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SHORT COMMUNICATIONS

On the effect of salicylate on incorporation of acetyl-CoA and malonyl-CoA into fatty acids and unsaponified compounds

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OUR PREVIOUS investigation[1] showed that salicylate inhibits the incorporation of [1-^{14}C]-acetate into cholesterol and fatty acids but has no effect on the incorporation of [2-^{14}C]-mevalonate into cholesterol by the supernatant fraction of rat liver homogenate. This suggests that the inhibitory effect of salicylate on the incorporation of acetate into cholesterol and fatty acids is probably related to the inhibition of acetyl-CoA carboxylase. To test this suggestion, the effect of salicylate on biosynthesis of cholesterol and fatty acids from [1-^{14}C]-acetyl-CoA and [2-^{14}C]-malonyl-CoA has been studied.

Methods and materials

The incorporation of [1-^{14}C]-acetyl-CoA and [2-^{14}C]-malonyl-CoA into cholesterol (unsaponified fraction) and fatty acids (saponified fraction) was studied by using 700 g supernatant fraction of rat liver homogenate. In each experiment the same homogenate was used for the study of incorporation both of acetyl-CoA and of malonyl-CoA.

The reaction mixture contained 2 ml of homogenate (70–80 mg of protein); NADPH, 3·5 μmoles; NADH, 3·5 μmoles; [1-^{14}C]-acetyl-CoA, 0·05 μmoles (0·5μc) or [2-^{14}C]-malonyl-CoA, 0·1 μmoles (0·5μc). $KHCO_3$ (3 mg) was added to the flasks containing the labelled acetyl-CoA and unlabelled acetyl-CoA (0·05 μmoles) to the flasks containing the labelled malonyl-CoA. Sodium salicylate was used at a concentration of 10^{-2} M. The total volume of mixture was 2·5 ml. It was incubated for 1 hr at 37° in a shaker. Other details of the method and technique used for the extraction of labelled unsaponified compounds and fatty acids were described earlier.[2] The radioactivity was measured with the help of T-25-BFL counter (USSR).

[1-^{14}C]-acetyl-CoA was received from New England Nuclear Corp. [2-^{14}C]-malonyl-CoA was synthesized from [2-^{14}C]-malonic acid and coenzyme A by the method of Trams and Brady[3] and purified after the method of Trams and Brady.[3]

Results and discussion

The data presented in the table show that salicylate at a concentration of 10^{-2} M almost completely inhibits the incorporation of [1-^{14}C]-acetyl-CoA into unsaponified fraction and strongly inhibits (by 65–79 per cent) that of this compound into fatty acids. However, the incorporation of [2-^{14}C]-malonyl-CoA into unsaponified fraction and fatty acids is not inhibited by salicylate. These results confirm the suggestion[1,4] that salicylate inhibits the carboxylation of acetyl-CoA; in other words, salicylate may be considered as an inhibitor of acetyl-CoA carboxylase.

These experiments also show that the incorporation of malonyl-CoA into fatty acids is much greater than into unsaponified compounds whereas acetyl-CoA is a better substrate for sterol synthesis than for fatty acids synthesis. This indicates that synthesis of cholesterol from acetyl-CoA is the main pathway. At the same time, the data obtained show that malonyl-CoA may be converted into sterol as shown earlier.[5–7] This conversion was not inhibited by salicylate.

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REFERENCES

1. A. N. KLIMOV, O. K. DOKUSOVA and E. D. POLIAKOVA, *Biochem. Pharmac.* **18**, 1251 (1969).
2. A. N. KLIMOV, E. D. POLIAKOVA, A. L. REMIZOV and L. A. PETROVA, *Vopr. med. Khimii* (*Moscow*) **11**, 101 (1965).
3. E. G. TRAMS and R. O. BRADY, *J. Am. chem. Soc.* **82**, 2972 (1960).

TABLE 1. EFFECT OF SALICYLATE (10^{-2} M) ON THE INCORPORATION OF [1-^{14}C]-ACETYL-CoA AND [2-^{14}C]-MALONYL-CoA INTO UNSAPONIFIED AND SAPONIFIED FRACTIONS

Substrate	No.	Condition	Unsaponified fraction (sterols) (counts/min/g of liver)	(% of inhibition)	Saponified fraction (fatty acids) (counts/min/g of liver)	(% of inhibition)
1-^{14}C-acetyl-CoA	1	control	4860	—	1300	—
		salicylate	200	96	290	78
	2	control	12,350	—	1590	—
		salicylate	120	99	340	79
	3	control	39,690	—	4050	—
		salicylate	170	99·6	1430	65
2-^{14}C-malonyl-CoA	1	control	8120	no inhibition	243,850	no inhibition
		salicylate	9850		300,440	
	2	control	9480		—	
		salicylate	15,040		—	
	3	control	14,460		348,670	
		salicylate	15,360		341,150	

4. P. GOLDMAN, *Biochem. Pharmac.* **16**, 47 (1967).
5. J. D. BRODIE, G. WASSON and J. W. PORTER, *J. biol. Chem.* **238**, 1294 (1963).
6. A. ICHIHARA, E. KOYAMA and Y. TAKEDA, *J. Biochem.* (*Tokyo*) **58**, 480 (1965).
7. H. RUDNEY, P. R. STEWART, P. W. MAJERUS and P. R. VAGELOS, *J. biol. Chem.* **241**, 1226 (1966).

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Ethanol binding to hepatic microsomes—Its increase by ethanol consumption*

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RECENT studies have established that, in addition to its direct hepatotoxicity, ethanol shares several properties with drugs such as phenobarbital.[1–3] Chronic administration of ethanol increases the following in the liver: (1) smooth endoplasmic reticulum; (2) microsomal protein (in male rats); (3) activities of microsomal drug-metabolizing enzymes; and (4) cholesterol biosynthesis.[4,5] Moreover, a hepatic microsomal system which oxidizes ethanol has been described.[6] For drugs to be oxidized by microsomes, it is generally thought that they must first bind to hemoprotein.[7,8] To determine whether ethanol shares with other drugs this property of binding to microsomal hemoprotein, we investigated the effect on ethanol of the spectral characteristics of hepatic microsomes.

Female Sprague–Dawley rats, weighing 150–200 g, were fed an adequate diet in liquid form[9] for 24 days. Pair-fed littermaters were given a similar diet in which ethanol, isocalorically substituted for carbohydrate, provided 36 per cent of total calories. With this regimen, blood ethanol concentrations, measured according to Bonnichsen,[10] in specimens obtained during periods of random access to ethanol, reached an average of 70 mg/100 ml and rarely exceeded 100 mg/100 ml. The rats were decapitated, livers were homogenized in isotonic KCl, and hepatic microsomes free from hemoglobin were prepared and suspended in 0·1 M phosphate buffer at a concentration of 3 mg of microsomal

TABLE 1. EFFECT OF CHRONIC ETHANOL PRETREATMENT ON THE BINDING OF CO, ETHYL ISOCYANIDE AND ETHANOL TO HEPATIC MICROSOMES

Rats*	CO difference spectra† ($\times 10^3$)	Ethyl isocyanide difference spectra 430 peak‡ ($\times 10^3$)	Ethyl isocyanide difference spectra 455 peak§ ($\times 10^3$)	Ethanol difference spectra‖ ($\times 10^2$)
Control	71·4 ± 3·7¶	49·8 ± 4·6	41·7 ± 2·7	0·238 ± 0·024
Ethanol-treated	129·8 ± 7·2	92·7 ± 6·5	71·2 ± 4·6	0·719 ± 0·065
	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

* Ethanol-treated rats were fed ethanol for 24 days. Control rats were fed a similar diet except that carbohydrate isocalorically replaced ethanol.
† Δ O.D. 450–500 nm/mg microsomal protein.
‡ Δ O.D. 430–500 nm/mg microsomal protein.
§ Δ O.D. 455–500 nm/mg microsomal protein.
‖ Ethanol concentration, 100 mM; Δ O.D. 415–500 nm/mg of microsomal protein.
¶ Mean ± standard error.

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