Activation of Insect Cholinesterase by *n*-Butanol, Autolysis, and Surfactants[1]

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Several workers have observed the enhancement of insect cholinesterase (ChE) activity by (1) water-miscible organic solvents, (2) autolysis, and (3) surfactants (e.g., sodium taurocholate and Triton X-100) (Metcalf and March 1950, Colhoun 1961, Dauterman et al. 1962, Shatoury 1963, Edwards and Gomez 1966, Lewis 1967, Krysan and Chadwick 1970, Krysan and Kruckeberg 1970). These same agents can solubilize insect ChE (Edwards and Gomez 1966, Krysan and Chadwick 1970, Krysan and Kruckeberg 1970). It has been concluded that all 3 of these treatments increase ChE activity by the same mechanism (Edwards and Gomez 1966, Lewis 1967) and further that activation is related to solubilization (Edwards and Gomez 1966). We record here ChE observations of the house fly, *Musca domestica* L.; the mayfly *Hexagenia bilineata* (Say); and the honey bee, *Apis mellifera* L., which show that the several phenomena cannot be explained by a single mechanism. Enzyme extraction conditions and ChE activity-measurement methods have been described (Krysan and Chadwick 1962, 1970). During autolysis, a drop of toluene was added to retard bacterial action. Each datum is the mean of 3 independent measurements.

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Table 1.—Activation of house fly ChE by *n*-butanol and sodium taurocholate.

Enzyme fraction	% activation: Sodium taurocholate	% activation: *n*-butanol
Soluble (105,000xg, 1 h)	0	48
Particulate (105,000xg, 1 h)	22	49
Autolyzed (96 h at 25°C) whole homogenate	0	—
Sephadex chromatographed	0	51

Table 2.—Effect of autolysis on the activation of mayfly ChE by *n*-butanol.

	% *n*-butanol	Activity (μmoles/0.3 g mayfly/hr)	% activation	Range
Freshly prepared homogenates	0	44.1	—	42.8–45.1
	1	66.2	+51	65.2–68.2
Homogenates incubated at 10°C for 24 h	0	45.8	—	45.1–46.0
	1	67.4	+48	65.7–68.3
Homogenates incubated at 25°C for 24 h	0	50.6	—	48.4–53.6
	1	76.0	+50	74.3–78.8

Table 1 summarizes the effect of *n*-butanol (3%) and sodium taurocholate (0.4%) on several preparations of house fly head ChE. The fact that sodium taurocholate activated the particulate fraction but did not activate the autolyzed whole homogenate is provocative. Autolysis of house fly homogenates results in considerable solubilization (Krysan and Chadwick 1970) and occasionally some activation. However, lack of reproducible autolytic activation precluded its systematic study in house fly preparations. We found, however, that 24-h autolysis of honey bee and mayfly homogenates led to highly reproducible levels of activation, and we therefore studied the activation effect of autolysis and *n*-butanol on enzyme from those sources. Tables 2 and 3 summarize the results. Butanol activates the autolyzed and nonautolyzed preparations to the same extent. Sodium taurocholate effects could not be studied with the ChE of the mayfly and honey bee, because it reduced the enzyme activity (Krysan and Norris, unpublished data), perhaps by denaturation (reviewed by Tanford 1968).

The observations presented here, taken in conjunction with other published results, can be summarized as follows: Sodium taurocholate and *n*-butanol do not activate house fly ChE by the same mechanism, because sodium

Table 3.—Effect of autolysis on activation of honey bee head ChE by *n*-butanol.

Homogenate	% *n*-butanol	Activity (μmoles/head/hr)	% activation	Range
Freshly prepared homogenate	0	9.0	—	8.9– 9.0
	3	12.6	39	11.9–12.9
Homogenate incubated at 10°C for 24 h	0	10.2	—	10.1–10.4
	3	14.1	38	13.8–14.3

taurocholate can activate only the particulate fraction; *n*-butanol activates, to the same extent, particulate, soluble, and Sephadex-chromatographed enzyme. Suggestions that *n*-butanol activates by solubilizing the enzyme are not compatible with our results, since the Sephadex-chromatographed enzyme, which is unquestionably soluble (Krysan and Chadwick 1966), is activated to the same extent as is the insoluble ChE. Furthermore, Randall[5] obtained evidence that with house fly head ChE *n*-butanol may activate by enhancing the deacetylation stop in the hydrolysis sequence.

If the activation caused by *n*-butanol and autolysis is due to the same mechanism, one would expect the 2 treatments to draw on the same pool of inactive enzyme, and it would follow logically that the degree of activation by *n*-butanol ought to be less with homogenates already activated by autolysis. On the contrary, the *n*-butanol activation of autolyzed and nonautolyzed homogenates is the same (Table 2 and 3), and therefore we conclude that *n*-butanol does not activate by the same means as does autolysis.

If we take the observations on the 3 species together, the activation effects of sodium taurocholate and autolysis are clearly different from that of *n*-butanol. The observation that sodium taurocholate could not activate autolyzed house fly ChE (Table 1) suggests that sodium taurocholate and autolysis draw on the same latent pool of ChE. Autolysis can solubilize the mayfly and the honey bee ChE (Krysan and Kruckeberg 1970), but whether such solubilization in these cases is related to activation is unknown.

We conclude (1) that activation of insect ChE by *n*-butanol, sodium taurocholate, and autolysis cannot all be explained by a single mechanism, (2) that autolysis and sodium taurocholate effects may be related, and (3) that activation and solubilization of ChE by *n*-butanol are not necessarily related events and therefore that activation by *n*-butanol probably is due to an effect on the hydrolytic event itself.

[5] R. F. Randall. 1970. House fly head (*Musca domestica* L.). Acetylcholinesterase activation by *n*-butanol. Unpublished Ph.D. thesis, University of Illinois, Urbana. 60 p.

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