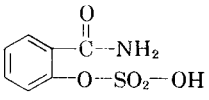
calcium ion, which could explain the halt in calcium stone growth observed in stone-formers when taking salicylamide daily. While the increase in solubilization is small [molar concentrations of salicylamide sulfate were used which approximate those to be expected in urines from the amount of salicylamide (2–3.6 Gm.) ingested by stone-formers on this therapy], it is sufficient to explain the failure of existing stones to accumulate the few mg. of calcium salts per day that ordinarily deposit in the kidneys of stone-forming patients.



I

EXPERIMENTAL

Potassium Salicylamide Sulfate.—Salicylamide (20 Gm., 0.146 mole) was dissolved in 40 ml. of distilled water containing 16 Gm. (0.285 mole) of potassium hydroxide. Finely powdered potassium pyrosulfate (34 Gm., 0.134 mole), previously heated to 350–400°, was added slowly, with stirring. The mixture was stirred for 6 hours at a temperature of 60–70°, and the pH was maintained between 8 and 9 by the addition of saturated potassium hydroxide solution.

The mixture was then shaken with 50 ml. of 95% ethanol and filtered. After cooling, the mixture was treated with 150–200 ml. of ether, with shaking. The aqueous layer was separated and neutralized to pH 7 by the dropwise addition of glacial acetic acid. Precipitated salicylamide was removed, and the filtrate was evaporated to dryness by means of a current of dry air. Absolute ethanol (200 ml.) was added to the residue, and a white, powdery product was filtered, washed with absolute ethanol, and air-dried for a few hours before being stored in a desiccator. The yield was 1.42 Gm. of product which softened at 145–150° and melted at 220–230°.

Anal.[1]—Calcd. for $C_7H_6KNO_5S$: C, 32.93; H, 2.37; S, 12.56. Found: C, 31.70; H, 2.35; S, 12.76.

The compound is extremely soluble in water and insoluble in absolute ethanol. Its aqueous solution has a nearly neutral pH and gave a violet color with ferric chloride T.S. only after hydrolysis with dilute hydrochloric acid. It gave no precipitate with barium chloride solution and dilute hydrochloric acid until the solution was heated for a few minutes. The barium sulfate was isolated, weighed, and the percentage of sulfur calculated. The total sulfur present was thus shown to be sulfate ester.

The compound gave a positive flame test for potassium. It is unstable in air at room temperature. An aqueous solution showed the same faintly fluorescent spot on a paper chromatogram, which did not migrate in the solvent system used, 1-butanol saturated with 3 *N* ammonia, as was obtained in the urines of patients on salicylamide therapy, but not in the absence of salicylamide ingestion. Hydrolysis of the material liberated salicylamide and sulfate ion.

Solubility Determinations.—Test tubes containing 50 ml. of distilled water with excess calcium hydroxide, and either 50 ml. of salicylamide sulfate solution (the appropriate concentration of the potassium salt neutralized with hydrochloric acid), or 50 ml. of sodium chloride solution of the appropriate normality were mechanically stirred in a constant temperature bath at 37°. Aliquots, 2 ml., were withdrawn at the indicated times by means of a pipet whose tip was covered with filter paper. From each aliquot, 1 ml. was used to determine calcium ion by a modification of the method of Hawk, Oser, and Summerson (8) (the calcium oxalate was centrifuged rather than filtered, and was washed with 20% ammonia water instead of distilled water) and 1 ml. was used to determine inorganic sulfate. The latter determinations showed no significant hydrolysis of the ethereal sulfate during the course of the experiment. For longer periods of time, appreciable hydrolysis of the salicylamide sulfate was observed, which prevented the determination of solubility products under equilibrium conditions.

[1] Carbon-hydrogen analysis was done by Carol K. Fitz, Needham, Mass.

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Digitoxin Antagonism by Visnadin

By D. D. VARONOS, P. C. VOUKYDES, and G. C. NIKITOPOULOU

Visnadin, a lactone derived from bishop's weed, has been found to have marked antidotal properties in animals poisoned with digitoxin. It increases survival on acute and chronic administration of the glycoside, prevents the appearance of bradycardia, and reverses cardiac arrhythmias.

DIGITALIS glycosides probably have the smallest margin of safety of any of our commonly used drugs and they appear to have resisted all attempts at improvement in this respect by chemical modification. A less desirable alternative would be to have available a reliable antidote, but this has not yet been found. Recently, a naturally occurring lactone, visnadin (1), has been introduced in Europe for the treatment of the anginal syndrome (2) and has been shown by Smith, *et al.* (1), to have 10 times

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the activity of khellin and four times that of papaverine as a coronary vasodilator (2). Since this drug will inevitably be used in digitalized patients, it seemed desirable to study its pharmacologic relationship to the glycosides.

METHODS AND RESULTS

The effect of visnadin was studied first on the acute LD_{50} of digitoxin in adult male, albino mice. Digitoxin was administered by subcutaneous injection using 10 animals for each dose level. The LD_{50} was calculated by the method of Miller and Tainter (3). Two mice of approximately equal weight were assigned to each cage. After several hours fasting, they were given a mixture of one-half of their usual daily food intake mixed intimately with the appropriate dose of visnadin. In this way, the dose was entirely consumed. Fresh food was given 12 hours later and any unconsumed portion removed from the cages after 4 hours. Finally the digitoxin was injected after another 4 hours. The results are given in Table I from which it appears that visnadin at the maximum feasible oral dose raised the LD_{50} of digitoxin approximately 70%.

TABLE I.—EFFECT OF VISNADIN ON THE LETHAL DOSE OF DIGITOXIN IN THE MOUSE

Dose of Visnadin, mg./Kg.	LD_{50} of Digitoxin ± S.E., mg./Kg.
0	14.4 ± 0.48
150	18.2 ± 0.79
300	24.4 ± 0.78
ca. 450[a]	24.2 ± 1.03

[a] Food-drug mixture not entirely consumed.

Visnadin was also found to modify the lethal dose of digitoxin on chronic administration in the mouse. Digitoxin was given subcutaneously at a dose of 2.5 mg./Kg. daily for 2 days and then 3.5 mg./Kg. daily until death occurred. One group of 10 mice received digitoxin only, while a second group of 10 were given the same dose of digitoxin and, simultaneously, 400 mg./Kg. of visnadin in the food as in the preceding experiments. The control group survived for an average of 9.3 days and the visnadin-treated group for an average of 12.8 days. The mean lethal doses were 25 and 35 mg./Kg. for control and treated group, respectively.

Digitoxin was administered daily by subcutaneous injection to adult albino rats with and without the addition of visnadin to the feed by a technique similar to that described above. Electrocardiograms were taken daily before the dose of digitoxin was given. The duration of the cardiac cycle was measured and the data are given in Table II. It is clear that visnadin strongly inhibited the development of bradycardia due to digitoxin.

DISCUSSION

The mechanism of the antagonistic action of visnadin on the toxicity of the cardiac glycosides is not known. It may possibly be related to its coronary vasodilator action since Lee (4) and Reiter (5) have reported that the positive inotropic action of the glycosides is followed by a deficiency of oxygen in the heart muscle. However, both visnadin and the digitalis glycosides contain unsaturated lactone rings and have certain other structural similarities, so that competitive antagonism must also be considered. Several unrelated lactones have been reported to have antidigitalis glycoside activity (6, 7), although results of other workers do not absolutely agree with this view (8). Nevertheless, it must be considered that the lactones of the cardiac glycosides, except scillaren, are furane forms, while the visnadin lactone belongs to the pyrane form.

It seems clear that visnadin merits further study as to mechanism of action and clinical application because of its great potential value in controlling the action of the digitalis glycosides in the treatment of heart disease.

TABLE II.—EFFECT OF VISNADIN ON BRADYCARDIA PRODUCED BY DIGITOXIN IN THE RAT

Time	Cardiac Cycle Duration, sec., + mean of 10 animals ± S.E. Group I Digitoxin, 2.5 mg./Kg. per day, s.c.	Group II Digitoxin, *ibid.*, plus Visnadin, 200 mg./Kg. per day, oral	Probability of Difference between Means Due to Random Sampling[a]
Control	0.138 ± 0.0040	0.139 ± 0.0039	0.8
Digitoxin started			
1st Day	0.170 ± 0.0039	0.168 ± 0.0037	0.8
Visnadin started			
2nd Day	0.183 ± 0.0032	0.158 ± 0.0039	0.001
3rd Day	0.198 ± 0.0035	0.149 ± 0.0046	0.001
4th Day	0.210 ± 0.0049	0.147 ± 0.0048	0.001
5th Day	Death	0.160 ± 0.0041	

[a] Based on Fischer's *t* test.

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