## Development of resistance to the lowering of plasma free fatty acids induced by pyrazole derivatives

SIR,—Several pyrazole derivatives, including 3,5-dimethylpyrazole (Gerritsen & Dulin, 1965a; Bizzi, Jori & others, 1964), 5-carboxy-3-methylpyrazole (Gerritsen & Dulin, 1965b) and 5-carboxamide 3-methylpyrazole (5CA3MP) (Bizzi, Codegoni & Garattini, 1967) are powerful inhibitors of free fatty acid (FFA) mobilization from adipose tissue. However, the blockade of lipolysis is frequently followed by an increase of plasma FFA above the normal level (Bizzi, 1966). Repeated daily treatments with 3,5-dimethylpyrazole or 5CA3MP tend to decrease the duration of the fall of plasma FFA. The onset of the resistance is, however, shortened if treatment with 5CA3MP in several doses far exceeding the amount necessary to produce a full effect are followed by a dose sufficient to give this effect.

In the experiments reported in Table 1 two groups of male Sprague Dawley rats, of 150 g average body weight, received saline or 5CA3MP, 50 mg/kg orally, twice daily for 3 days. The day after, a dose of 5CA3MP (7·5 mg/kg, orally) was markedly effective in the control group and completely ineffective in the treated group. When 5CA3MP was ineffective on plasma FFA, it did not show any capacity to lower plasma and liver triglycerides.

One of the possible reasons for explaining the onset of resistance relates to the observation that adipose tissue of animals pretreated with 5CA3MP shows *in vitro* a supersensitivity to lipolytic agents such as noradrenaline, ACTH or

TABLE 1. RESISTANCE TO THE LIPOLYTIC INHIBITOR EFFECT EXERTED BY 5-CARBOXAMIDE-3-METHYLPYRAZOLE (5CA3MP). Rats received saline or 5CA3MP for 3 days twice a day. On the 4th day they received saline or 5CA3MP 7·5 mg/kg orally

| Treatment mg/kg orally | | Plasma | | | Liver | Adipose tissue |
|---|---|---|---|---|---|---|
| from the 1st to the third day (twice a day) | 4th day | FFA** μ-equiv./litre | Glycerol μM/litre | Triglycerides mg/100 ml | Triglycerides mg/100 ml | FFA μ-equiv./g |
| Saline | Saline | 613 ± 27 | 153 ± 7 | 55 ± 3 | 419 ± 30 | 5·0 ± 0·2 |
| ,, | 5CA3MP 7·5 (15)* | 199 ± 20 | 61 ± 4 | 36 ± 3 | 257 ± 59 | 3·5 ± 0·1 |
| ,, | ,, (30) | 214 ± 14 | 64 ± 3 | 42 ± 3 | 439 ± 72 | 2·9 ± 0·1 |
| ,, | ,, (60) | 135 ± 7 | 54 ± 7 | 36 ± 2 | 359 ± 17 | 2·9 ± 0·1 |
| ,, | ,, (120) | 214 ± 8 | 50 ± 2 | 29 ± 3 | 224 ± 34 | 2·9 ± 0·1 |
| 5CA3MP | Saline | 728 ± 94 | 183 ± 2 | 77 ± 7 | 804 ± 71 | 5·8 ± 0·1 |
| ,, | 5CA3MP 7·5 (15) | 749 ± 18 | 169 ± 8 | 64 ± 11 | 927 ± 132 | 7·4 ± 0·6 |
| ,, | ,, (30) | 654 ± 82 | 140 ± 12 | 68 ± 12 | 1061 ± 356 | 5·8 ± 0·5 |
| ,, | ,, (60) | 664 ± 41 | 131 ± 6 | 58 ± 5 | 717 ± 106 | 5·8 ± 0·2 |
| ,, | ,, (120) | 721 ± 89 | 141 ± 10 | 55 ± 8 | 1140 ± 193 | 6·1 ± 0·4 |

* The time (min) between the last dose and killing the animals is in parentheses.
** Plasma FFA determinations were made according to Trout, Estes & Friedberg (1960) with minor modifications; glycerol according to Wieland (1957) and triglycerides according to Van Handel & Zilversmit with minor modifications (1957).

TABLE 2. EFFECT OF LIPOLYTIC AGENTS ON ADIPOSE TISSUE OBTAINED FROM NORMAL and 5-CARBOXAMIDE-3-METHYLPYRAZOLE (5CA3MP) RESISTANT RATS

| Additions μg/ml | FFA μ-equiv./g/hr | | Glycerol μM/g/hr | |
|---|---|---|---|---|
| | controls | 5CA3MP | controls | 5CA3MP |
| — .. .. .. .. | 8·6 ± 0·3 | 8·99 ± 0·3 | 3·57 ± 0·4 | 3·8 ± 0·3 |
| Noradrenaline[1] 0·25 .. .. | 21·6 ± 0·6 | 30·9 ± 1·0 | 8·4 ± 0·2 | 13·6 ± 0·5 |
| Theophylline[2] 75 .. .. | 32·0 ± 0·9 | 37·0 ± 0·8 | 12·2 ± 0·4 | 15·0 ± 0·7 |
| ACTH[3] 0·05 .. .. .. | 23·0 ± 1·0 | 32·0 ± 1·0 | 9·2 ± 0·6 | 14·0 ± 0·7 |

200 ± 10 mg of pooled and minced epididymal adipose tissue were incubated in 4 ml of Krebs Ringer bicarbonate medium pH 7·4, containing albumine 3%. Preincubation time 30 min at room temperature. Incubation time 60 min at 37° in air.
[1] As bitartrate monohydrate. [2] Monohydrate. [3] Synacthen.

theophylline (see Table 2). Since these agents are known to act by increasing the level of 3′,5′-cyclic AMP in the adipose tissue although not by the same means (Butcher, 1966), it may be possible that an elevation of 3,5-cyclic AMP in the adipose tissue is the cause of the resistance.

One of us (A.B.) has found that 3′,5′-cyclic AMP (dibutyryl ester) also antagonizes *in vitro* the effect of 5CA3MP and other pyrazole derivatives.

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## Interaction of aspirin with urea in water

SIR,—Previously it has been shown that urea increases the aqueous solubility of benzoic and salicylic acids (Bolton, 1963). As an extension of this, we have observed the effect of urea on aspirin solubility and stability.

Excess aspirin in water was shaken at 30° in the presence of varying amounts of urea for 5 hr. Clear aliquots were then analysed spectrophotometrically for aspirin content (Bolton, 1960). At pH 2·0, hydrochloric acid was used as a buffer and, at pH 3·5, formic acid-sodium formate. The pH of all solutions was carefully checked before and after equilibration.

Kinetic studies were made at pH values of 2·0, 2·5, 2·75, 3·0 and 3·5 at 30° ±0·2°. Formate buffers were used at pH values above 2·0 and hydrochloric acid was the buffer at pH 2·0.

The effect of urea on aspirin solubility at pH 2·0 and 3·5 is shown in Fig. 1. Although the increased solubility observed may be due to other than complexing effects, e.g. solvent effects, the solubility curve may well be described by two constants, $K_1$ and $K_2$, corresponding to the formation of 1:1 and 2:1 urea-aspirin complex species.

$K_1$ and $K_2$ can be determined graphically (Higuchi & Bolton, 1959). The values of the constants are in Table 1.

It is surprising that the values of $K_1$ and $K_2$ for the unionized and ionized complexes are of the same order of magnitude. However the calculations involved approximations as well as the neglect of other factors which may be responsible for the solubilization. The stronger solubilization of the unionized species is to be expected because of the weak basic nature of the urea molecule.

The first order rate constants from the kinetic studies are in Table 2.

Urea increases the rate of hydrolysis below pH 2·75 and decreases the rate at pH values greater than 2·75. It is interesting to note that this "crossover"