Intestinal Absorption of Cardiac Steroids. (27297)

ROY G. HERRMANN, R. J. PARKER, FRANCIS G. HENDERSON AND K. K. CHEN
The Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, Ind.

After Withering(1) introduced digitalis into the practice of medicine numerous attempts were directed to the skillful use of its galenical preparations(2). Gold and associates(3,4) demonstrated in patients that digitoxin when given by mouth is almost completely absorbed whereas only one-fourth of the total glycosides of digitalis powder cross the intestinal membrane to exert the clinical effect(5). Although glycosides of other species are absorbed by man *per os* none appear to be as completely absorbed as digitoxin. For more than 30 years studies with digitalis-like principles from various sources have been undertaken in these laboratories(6,7). Because of the great expense of time and money involved in clinical investigations, as well as unforeseen dangers, we attempted to assess in dogs the absorbability of 6 substances from the intestine—digitoxin, ouabain, lanatoside E, bovoside A, bufalin, and acetyl strophanthidin. The first 4 compounds are glycosides and the last is an ester; bufalin is a natural aglycone of toad venom.

Methods. A chronic jejunal loop, made by pulling a portion of the intact jejunum up between the skin and abdominal muscles, was prepared in 22 registered beagle dogs weighing 7-11 kg. Rigid asepsis and control of bleeding as described by Markowitz(8) were observed. The animals were anesthetized with secobarbital sodium, 30 mg/kg intraperitoneally, for the surgical operation. An abdominal incision of about 10 cm was made along the midline from the xiphoid process through the fascia, muscle borders, linea alba, and peritoneum. A segment of the jejunum just below the duodeno-jejunal juncture was drawn up through the incision. The cephalic end of the intestinal loop was anchored to the rectus muscles by a stitch through both abdominal and intestinal muscles, approximately 1 cm below the xiphoid process. A retaining stitch was placed to either side close to the mesentery. The peritoneum and the muscle layers were closed with double stitches through the openings of mesentery between blood vessels. Care was taken not to have the stitches too close in order to avoid constriction of mesenteric vessels. The loop of jejunum, about 8 cm long, now lay loosely on the abdominal muscle. The caudal end was anchored to the muscles as at the cephalic end. The subcutaneous tissue was sewed together in the usual manner with individual stitches. Finally, the skin was closed by a running stitch. After the wound had healed the dog was ready for experimentation. The loop was so superficial that peristaltic movements could easily be observed.

We originally planned to record electrocardiographic changes as evidence of absorption following administration of the cardiotonic steroids, but soon found that vomiting occurred before cardiac alterations. A dose effective on the electrocardiogram was greater than the emetic dose. We therefore decided to determine the median emetic dose (EmD_{50}). With the exception of ouabain the cardiac steroids, in 0.1% solution with 47.5% ethanol, were injected either into the femoral vein or directly into the jejunal loop, and the dogs were observed for 4 hours to record the vomiting response. The largest volume of ethanolic solution injected was 0.175 ml/kg intravenously and 0.3 ml/kg intrajejunally. For ouabain a 0.1% aqueous solution was used. Control experiments with the same volumes of ethanolic solution without cardiac steroids brought about no response. The dogs were used not oftener than at 2-week intervals. The same animal was employed for both intravenous and intrajejunal administration. Because of the limited supply of some of the steroids very few dogs could be studied for each dose level.

Results and discussion. The results are summarized in Table I. In most instances the accuracy of EmD_{50} was limited by the small number of observations. Examination of the intrajejunal-intravenous ratios (last column, Table I) revealed that the degree of intestinal absorption of the 6 steroids varied in dogs. Digitoxin appeared to be more

easily absorbed than lanatoside E. Acetyl strophanthidin was absorbed as well as digitoxin. Ouabain was least active by intrajejunal injection. For bufalin, twice the intravenous dose had to be given by jejunum to cause the same effect. Bovoside A was exceptional in that it produced persistent loss of appetite and the dogs gradually became emaciated. The pathological findings were those of inanition. Two animals given the drugs by intravenous injection died the day after receiving a dose of 0.075 mg/kg, 2 died 2 and 3 days after 0.0625 mg/kg, 2 died 3 and 4 days after 0.05 mg/kg, 2 died 26 days and one 60 days after 0.025 mg/kg. Following intrajejunal administration one death occurred 8 days after the 0.025 mg/kg dose. Two dogs in this series were stomach fed over an extended period. They survived. No loss of dogs was encountered with the other

TABLE I. Emetic Effect of Cardiac Steroids after Intravenous or Intrajejunal Administration.

Steroid	Route	Dose, mg/kg	Dogs vomited/Dogs used	Avg time before vomiting, min.	EmD_{50}, mg/kg	Intrajej./ Intrav. ratio
Digitoxin	Intrav.	.05	1/4	104		
		.075	0/3			
		.125	3/4	75	.095 ±.017	
		.135	3/4	72		
		.150	3/4	24		
		.175	4/4	37		
	Intrajej.	.08	3/6	47		1.36
		.10	0/0			
		.15	5/6	54	.129 ±.14	
		.18	2/2	24		
		.20	2/2	25		
		.24	2/2	27		
Lanatoside E	Intrav.	.05	0/2			
		.08	1/2	214	.100 ±.010	
		.11	1/2	228		
		.15	2/2	94		
	Intrajej.	.10	0/2			2.12
		.20	1/2	221	.212 ±.034	
		.25	1/2	157		
		.30	2/2	250		
Acetylstrophanthidin	Intrav.	.025	0/2		.042 ±.003	
		.04	1/4	5		
		.045	1/4	9		
		.05	4/4	5		
	Intrajej.	.05	1/4	204	.058 ±.004	1.38
		.0625	2/4	201		
		.075	4/4	13		
Ouabain	Intrav.	.005	2/6	73		
		.01	1/7	63	.035 ±.028	
		.025	1/7	82		
		.05	4/4	43		
	Intrajej.	.125	0/2			20.54
		.15	1/4	42		
		.175	0/2			
		.20	0/2		.719 ±.585	
		.50	1/4	30		
		.75	1/2	38		
		1.0	2/4	67		
		2.0	2/2	79		

TABLE I (continued).

Steroid	Route	Dose, mg/kg	Dogs vomited/Dogs used	Avg time before vomiting, min.	EmD_{50}, mg/kg	Intrajej./ Intrav. ratio
Bufalin	Intrav.	.03	1/4	5	.04 to .07 (Est.) †	
		.05	2/5*	9		
		.075	1/2*			
						ca. 2
	Intrajej.	.05	0/2			
		.10	1/4	141	.09 to .13	
		.15	2/2	71		
Bovoside A	Intrav.	.02	0/2			
		.025	1/4	81	.027 ±.004 (Est.)	
		.05	2/2	12		
		.063	2/2	60		
		.075	2/2	5		
						ca. 67
	Intrajej.	.0125	0/4			
		.015	2/4	53	.018 ±.002	
		.0175	2/4	58		
		.025	3/4	134		

* Convulsions † Est. = estimated

5 cardiac steroids.

After intravenous injection of acetyl strophanthidin or bufalin vomiting took place in less than 10 minutes, but following intrajejunal injection the latent period exceeded 1-3 hours except with the largest dose of acetyl strophanthidin, which induced vomiting in an average time of 13 minutes (Table I). With the other steroids the time required for vomiting did not vary greatly between the two methods of administration. The delayed action of lanatoside E was particularly noticeable. The fact that intravenous administration elicited vomiting indicates that vomiting was due to the central stimulation, as elucidated by Wang and Borison(9). Bufalin was the only member of the 6 steroids which had a convulsant action that was obviously separated from the cardiac and emetic effects.

The structures of the cardiac steroids are shown in Fig. 1. It is apparent that the sugar component coupled with the secondary hydroxy group at C_3 was not important for intestinal absorbability because bufalin, an aglycone, and acetyl strophanthidin, an ester, both caused vomiting by the jejunal route. Furthermore, ouabain, a glycoside, was absorbed from jejunum to the extent of less than 5%. The larger lactone ring in bufalin and bovoside A did not hinder jejunal absorption. The convulsant action of bufalin reminds one of that of digitoxigenin(10).

The results obtained from the chronic jejunal loop of the dog may serve as useful guides for clinical study with any cardiotonic steroid. The recording of vomiting as the end-point was necessary because vomiting always accompanied and preceded the cardiac

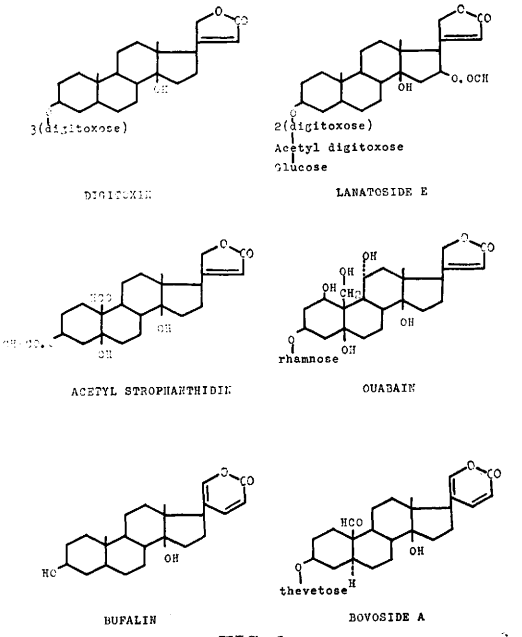

FIG. 1

changes in conscious dogs. The chronic preparation was especially valuable for comparison of dose response in the same animal by both intravenous and intrajejunal injections in order to measure degree of absorption through the intestinal tract. The data presented in Table I offered certain interesting features. The ease of absorption of digitoxin as indicated by the small intrajejunal-intravenous ratio substantiated the conclusion in man(3). The poor absorption of ouabain from jejunum agreed with the clinical observation that by mouth its efficacy is negligible (11). Acetyl strophanthidin surprisingly had about the same intrajejunal-intravenous ratio as digitoxin; it deserves a clinical trial for verification. Bufalin and lanatoside E may require twice the intravenous dose for oral administration. The loss of 10 dogs after bovoside A, due to persistent anorexia, makes a clinical investigation of this steroid unjustifiable. The presence of convulsant action of bufalin similarly rules out any consideration of application to human beings.

Summary. 1. Six cardiotonic steroids—digitoxin, lanatoside E, acetyl strophanthidin, ouabain, bufalin and bovoside A—have been compared with reference to intestinal absorption in dogs with a chronic jejunal loop. 2. Because of the dominance of vomiting before electrocardiographic changes took place the median emetic dose was estimated after intravenous and intrajejunal injections in order to appraise the absorbability of each steroid from the intestine. 3. In the dog digitoxin and acetyl strophanthidin were more easily absorbed than lanatoside E and bufalin. Ouabain was least absorbed—less than 5%. Although bovoside easily crossed the intestinal membrane it produced persistent loss of appetite resulting in death.

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Development of Tumors in Hamsters Inoculated in the Neonatal Period with Vacuolating Virus, SV_{40}* (27298)

A. J. GIRARDI, B. H. SWEET, V. B. SLOTNICK AND M. R. HILLEMAN

Division of Virus & Tissue Culture Research, Merck Institute for Therapeutic Research, West Point, Pa.

Previous reports(1-2) from this laboratory described the recovery from cell cultures of rhesus and of cynomolgus monkey kidney of a previously undescribed virus. This agent was unique among simian viruses since it grew but did not cause a cytopathic effect in the rhesus or cynomolgus kidney cell cultures from which it was derived. Instead, it propagated and caused marked cytopathic changes in kidney cell cultures of a heterologous species, *i.e.,* the grivet monkey, *Cercopithecus aethiops,* of Equatorial East Africa. The new virus was called "vacuolating virus" by us because of the prominent cytoplasmic

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