One uninfected subject failed to react to the polysaccharide either before or after the intravenous administration of horse antiserum. Two weeks later the reaction was still negative. She was then given anti-*H. influenzae* rabbit antiserum intravenously. Subsequent intracutaneous injection of the polysaccharide gave a marked immediate reaction. All patients prior to serotherapy, and all uninfected controls in the same age groups failed to react to the test. It has been demonstrated[8] that the blood of normal adults contains bactericidal antibodies for this organism. Ten such normal adults showed negative skin reactions to the polysaccharide. Further work is necessary to determine adequately the specificity and sensitivity of this reaction.

Summary. Positive immediate skin reactions to the specific polysaccharide may be obtained in patients with influenza bacillus meningitis and in normal individuals following the administration of anti-*H. influenzae* rabbit serum in amounts sufficient to establish an excess antibody concentration in the blood. The test is proposed for further investigation as a simple clinical method of evaluating the adequacy of serotherapy.

The authors gratefully acknowledge the suggestions and encouragement of Drs. J. A. V. Davies and L. D. Fothergill.

11880 P

Toxicity and Fate of the Lethal Effect of Theophyllinated Scillaridin* in the Cat.

ARTHUR C. DEGRAFF AND ROBERT A. LEHMAN.

From the Department of Therapeutics, New York University, College of Medicine.

Riseman and London[1] have recently described some of the properties of a substance which is obtained by condensing a disodium salt of a mixture of genins isolated from Squills (presumably scillaridin A and B) with 2 molecules of theophylline. They demonstrated by electrocardiographic studies that the substance so obtained differs in its action from either a mechanical mixture of theophylline with the genins, or from the original glycosides, thus giving physio-

[8] Fothergill, L. D., and Wright, J., *J. Immunol.*, 1933, **24**, 273.

* Supplied by Parker Dorn, Inc., Worcester, Mass.

[1] Riseman, J. E. F., and London, S. B., *Science*, 1940, **92**, 384.

logical confirmation for a discrete compound. Since this appears to be the first report of an attempt to modify the action of a cardiac glycoside by chemical combination with a xanthine derivative, it seemed to be of considerable interest to determine the toxicity of the substance and the fate of the lethal effect of sublethal doses in the cat. The ratio of genins present in the compound has not been determined, but the empirical formula may be approximated as $C_{24}H_{28}O_3 \cdot (C_7H_7O_2N_4)_2 \cdot 2H_2O$. It should be pointed out however, that by the method of preparation used, structural rearrangement of the genin may well have taken place. The influence of structure on physiological action is discussed further by Lendle.[2]

The preparation was assayed by the original Hatcher and Brody procedure using a 1:714 solution in normal saline and a rate of infusion such that the animals died within 30 to 90 minutes. The geometric mean lethal dose on the basis of 10 cats was found to be 21.4 mg per kg (or 28.2 micromoles per kg) and the standard deviation was 4.2. By comparison, the lethal doses[3] of Scillaridin A and of Scillarin A are respectively 0.150 and 1.72 mg per kg (0.217 and 4.73 micromoles per kg). The toxicity of the substance is thus much less than that of two of the substances related to it chemically. Calculation of the lethal doses in micromoles per kg shows that this decrease in toxicity cannot be explained on the basis of difference in molecular weight.

The fate of the lethal effect of sublethal doses was determined by the procedure described previously.[4] A preliminary injection of 13 mg per kg (60% of the lethal dose) of theophyllinated Scillaridin (1.3%) was given intravenously to each of 31 cats. These were divided into 3 approximately equal groups and sacrificed as follows: the first group was killed 3 hours after injection by an infusion of ouabain (1:200,000) at one to 2 cc per minute. The second group was also killed 3 hours after injection, but with the preparation itself (1:714) and the third group received the theophyllinated Scillaridin (1:714) infusion 24 hours after the initial injection. The results are given in the table. The geometric mean lethal dose of ouabain was previously reported to be 0.10 mg per kg.[4]

It will be seen from the table that 3 hours after administration

[2] Lendle, L., *Handbuch der Experimentellen Pharmakologie*, supplementary v. 1, Springer, Berlin, 1935, p. 77.

[3] Chen, K. K., Robbins, E. B., and Worth, H., *J. Am. Pharm. Assn.*, 1938, **27**, 189.

[4] DeGraff, A. C., and Lehman, R. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 323.

TABLE I.
Dissipation of the Lethal Effect After 3 and 24 Hours.

Hrs	No. of animals	Mean fraction of lethal dose required in final infusion	σ	P	% dissipation of initial dose
3	10	0.76 (Ouabain)	0.139	<0.01	60
3	9	0.79 (Theophyllinated Scillaridin)	0.208	<0.05	65
24	12	0.77 (Theophyllinated Scillaridin)	0.112	<0.01	62

of 60% of a lethal dose of the new compound, the lethal dose of ouabain has fallen to an average of 76% of its original value. Essentially the same result was obtained when theophyllinated Scillaridin was used after 3 and 24 hours, that is, 79 and 77% of the mean lethal dose respectively was required. The significance of the differences between these mean values and the means for the assays of ouabain and theophyllinated Scillaridin was determined by calculating the statistics s, t, and P according to Fisher's notation.[5]

Thus, P in the table gives the probability that the assay values obtained with and without premedication are drawn from the same population. P is found to lie well below 0.05 and therefore, the means listed in the table may be said to differ significantly from the corresponding mean results of bioassay. In the last column of the table the data have been calculated in terms of the initial dose and are expressed as percent dissipation as follows:

$$\frac{100\ (0.60 + \text{final dose} - 1)}{0.60}$$

where the final dose is expressed as a fraction of the Hatcher and Brody lethal dose.

Since the same percentage dissipation was found for theophyllinated Scillaridin when the titration was done with ouabain as with the drug itself, it is probable that the lethal action of these two drugs is qualitatively similar. It may also be concluded by comparison with the results on the lanata glycosides[4] that the rate of dissipation is relatively rapid, but does not resemble that of digoxigenin as might have been expected. It would seem, then, that substitution of xanthine for carbohydrate in the squills glycoside molecule lowers the toxicity but does not remove the so-called "cumulative" property.

[5] Fisher, R. A., *Statistical Methods for Research Workers*, Oliver and Boyd, London, 7th ed., 1938, p. 128.