, 264–268.
Donnelly, P., Lloyd, K. & Campbell, H. (1967). *Ibid.*, **1**, 69–74.
Englund, D. W. (1966). *Arthritis Rheum.*, **9**, 502–503.
Gaspardy, G., Gaspardy, G. Jr., Balint, G. & Kemeny, V. (1966). *Z. Rheumaforsch.*, **25**, 199–204.
Mainland, D. (1967). *Clin. Pharmac. Ther.*, **8**, 11–37.
Pinals, R. S. & Frank, S. (1967). *New Engl. J. Med.*, **276**, 512–514.
Pitkeathly, D. A., Banerjee, N. R., Harris, R. & Sharp, J. (1966). *Ann. rheum. Dis.*, **25**, 334–339.
Rothermich, N. O. (1966). *J. Amer. med. Ass.*, **195**, 1102–1106.
Smith, M. J. H. & Smith, P. K. (1966). *The Salicylates*, P. 203–232, New York: Interscience.
Smyth, C. J. (1965). *Arthritis Rheum.*, **8**, 921–942.
Winter, C. A., Risley, E. A. & Nuss, G. W. (1962). *Proc. Soc. exp. Biol. Med.*, **111**, 544–547.

## The reactivity of the pregnant rat myometrium

SIR,—It has been demonstrated (Schofield, 1957) that the actomyosin concentration of the rabbit myometrium, measured by the tension developed to optimal electrical stimulation, increases from mid-term to parturition and that the synthesis of actomyosin is regulated by oestrogens (Csapo, 1950). For the greater part of pregnancy, the rabbit myometrium is progesterone-dominated and refractory to oxytocin (Schofield, 1957). A possible mechanism by which progesterone exerts its "blocking" action may be due to the greater effectiveness of calcium binding which the progestrone-dominated uterus shows when compared with the oestrogen-dominated uterus (Csapo, 1961). The present work was undertaken to assess the influence of the female sex hormones on the pregnant rat myometrium.

Wistar rats 13–15 weeks old were with males for 4 days and subsequently separated into groups at different periods of gestation; each group therefore represented a 4 day period of gestation as shown in Table 1. Those animals used on the day of parturition were killed within 6 hr of completion of delivery and further animals were used for 3 days post-partum. A group of 10 non-pregnant animals served as controls.

The animals were stunned, decapitated, and the uteri rapidly dissected into a dish of modified Krebs solution (Knifton, 1966) at 4°. One uterine horn from each animal was incised longitudinally and the foetuses removed. A strip of uterus 25 mm × 7 mm was cut, transferred to a 10 ml tissue bath and assembled for electrical stimulation and isometric recording as previously described (Knifton, 1966).

All experiments were made with the tissues adjusted to resting length. After a 30 min resting period to allow the pattern of spontaneous motility to become apparent, the minimum dose of oxytocin (Syntocinon, Sandoz) which caused a uterine contraction (oxytocin threshold) was determined. The tissue was then stimulated electrically at 1 min intervals at optimum voltage, each stimulus of 5 sec duration. When the contractions attained a steady state tension, the tissue was washed repeatedly in calcium-free Krebs solution and the time when the tension was reduced to 50% of the steady state tension (T50) was measured.

The results are summarized in Table 1.

The values for steady state tension increased as gestation advanced to reach a maximum at the time of parturition. When the mean steady state tension of each group is compared with that of the controls however, the difference in tension is not significant until the 17–20 day period of gestation. That is, the steady state