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Genetic and histological aspects of stomach lesions induced by systemic injection of phenylbutazone in the rat

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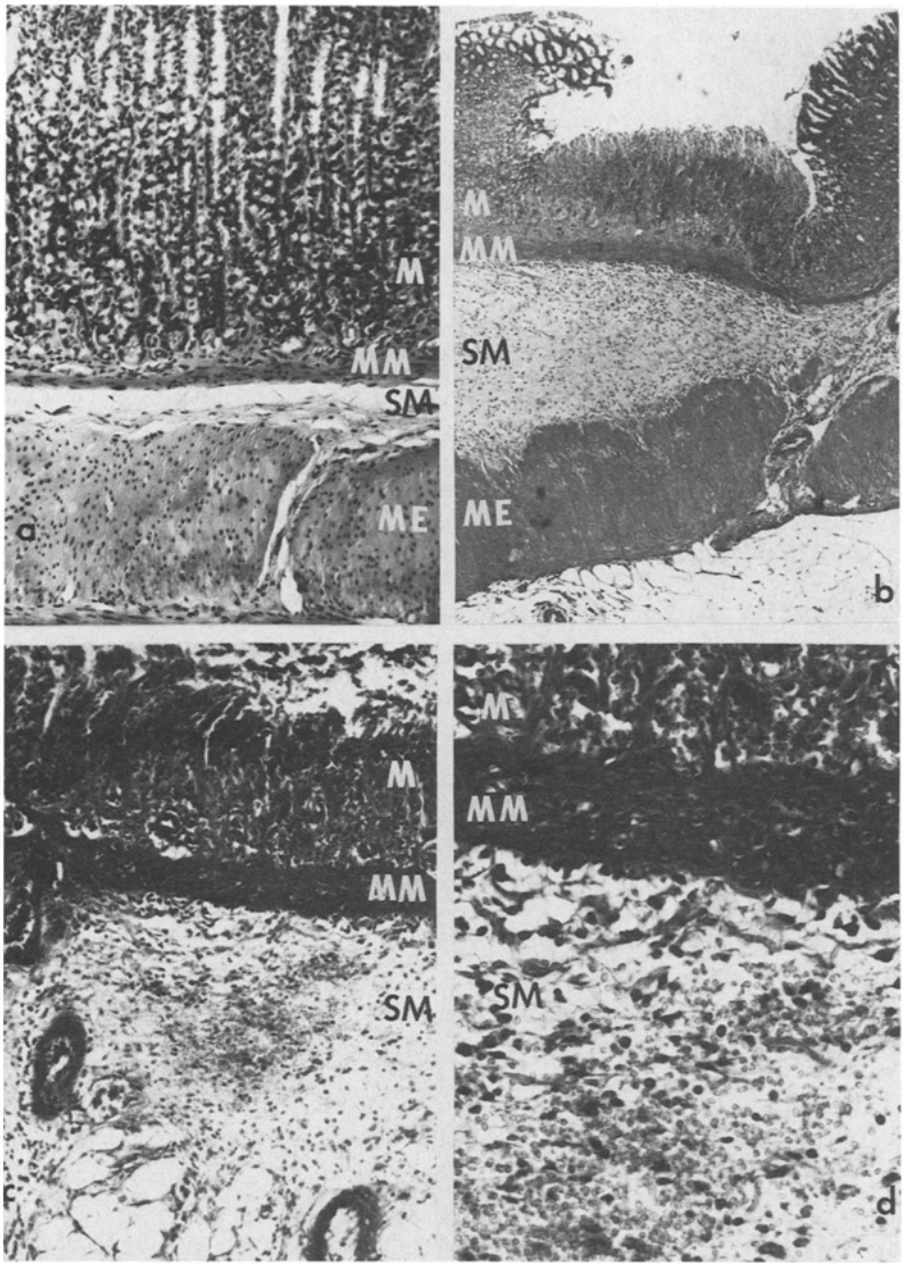
Summary. Roman high-avoidance (RHA/Verh) rats, food-deprived (F–D) for 5 days, had higher stomach lesion scores than did F-D Roman low-avoidance (RLA/Verh) rats. F–D RLA/Verh rats which were injected i.p. with phenylbutazone (PBZ) 24 h before examination, however, had higher scores than did PBZ-treated, F–D RHA/-Verh rats. Histologically, extensive edema and cellular infiltration (including numerous erythrocytes) were seen below the lesions, in the submucosa, denoting vascular damage. An attenuating influence of food on the ulcerogenic effects of PBZ, which were much more severe in F–D than in fed rats, was also indicated.
Key words. Phenylbutazone; stomach ulcers; food-deprivation; rats, food-deprived; stomach lesion scores; ulcer susceptibility, genetic differences in.

Roman high-avoidance (RHA/Verh) and low-avoidance (RLA/Verh) rats are bred, respectively, for rapid versus non-acquisition of a 2-way, active avoidance response. In addition to showing differences in that, and other, behavioral tests[2], various neurochemical and pharmaco-physiological differences have been found between these psychogenetically-selected lines of rats. For example, RLA/Verh rats were more sensitive to the toxic effects of several drugs, such as pentobarbital[3] and oxotremorine[4], probably due in large part to a less active hepatic microsomal system, leading to a reduced metabolism of these substances[3,5]. Other experiments, concerned with food-deprivation induced gastric lesions, have accentuated potential differences in metabolism between the 2 rat lines. When RHA/Verh and RLA/Verh rats were housed in plastic cages with sawdust bedding, and food-deprived (F–D) for 4–5 days, it was seen that F–D RHA/Verh rats had higher stomach lesion scores that did their unfasted controls and higher lesion scores than did F–D RLA/Verh rats. It was suggested that RHA/Verh rats were more sensitive to this type of stress than were RLA/Verh rats, possibly due to their normally larger appetite and metabolic requirements[6]. The present study sought to investigate these genetically-based differences in ulcer-susceptibility in combination with injections of the anti-inflammatory agent phenylbutazone (PBZ), which is also known to be biotransformed by the hepatic microsomal system. In addition, as PBZ has long been recognized to be extremely ulcerogenic in man[7,8], as well as in the rat[9], its effects were studied in further detail, through histological examination of the lesion area.

Methods. 48 naive, male RHA/Verh rats and 48 naive, male RLA/Verh rats, between 6 and 7 months of age, were used, with each animal being housed individually in a 40 × 25 × 16 cm plastic cage with sawdust bedding during the course of the experiment. The 1st group (N = 16 of each line) was given food and water ad libitum, whereas the 2nd group (N = 32 of each line) was F–D for 5 days with water ad libitum. Half of the rats of each line, in each of the groups, were given an i.p. injection of 100 mg/kg PBZ, 24 h before sacrifice, and the other half were given an i.p. injection of physiological saline (NaCl) solution. At the termination of the 5-day experimental period, each rat was sacrificed with chloroform and its stomach was removed, opened, rinsed and examined under magnification by 2 independent observers (one of which was unaware of the subjects status), for lesions. The following scoring system[9] was used: petechial lesion = 1 point, erosion less than 1 mm = 2 points, erosion 1–2 mm = 3 points, erosion 2-4 mm = 4 points, and erosion greater than 4 mm = 5 points. Due



Histological sections of the pyloric region of 3 RLA/Verh rat stomachs (see text for explanations). *a* Control rat No. 251, ×56, *b* PBZ-treated, F–D rat No. 95, ×26.4, *c* PBZ-treated, F–D rat No. 29, ×56, and *d* rat No. 29, ×140. MM, muscularis mucosae; SM, submucosa; and ME, muscularis externa.

to the large number of zero scores, comparisons between the lines of rats and experimental conditions were made with the Kruskal-Wallis analysis of variance test, followed by 2-tailed, Mann-Whitney U-tests, when appropriate. All stomachs were placed in separate bottles of formalin solution, following the macroscopic examinations, and several were selected later for sectioning and H–E staining.

Results. The table shows that F–D RHA/Verh rats had significantly higher lesion scores than did fed RHA/Verh rats, whether injected with PBZ or not. The same was true for PBZ-treated, F–D RLA/Verh rats, as compared to PBZ-treated, fed F–D RLA/Verh rats. Whereas untreated, F–D RHA/Verh rats had higher lesion scores than did untreated, F–D RLA/Verh rats, however, these results were reversed in the PBZ-treated F–D rats, i.e. PBZ-treated, F–D RLA/Verh rats had higher scores than did PBZ-treated, F–D RHA/Verh rats. It should be mentioned that the scores represent the values agreed upon by both observers, whose estimations were generally identical

Mean lesion scores for all test conditions, ± SEM

Treatment	RHA/Verh		RLA/Verh	
	Fed (8,8)	Food-deprived (16,16)	Fed (8,8)	Food-deprived (16,16)
NaCl	0	4.7 ± 1.4	0	1.4 ± 0.6
PBZ	2.9 ± 1.3	12.0 ± 2.6	2.1 ± 2.0	22.1 ± 3.9

*$p < 0.05$; **$p < 0.01$ (N).

to one another. When discrepancies did exist (as in some of the extensively lesioned, high score cases), the final scores were arrived at by consultation.

With the exception of 4 PBZ-treated RLA/Verh animals, which also showed some rumenal lesions (in addition to numerous pyloric lesions), all lesions were found in the glandular portion of the stomach, i.e. the pyloric region. Two of these lesions are shown in the figure. Figure a shows a section through the glandular stomach wall of an untreated, fed RLA/Verh rat (no lesions). In comparison, figure b shows a circumscribed, necrotic lesion of the mucosa from a PBZ-treated, F–D RLA/Verh rat. An extensive edema was found in the area of the lesion, especially in the submucosa, where cellular infiltration was also prominent. Figure c, showing a more highly magnified section through the glandular stomach wall of another PBZ-treated, F–D RLA/Verh rat revealed, also in the area of the mucosal lesion, an edematous, infiltrated submucosa containing abnormal blood vessels. Figure d shows the same section under yet higher magnification. Here it was clearly seen that the cellular infiltration consisted largely of erythrocytes, with many lymphocytes and granulocytes also present.

Discussion. Several studies in addition to the present one, which have demonstrated genetic differences in rats[6, 10, 11], as well as others which have not dealt with genetic factors[12, 13], have consistently emphasized the importance of food-deprivation in the experimental production of stomach lesions in rats. The present study has shown that, although it was possible to increase the lesion scores significantly in fed RHA/Verh rats by injecting PBZ, the scores were elevated even more when rats of this line were deprived of food. This difference in the effect of PBZ was even more remarkable in fed, versus F–D, RLA/Verh rats. Indeed, whereas untreated F–D RHA/Verh rats had higher lesion scores than did untreated F–D RLA/Verh rats, these results were reversed when F–D rats of both lines were injected with PBZ. Although the stomachs of RHA/Verh rats were basically more sensitive to food-deprivation, injections of PBZ in the present study produced an 11-fold increase in lesion scores in F–D RLA/Verh rats, as compared to a 4-fold increase in F–D RHA/Verh rats.

Why was systemically-injected PBZ more toxic in F–D RLA/Verh rats than in F–D RHA/Verh rats, and why was it more toxic in F–D rats than in fed rats? The 1st question is perhaps easy to answer since, as we have seen earlier, it appears that RLA/Verh rats show an attenuated metabolism (in comparison to RHA/Verh rats) of certain substances, such as PBZ, which are biotransformed by the hepatic microsomal system. PBZ would, therefore, probably accumulate more extensively in the gastric mucosa of FD-RLA/Verh rats, due to its increased availability. In regard to the 2nd question, it must be acknowledged that the ulcerogenic, and even the anti-inflammatory, mechanisms of action of PBZ are sill unknown. PBZ has been shown, for example, to be equally toxic to the rat stomach when given orally or systemically[14]. Those investigators suggested that its gastric toxicity (as well as that of indomethacin), would thereby be difficult to reduce, as it is closely connected to the systemic acitivity of the drug(s).

The possiblity exists that PBZ may inhibit prostaglandin synthesis in the mucosa, as does aspirin, and this would reduce the resistance of the stomach wall to subsequent erosions. In this regard, food is known to stimulate prostaglandin synthesis, thereby protecting the stomach wall. It is also known that gastric acid secretion plays a role in the pathogenesis of stomach lesions in F–D rats[9, 15] and that food, or even non-nutritive, bulky substances, such as sawdust, can reduce the incidence of lesions[6, 12, 16], possibly by buffering, or diluting, the acid. It would then be expected that the ulcerogenic effects of PBZ would be reduced by the presence of food, which was indeed the case in the present experiment. A further possibility is that PBZ induces a 'glucoprivic state' in F–D rats, which results in an ulcer-inducing, gastric contractile response in the pyloric region, adjacent to the acid-secreting mucosa. This syndrome, which has been produced by the intraileal application of PBZ, may be prevented or reduced by glucose feeding or infusion[17]. Examination of the photographs in the figure (keeping in mind that PBZ was administered i.p. in the present study) might also lead one to inquire whether the systemic ulcerogenic effects of PBZ are not actually more important than eventual direct effects on the gastric mucosa, and whether this might not be the case even if this drug, as well as other anti-inflammatory drugs, were to be given orally, as it usually is in practice. Most studies which have included a histological examination of the stomach wall have concentrated on the mucosal lesions only, whithout considering other, possibly important, pathology in the area of the lesions. One notable exception which utilized electron microscopy following a relatively low, oral dose of aspirin, noted that the first structural change in the rat stomach was a rupture of the basement membrane of the endothelial cells of the capillaries and post-capillary venules[18]. That is, a break-down of small blood vessels occurred before any other cytolytic effect. It was proposed further that the subsequent mucosal erosion develops as an ischemic infarct. Hemodynamic factors have also been implicated in the etiology of stomach ulcers in genetically-hypertensive rats[11]. The type of lesions mentioned above, in small blood vessels, cannot be ascertained by light microscopic means. However, the extensive edema and cellular infiltration containing, most importantly, an abundance of erythrocytes, as seen in the present study, provide a strong indication of vascular damage. This potentially primary, toxic effect of PBZ might be compounded by a known circulatory effect of that drug, i.e. the retention of Na^+ and Cl^-, which generally leads to edema and an increase in plasma volume.

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