Production of Perforating Duodenal Ulcers by 3,4-Toluenediamine in the Rat[1] (37206)

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The production of gastric ulcers in rats by stress—alone (1) or in combination with various hormones, particularly glucocorticoids (2, 3)—has been the subject of many investigations, and several modifications of these techniques are currently employed as models for studies on the therapy of peptic ulcers. However, rats are unusually resistant to the induction of duodenal ulcers. The few techniques developed for this purpose are not readily reproducible because of individual variations in sensitivity and the comparative complexity of the procedures used, most of which depend upon concurrent treatment with several agents.

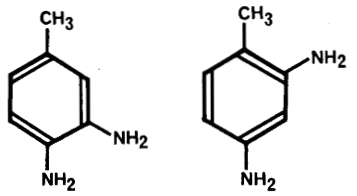
Until a few years ago, the only practical method available for the induction of duodenal ulcers was chronic pantothenic acid deficiency which, according to one of the leading authorities in this field (4) is unsatisfactory because of the low incidence of duodenal ulcers and the time (13 weeks) required for their production. By far the most rapid and reliable method described to date is that of Robert *et al.* (4–6). It depends essentially upon the continuous subcutaneous infusion of various secretagogues, such as histamine, carbachol, and pentagastrin. However, to obtain a high incidence, several of these agents must be administered simultaneously during fasting; normally-fed rats are virtually resistant even to this combination treatment (5). Nicotine, given during conjoint treatment with pentagastrin and carbachol, sensitizes rats to duodenal ulcer production, perhaps by reducing pancreatic secretion which, being alkaline, normally neutralizes the acid gastric juice (7). Such sensitization by nicotine has also been observed using another, somewhat more complex, means for the induction of duodenal ulcers: the constant perfusion of the stomach and duodenum with acid after introduction of a permanent catheter through a skin-incision in fasted and immobilized rats (8).

While studying the effects of hormones upon resistance to various intoxications (9), we noted accidentally that duodenal ulcers developed almost invariably in rats given 3,4-toluenediamine. For almost a century, this compound has been known to produce icterus—presumably owing to its hemolytic and hepatotoxic properties—in man and some experimental animals, including the rat (10–14); yet, apparently, its effect upon the duodenum has never been observed. At autopsy, it is virtually impossible to overlook a perforated duodenal ulcer, but the material used in the early toxicity studies was variously described as "Toluenediamine" or "Toluylenediamine" without indicating the position of the amine groups. Up to now, we have been unable to produce duodenal ulcers with 2,4-toluenediamine, whereas 3,4-toluenediamine is extremely active in this respect; possibly most of the earlier investigators used the former isomer, which is easier to synthetize.

The technique to be described here permits the reproducible production of well-demarcated, usually perforating duodenal ulcers in rats allowed to move about freely and having unlimited access to food and water.

Materials and Methods. All our experiments were performed on Sprague–Dawley rats (Canadian Breeding Farms). For the main experiment, we exclusively used females averaging 100 g (range 90–110 g) in body

[1] This work was supported in part by the Medical Research Council of Canada (Block Term Grant MT-1829) and the Ministère des Affaires Sociales, Quebec.



3, 4-Toluenediamine 2, 4-Toluenediamine

FIG. 1.

weight. However, a few complementary observations were made on males and females of different weight groups, as indicated later in the text. Throughout the experiments, all animals had free access to Purina Laboratory Chow (Ralston Purina Company of Canada) and tap water.

Unless otherwise stated, 3,4-toluenediamine ["3,4-TDA", 97% pure (Aldrich Chemical Co.)] or 2,4-toluenediamine ["2,4-TDA", practical grade (Matheson Coleman & Bell)] was administered in 2 ml water, twice daily by stomach tube, at the individual dose of 50 mg/100 g body weight (Fig. 1).

Results. In the main experiment, 13 female rats with an average body weight of 100 g were treated with 3,4-TDA, as indicated above. The experiment was terminated on the 5th day, by which time 7 rats had died, 6 of them with macroscopically-obvious and usually perforated duodenal ulcers. Simultaneously, in this experiment, 157 additional females in the same weight range received the same dose of 3,4-TDA. These rats had been pretreated with various steroidal and nonsteroidal hepatic microsomal enzyme inducers according to a previously-described screening technique for drug detoxication (9), but, since none of these compounds appeared to have had any effect upon the course of 3,4-TDA intoxication, we need not report these experiments in detail. Suffice it to state that, during the same period of observation, 93 animals died and, among these, 83 had grossly-evident duodenal ulcers. In general, the lesions began with the formation of an oval reddish brown spot, about 3 × 5 mm in size, just below the pylorus. Subsequently, this entire region became liquefied, and the pyloric contents extruded into the peritoneum. Yet, generalized peritonitis was sometimes avoided through the formation of adhesions between the pylorus and the liver; in these instances, a deep cavity developed in the adjacent hepatic parenchyma as a consequence of erosion by the duodenal digestive juices (Fig. 2).

The experiment was subsequently repeated on three groups, each consisting of 10 male and 10 female rats, weighing 50, 100, and 250 g, respectively. Since there was no striking difference referable to sex or age, it would be redundant to describe these observations in detail. The incidence of ulcers and the frequency of perforations were essentially the same as in the main experiment. Finally, under otherwise identical conditions, 3-4-TDA was approximately equally ulcerogenic,

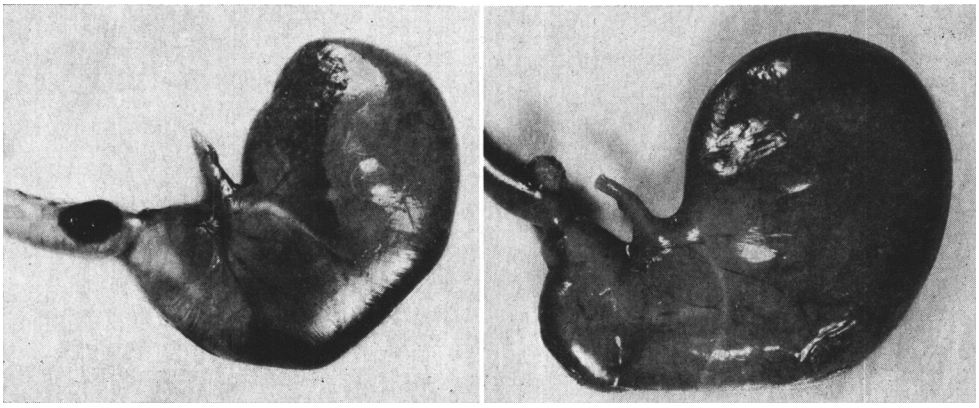


FIG. 2. Duodenal ulcers produced by 3,4-TDA. Left: Hemorrhagic spot in typical post-pyloric location. Right: More advanced stage after perforation and extrusion of duodenal contents.

when given orally in 0.5 ml peanut oil, 0.2 ml dimethylsulfoxide, 0.2 ml propylene glycol, or water, and only doubtfully less efficacious, when administered parenterally (ip, sc).

Repetition of the main experiment on 20 females with an initial body weight of 100 g showed that (with a single possible exception) no duodenal ulcers were produced by the isomeric 2,4-TDA, given orally at the same dose level. However, it should be noted that both 2,4-TDA and 3,4-TDA caused severe icterus, a fact which had been reported in all earlier communications on the toxicity of these compounds.

Discussion. It is evident that 3,4-TDA (unlike the 2,4-homologue), given orally or parenterally, is highly active in producing circumscribed and usually perforating duodenal ulcers in rats of both sexes and of various age groups. This effect is obtained in animals allowed free motion and access to food and water during the period of observation. In the course of our systematic studies on the effects of hormones and nonhormonal microsomal drug-metabolizing enzyme inducers upon the toxicity of various agents, we have noted (in collaboration with Dr. S. Szabo) (15) the occasional occurrence of duodenal ulcers after poisoning with acetanilide, allylchloride, acetaminophen, and 4,4-diaminodiphenylmethane. These observations, as well as more systematic studies concerning factors that might influence the development of drug-induced duodenal ulcers, will be described later.

Here, we merely wanted to call attention to the fact that such an easily-obtainable, inexpensive compound as 3,4-TDA provides us with a reproducible model of rapidly-developing, perforating duodenal ulcers in the rat.

Summary. In rats, 3,4-toluenediamine (3,4-TDA), unlike its 2,4-homologue (2,4-TDA), produces a very high incidence of perforating duodenal ulcers within a few days. These lesions develop, following oral or parenteral administration, in rats of both sexes and of various age groups.

1. Selye, H., Nature (London) **138**, 32 (1936).

2. Selye, H., "Stress," p. 822. Acta Inc., Med. Publ., Montreal, Quebec (1950).

3. Robert, A., *in* "Endocrine Aspects of Disease Processes" (G. Jasmin, ed.), p. 175. Warren H. Green, Inc., St. Louis, MO (1968).

4. Robert, A., and Stout, T. R., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **28**, 323 (1969).

5. Robert, A., and Dale, J. E., Proc. Soc. Exp. Biol. Med. **136**, 439 (1971).

6. Robert, A., Stout, T. J., and Dale, J. E., Gastroenterology **59**, 95 (1970).

7. Robert, A., Proc. Soc. Exp. Biol. Med. **139**, 319 (1972).

8. Robert, A., Stowe, D. F., and Nezamis, J. E., Nature (London) **233**, 497 (1971).

9. Selye, H., "Hormones and Resistance," Part 1, p. 566; Part 2, p. 567. Springer-Verlag, Heidelberg (1971).

10. Joannovics, G., and Pick, E. P., Ztschr. Exp. Pathol. Therap. **7**, 1 (1909).

11. Ohno, Y., Med. Klin. **23**, 1639, 1685 (1927).

12. Petri, E., *in* "Handbuch der Speziellen Pathologischen Anatomie und Histologie" (F. Henke and O. Lubarsch, eds.), Vol. 10, p. 7, Julius Springer, Berlin (1930).

13. Bollman, J. L., and Mann, F. C., Amer. J. Physiol. **116**, 214 (1936).

14. Stadelmann, E., Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmak. **14**, 231, 422 (1881).

15. Szabo, S., and Selye, H., Arch. Pathol. **93**, 390 (1972).

Received Dec. 6, 1972. P.S.E.B.M., 1973, Vol. 142.