Production of Duodenal Ulcers in the Rat by Administration of Digitoxin

The rat is sensitive to the formation of gastric ulcers, but by comparison with other species (guinea pig, cat, dog, man) is particularly resistant to duodenal ulcers. Recently, however, methods for producing duodenal ulcers in the rat were reported (pantothenic acid deficiency[1,2]; infusion of secretogogues such as histamine, pentagastrin and carachol[3]; injections of propionitrile 3 times a day for 4 days[4]; a single administration of cysteamine[4a]; a single s.c. injection of histamine[5]). We describe here another method of producing duodenal ulcers in rats, by administering digitoxin.

Materials and methods. 100 male and 100 female rats (average body weight 222 g) were fed ad libitum with pellet food, and given water to drink. Digitoxin, in saline with 5 drops of Tween 80 per 50 ml, was given i.p. at 0.75 mg/rat in 0.5 ml, once a day. A few animals died after 3 days, and these were autopsied as soon as possible after death. The rest were killed with chloroform after 4 days of treatment. Their stomach and duodenum were dissected out as a single unit, opened along the mesenteric attachment for the duodenum and the greater curvature for the stomach and examined with a 2× binocular magnifier for the presence of ulcerations. The incidence of animals with ulcers was recorded. In a separate experiment, groups of 10 animals were sacrificed after 1, 2, 3 and 4 days of treatment to study the evolution of the duodenal ulcers. In each experiment, 5 control animals of each sex received 0.5 ml saline containing Tween 80.

Fig. 1. Gross appearance of digitoxin-induced duodenal ulcers. Stomach and duodenum before (top) and after (bottom) opening. Left: control. Right: digitoxin for 4 days. Marked distention of the stomach due to food retention in digitoxin treated animal. Also, well demarcated duodenal ulcer. The stomach is not ulcerated.

Results. Only 2% of the males died during digitoxin treatment compared with 18% of the females (Table). The females also lost more weight than the males. 8% of the males developed convulsions during treatment, as compared to 83% of the females. Duodenal ulcers were present almost exclusively in females (50% of the females versus 2% of the males). Usually a single ulcer was found, but sometimes 2 were present, located within 5 mm from the pylorus and opposite to the mesenteric attachment. No ulcers were found in control animals.

In the animals killed after 1 to 4 days of treatment, the duodenal lesion first appear, after 2 days, as tiny parallel red lines on the serosal surface, perpendicular to the duodenal axis and opposite to the mesenteric attachment. These red lines seemed to consist of thrombotic vessels since they did not fade upon pressure with fingers (however, thrombosis could not be verified histologically). With time, the red lines increased in number and covered a progressively larger area (2–3 mm). After opening the duodenum, a small ulcer was seen in the mucosa, exactly where this network of red lines was located. After 3 to 4 days of treatment, a well formed ulcer had developed that could be seen from the outside (Figure 1), sometimes bulging like a blister and consisting of a punched out crater. The lesion became deeper with time and, in a few cases, reached the muscle layer. A duodenal perforation was seen in only 1 animal.

Histologically, the ulcer first involved only the duodenal mucosa (localized necrosis). In some animals, the crater penetrated the muscularis mucosae, invaded the Brunner's glands and the submucosa (Figure 2). Polymorphonuclear cells were present within and under the necrotic layer, but no edema developed. On each side of an ulcer, the duodenal mucosa appeared normal. The ulcers were invariably covered with a PAS positive amorphic substance that appeared to be mucus. This mucus material, abundant and adhering to the lesion, did not extend beyond the ulcer site (Figure 2, C and D).

Already after 1 day of treatment, that is, prior to duodenal ulcer formation, the stomach became distended with food, being 3 to 5 times as large as that of control animals. So much food was present that the shape of the stomach was deformed (Figure 1).

Gastric ulcers were rare (20%) (Table) and found only in animals that had lost much weight, were convulsing and were considered moribund or had died. Such ulcers were located in the gastric corpus, according to a terminology of the anatomy of the rat stomach previously described[6].

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Discussion. The mechanism by which digitoxin induces duodenal ulcers is not known. a) It is unlikely that these ulcers resulted from gastric hypersecretion, since the stomach was replete with dry food. Food is known to buffer gastric acidity. b) Since the lesion starts with transverse red lines, it may have a vascular origin, such as hemorrhage or thrombosis, although such changes were not observed histologically. Cardiac glycosides (strophantin, strophantidin) were reported to produce vasoconstriction both in vitro[7] and in vivo[8]. c) It is also possible that digitoxin exerts a specific cytotoxic effect on certain layers of the duodenum, in the same sense as alloxan is cytotoxic for the beta cells of the pancreas. d) The marked food retention, so pronounced that it distorted the natural shape of the stomach, may play a role in the development of duodenal lesions. The presence of such large amounts of food in the stomach might mechanically interfere with the proper blood flow to the duodenum. e) Duodenal ulcers can also be produced, as mentioned earlier, by s.c. administration of propionitrile[4]. Both digitoxin and propionitrile induce tremor and convulsions when given at toxic doses. Whether convulsions and duodenal ulcer formation are coincidental or interrelated cannot be determined at the present.

The sex difference is unexplained, and was a surprise finding. Male rats are definitely more resistant to digitoxin intoxication, since they showed almost none of the signs of overdose, namely, marked loss of weight, convulsions, duodenal ulcers, and mortality. Whether this resistance is due to androgenic hormones is unknown.

Toxic and ulcerogenic effects of digitoxin in male and female rats

	Males	Females
No. of animals	100	100
Body weight		
Initial (g)	222	223
After 4 days (g)	196	185
Difference (g)	— 26	— 38
Mortality (%)	2	18
Convulsion (%)	8	83
Duodenal ulcers (%)	2	50
Gastric ulcers (%)	0	20

Digitoxin: 0.75 mg per rat, i.p. 2 males and 18 females died after 3 days. The rest were sacrificed after 4 days.

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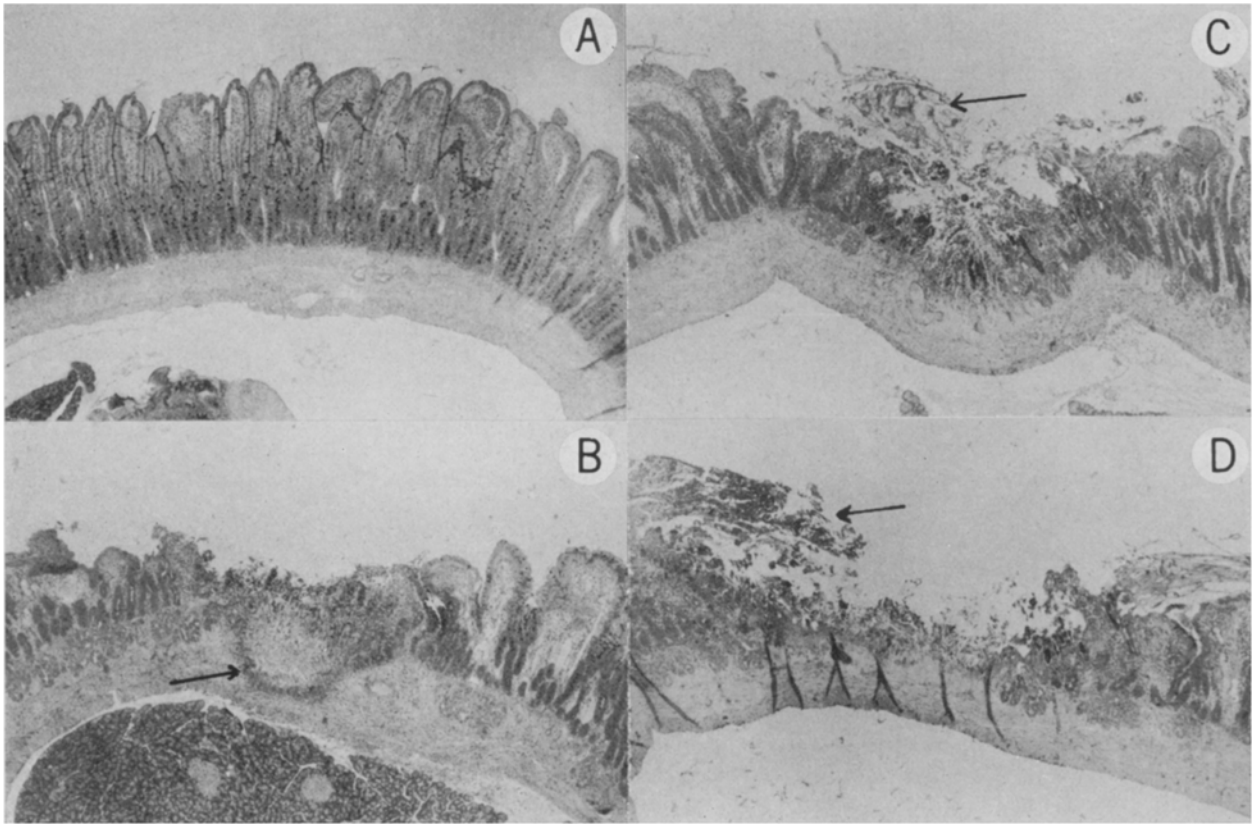

Fig. 2. Microscopic appearance of digitoxin-induced ulcers. Sections stained with PAS technique. ×25. A) Normal duodenum. The black spots represent mucus contained in goblet cells. B) Ulcer involving the mucosa and part of the submucosa. Little tissue has been lost; the lesion consists mainly of a necrotic mass surrounded by an inflammatory barrier (arrow). C) Ulcer in a more advanced stage. Much of the mucosa has been lost and the surface of the lesion contains free mucus (arrow). D) The mucosa has disappeared and, again, free amorphous mucus (arrow) covers the sides of the large crater. Only the muscle layer and part of the submucosa are left at the center of the defect.

The gastric ulcers, found in a small percentage of animals, appeared to be typical stress ulcers, similar to those produced by exertion[9] and restraint[10], and probably resulted from the severe stress of digitoxin intoxication. On the other hand, the duodenal ulcers produced by digitoxin are not due to stress, since other types of stress, e.g. restraint, exertion, exposure to cold, starvation, fail to produce duodenal ulcers while regularly producing gastric ulcers. Therefore, the duodenal ulcers described in the present study are due to a specific effect of digitoxin.

Résumé. L'administration i.p. de digitoxine (0,75 mg) à des rats femelles de 220 g produit en 3 à 4 jours un ulcère duodénal chez 50% des animaux. L'estomac reste intact. Les rats mâles sont presque totalement réfractaires. La digitoxine distent aussi l'estomac par rétention alimentaire. La pathogénie de l'ulcère duodénal par la digitoxine reste inexpliquée.

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Anhydrotic Effect of Benzodiazepines in Mice

Benzodiazepines are not believed to inhibit secretion; however we have been able to show an inhibitory effect of the drugs on palmar sweating in mice.

Methods. Swiss mice weighing 16–20 g, randomized into groups of 10, were used in accordance with a method already fully described[1]. The anhydrotic effect was assessed by the inhibition of palmar skin conductivity (IPSC %). The benzodiazepines tested were administered in increasing doses; the animals of each group all received the same concentration. The drugs were given i.p. as a suspension in carboxymethylcellulose 20 min before reading the PSC.

Results. Administration of benzodiazepines to mice resulted in IPSC secondary to an inhibition of sweating. This IPSC is dosebound, as can be seen from the graphs of the regression equations in the Figure.

Calculated from the corresponding regression equations, the doses (mg/kg) producing a 50% inhibition of the conductivity (IPSC 50) as follows: nitrazepam: 3.82 (3.09–4.95); lorazepam: 4.87 (3.62–7.50); diazepam: 5.21 (3.57–9.56); clorazepate: 37.61 (15.04–129.30); oxazepam: 46.98 (22.0–128.90); chlordiazepoxide: 54.19 (24.70–143.10); tetrazepam: 72.10 (37.52–156.90); medazepam: 105.10 (25.55–866.90).

Discussion. The occurrence of IPSC by benzodiazepines is established by the preceding regression equations. Now, many writers agree that PSC depends on the intensity of sweating[2–7], so this IPSC can be interpreted as an inhibition of palmar sweating (IPS). If this is so, what mechanism is involved in the phenomenon?

Actually, it can hardly be said that benzodiazepines have a noticeable anticholinergic effect. Indeed the antagonistic activity of diazepam on acetylcholine in the guinea-pig ileum test is 20,000 times lower than that of atropine[8].

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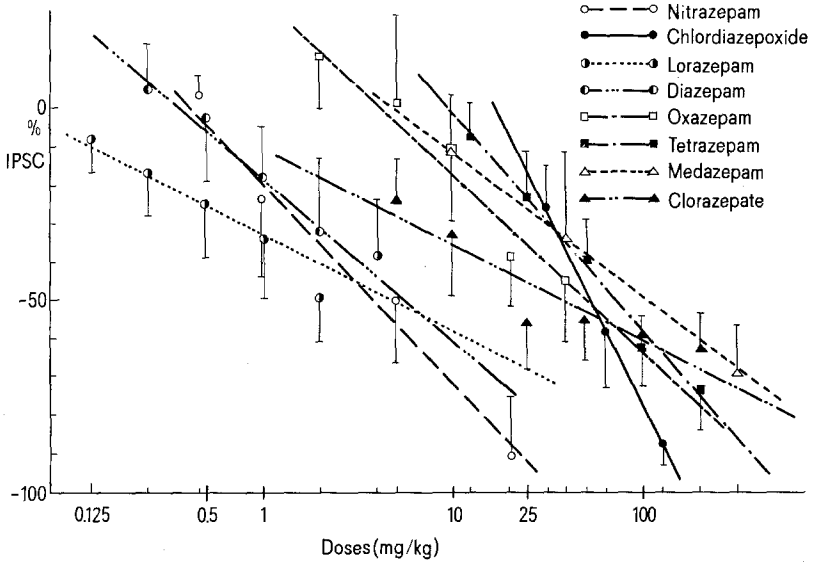

Dose-related inhibition of palmar skinconductivity (IPSC %) by benzodiazepines.