In vitro assessment of anti-inflammatory activity

SIR,—Whitehouse & Skidmore (1965) have reported that many acidic anti-inflammatory drugs inhibit histidine decarboxylases *in vitro*, presumably by displacing the pyridoxal phosphate coenzyme from the apoenzyme, while analogues of these compounds with little or no anti-inflammatory activity do not inhibit these enzymes. At the same time, it was reported (Skidmore & Whitehouse, 1965) that these histidine decarboxylase inhibitors displaced pyridoxal phosphate from some of its binding sites on bovine plasma albumin, in a manner essentially parallel to their effect on histidine decarboxylases. These authors further found that the same drugs inhibited the binding of 2,4,6-trinitrobenzaldehyde to bovine plasma albumin. In view of the good correlation between clinical antirheumatic activity and inhibition of trinitrobenzaldehyde binding to albumin, it was suggested that the system might serve as an *in vitro* model for the assay of potential anti-inflammatory activity. The purpose of this report is to present findings based on extensive use of the system which suggest a poor correlation between *in vitro* and *in vivo* anti-inflammatory activity.

In vitro anti-inflammatory activity was determined by the trinitrobenzaldehyde assay described by Skidmore & Whitehouse (1965). *In vivo* anti-inflammatory activity was assayed using the carrageenan foot oedema method (Winter, Risley & Nuss, 1962) or the azovan blue-carrageenan pleural effusion method (Sancilio & Rodriguez, 1965) or both, at doses ranging from 75 to 316 mg/kg. Of 57 diverse organic acids screened *in vitro*, 42 (74%) gave results equivalent to or greater than acetylsalicylic acid. In most instances comparable activity could not be demonstrated *in vivo*; some representative results are summarized in Table 1.

TABLE 1. EFFECT OF VARIOUS ACIDIC COMPOUNDS IN *in vitro* AND *in vivo* ANTI-INFLAMMATORY ASSAYS

Compound	Anti-inflammatory assay		
		in vivo†	
	in vitro % inhibition*	Foot oedema	Pleural effusion
α,5-Diphenyl-2H-tetrazole-2-acetic acid	58·0	0	
m-[(α-Phenylphenacyl)amino]-benzoic acid	49·8	0	
m-[3-(2,4-Dioxo-1H,3H-quinazolinyl)]-benzoic acid	21·4	0	
5,5′-Selenobis-salicylic acid	76·7	0	0
3-Octylsalicylic acid	74·9		0
N-Acetyl-p-aminophenol	86·4	0†	
3-*O*-(β-Carboxypropionyl)-11-oxo-18β-olean-12-en-30-oic acid	59·1		0
Acetylsalicylic acid	19·1	+	+

* Measured at 430 mμ.
† 0—inactive, +—active.
‡ Marginal activity observed at 400 mg/kg.

These results indicate that with a variety of acidic structures (including tetrazole alkanoic, substituted aminobenzoic, substituted benzoic, substituted salicylic, and triterpene acids, and a phenolic compound) significant *in vitro* anti-inflammatory activity does not correlate with *in vivo* anti-inflammatory activity. Thus, while the method may be useful in the preliminary evaluation of anti-inflammatory activity, since clinically active compounds are detected, many false positive results will arise from such a screen. The fact that clinically active compounds do interfere with trinitrobenzaldehyde binding to serum albumin, while certain closely related but clinically inactive compounds do not,

has been interpreted as suggesting a possible relation to the mechanism of anti-inflammation; however, these results suggest that any such relation is not specific.

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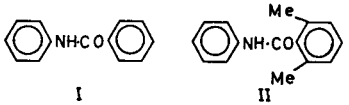
The influence of the stability of the amide link on the formation of methaemoglobin by anilides

SIR,—It is considered that the reactions involved in the formation of methaemoglobin by aromatic amides are (*a*) hydrolysis of the amide, (*b*) metabolism of the amine produced to the appropriate species, and (*c*) oxidation of the haemoglobin by the amine metabolite (McLean, Murphy & others, 1967). The stability of the amide link in anilides may be modified by (*a*) substitution on the aromatic ring, and (*b*) substitution on the acyl group. McLean & others (1967) examined a wide range of derivatives of acetanilide which were substituted on both the acyl and aromatic moieties and found no correlation between the stability of the amide group and the ability of the compounds to induce the formation of methaemoglobin in cats. They came to the conclusion that the nature of the amine formed by hydrolysis of the aromatic amide was of prime importance in determining the amount of methaemoglobin formed by the compounds. We wish to report an example in which the hydrolysis of an anilide is the rate determining step in the formation of methaemoglobin. It is well known that disubstitution in the 2,6-positions of an aromatic amide or ester confers considerable stability on the amide or ester group because of the so-called "*ortho*-effect". McLean & others (1967) attempted to determine the importance of the "*ortho*-effect" in controlling the ability of an aromatic amide to form methaemoglobin by examining a series of 2,6-dimethylanilides. These amides formed much less methaemoglobin than the corresponding unsubstituted anilides but the influence of the "*ortho*-effect" could not be assessed because 2,6-dimethylaniline was also a poor former of methaemoglobin. To obtain evidence that the rate of hydrolysis of an anilide can be the rate determining step in the formation of methaemoglobin, benzanilide (I) and 2′, 6′-dimethyl-



I II

benzanilide (II) have now been examined. The compounds were prepared by condensing the appropriate acid chloride with aniline. 2,6-Dimethylbenzoic acid was prepared by the method described by Thomas & Canty (1962) and converted to 2,6-dimethylbenzoyl chloride by treatment with thionyl chloride. Benzanilide was recrystallized from benzene-ethanol as white crystals, m.p.