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Effect of non-steroid anti-inflammatory drugs on aldehyde binding to plasma albumen: a novel *in vitro* assay for potential anti-inflammatory activity

SIR,—Dempsey & Christensen (1962) showed that pyridoxal phosphate binds to the lysyl ϵ-amino-groups of plasma albumen and that certain aromatic acids, including salicylic acid, partly reversed this association. We have confirmed and extended their observations as follows. When bovine plasma albumen (Armour Pharmaceuticals Ltd., Eastbourne) was treated with 2,4,6-trinitrobenzene sulphonic (picryl sulphonic) acid (TNBS) in aqueous solution at pH 7·5, at least 50 amino-groups per albumen molecule (number average) were substituted by the trinitrophenyl (TNP) group. The product, "TNP-albumen", no longer bound salicylate anions or pyridoxal phosphate. A similar loss of binding capacity occurred when albumen was treated with 1-fluoro-2,4-dinitrobenzene (FDNB, Sanger's reagent) in dilute sodium bicarbonate solution at pH 8·5. Albumen which had been dialysed against a solution of sodium salicylate at pH 7·5 and had bound *several* molecules of salicylate per albumen molecule, lost all the bound salicylate on treatment with TNBS or FDNB. These findings establish the lysyl ϵ-amino-group as a common binding site for both the salicylate ion and pyridoxal phosphate. The single *N*-terminal aspartyl α-amino-group which also reacts with TNBS and FDNB, could only bind one molecule of drug or coenzyme. [TNBS and FDNB do not react with the guanidino-group of arginine, the only other basic residue in the albumen molecule].

Many acidic anti-inflammatory drugs inhibit mammalian substrate-specific histidine decarboxylases *in vitro* (Whitehouse & Skidmore, 1965); this is believed

to occur by displacing the pyridoxal phosphate coenzyme from the apoenzyme, to which it is probably bound through a lysyl ϵ-amino-group. Furthermore, chemical analogues of these drugs with little or no anti-inflammatory activity do not inhibit these enzymes. We have now found that these anti-inflammatory acids (histidine decarboxylase inhibitors) will displace pyridoxal phosphate from some of its binding sites on bovine plasma albumen (Table 1). The effectiveness of these drugs and their chemical analogues in displacing albumen-bound pyridoxal phosphate largely parallels their relative activity in inhibiting histamine formation *in vitro*.

TABLE 1. EFFECT OF ANTI-INFLAMMATORY ACIDS ON BINDING OF PYRIDOXAL PHOSPHATE AND 2,4,6-TRINITROBENZALDEHYDE TO BOVINE PLASMA ALBUMEN

Drug added	% inhibition with pyridoxal phosphate		% inhibition with trinitrobenzaldehyde	
	at 332 mμ	% shift (410 − 387 mμ	at 425 mμ	at 526 mμ
Sodium salicylate	80	72	31	31
Sodium *p*-hydroxybenzoate	40	55	22	20
Sodium gentisate	50	61	31	27
γ-Resorcylic acid	100	83	41	42
Sodium benzoate	60	55	0	3
Acetylsalicylic acid	80	61	9	9
Phenylbutazone	62	72	45	37
Oxyphenbutazone	100	50	50	44
Sulphinpyrazone	50	61	0	0
Phenazone	0	0	5	5
Amidopyrine	5	0	0	0
Flufenamic acid	100	70	74	73
Mefenamic acid	100	50	57	55
N-Methylanthranilic acid	100	50	35	30
Anthranilic acid	70	—	9	6
(5-Methyoxy)indomethacin (IM)	100	100	75	81
5-Fluoro analogue of IM	100	90	70	76
Indomethacinamide*	0	0	0	0
5-Methoxyindole-3-acetic acid	0	10	23	23
Cinchophen	100	61	54	55
Hydrocortisone*	0	0	8	12
Chloroquine phosphate	100	0	18	22

*Sat. soln., insoluble at 1 mM.

0·67% Albumen (0·1 mM), 0·1 mM aldehyde, 1·0 mM drugs all in 0·1 M sodium phosphate pH 7·5. Inhibition of pyridoxal phosphate (PXALP) binding given as percentage decrease in light absorption at 332 mμ (drug free controls as 0% and unbound PXALP as 100%) and also by the percentage shift of the absorption maximum of bound PXALP (at approximately 410 mμ) towards the absorption maximum of free PXALP (at 387 mμ). Inhibition of trinitrobenzaldehyde (TNBAL) binding given as the percentage decrease in light absorption at 425 mμ and 526 mμ due to bound TNBAL. All values were corrected for light absorption by plasma albumen. Where necessary, drugs were added in solution in *NN*-dimethylformamide (DMF) giving a final DMF concentration of 3% v/v. (also in controls).

These findings indicate the possible value of an *in vitro* assay for potential anti-inflammatory activity, based on spectrophotometric measurements of the extent of pyridoxal phosphate binding to plasma albumen in the presence of the drug. However, the combination of pyridoxal phosphate with plasma albumen involves the formation of several complexes with different absorption characteristics, some of which overlap the absorption bands of aromatic anti-inflammatory drugs. We therefore devised a simpler assay for determining the ability of a drug to bind to protein lysyl ϵ-amino-groups using 2,4,6-trinitrobenzaldehyde (Aldrich Chemical Co., Milwaukee), which rapidly forms a reddish complex with plasma albumen (but not with TNP-albumen or *N*-acetyl-albumen) exhibiting two absorption maxima in the regions 425-435 mμ and 525-535 mμ at pH 7·5. The formation of this complex is prevented by many drugs (see Table 1) and this drug effect is readily seen by eye.

These experiments indicate that there is a remarkable variation in the capacity of individual aromatic acids to displace these two aldehydes when they are bound to albumen, and, also, indomethacin is particularly effective and must have a high affinity for protein lysyl ϵ-amino-groups. Neutral and basic anti-inflammatory drugs such as amidopyrine, hydrocortisone and chloroquine, did not appreciably affect the binding of these aldehydes to albumen. We have the impression that drug antagonism of trinitrobenzaldehyde binding corresponds more closely with clinical antirheumatic activity (*vide* inactivity of benzoic and anthranilic acids) than does the effect of these acids upon pyridoxal phosphate binding to plasma albumen.

Mizushima (1964; 1965) reported that 1 mM antirheumatic (acidic) drugs and sodium dodecyl (lauryl) sulphate, stabilised a bovine plasma albumen fraction against heat coagulation. Dodecyl sulphate binds to at least 14 lysyl ϵ-amino groups per molecule of bovine plasma albumen (Markus, Love & Wissler, 1964). We found that both TNP- albumen and *N*-acetyl-albumen could not be protected from heat denaturation in this way and, furthermore, neither of these modified proteins would react with trinitrobenzaldehyde. We therefore believe that Mizushima's method of screening for potential anti-inflammatory drugs *in vitro* affords a measure of the protein-binding, or more specifically the lysine-complexing, ability of the compounds being tested. Measuring aldehyde binding in the presence of potential anti-inflammatory drugs affords another quantifiable index of potency in associating with protein (lysyl) ϵ-amino-groups, the importance of which is discussed in another communication (Whitehouse & Skidmore, 1965).

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8β-Carbobenzyloxyaminomethyl-1,6-dimethyl-10α-ergoline

SIR,—We wish to draw attention to an error in the chemical name of the compound we examined in our paper entitled: Antagonism of 5-hydroxytryptamine-induced bronchospasm in guinea-pigs by 8β-carbobenzyloxyaminomethyl-l-methyl-10α-ergoline (*J. Pharm. Pharmacol.*, 1965, **17**, 423–428).

We are informed that the stated compound should be 8β-carbobenzyloxyaminomethyl-1,6-dimethyl-10α-ergoline.

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