conscious selection may be carried on.

The findings present an interesting picture of reproduction of long-day, temperature zone plants introduced to the tropics. It is shown that low night temperatures, or changes of temperature associated with high elevations, constitute a mechanism for inducing flowering. The ability of such plants to persist is, therefore, apparent (*5*).

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References and Notes

1. F. W. Went, *The Experimental Control of Plant Growth* (Chronica Botanica, Waltham, Mass., 1957), p. 343.
2. A. C. Leopold, *Quart. Rev. Biol.* **26**, 247 (1951).
3. R. H. Roberts and B. E. Struckmeyer, *J. Agr. Research* **56**, 633 (1938); **59**, 699 (1939).
4. W. M. Hiesey. *Am. J. Botany* **40**, 205 (1953).
5. This study was supported, in part, by Western Regional Technical Committee W-58. Published with the approval of the director, Hawaii Agricultural Experiment Station, as miscellaneous paper No. 113.

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Mammalian Liver β-Glucuronidase for Hydrolysis of Steroidal Conjugates

Abstract. Although the rate of hydrolysis by mammalian β-glucuronidase appears to be inhibited by methylene chloride or carbon tetrachloride with the standard technique (phenolphthalein glucuronide as a substrate), the release of steroidal conjugates under conditions generally employed does not appear to be affected.

Gautney *et al.* (*1*) have recently described the potentiating effect of several organic solvents (aromatic hydrocarbons, chlorinated aliphatic hydrocarbons, aliphatic alcohols) on bacterial β-glucuronidase, thus extending an earlier report concerning chloroform (*2*). The authors, however, found that dichloromethane and chloroform inhibit the hydrolysis of phenolphthalein glucuronide by mammalian liver enzyme. This raises the question whether mammalian liver β-glucuronidase is suitable for hydrolysis of steroidal glucuronides in biological fluids which have previously been extracted with such solvents.

Table 1. Effect of solvents on hydrolysis by liver β-glucuronidase of various glucuronides.

Enzyme units/ml	Control (No solvent)	Dichloromethane (% Aglycon)	Carbon Tetrachloride (% Aglycon)
	Phenolphthalein		
6.25	2.2	1.0	2.8
12.5	5.4	2.2	4.9
25.0	15.0	4.7	12.7
50.0	32.0	9.7	30.0
250.0	69.0	54.0	71.5
1000.0	100.0	98.0	100.0
	17-Ketosteroids		
10.0	16.0	9.4	10.7
100.0	45.2	43.3	41.0
500.0	100.0	96.0	94.0
	Corticoids		
10.0	16.0	14.0	13.3
100.0	58.5	67.5	62.3
250.0	85.5	81.3	81.0
500.0	100.0	99.5	100.0

Previous studies in our laboratory had demonstrated quantitative release of the free steroid when pregnane-3α, 17α, 21-triol-11, 20-dione monoglucuronide, added to human plasma previously extracted with dichloromethane, was incubated with 300 to 500 Fishman units of mammalian β-glucuronidase per milliliter (*3*). In view of the implications of Gautney's (*1*) results, this matter was reinvestigated.

A pool of human urine was divided into aliquots of 10 ml, and duplicates were equilibrated with either dichloromethane or carbon tetrachloride. Thereafter the urine was incubated with beef liver β-glucuronidase (*4*) in doses of 10 to 500 Fishman units per milliliter at *p*H 4.5, 37°C for 48 hours. The 17-ketosteroids were extracted and measured as reported elsewhere (*5*), and the urinary corticoids were determined by the method of Silber and Porter (*6*). In addition, the mammalian enzyme was studied by use of phenolphthalein glucuronide as substrate after the manner of Talalay *et al.* (*7*), with the addition of 0.1 ml of the organic solvent to replicate digestion mixtures employed by Gautney (*1*) in his investigation.

The results (Table 1) are expressed as percentage of maximal hydrolysis achieved with each substrate. In agreement with Gautney *et al.* (*1*), the solvents inhibited hydrolysis of phenolphthalein glucuronide by the low concentrations of enzyme often employed. On the other hand, under the conditions of incubation with high enzyme concentrations for 48 hours, as generally described for hydrolysis of steroid conjugates (*3, 8*), no significant inhibition was apparent. At lower concentration of enzyme the solvents only slightly reduced the partial hydrolysis of steroid glucuronides. These results do not support the assertion by Gautney *et al.* (*1*) that such solvents must not be used for prior extraction if liver β-glucuronidase is to be employed for subsequent hydrolysis of steroid glucuronides.

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References

1. M. C. Gautney, S. B. Barker, S. R. Hill, Jr., *Science* **129**, 1281 (1958).
2. Sigma Chemical Co., "Urgent bulletin re bacterial glucuronidase" (St. Louis, Mo., Nov. 1958).
3. A. M. Bongiovanni and W. R. Eberlein, *Proc. Soc. Exptl. Biol. Med.* **89**, 281 (1955).
4. Ketodase (Warner-Chilcott).
5. A. M. Bongiovanni, W. R. Eberlein, P. Z. Thomas, *J. Clin. Endocrinol. and Metabolism* **17**, 331 (1957).
6. R. H. Silber and C. C. Porter, in *Methods of Biochemical Analysis*, D. Glick, Ed. (Interscience, New York, 1957), vol. 4, p. 139.
7. P. Talalay, W. H. Fishman, C. Huggins, *J. Biol. Chem.* **166**, 757 (1946).
8. D. K. Fukushima and T. F. Gallagher, *J. Clin. Endocrinol. and Metabolism* **18**, 694 (1958); A. M. Bongiovanni and W. R. Eberlein, *Anal. Chem.* **30**, 338 (1958); E. M. Glenn and D. H. Nelson, *J. Clin. Endocrinol. and Metabolism* **13**, 911 (1953).

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Glucuronidase Activation: Enzyme Action at an Interface

Abstract. The potentiating action of chloroform on bacterial β-glucuronidase has been shown to increase as the interface area between the two liquid phases increases. Prior extraction of the enzyme with chloroform causes a loss rather than an increase in activity. It is tentatively suggested that the correlation between activity and interface area may reflect a phenomenon of enzyme action at a liquid/liquid interface.

In an investigation of the "paradoxical" effect of organic solvents on the activity of animal and bacterial β-glucuronidase, which was first observed with chloroform (*1*), and more recently with other solvents by Gautney, Barker, and Hill (*2*), we have also observed an activation of the bacterial enzyme (*3*) varying in degree with the solvent used and a similar inactivation of the animal enzyme (*4*). We have further noted that the activation of the bacterial enzyme seems not to be due to the removal of an inhibitor, since prior extraction of the enzyme solution by shaking with one volume of chloroform for 5 minutes causes a 40 percent loss rather than an increase in activity. An activation such as that noted by Bernfeld, Jacobsen, and Bernfeld (*5*) has been ruled out by the work of Gautney, Barker, and Hill. Moreover, the continuous removal of phenolphthalein from the reaction site cannot account for the inverse effect on the animal and bacterial enzymes. These facts suggest that some physicochemical property of these enzymes is involved in the activation by solvents. The preliminary results reported here relate to this problem.

The assay method used in this study is that suggested by the Sigma Chemical