Interaction of Salicylates with Rat Liver Lysosomes

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A number of anti-inflammatory agents are known to stabilize the lysosomal membrane (Weissmann, 1966), and there is conflicting evidence for the function of salicylates in this way (Miller & Smith, 1966; Weissmann, 1968; Tanaka & Iizuka, 1968).

Lysosomes contain esterases, possibly bound to the membrane (Shibko & Tappel, 1964), and thus any effect of aspirin could be due to products of 'in vivo' hydrolysis. The breakdown of aspirin by lysosomal esterases was demonstrated, by using lysosomes from the livers of rats previously injected with Triton WR-1339. A crude mitochondrial fraction prepared by differential centrifugation of liver homogenate was subjected to zonal centrifugation in an MSE type 'A' rotor by using a linear 15–40% (w/w) sucrose gradient. After 18 hr. at 6000 rev./min. the low-density lysosomes were clearly separated from mitochondria and peroxisomes, although contaminated with plasma-membrane fragments as indicated by the presence of 5'-nucleotidase. The fractions collected were also monitored for urate oxidase (peroxisome marker), cytochrome *c* oxidase (mitochondria) and acid phosphatase (lysosomes) as well as for esterases by using *p*-nitrophenyl acetate, β -naphthyl acetate and acetylsalicylic acid both in the presence and absence of 10 μ M-E 600 (Shibko & Tappel, 1964). The results confirmed the finding by Shibko & Tappel (1964) that the lysosomal fraction shows an enrichment of E 600-resistant esterases. The optimum pH for aspirin hydrolysis was 5.0, compared with 7.0 for *p*-nitrophenyl acetate and β -naphthyl acetate.

The effect of acetate, salicylate and aspirin on the integrity of rat liver lysosomal membranes was examined by using a cell fraction prepared by the procedure of de Duve, Pressman, Gianetto, Wattiaux & Appelmans (1955). The compounds were incubated with lysosomes for 45 min. at 37° at pH 5.0, and for 90 min. at 37° at pH 7.4. Subsequently the latency of particulate acid phosphatase was measured by assaying before and after the addition of 0.1% Triton X-100, and the release of enzyme into the supernatant was also measured. The results confirmed the stabilizing effect of cholesterol in terms of increased latency compared with control experiments, but no such effect was detected with acetate, salicylate or aspirin up to 1 mM.

There have been reports of direct interaction between anti-inflammatory drugs and lysosomal enzymes (Anderson, 1968; Ennis, Grada & Posner,

1968). Locally high concentrations of acetate and salicylate could arise *in situ* by the action of esterases on aspirin, but no inhibition of lysosomal β -galactosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase or acid phosphatase could be detected with aspirin, salicylate or acetate at 1 mM.

We therefore conclude that a direct interaction between aspirin or its hydrolysis products and either the lysosomal membrane or its enzymic contents is unlikely to be a major factor in its pharmacological action.

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- Anderson, A. J. (1968). *Biochem. Pharmacol.* **17**, 2253.
 de Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R. & Appelmans, F. (1955). *Biochem. J.* **60**, 604.
 Ennis, R. S., Grada, J. L. & Posner, A. S. (1968). *Arthr. Rheum.* **11**, 756.
 Miller, W. S. & Smith, J. G. (1966). *Proc. Soc. exp. Biol., N.Y.*, **122**, 634.
 Shibko, S. & Tappel, A. L. (1964). *Arch. Biochem. Biophys.* **106**, 259.
 Tanaka, K. & Iizuka, Y. (1968). *Biochem. Pharmacol.* **17**, 2023.
 Weissmann, G. (1966). *Arthr. Rheum.* **9**, 834.
 Weissmann, G. (1968). In *The Interaction of Drugs and Subcellular Components in Animal Cells*, p. 203. Ed. by Campbell, P. N. London: J. and A. Churchill Ltd.

Effects of Suramin and Aurothiomalate on Rat Liver Lysosomes

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By using a technique developed from that of Mego, Bertini & McQueen (1967) we have recently been able to show a decreased digestive capacity in intact rat liver lysosomes containing Trypan Blue (Davies, Lloyd & Beck, 1969). The present experiments use the same technique to investigate the action of two drugs, for both of which there is evidence of uptake into lysosomes and of enzyme inhibition *in vitro*.

Suramin, a trypanocide, is concentrated in the lysosomes of both rat liver and trypanosome cells (see Allison, 1968) and inhibits some lysosomal enzymes (M. Davies, unpublished results). Sodium aurothiomalate, a drug used clinically in rheumatoid arthritis, was found in a lysosomal fraction of polymorphonuclear leucocytes after injection into rabbits (Persellin & Ziff, 1966). It inhibits the acid phosphatase and β -glucuronidase activities in these cells (Persellin & Ziff, 1966). More recently Ennis, Granda & Posner (1968) have demonstrated inhibition of both these enzymes and of cathepsin from rabbit liver lysosomes or human synovial fluid.