Biochemical effects of some newer salicylic acid congeners

SIR,—The exact mechanism of anti-inflammatory activity of drugs is not well understood. The anti-inflammatory agents like salicylates, butazolidine, resorcylic acid, hydrocortisone, glycyrrhetic acid and imipramine have been shown to inhibit aminotransferases (Moses & Smith, 1961; Huggins, Bryant & Smith, 1961; Huggins, Smith & Moses, 1961; Tangri, Seth, Parmar & Bhargava, 1965; Tangri, Saxena, Seth & Bhargava, 1966), uncouple oxidative phosphorylation (Whitehouse & Haslam, 1962) and stimulate ATP phosphohydrolase activity (Whitehouse & Haslam, 1962; Tangri & others, 1965, 1966); all of these actions may be related to their anti-inflammatory property. Some newer salicylic acid congeners like 2,4-diacetoxybenzoic acid, *m*-cresotinic acid and 5-ethyl-2-hydroxybenzoic acid were reported to have potent anti-inflammatory activity (Tangri & Bhargava, 1964). The effect of these agents on serum aminotransferases and tissue ATP phosphohydrolase has been examined to correlate their biochemical effects with their anti-inflammatory activity.

Enzyme assays were made both in normal and arthritic albino rats with or without drug treatment. Serum for the estimation of aminotransferase activity was obtained from the blood of decapitated rats. The livers were removed immediately and pooled for the estimation of ATP phosphohydrolase (EC. No. 3.6.1.4.) activity. Serum L-aspartate: 2-oxoglutarate aminotransferase (EC. No. 2.6.1.1, aspartate aminotransferase) and serum L-alanine: 2-oxoglutarate aminotransferase (EC. No. 2.6.1.2, alanine aminotransferase) were assayed by the method of Reitman & Frankel (1957). One unit of enzyme activity was the change in the extinction of 0·001/min/ml of serum, which was measured in a Bausch & Lomb Spectronic '20' colorimeter at 505 mμ. ATP phosphohydrolase activity was estimated in 10% (w/v) pooled liver homogenate prepared in 0·25 M sucrose. The reaction mixture consisted of 0·05 M Tris pH 8·0, 1 mM ATP and 0·1 ml of 10% tissue homogenate in a final volume of 2 ml. Release of P_1 (inorganic phosphorus) from ATP was measured according to Fiske & Subbarow (1925). The release of 1 μM of P_1/100 mg of tissue in 15 min at 37° was considered as one unit of enzyme activity.

The arthritis in the ankle joints of albino rats (100–110 g) was produced by injecting 0·1 ml of 2% (v/v) formaldehyde subcutaneously under the plantar aponeurosis according to Brownlee (1950). The animals were treated with daily intraperitoneal injections of 2,4-diacetoxybenzoic acid, *m*-cresotinic acid, 5-ethyl-2-hydroxybenzoic acid (2 mg/100 g bodyweight) and hydrocortisone (0·5/100 g bodyweight) for 10 consecutive days.

The results are in Tables 1 and 2. The newer salicylic acid congeners inhibited the normal alanine aminotransferase but did not significantly alter the normal aspartate aminotransferase. A greater sensitivity of alanine aminotransferase to anti-inflammatory agents (viz. salicylates, glycyrrhetic acid, hydrocortisone and imipramine) compared to aspartate aminotransferase has been reported (Huggins & others, 1961a, b; Tangri & others, 1965, 1966). Furthermore, the inflammatory reaction increased the levels of both serum aminotransferases but particularly aspartate aminotransferase. This increase in the enzymic activities due to inflammation was prevented by the salicylic acid congeners to the same degree as hydrocortisone. Other anti-inflammatory agents, glycyrrhetic acid, methyl glycyrrhetic acid and imipramine, also prevented the rise in the serum aminotransferases caused by inflammation (Tangri & others 1965, 1966).

Since the anti-inflammatory agents significantly reduced the enhanced serum aspartate aminotransferase level induced by the inflammatory stimulus and since they failed to alter the normal aspartate aminotransferase activity, it may be

TABLE 1. EFFECT OF HYDROCORTISONE, 2,4-DIACETOXYBENZOIC ACID, *m*-CRESOTINIC ACID AND 5-ETHYL-2-HYDROXYBENZOIC ACID ON SERUM ASPARTATE AMINOTRANSFERASE AND ALANINE AMINOTRANSFERASE IN NORMAL AND ARTHRITIC RATS.
(One unit of enzyme activity is the change in extinction of 0·001/min/ml of serum)

		Control	Hydrocortisone*	2,4-Diacetoxybenzoic acid	*m*-Cresotinic acid	5-Ethyl-2-hydroxybenzoic acid
Serum aspartate aminotransferase	Normal	28·27 ± 1·19	28·1 ± 0·68 (P = 0·9)	31·2 ± 0·63 (P = 0·2)	28·0 ± 1·15 (P = 0·9)	29·8 ± 0·63 (P = 0·5)
	Arthritic	50·0 ± 1·1	28·6 ± 1·31 (P = 0·001)	39·8 ± 0·5 (P = 0·001)	31·6 ± 0·6 (P = 0·001)	37·2 ± 0·5 (P = 0·001)
	Percent increase in inflammation	76·7	1·77	27·5	13·8	25·0
Serum alanine aminotransferase	Normal	32·13 ± 1·5	22·5 ± 1·25 (P = 0·001)	23·8 ± 1·4 (P = 0·02–0·01)	25·2 ± 0·8 (P = 0·05–0·02)	28·0 ± 0·8 (P = 0·2)
	Percent decrease with drug	—	30·0	26·0	21·4	15·7
	Arthritic	39·8 ± 1·7	25·7 ± 1·56 (P = 0·001)	20·0 ± 0·9 (P = 0·001)	25·0 ± 0·6 (P = 0·001)	26·8 ± 0·8 (P = 0·001)
	Percent decrease with drug	—	39·9	49·9	37·9	32·6

* Data reported by Tangri & others (1965).

TABLE 2. EFFECT OF HYDROCORTISONE, 2,4-DIACETOXYBENZOIC ACID, *m*-CRESOTINIC ACID AND 5-ETHYL-2-HYDROXYBENZOIC ACID ON THE ATP PHOSPHOHYDROLASE ACTIVITY IN THE POOLED LIVER HOMOGENATES OBTAINED FROM NORMAL AND ARTHRITIC RATS

		Control	Hydrocortisone*	2,4-diacetoxybenzoic acid	*m*-Cresotinic acid	5-Ethyl-2-hydroxybenzoic acid
Liver ATP phosphohydrolase	Normal	11·62	16·96	16·09	15·21	15·21
	% increase with drug	—	46·1	38·4	30·9	30·9
	Arthritic	11·62	16·99	16·99	16·09	16·99
	% increase with drug	—	46·2	46·2	38·4	46·2

* Data reported by Tangri & others (1965).

implied that the extra aspartate aminotransferase activity caused by inflammation is more sensitive to these drugs and that a correlation for anti-inflammatory action and reduction in enhanced aspartate aminotransferase activity exists. It may well be that there is an enzyme or isoenzyme differing from the ordinary circulating aspartate aminotransferase. On the contrary, no correlation can be made for the inhibition of enhanced alanine aminotransferase activity and the inhibitory effects of the agents since they inhibited both enhanced and normal serum enzyme activity.

Salicylic acid congeners were also found to stimulate the liver ATP phosphohydrolase activity both in the normal and arthritic rats. The anti-inflammatory

activity of these agents is not dependent upon the increased ATP phosphohydrolase, since the inflammatory reaction *per se* did not influence the hepatic ATP phosphohydrolase activity.

Acknowledgements. Grateful acknowledgement is made to Council of Scientific and Industrial Research for the award of Senior Fellowship to P. K. Seth.

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October 7, 1966

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Potentiation of the anticonvulsant action of acetazolamide

SIR,—Certain α- or β-adrenergic blocking agents antagonize the anticonvulsant action of acetazolamide (Rudzik & Mennear, 1966a), but dichloroisoprenaline enhances it. Since the anticonvulsant action of acetazolamide is mediated through a catecholamine mechanism (Gray, Rauh & Shanahan, 1963; Rudzik & Mennear, 1966b), the enhancement of acetazolamide by dichloroisoprenaline may be related to its ability to produce adrenergic stimulation before blockade. We now report the effects of several central nervous system stimulants as well as amine-releasing agents on the anticonvulsant action of acetazolamide.

Male albino mice (Harlan Industries), 18–26 g were dosed with the various agents under investigation 30 min before the intraperitoneal injection of acetazolamide. The ED 50 value (Litchfield & Wilcoxon, 1949) for the anticonvulsant effect of acetazolamide was determined 30 min after the injection of acetazolamide. Maximal electroshock siezures were given (Swinyard, Brown & Goodman, 1952) and the criterion for protection against the seizure was abolition of hind leg extension. The results are in Table 1.

As reported earlier (Rudzik & Mennear, 1966b) dichloroisoprenaline potentiated the anticonvulsant effect of acetazolamide while MJ-1999 [4′-(1-hydroxy-2-isopropylamino) methanesulphonanilide] antagonized it. Dichloroisoprenaline has also been reported to produce a stimulant effect upon the grossly observed behaviour of rats (Randrup, Munkvad & Udsen, 1963), to antagonize reserpine-induced ptosis in mice (Aceto & Harris, 1963) and to enhance the lethal effect of amphetamine in aggregated mice (Mennear & Rudzik, 1965). Intrinsic (adrenergic stimulatory) activity has not been reported for MJ-1999 (Dugan & Lish, 1964).

Since amine-depleting agents have been reported to antagonize the action of