bral in mice(23) and in rabbits(24). Influenza virus and the other hemagglutinating viruses lend themselves particularly well to this type of investigation, because both, the soluble complement-fixing antigen and the hemagglutinin, can be measured apart from the infectivity as specific phases of viral activity. It is fascinating to speculate upon the possibility that intermediate stages in the reproductive cycle of other viruses may also become recognizable.

Summary. Mouse brain, previously considered as insusceptible to infection with non-neurotropic strains of influenza virus, has been found to support a single cycle of viral reproduction in which hemagglutinin and complement-fixing antigen increase without concomitant rise in infective titer. Rather, the infective titer decreases progressively in mouse brain. The newly formed, incomplete virus is non-infectious, but is produced only if the intracerebral inoculum contains fully infectious virus. The yield of incomplete virus is proportional to the amount of infectious virus inoculated. In mouse brain, as in the allantoic membrane, the constant period preceding a rise in hemagglutinin titer is longer for the Lee than for the PR8 strain. These findings appear to have significance in relation to the "toxicity" of non-neurotropic influenza viruses and to their ability to interfere with unrelated neurotropic viruses, and to the problem of viral adaptation to experimental hosts.

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Absence of Digitalis-like Cardiac Effects in the Action Pattern of Several Simple Lactones.* (17967)

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The cardiac glycosides are currently being subjected to investigative studies aimed at recognition of various relations of chemical structure and biological activity. Interest has centered on the lactone ring moiety and degradation of this component has been described as abolishing the typical digitalis effects(1). Further, various series of the simple lactones have been described from several laboratories as exhibiting positive inotropic effects when tested with cold-blooded hearts(1-10). At the same time, one of these reports by Chen *et al.*(3) presents evidence that a lactone producing digitalis-like effects on frog-heart preparations fails to exhibit such effects when tested on mammalian hearts. This observation made by electrocardiographic recordings in cats has been

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confirmed in the present report and has been extended by the application of a method determining the contractile force changes of mammalian hearts *in situ*. This same procedure of characterization has also been applied to other lactones previously described as exhibiting digitalis-like effects in frog-heart preparations. The essential objective of this study has been a determination of the suitability of the biologic characterization methods which may be used in the study of structure-activity relationships of this drug group.

Method. The contractile force of a segment of the right ventricle in open-chest dog preparations was measured according to procedures previously described(11). Factors influencing such determinations have been recently studied and described(12). Concomitant recordings of arterial pressures and electrocardiograph effects were made as before. Results obtained with lactones of the present study were compared against separate characterization studies which have been made under the same conditions with several cardiac glycosides, sympathomimetic amines, veratrine alkaloids and erythrophleum alkaloids. Each of these groups has been found to exhibit distinct and relatively consistent increments in contractile force when administered in favorable dose ranges. In addition to these observations with an *in situ* preparation, experiments were conducted with a modification of the usual Langendorff-Martin arrangement for coronary perfusion of the isolated rabbit heart.

The lactones studied were β,γ-angelica lactone(2-4); l-ascorbic acid(5,6); Ψ-santonin(1,7); the di-lactone of pulvinic acid(8) and the di-lactone, dicumarol(9).

Procedure and results. In 2 experiments, β,γ-angelica lactone was initially administered in doses of 400 mg/kg by intravenous infusion of a 4% aqueous solution over a period of 30 minutes. There were no effects, or slight depression from which recovery was prompt. Subsequent administration of the same doses and of larger doses produced

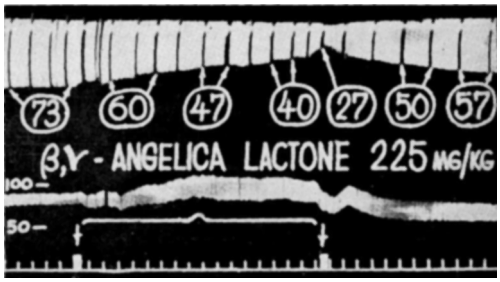

FIG. 1.
Myocardiograph and arterial pressure tracing. Open-chest dog preparation with Cushny myocardiograph levers attached to anterior wall of right ventricle. Encircled figures represent contractile force in grams determined by spring tension necessary to bring lever stroke to near standstill. Downstroke produced by systolic contraction. Time marker in minutes. The animal had previously received 1200 mg/kg of the lactone in 4 instalments over a total experimental period of 5 hours. During this 5th instalment the cardiac depression was sufficiently marked to require the interruption of the usually attempted 30-minute infusion period.

significant depression of the contractile force and, commonly, the 30 minute infusion procedure had to be discontinued because of marked cardiac depression. In each experiment, total amounts of about 1500 mg/kg were administered over periods of 3½ and 5½ hours. (Fig. 1) Virtually complete recovery occurred and the experiments were subsequently terminated by other means. Systemic arterial pressure changes were not marked or constant in direction. Most frequently the pressure was slightly increased, an effect evidently influenced by the volumes of fluid administered. ECG changes throughout suggested those which might have been obtained with any general depressant.

Several injections of aqueous solutions of the lactone into the perfusion fluid of two isolated rabbit heart preparations consistently produced depression with, usually, prompt recovery to approximately control levels. Varying strengths of solution from 0.5 to 4.0% were used, this highest concentration bringing about complete diastolic standstill with recovery occurring after several minutes. This compound was prepared according to the method of Gilmour(13), observed physical constants being in agreement (b.p. 64-66°

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at 15 mm).

L-ascorbic acid was administered in 5% solutions in water by intravenous infusions over periods of 30 minutes. In 3 separate experiments the single doses were 50, 150 and 250 mg/kg respectively. The observations were continued for 3 to 4 hours after the drug was administered. Aside from the fact that the largest dose produced a moderate depression of contractile force (about 20% of that in the control period) there were no significant effects on contractile force, systemic arterial pressure or ECG tracings. It should be noted that the earlier reported cardiotonic actions of these two lactones in frog heart preparations have subsequently been ascribed to the formation of hydrogen peroxide in the solutions used(14-16).

In one experiment Ψ-santonin was administered by intravenous infusion over a period of 30 minutes using a saturated aqueous solution which constituted a dose of about 35 mg/kg. No significant effects were noted. In another experiment, 150 mg/kg in a 20% ethyl alcohol solution was administered in two instalments over a period of 40 minutes. Because of the distinct depression of both systemic arterial pressure and contractile force, the infusion could not be given directly in the usual 30 minute infusion period. The depression was distinctly greater than was obtained from the same amount of ethyl alcohol alone. The condition of the circulation returned to approximately control level 10 minutes after discontinuance of the infusions. No significant ECG changes were noted in either of these 2 experiments. We are obliged to Prof. G. R. Clemo, University of Durham, Newcastle-upon-Tyne, for kindly supplying us with 2 g of this compound.

The di-lactone of pulvinic acid, administered by intravenous infusion to two dogs over a 30-minute period in doses of 20 cc/kg of a saturated aqueous solution (Ringer-Locke), produced no significant changes. Similarly, no significant effects were obtained with doses of 1 and 2 mg/kg administered by immediate intravenous injections of a 1% solution in dioxane. A single injection of 8 mg/kg of the di-lactone of pulvinic acid in the dioxane solution resulted in prompt heart failure. This effect was somewhat greater than that obtained with equivalent volumes of dioxane judged on the basis of a few observations with dioxane alone. In every case, the drug was precipitated as the dioxane entered the saline of the vein cannula system. This di-lactone of pulvinic acid was prepared by the method of Volhard(17) by condensing benzyl cyanide with ethyl oxalate in the presence of metallic sodium to produce oxalyl-bis-benzylcyanide. The oxalyl-bis-benzylcyanide was hydrolysed and condensed to form the di-lactone by heating with three parts of aqueous 60% sulphuric acid. The precipitate was recrystallized from dioxane giving a bright yellow crystalline product agreeing in physical characteristics with recorded data(17).

Dicumarol exhibited no basic similarity to the cardiac glycosides. Unlike the other lactones, however, it did, on occasion, produce marked increments in contractile force of the *in situ* heart preparations. This action has been identified as being due to a general calorigenic action closely related to that produced by dinitrophenol. This study has been reported in abstract(18).

Conclusions. Some relatively simple compounds with the lactone structure have been shown to be lacking in characteristic digitalis-like actions when tested in mammalian heart preparations. Each of these compounds had been previously shown to produce digitalis-like effects in frog heart preparations. The present experiments emphasize the low prediction value of frog-heart preparations in any screening procedure intended for application to mammalian hearts.

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