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Sodium salicylate and L-glutamic dehydrogenase activity of the brain

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IT HAS been shown by Gould *et al.*[1] that sodium salicylate inhibits the activity of L-glutamic dehydrogenase (L-glutamate NAD (P) oxido reductase EC 1.4.1.2)* when added to purified enzyme preparations of bovine liver. It also inhibits glutamate–pyruvate transaminase in rat serum and tissue extracts and the drug is active against xanthine dehydrogenase of the liver.[2, 3]

The important role played by GDH in brain metabolism suggested to us to study the effect of salicylate on the enzymes involved in the formation and breakdown of glutamic acid in the brain.

Normal white mice of known pedigree were used throughout this study. The animals were killed by cervical dislocation and the brain immediately removed, blotted, weighed and immersed in 1 ml of ice-cold distilled water. The homogenates were prepared in a glass Potter–Elvehjem apparatus with a Teflon pestle for 3 min and sufficient cold distilled water to give a 10 per cent homogenate and then centrifuged at 0° in an International apparatus at −2° for 20 min and the cell-free supernatant used for the assays according to the method of Olsen and Anfinsen.[4] GDH being confined to the mitochondria, the homogenates were prepared with distilled water instead of buffer and incubated 1 hr at 0° in the way to obtain full activity by disruption of the mitochondria membrane.[5] The results are expressed in micromoles of NADH oxidized per milligram protein per minute.[6] The values were recorded by observing the changes in absorption at 340 nm in a Zeiss spectrophotometer PQMII. Protein was determined spectrophotometrically.[7]

Cell-free extracts were incubated for 60 min with different concentrations of sodium salicylate and the enzymatic activity measured as shown in Fig. 1. The percentages of inhibition increased with the concentration of salicylate and the time of incubation at 0°. After 24 hr there is a 100 per cent inhibition even with 0·1 ml of the 0·3 M solution (Table 1).

It has been shown by Tomkins *et al.*[8] that crystalline GDH of bovine liver (molecular weight 1,000,000) can be dissociated in four subunits when inactivated by diethylstilbestrol and other compounds. These subunits are devoid of GDH activity but showed ADH activity. We were therefore interested to know if the *in vitro* inactivation of this enzyme in brain extracts could be explained by the disaggregation of the molecule. We found that when the activity decreased there was a marked

* Abbreviations used: GDH = L-glutamic dehydrogenase; ADH = alanine dehydrogenase; ADP = adenosine 5-diphosphate.

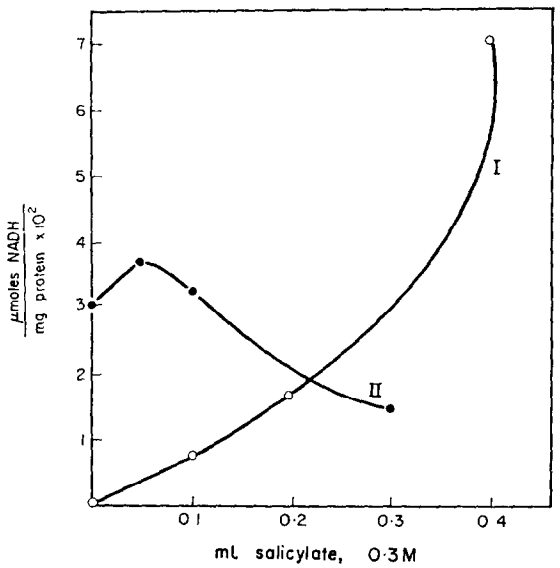

FIG. 1. Effect of sodium salicylate on pyruvate reduction. The reaction mixture contained 0·2 ml of sodium pyruvate 0·05 M, NH_4Cl 3 M, NADH $1{\cdot}4 \times 10^{-3}$ M, Tris buffer 0·02 M pH 8·0. The enzyme preparation contained 2·10 mg protein measured spectrophotometrically. Sodium salicylate 0·2 M was added as indicated in the graph. ΔE = differences in optical densities at 340 nm, per minute. Curve I refers to pyruvate reduction and curve II to GDH activity measured at 340 nm with oxoglutarate $1{\cdot}6 \times 10^{-3}$ M, NH_4Cl 3 M, NADH $1{\cdot}4 \times 10^{-3}$ M. Protein content = 3·1 mg.

TABLE 1. INHIBITION *IN VITRO* OF GDH BY SODIUM SALICYLATE

NADH oxidised (units/min × 10)	Time of incubation (0°)	Inhibition (%)	Salicylate added (μM)
30	1	25·35	0·0312
73	1	61·97	0·0624
93	1	78·87	0·1248
113	—	—	—
—	24	100·0	0·0312

TABLE 2. EFFECT OF SODIUM SALICYLATE AND ADP ON GDH OF THE BRAIN

System	GDH (μmoles/mg protein)	ADH
Enzyme* plus sodium salicylate	2·53	7·68
Enzyme* plus salicylate† and ADP‡	3·75	inactive
Enzyme* plus ADP‡	6·90	inactive

* 2·10 mg protein.
† = 0·4 ml of 0·3 M solution.
‡ 0·4 ml of 5×10^{-3} M solution. Adenosine 5-diphosphate, barium salt (Sigma, St. Louis, U.S.A.) NADH (Sigma) disodium salt, grade III. Sodium salicylate (Merck, Rahway, U.S.A.)

activity of ADH measured by the reduction of pyruvate in the presence of ammonium chloride. These results are shown in Fig. 1 and indicate that salicylate dissociates the molecule of GDH into smaller units, a mechanism similar to that reported for other compounds. ADP was able to stimulate the GDH activity of GDH and prevents the dissociation of its molecule.[9] In some experiments summarized in Table 2 ADP reverse the effect of salicylate.

However, salicylate injected intraperitoneally causes a marked increase of the GDH activity. These results are shown in Fig. 2. This observation suggests that salicylate *in vivo* may have an effect on the enzymatic complex in the cell without any dissociation of its molecule. As expected, in this case there is no ADH activity.

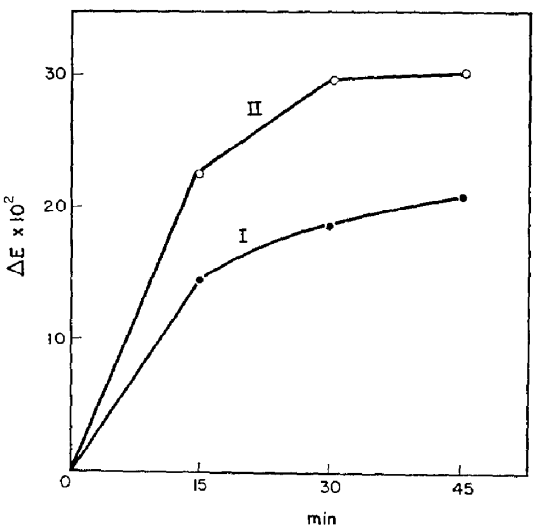

FIG. 2. *In vivo* experiments of the effect of sodium salicylate injected intraperitoneally with 0·4 ml of a 0·320 mM of the drug. Each point correspond to four mice weighing 20 g ± 1·0 (36 mice). GDH measured as reported in Fig. 1. I = increase in the activity of GDH after 15, 30 and 45 min of the injection. II. = The same measured after the extract was freezed for 24 hr and thawed just before the assay.

We can conclude that sodium salicylate promotes the dissociation of GDH in cell-free extracts of brain with a concomittant increase of the ADH activity. On the other hand, salicylate *in vivo* stimulates the GDH by a mechanism not yet explained.

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Experimental errors resulting from uptake of lipophilic drugs by soft plastic materials

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FORREST *et al.*[1] reported a few years ago that soft plastic containers adsorb measurable quantities of chlorpromazine and suggested the use of the plastic material, nalgene. Although more observations of this kind have become known, too little is known about the extent of uptake by plastic materials and the pitfalls of experimental work with highly lipophilic drugs.

A study on the metabolism of imipramine in the isolated perfused rat liver was initiated with a perfusion apparatus previously used in our laboratory.[2] In preliminary experiments the livers were perfused with 10-^{14}C-imipramine. The label in this position is known not to be eliminated as $^{14}CO_2$.[3] After perfusion the recovery of ^{14}C-activity in the total of the three compartments (perfusion medium, liver, bile) amounted to 5 to 10 per cent only. Experiments, summarized in Table 1, showed that the

TABLE 1. UPTAKE OF LIPOPHILIC DRUGS BY PLASTIC MATERIALS

			% ^{14}C-IP†		% ^{3}H-CTP‡	
			(15 min)	(60 min)	(30 min)	(120 min)
Silicone rubber	(tube) 250 · 2·5 mm	A*	98	99	96	98
PVC	(tube) 250 · 2·0 mm	A	98	99	97	99
Rubber	(tube) 250 · 2·5 mm	A			93	96
Silicone rubber	(tube) 200 · 5·0 mm	B		30		
Teflon	(tube) 200 · 5·0 mm	B		3		
Teflon	(tube) 300 · 2·0 mm	B		0		
Nylon	(filter) 1 cm^2	C		2		

* A: ring-shaped tube in roller pump, 4 ml.
B: ring-shaped tube on rotating disc, 3–5 ml.
C: agitated in 5 ml.
† imipramine
‡ clothiapine, 2-chloro-11(4-methyl-1-piperazinyl)-dibenzo [b,f][1,4] thiazepine.
The two silicone rubber tubes are from different manufacturers.

remaining imipramine could have been rapidly taken up by the silicone rubber tubing used in the perfusion apparatus. The uptake was increased to near-total if the silicone rubber was treated with a roller pump as in the perfusion apparatus. Similar results were obtained with the lipophilic drug, clothiapine, and with the soft plastic, polyvinylchloride, or rubber, but not with the hard plastic, teflon. Based on these results the perfusion apparatus was modified in the following way: The roller