volume were unchanged during absorption of erythrocyte antibodies and purification. The final preparation was electrophoretically pure, gave a single 6.76 peak on the ultracentrifuge, and was structurally unaltered as evidenced by *in vivo* traces studies, and immunoelectrophoretic analysis. *In vivo* toxicity did not occur during long term intravenous administration of the purified preparation. The resultant lymphopenia was equal to that achieved with whole serum containing two times the gamma globulin and 7 times the total proteins.

1. Abaza, H. M., Nolan, B., Watt, J. G., Woodruff, F. A., Transplantation, 1966, v4, 618.

2. Monaco, A. P., Wood, M. L., Gray, J. G., Russell, P. S., J. Immunol., 1966, v96, 229.

3. Sacks, J. H., Filippone, D. R., Hume, D. M., Transplantation, 1964, v2, 60.

4. Huntley, R. T., Iwasaki, Y., Marchioro, T. L., Jeejeebhoy, H., Porter, K. A., Starzl, T. E., Surg., Forum, 1966, v17, 230.

5. Iwasaki, Y., Porter, K. A., Amend, J. R., Marchioro, T. L., Zühlke, V., Starzl, T. E., Surg. Gynec. & Obstet., 1967, v124, 1.

6. Monaco, A. P., Abbott, W. M., Othersen, H. B., Simmons, R. L., Wood, M. L., Flax, M. H., Science, 1966, v153, 1264.

7. Dixon, F. J., Harvey Lectures, 1962-1963, p21, Series 58.

8. Baumstark, J. S., Laffin, R. J., Bardawil, W. A., Arch. Biochem. & Biophys., 1964, v108, 514.

9. James, K., Stanworth, D. R., J. Chromat., 1964, v15, 324.

10. Ellis, F. G., Surgery, 1966, v60, 1251.

11. Walford, R. L., Gallagher, R., Troup, G. M., Transplantation, 1965, v3, 387.

12. Hildemann, W. H., Transpl. Bull., 1957, v4, 148.

13. Perper, R. J., Najarian, J. S., Transplantation, in press.

14. Kabat E. A., Mayer, M. M., Experimental Immunochemistry, 2 ed., C. C Thomas, Springfield, Ill., 1966, p762.

15. Najarian, J. S., Perper, R. J., Surgery, in press.

16. Rockey, J. H., Klinman, N. R., Karush, F., J. Exp. Med., 1964, v120, 589.

17. Cohn, E. J., Chem. Rev., 1941, v28, 395.

18. Klinman, N. R., Rockey, J. H., Frauengerger, G., Karush, F., J. Immunol., 1966, v96, 587.

19. Gray, J. G., Monaco, A. P., Wood, M. L., Russell, P. S., ibid., 1966, v96, 217.

Received February 17, 1967. P.S.E.B.M., 1967, v125.

## Antagonism of Intravenous Digitoxigenin Lethality by Reserpine Pretreatment in the Mouse.* (32151)

G. L. LAGE AND J. L. SPRATT† (Introduced by G. L. Plaa)

*Department of Pharmacology, College of Medicine, University of Iowa, Iowa City*

Dick *et al*(1) reported increased arrhythmias from digitalis in patients receiving reserpine therapy. Roberts *et al*(2) reported that reserpine treatment increased the amount of acetylstrophanthin required to produce ventricular arrhythmias in the dog. On the other hand, Nash *et al*(3) reported that ouabain lethality was decreased with reserpine pretreatment in rats. Takagi *et al*(4) also reported decreased lethality of digoxin with reserpine pretreatment in dogs. From these various reports, it would appear that although reserpine may increase the possibility of arrhythmias induced by digitalis glycosides it also decreases lethal potential of the glycosides.

Considering the known central and peripheral effects of reserpine, the current study was therefore undertaken to investigate possible effects of reserpine on the lethality of digitoxigenin. The latter compound was selected since it is a potent convulsant and was the most toxic of various structurally-related compounds when intravenously administered to the mouse(5).

*Methods.* Crystalline digitoxigenin was obtained from Lachat Chemicals, Inc., Chicago, Ill., and was dissolved in a final solution of 47.5% ethanol in 0.9% saline. Solubilized

* Supported by USPHS Research Grant GM-09784 and Training Grant 5T1-GM-141.

† Research Career Development Awardee of US PHS (GM-K3-5271) and Markle Scholar in Academic Medicine.

reserpine, supplied as Serpasil in water for injection, was supplied by Ciba Pharmaceutical Co., Summit, N. J. Norepinephrine was freshly prepared in 0.9% saline as a 100 μg per ml solution.

Adult male Swiss-Webster mice, weighing 20-30 g, were maintained in local animal facilities for at least 4 days prior to use. Wayne Lab Blox and tap water were allowed *ad libitum* both before and after injection and each animal was used only once. All agents except norepinephrine were injected in a dose volume of 2 ml/kg. Digitoxigenin and norepinephrine were administered by tail vein, whereas the reserpine was injected subcutaneously in the scruff of the neck. The norepinephrine dose was 100 μg/kg.

At least 3 dose levels of digitoxigenin with at least 20 animals per dose level were used for each comparative study. Control injections with the digitoxigenin vehicle caused only mild depression and any animals with questionable tail vein injections were immediately discarded. All animals used in a given comparison were injected with digitoxigenin at 24 hours following their reserpine or saline pretreatment. When it was necessary to pool data, numbers of animals and the digitoxigenin doses were equally represented (a) during a given 24-hour experimental period and (b) from one 24-hour experimental period to another. All animals were observed for 24 hours following the digitoxigenin injection. Median dose 50 values and potency ratios were computed on the accumulated data using probit analyses as described by Finney (6). Fisher exact probability analysis(7) was performed on the data shown in Table I.

*Results.* Reserpine was administered in varying doses 24 hours prior to a known lethal dose of digitoxigenin (5.75 μmoles/kg). The effect of this pretreatment on the lethality of digitoxigenin is shown in Fig. 1. The reserpine pretreatment had a protective effect on digitoxigenin lethality at the lower dose, reached a peak at 1.25 mg/kg of reserpine and then declined to no protection at 10 mg/kg. Thus 1.25 mg/kg of reserpine was used in further studies of the protective action of this compound on the lethality of digitoxigenin.

TABLE I. Comparison of 24 Hr Adrenalectomy *vs* 24 Hr Reserpine Pretreatment on Digitoxigenin Lethality in the Mouse.

| Treatment | No. dead / No. animals |
|---|---|
| Reserpine pretreatment | 5/20 )* |
| Saline control | 18/20 ] |
| Adrenalectomized | 17/20 ] |
| Sham operated | 19/20 ] |

* Reserpine significantly different from all other treatments at $p < 0.05$. No other significant comparisons.

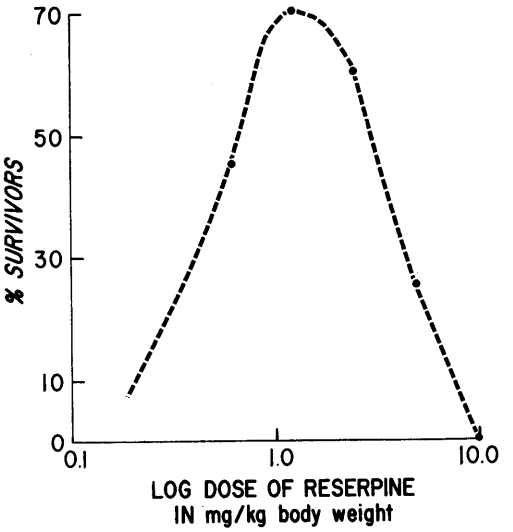


FIG. 1. Effect of 24-hour reserpine pretreatment in antagonizing the 24-hour lethality of digitoxigenin in adult male mice. Curve extrapolated toward zero survival since 100% mortality was obtained when saline replaced the reserpine pretreatment.

Fig. 2 shows the 24-hour $LD_{50}$ values for digitoxigenin with and without reserpine pretreatment. The reserpine significantly increased ($p<0.05$) the $LD_{50}$ approximately 2-fold. It was assumed from these data that a possible explanation for the reserpine protection was the depletion of catecholamines. This was then tested using 2 groups of mice, both pretreated with reserpine. One group received digitoxigenin alone and the other group received intravenous norepinephrine (100 μg/kg) immediately before the digitoxigenin. The 24-hour $LD_{50}$'s are shown in Fig. 3. It can be seen that the intravenous norepinephrine administration did significantly ($p< 0.05$) antagonize the protective effect of reserpine. Although the $LD_{50}$'s are not identi-

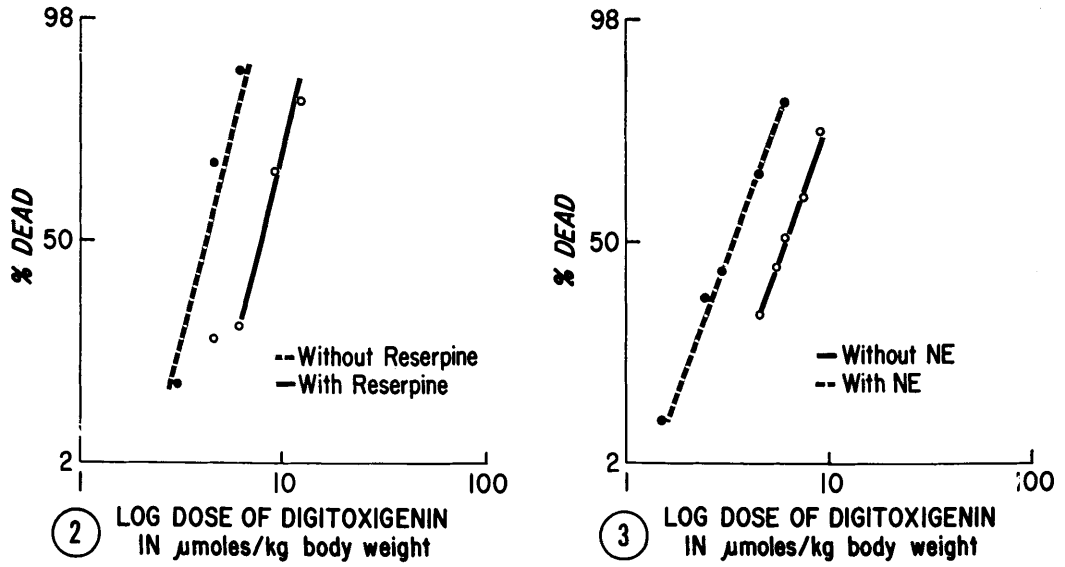


FIG. 2. Mortality produced by intravenous digitoxigenin with and without 24-hour reserpine pretreatment in adult male mice. The $LD_{50}$ without reserpine was 4.0 μmoles/kg with 95% confidence limits of 4.4 and 3.6. The $LD_{50}$ with reserperine was 7.5 μmoles/kg with 95% confidence limits of 8.2 and 6.7. The potency ratio was 1.9 with 95% confidence limits of 2.1 and 1.6.

FIG. 3. Effect of norepinephrine on the reserpine antagonism of digitoxigenin lethality in adult male mice. The $LD_{50}$ with reserpine and with norepinephrine was 3.3 μmoles/kg with 95% confidence limits of 3.7 and 3.0. The $LD_{50}$ with reserpine but without norepinephrine was 6.1 μmoles/kg with 95% confidence limits of 6.7 and 5.5. The potency ratio was 1.8 with 95% confidence limits of 2.1 and 1.6.

cal to those reported in Fig. 2, the potency ratios are approximately the same as those of Fig. 2.

The possibility of synergism between digitoxigenin and norepinephrine was ruled out by another experiment with 2 groups of mice. One group received an intravenous saline injection followed by digitoxigenin, while the other group received intravenous norepinephrine (100 μg/kg) followed by digitoxigenin. Probit analysis resulted in no significant difference between the two $LD_{50}$'s, thereby indicating that this dose of norepinephrine did not alter the lethality of digitoxigenin.

Another question tested was whether the depletion of catecholamines from the adrenal medulla was the primary source of protection. Adrenalectomized mice, sham operated mice, reserpine treated mice (1.25 mg/kg), and mice treated with subcutaneous saline were injected with digitoxigenin (5.75 μmoles/kg) 24 hours later. The results shown in Table I indicate that only reserpine preatreatment significantly ($p<0.05$) altered the lethality of the digitoxigenin.

*Discussion.* As presented in Fig. 1 and 2, it can be seen that 24-hour reserpine pretreatment significantly lowered the lethality of intravenous digitoxigenin. The most effective dose for the antagonism of digitoxigenin lethality was 1.25 mg/kg although there is no obvious reason for the peak reserpine effect at this dosage. The convulsant action of digitoxigenin was also antagonized. All of the animals that died from the digitoxigenin convulsed violently prior to death, whereas survivors did not convulse. Since the mechanism of digitoxigenin convulsions is still subject to discussion(5) these data cannot necessarily be interpreted as indicating a specific central protective effect of reserpine. In this regard, Foerster *et al*(8) did not observe any effect of reserpine on the neurotoxicity of g-strophanthin or cymarin in the mouse.

The antagonism of reserpine's protective effect by the injection of a large dose of norepinephrine immediately prior to the digitoxigenin (Fig. 3) would suggest that the depletion of catecholamines by reserpine may

be responsible for the protection. The studies of Iversen *et al*(9) showing decreased cardiac uptake of $H^3$-norepinephrine in reserpinized rats at 24 hours were performed with a comparable dose of norepinephrine. Likewise, Pokrovskaya(10) found decreased myocardial sensitivity to cardiac glycosides in both intact and decapitated frogs which were treated with reserpine. However, Tanz and Marcus(11) have questionel whether the myocardial catecholamine content necessarily reflects the physiological responses of this organ. In addition, Spann *et al*(12) have more recently noted that depletion of cardiac norepinephrine stores by reserpine did not alter the inotropic response of cat papillary muscles to strophanthidin.

The data of Table I indicate that the depletion of catecholamines from the adrenal medulla cannot suffice as the sole explanation for these observations. The depletion of brain catecholamines by reserpine may explain this protection although other mechanisms cannot yet be disregarded.

The competent technical assistance of Mr. Gene Rostermundt is gratefully acknowledged.

1. Dick, H. L. H., McCawley, E. L., Fisher, W. A., Arch. intern. Med., 1962, v109, 503.

2. Roberts, J., Ito, R., Reilly, J., Cairoli, V. J., Circ. Res., 1963, v13, 149.

3. Nash, C. B., Alley, J. H., Manley, E. S., Toxic. Appl. Pharmac., 1964, v6, 163.

4. Takagi, M., Zanuttini, D., Khalil, E., Bellet, S., Am. J. Cardiol., 1965, v15, 203.

5. Lage, G. L., Spratt, J. L., J. Pharm. Exp. Ther., 1966, v152, 501.

6. Finney, D. J., Probit Analysis, 3rd, ed., Cambridge Univ. Press, London, 1962.

7. Siegel, S., Non-parametric Statistics for the Behavioral Sciences, McGraw-Hill, New York, 1956.

8. Foerster, W., Sziegoleit, W., Guhlke, I., Arch. int. Pharmacodyn. Ther. 1965, v155, 165.

9. Iversen, L. L., Glowinski, J., Axelrod, J., J. Pharm. Exp. Ther., 1965, v150, 173.

10. Pokrovskaya, V. A., Farmakol. i Toksikol., 1965, v28, 665. Abstracted in Chem. Abst. 1966, v64, 10265.

11. Tanz, R. D., Marcus, S. M., Proc. Soc. Exp. Biol. & Med., 1966, v121, 853.

12. Spann, J. F., Sonnenblick,, E. H., Cooper, T., Chidsey, C. A., Willman, V. L., Braunwald, E., Circ. Res., 1966, v19, 326.

Received February 23, 1967. P.S.E.B.M., 1967, v125.

## Antibody Formation at Various Times After Previous Treatment of Mice With Endotoxin. (32152)

HENRY H. FREEDMAN, ALFRED E. FOX, AND BENJAMIN S. SCHWARTZ
*Department of Microbiology, Warner-Lambert Research Institute, Morris Plains, N. J.*

The number of hemolysin-forming spleen cells increases after injection of bacterial endotoxin into normal mice(1). This increase fails to occur in mice previously made endotoxin-tolerant by a series of daily doses, although the response to specific antigen, sheep red blood cells, is unimpaired(2). In addition, endotoxin has an adjuvant effect, elevating the specific immune response when injected together with sheep red blood cells(2). There are reasons for believing that these stimulatory effects of endotoxins on pre-existing hemolysin-forming cells may not be attributable to cross-reacting antigens, but rather to the cytotoxicity of endotoxins with release of stimulatory DNA breakdown products(2-5). Whether cytotoxicity of endotoxins reflects a direct primary toxicity or has an immune basis is presently unresolved(6). The present report extends previous studies on the influence of endotoxin on antibody formation and supports the hypothesis that reactivity to these ubiquitous antigens is conditioned by previous exposure.

*Materials and methods.* Female CD-1 or C57Bl/6 mice, weighing 18-20 g, were used. Protein-containing Boivin type endotoxins (ET) of *Salmonella abortus equi, Salmonella typhosa, Serratia marcescens* and *Escherichia coli* (Difco), and protein-containing and pro-