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Strain and Sex Differences in Gastric Ulceration in Restrained Rats

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Physical restraint has been shown to produce gastric ulceration in rats (Bonfils *et al.* 1957, 1959*a*,*b*,*c*; Sines, 1959; Brodie and Hanson, 1960; Hanson and Brodie, 1960; Bonfils and Lambling, 1963; Wilson, 1964, 1966*a*,*b*). Although these workers have used different strains of rats, the importance of strain as a factor affecting ulcer severity cannot be ignored, as the work of Sines (1959), Ader *et al.* (1960*a*,*b*) and Sawrey and Long (1962) has shown.

In a similar manner sex has been shown to affect restraint ulcer severity in rats (Ader *et al.*, 1960*a*,*b*,*c*; Wilson, 1964, 1966*a*), although the importance of this appears to have often been ignored also. However, when the experimental data reported separately on these two variables are studied, disagreement between them is readily observed. The present study was therefore undertaken in order to compare systematically the effects of strain and sex difference on restraint-ulcer susceptibility among three strains of rats.

Methods

Male and female rats from three strains, and all taken from the same colony, were employed. All were within the weight-range 100-120g, this being the optimum body-weight for restraint-ulcer susceptibility in rats (Wilson, 1964, 1966*a*). Strains used were Wistar, Hooded Wistar and Sprague-Dawley. All animals were fed on the same diet (Cube Diet 41B) from weaning until the time of the experiment.

All rats were housed in cages of a standard type, and were grouped eight per cage according to strain and sex. Each cage consisted of a tin box 9″ × 9″ × 4½″ covered with a wire mesh lid. The base of the cage was covered with a thin layer of sawdust. Ambient laboratory temperature was maintained at 72°F (22°C).

Production of gastric ulcers

Gastric ulcers were produced by a modification of the method of Rossi *et al.* (1956) which has been successfully employed elsewhere (Martindale *et al.* 1960; Wilson, 1964, 1966*a*,*b*). All rats were deprived of food for 24 hours before the experiment began, but water was allowed. Rats in experimental groups were then anaesthetised with ether and bound with a plaster of Paris bandage measuring 2″ wide and 4′ 6″

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long. The bandage extended from the base of the thorax to the hind legs, rendering them immobile. Movements of the front legs were unrestricted. Control rats were subjected to the same conditions as experimental animals, except that restraint bandages were not applied.

Evaluation of gastric ulceration

After 24 hours of restraint the rats were killed with ether; the abdomen was incised, and the stomach removed and washed with warm water through a polythene tube inserted into the cardiac end. The pyloric end was then tied, and the stomach slowly inflated with formal saline (Formaldehyde 4%, Sodium Chloride 0.9%, Distilled water to 100% until it was fully distended. The polythene tube was then removed, and the cardiac end of the stomach tied with a ligature.

Severity of gastric ulceration was assessed in each case by macroscopic examination of the stomachs under transmitted light, when ulcers appear as white spots or lines having a brightness equivalent to that of the forestomach (Bonetti and Wilson, 1964; Wilson, 1964, 1966*a*,*b*). The degree of gastric ulceration produced by restraint in each rat was expressed in terms of an ulcer " score ", based on the number and size of the ulcers present. Details of the scoring are shown in the following table (Tab. 1).

Tab. 1. Classification and scoring of gastric ulcers produced by restraint

Small ulcers (not exceeding 0.5 mm diameter) N. of ulcers	Intermediate ulcers (dimensions not exceeding 0.75 × 5 mm) N. of ulcers	*Score indicative* of gastric damage
5	—	0.25
10	—	0.50
15	—	0.75
20	1	1.00
25	2	1.25
30	3	1.50
35	4	1.75
40	5	2.00
	6	2.25
	7	2.50

The ulcer " score " for the gastric damage in the stomach is found by selecting the type of ulcer which by its numbers will produce the highest score in the tables. This method has advantages in that it performed quickly, and produces a small variance in the results when gastric damage is being evaluated in a group of rats.

In these experiments no gastric damage exceeding seven intermediate ulcers was found. Fuller details of the method and scoring for ulcers larger than this are given by Wilson (1964).

Results

The results of this experiment are shown in Tab. 2, from which it will be seen that for Wistar rats, ulcer severity is highly significant for both males and females ($p < 0.001$); for Hooded Wistar rats, ulcer severity is highly significant for females ($p < 0.001$) but slightly less so for males ($p < 0.01$). For Sprague-Dawley rats, ulceration was not significantly greater between experimental and control groups of either sex.

Tab. 2. Ulcer scores for male and female rats showing effect of strain differences on gastric ulceration produced by restraint

Strain	Male rats			Female rats		
	Experimental groups (Restrained)	Control groups (Free)	p	Experimental groups (Restrained)	Control groups (Free)	p
Wistar	$\bar{x} = 1.85$ $\sigma = 0.085$	$\bar{x} = 0.89$ $\sigma = 0.107$	< 0.001	$\bar{x} = 1.88$ $\sigma = 0.072$	$\bar{x} = 0.83$ $\sigma = 0.096$	< 0.001
Hooded Wistar	$\bar{x} = 1.05$ $\sigma = 0.089$	$\bar{x} = 0.56$ $\sigma = 0.103$	< 0.01	$\bar{x} = 1.48$ $\sigma = 0.084$	$\bar{x} = 0.53$ $\sigma = 0.107$	< 0.001
Sprague-Dawley	$\bar{x} = 0.58$ $\sigma = 0.109$	$\bar{x} = 0.33$ $\sigma = 0.067$	Not. Sig.	$\bar{x} = 0.50$ $\sigma = 0.094$	$\bar{x} = 0.29$ $\sigma = 0.049$	Not. Sig.

N = 16 in all cases.

There are no significant differences between male and female scores for either experimental or control groups (regardless of strain), except for Hooded Wistar experimental groups ($p < 0.01$)

Tab. 2 also shows there was no significant difference between the mean scores of male and female groups under all experimental and control conditions, except for experimental groups of Hooded Wistar rats, where the difference was significant ($p < 0.01$).

Discussion

Differences in ulcer severity were observed between the three strains employed in this study. These differences are unlikely to be due to dietary factors, since all rats were fed on the same diet from weaning until the time of the experiment. Neither is parasitic infection likely to be a contributing factor, since all animals were kept under hygienic conditions. It is therefore likely that these differences in ulcer severity observed between the three strains of rats reflect some underlying genetic difference

in susceptibility between them. Genetic differences in response to drugs have been demonstrated (Broadhurst, 1964) and therefore if stress is mediated through neurohormonal mechanisms, similar differences in reponse to it may occur.

A significant degree of gastric ulceration occurred in male and female rats of both Wistar and Hooded Wistar strains. However, the overall severity of ulceration in both experimental and control animals, was greater in the Wistar rats.

Sprague-Dawley rats of both sexes were resistant to gastric ulcer formation. It is inretesting to note the low scores of control animals in this strain, which when compared with control scores of the other two strains, would seem to indicate a general resistance to stress in Sprague-Dawley animals.

These results confirm the findings of Ader *et al.* (1960*a*,*b*,*c*) that Wistar rats are more susceptible to restraint ulcer formation than are Sprague-Dawley rats. Their report that female animals of both strains were more ulcer-susceptible than the males could not be confirmed by the present study, but earlier findings (Wilson, 1964, 1966*b*) of no significant sex differences in restraint-ulcer severity in Wistar rats were upheld.

It is interesting to note that the only instance of a significant sex difference in ulcer-susceptibility occurred in Hooded Wistar rats. This result compares favourably with that of Sawrey and Long (1962) who used Nebraska Hooded rats. Their different technique for producing gastric ulcers renders direct comparison of experimental results impossible, however.

It may be observed that these experimental results appear to be in direct contrast to the difference in gastric ulcer incidence in humans, which is approximately four times greater in males than in females (Cleave, 1962; Beeson and Macdermott, 1963; Aagaard, 1963). However, stress ulcers in man arising from a different cause (*Brit. Med. J.*, 1965) may be more relevant to this particular type of experimental gastric ulcer.

When data in Tab. 2 are used to make statistical comparisons between strains, it can be shown that Wistar rats of both sexes are significantly more ulcer-susceptible than are Sprague-Dawley rats under both experimental and control conditions. When they are compared with Hooded Wistar rats, however, ulceration in Wistar rats is significantly greater only for experimental animals; scores for control groups do not differ. A similar result is obtained when Hooded Wistar and Sprague-Dawley groups are compared.

It is quite clear that there are marked differences between strains of rats in their susceptibility in induction of gastric ulcers by restraint. In the design of such experiments, therefore, the strain of animals used is of paramount importance. Although different degrees of " emotionality " in rats of different strains has been observed (Sines, 1959; Ader *et al.*, 1960*a*,*b*) which may serve as a partial explanation of these results, it is likely that genetic factors may ultimately be responsible. However, the mechanisms by which they operate are not understood.

Summary

Male and female rats of three different strains were subjected to physical restraint, when it was demonstrated that there is a relationship between the strain of the rat and its susceptibility to gastric ulcer formation. Severity of ulceration was greatest in Wistar rats. Hooded Wistar rats were also ulcer-susceptible, but not to such a great extent as Wistar animals. Sprague-Dawley rats were resistant to ulcer formation. Sex differences in ulcer severity were found only with restrained Hooded Wistar rats.

The results of this investigation compare favourably with results published for individual strains of rats by other workers. It is possible that genetic influences may be responsible for the differences observed, but the way in which they operate is not understood.

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RIASSUNTO

In ratti maschi e femmine mantenuti in stato di costrizione è stata stabilita l'esistenza di rapporti tra ceppo di appartenenza e sviluppo di ulcere gastriche con questo metodo prodotte. Le ulcere mostrarono un massimo di sviluppo nei ratti del ceppo Wistar, un grado intermedio nei Wistar-Hooded. I ratti Sprague-Dawley si mostrarono resistenti all'induzione di ulcere gastriche da costrizione. Una dipendenza della gravità delle ulcerazioni dal sesso fu osservata soltanto nei Wistar-Hooded.

Le osservazioni qui riportate concordano con risultati pubblicati da altri autori per singoli ceppi di ratti. Anche attribuendo la responsabilità di questo diverso comportamento ad influenze di ordine genetico occorre ammettere che il loro meccanismo d'azione rimane incompreso.

RÉSUMÉ

Des rats des deux sexes, de souches différentes, ont été soumis à la technique de la contrainte. On a démontré l'existence d'une relation entre la souche et la sensibilité à développer des ulcères gastriques. On a observé une plus grande sévérité de l'ulcération chez les rats Wistar, moins grande chez les Hooded-Wistar. Les rats Sprague-Dawley se sont montrés résistants à la formation d'ulcères. Des différences entre les sexes en ce qui concerne la gravité des ulcères, ont été trouvées seulement parmi les rats Hooded-Wistar.

Les résultats de cette investigation sont favorablement comparables aux résultats publiés par d'autres auteurs pour des souches individuelles de rats. C'est possible que des influences génétiques soient responsables des différences observées, mais leur mode d'action n'est pas compris.

ZUSAMMENFASSUNG

In männlichen und weiblichen Ratten dreier Zuchtstämme konnte bei Einengung eine Abhängigkeit der damit erzeugten Magenulcera von der Stammzugehörigkeit beobachtet werden. Den grössten Ulcerationsgrad wiesen die Wistarratten auf, bei den Wistar-Hooded waren die Ulcera weniger ausgeprägt. Die Sprague-Dawley-Ratten erwiesen sich als resistent. Eine Abhängigkeit der Schwere der Ulcera vom Geschlecht konnte nur bei den Wistar-Hooded beobachtet werden.

Diese Resultate lassen sich gut mit solchen vergleichen, die von anderen Verf. mit einzelnen Zuchtstämmen erhalten worden sind. Auch wenn man die beobachteten Empfindlichkeitsunterschiede genetischen Einflüssen zuschreiben will bleibt eine Erklärung ihrer Wirkungsweise noch aus.