accompanied by protein. Thus, both glucose and mannitol, but particularly the latter, increased the blood volume in hypovolemia.

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Effect of Acetylsalicylic Acid on Lysosomes.* (31212)

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Since the description of lysosomes over 10 years ago(1), their occurrence in various tissues and important role in cellular metabolism have been the subject of much investigation(2,3). A number of agents, particularly corticosteroids(4) and chloroquine(5) have been found to stabilize lysosomes *in vitro* and this action has been proposed as an explanation of certain of their therapeutic effects in various clinical diseases.

Duthie(6) has hypothesized that salicylates might stabilize lysosomes. This study was carried out to investigate the influence of acetylsalicylic acid (ASA) on rat liver lysosomes.

Materials and methods. Adult white rats (Wistar strain, Charles River Laboratories) were sacrificed by a blow to the base of the skull. The liver was dissected free, weighed, and placed in ice cold 0.25 M sucrose. In most experiments, 2 livers were used. The livers were homogenized in a Rosett grinder chilled with ice(7). All centrifugations were done in a Servall RC-2 centrifuge with a SS-34 rotor at 5°. The liver homogenate was centrifuged at 2880 rpm for 10 minutes (10,000 *g* minutes) to remove unruptured cells and nuclear debris. The residue was discarded and the supernate was centrifuged at 14,400 rpm for 10 minutes (250,000 *g* minutes) to obtain a lysosomal residue. The supernate was discarded, the residue resuspended in cold 0.25 M sucrose and centrifuged again at 14,400 rpm for 10 minutes (250,000 *g* minutes). Acetylsalicylic acid (USP-Mallinckrodt) was dissolved in water in a 10^{-2} M concentration and in 95% ethanol in a 10^{-1} M concentration and serially diluted with water and 95% ethanol, respectively, to 10^{-4} M. ASA was prepared fresh prior to each experiment to minimize hydrolysis. Hydrocortisone sodium succinate (Solu-cortef, Upjohn) and chloroquine phosphate (Winthrop) were prepared in aqueous solutions similarly. The lysosomal residue was resuspended in 0.25 M sucrose and 0.9 ml of the lysosomal suspension was added to 0.1 ml of water or 0.1 ml of 95% ethanol as a control (final ethanol concentration 9.5%). 0.1 ml of various concentrations of ASA, chloroquine, and hydrocortisone in water and ASA in ethanol were added to 0.9 ml aliquots of the lysosomal suspension. After mixing, the lysosomal suspensions were incubated in a waterbath at 37° for 2 hours to labilize the lysosomes(8). Following incubation, the lysosomal suspensions were centrifuged at 14,400 rpm for 10 minutes (250,000 *g* minutes) to remove mitochondria, unruptured lysosomes,

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TABLE I. Acid Phosphatase and Beta Glucuronidase Release from Liver Lysosomes (2 Hr Incubations at 37°).

	ASA		Hydrocortisone		Chloroquine	
	Acid phosphatase	Beta glucuronidase	Acid phosphatase	Beta glucuronidase	Acid phosphatase	Beta glucuronidase
Control	100	100	100	100	100	100
10^{-3} M	30	30	40	38	42	40
10^{-4} M	52	57	59	57	59	59
10^{-5} M	84	80	93	95	96	95

Results are expressed as percent of controls. Average of 3 experiments.

and lysosomal membranes. Lysosomal suspensions prepared in the same manner were incubated at 37° with water as a control and various concentrations of aqueous ASA (10^{-3}-10^{-6} M) in a ratio of 9:1, lysosomal suspension:water as a control or aqueous ASA. At 1-, 2-, and 3-hour intervals, aliquots were removed and centrifuged at 14,400 rpm for 10 minutes (250,000 *g* minutes). The clear supernates were assayed for acid phosphatase (9) at pH 5.4 using phenolphthalein diphosphate (Sigma) as a substrate. The optical density of the samples was read at 530 mμ against blanks containing all reagents at zero time incubation. Standards contained 5-20 μg phenolphthalein. Results were expressed as percent phenolphthalein released in comparison with the control which was equated to 100%. β-glucuronidase was assayed by a modification of the method of Fishman *et al* (10) at pH 4.5 using phenolphthalein mono-β-glucuronic acid (Sigma) as a substrate. Optical density was read at 540 mμ and the results expressed as for the acid phosphatase.

To evaluate the effects of ASA on free acid phosphatase and β-glucuronidase, suspensions of rat liver lysosomes were prepared as above. These suspensions were freeze-thawed 10 times to labilize the lysosomal enzymes(11) and centrifuged at 14,000 rpm for ten minutes (250,000 *g* minutes) to remove mitochondria, lysosomal membranes, and unruptured lysosomes. Aliquots of the supernates were incubated with various concentrations of aqueous and ethanolic ASA with controls and acid phosphatase and β-glucuronidase activity assayed.

Results. The effects of aqueous ASA, hydrocortisone, and chloroquine in stabilizing rat liver lysosomes are given in Table I. Aqueous ASA stabilized the lysosomes slightly better than equimolar concentrations of hydrocortisone and chloroquine. The effects of ethanolic ASA on lysosomes are given in Table II with some stabilization apparent up to a 10^{-4} M ASA concentration. Table III gives the acid phosphatase and β-glucuronidase activity of supernates obtained from timed incubations of lysosomes in ASA solutions at 1, 2, and 3 hours. A stabilizing effect is apparent at all 3 time intervals in concentrations of ASA as dilute as 10^{-5} M.

Neither aqueous ASA (10^{-3}-10^{-5} M) nor ethanolic ASA (10^{-2}-10^{-5} M) had any significant inhibitory effect on free acid phosphatase or β-glucuronidase activity (Table IV).

Discussion. These results demonstrate that ASA stabilizes rat liver lysosomes *in vitro* and suggest that the anti-inflammatory and anti-pyretic effects of ASA may be mediated by a lysosomal mechanism. ASA has been demonstrated in plasma at levels of 0.2-1.4 mg/100 ml ($<10^{-4}$-$>10^{-5}$ M) 30 minutes after oral administration of 1.2 g of ASA(12). ASA has also been recovered in unhydrolyzed form in the urine(13) indicating that complete hydrolysis of ASA *in vivo* does not occur. Thus, these concentrations which have

TABLE II. Acid Phosphatase and Beta Glucuronidase Release from Liver Lysosomes in the Presence of Ethanolic ASA (2 Hr Incubations at 37°).

	Acid phosphatase	Beta glucuronidase
Control (9.5% ethanol)	100	100
10^{-2} M ASA	23	33
10^{-3} M ASA	45	45
10^{-4} M ASA	79	78
10^{-5} M ASA	91	92

Results are expressed as percent of controls.
Acid phosphatase average of 6 experiments.
Beta-glucuronidase average of 3 experiments.

TABLE III. Acid Phosphatase and Beta Glucuronidase Release from Timed Incubations of Liver Lysosomes at 37°.

	1 hr		2 hr		3 hr	
	Acid phosphatase	Beta glucuronidase	Acid phosphatase	Beta glucuronidase	Acid phosphatase	Beta glucuronidase
Control	44	64	78	88	100	100
10^{-3} M ASA	17	29	24	33	52	54
10^{-4} M ASA	24	39	30	45	68	66
10^{-5} M ASA	27	46	39	50	76	79
10^{-6} M ASA	44	62	76	87	100	97

Results are expressed in percent of 3 hr control.
Results are average of 2 experiments.

TABLE IV. Effect of Aqueous and Ethanolic ASA on Free Acid Phosphatase and Beta Glucuronidase.

	Aqueous ASA		Ethanolic ASA	
	Acid phosphatase	Beta glucuronidase	Acid phosphatase	Beta glucuronidase
Control (aqueous)	100	100	100	100
9.5% Ethanol	—	—	102	101
10^{-2} M ASA	—	—	96	97
10^{-3} M ASA	98	100	96	96
10^{-4} M ASA	97	95	100	97
10^{-5} M ASA	96	92	101	90

Values are expressed as percent of aqueous control.

stabilizing effects on lysosomes *in vitro* can be achieved *in vivo* following administration of a single dose of 1.2 g of ASA orally.

Summary. Acetylsalicylic acid (ASA) stabilizes rat liver lysosomes *in vitro* from the labilizing influence of incubation at 37°. On a molar concentration basis, this stabilization is slightly greater than that achieved with hydrocortisone and chloroquine. Concentrations of ASA of 10^{-2} M and below do not inhibit either free acid phosphatase or β-glucuronidase enzyme activity.

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