

## N O T I C E

THIS DOCUMENT HAS BEEN REPRODUCED FROM  
MICROFICHE. ALTHOUGH IT IS RECOGNIZED THAT  
CERTAIN PORTIONS ARE ILLEGIBLE, IT IS BEING RELEASED  
IN THE INTEREST OF MAKING AVAILABLE AS MUCH  
INFORMATION AS POSSIBLE

THE ULCEROGENIC EFFECT OF BILE AND BILE ACID IN RATS  
DURING IMMOBILIZATION STRESS

J. Weisener

Translation of "Zur ulzerogenen Wirkung von Galle und Gallensäuren bei Ratten im Immobilisationsstress," Zeitschrift fuer Gastroenterologie (West Germany), Vol. 12, No. 7, 1974, pp. 539-546.

(NASA-TM-76054) THE ULCEROGENIC EFFECT OF  
BILE AND BILE ACID IN RATS DURING  
IMMOBILIZATION STRESS (National Aeronautics  
and Space Administration) 14 p  
HC A02/MF A01

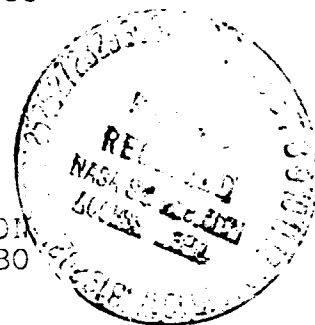
N80-22960

Unclas

CSCL 06C G3/51 47663

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION  
WASHINGTON, D.C. 20546

FEBRUARY 1980



## STANDARD TITLE PAGE

1. Report No. NASA-TM -76054	2. Government Accession No.	3. Recipient's Catalog No.	
4. Title and Subtitle THE ULCEROGENIC EFFECT OF BILE AND BILE ACID IN RATS DURING IMMOBILIZATION STRESS		5. Report Date February 1980	
		6. Performing Organization Code	
7. Author(s) J. Wiesener Pathology Institute, Free University, Berlin		8. Performing Organization Report No.	
		10. Work Unit No.	
9. Performing Organization Name and Address Leo Kanner Associates Redwood City, California 94063		11. Contract or Grant No. NASW-3199	
		13. Type of Report and Period Covered Translation	
12. Sponsoring Agency Name and Address National Aeronautics and Space Administration, Washington, D.C. 20546		14. Sponsoring Agency Code	
15. Supplementary Notes Translation of "Zur ulzerogenen Wirkung von Galle und Gallensäuren bei Ratten im Immobilisationsstress," Zeitschrift fuer Gastroenterologie (West Germany), Vol. 12, No. 7, 1974, pp. 539-546.			
16. Abstract The present work studies the effect of oxen bile and individual bile acids, or their sodium salts, in differing concentrations on the gastric mucosa of rats in combination with immobilization stress. A statistically significant higher frequency of ulcers was only determined in the application of 10 % oxen bile. Dosages on 10 % sodium glycocholic acid demonstrated strong toxic damage with atonic dilation of the stomach and extensive mucosal bleeding. The results gained are explained in connection with published clinical and animal experiments.			
17. Key Words (Selected by Author(s))		18. Distribution Statement Unclassified- Unlimited	
19. Security Classif. (of this report) Unclassified	20. Security Classif. (of this page) Unclassified	21. No. of Pages 14	22. Price

# THE ULCEROGENIC EFFECT OF BILE AND BILE ACID IN RATS DURING IMMOBILIZATION STRESS

J. Wiesener

Pathological Institute of the Free University in Berlin

The duodenal reflux has been discussed for some time as a cause in /539\* the pathogenesis of the gastric ulcer. The reflux of alkaline duodenal juice may destroy the gastric mucosal barrier [15] , so that the acid may directly affect the mucosa. When examining the juices of the empty stomach, high concentrations of bile and/or bile acids were found 33 .

In the present study the effect of oxen bile and bile acid on the gastric mucosa is to be studied under immobilization stress conditions.

## Method

Male Wistar rats (AFH Hanover, Institute of Dr. Hageman) weighing 200-240 g. served as trial animals. The rats were held in plastic boxes (55 x 35 x 20 cm. in size) in groups of 12 animals before trials were made. They were fed Altromin solid fodder and water ad libitum. Immobilization was carried out in cylindrical wire cages 16 cm. long and 5.5 cm. base diameter.

Pretrials with groups of 10 or 12 animals for determination of an approx. 50% ulcer frequency resulted in rats, which had not previously fasted, 30% after 8 hours, 33% after 12 hours, 50% after 16 hours and 75% after 24 hours erosive or ulcerogenic mucosal lesions. An ulcer frequency of 42% resulted after a 4 hour immobilization of the animal when they had not been fed for 24 hours but had received sufficient water.

---

\* Numbers in the margin indicate pagination in the foreign text.

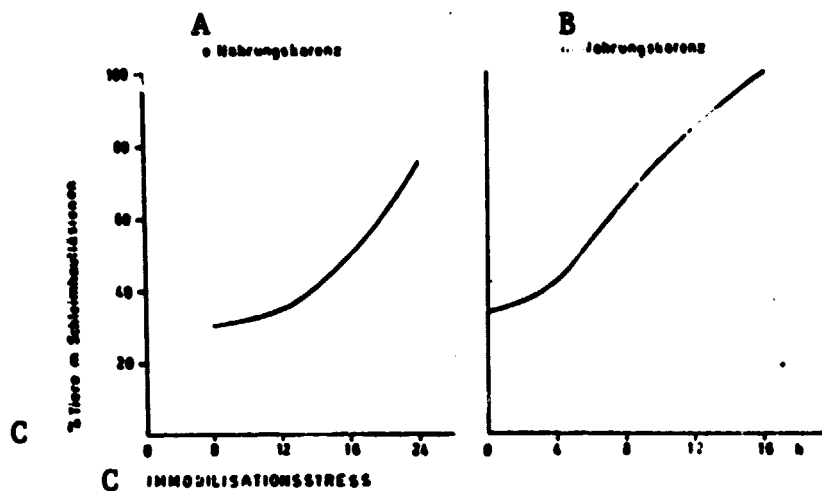


Figure 1: Frequency of Ulcers After Several Hours of Immobilization Stress With and Without Feeding

Key

- |   |                 |   |                                   |
|---|-----------------|---|-----------------------------------|
| A | With Feeding    | C | % of Animals with Mucosal Lesions |
| B | Without Feeding | D | Immobilization Stress             |

In further pretrials with 50% and 10% oxen bile, with 3.3% crystalline cholic acid and physiological salt solution after a 4 hour immobilization stress, 90% of the animals receiving 10% oxen bile demonstrated mucosal lesions. In the group receiving 50% oxen bile in the stomach, a 50% frequency of ulcers resulted with a high degree of dilated and atonic stomachs. In the case of the animals receiving 3.3% crystalline cholic acid, 30% mucosal lesions resulted; in the control group, receiving physiological salt solution, 20% of the animals demonstrated mucosal lesions.

Defined trial conditions were therefore employing rats weighing 200-240 g., giving the animals no food, but sufficient water, for a 24 hour period and carrying out a 4 hour immobilization after application of oxen bile and bile acids via a stomach tube.

The stomach tube was introduced into the stomach; 2 ml. each pre-warmed to 35-37° C of oxen bile, crystalline cholic acid, Na-deoxycholic acid, Na-glycocholic acid, Na-taurocholic acid and lithocholic acid

were applied at a concentration of 1%, 5%, and 10% in a physiological salt solution. A further group of rats received 2 ml. of physiological salt solution, another received nothing.

### Evaluation

After 4 hours of immobilization the animals were anesthetized by means of ether and killed with the application of a pneumothorax. The stomach was separated 5 mm. below the pylorus, filled with 2 ml. water and fixed for 3-5 minutes in a 4% Formalin solution. The stomachs were subsequently cut open carefully along the large curvature without damaging mucosa and spread on a cork plate. The preparations were immediately examined with the naked eye and with the microscope. Localization and size of mucosal lesions were registered. Only those mucosal lesions microscopically visible were evaluated. A group comparison was made of the animals with gastric mucosal lesions and without these findings. No determination of an ulcer index was made.

The % test was applied, where necessary, for checking results.

### Results

The mucosal lesions were found almost exclusively in the glandular stomach - in only one case an ulcer having the dimensions of a large-sized pinhead was found in the stomach. The lesions were evenly distributed on the front and back wall. The antrum was seldom affected, the duodenum was never affected. The lesions were usually found in the folds, having a deep, wide hemorrhagic base. Microscopically only the mucosa were affected, never the lamina musculavis mucosa.

In addition to the alterations in mucosa already described, in the case of the rats receiving 50% oxen bile and in those receiving 10% Na-glycocholic acid, an extreme dilation of the stomach occurred. In addition, all animals of the 2 groups mentioned were affected by diarrhea.

Frequency of acute mucosal lesions in the glandular stomach of rats where oxen bile and various bile acids were applied in differing concentrations is shown in Table 1.

Table 1: Frequency of Mucosal Lesions in the Glandular Stomach of Rats Where Oxen Bile and Varying Bile Acids Were Applied in Differing Concentrations.

/541

A Gruppe	B Substrat	C Löslich in 0,1% NaCl (0,9% w/v)		D Ulcerationen I pro Gruppe G punktf. signif.	E Erosionen H punktf. signif.	I o. B.	J stat. Signif. (%)
		n	pH				
A <sub>1</sub>	K Künderzähne	1	+	5	6	3	-
A <sub>2</sub>	Künderzähne	5	+	5	6	6	-
A <sub>3</sub>	Künderzähne	12	+	7	16	4	+
B <sub>1</sub>	L Cholesterin	1	Suspension	1	3	9	-
B <sub>2</sub>	Cholesterin	5	Suspension	-	8	9	-
B <sub>3</sub>	Cholesterin	12	Suspension	2	12	6	-
C <sub>1</sub>	M Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	1	+	1	3	9	-
C <sub>2</sub>	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	5	+	1	-	11	-
C <sub>3</sub>	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	10	+	1	-	11	-
D <sub>1</sub>	N Glykocholsäure	1	+	-	2	12	-
D <sub>2</sub>	Na <sub>2</sub> Glykocholsäure	5	+	-	-	12	-
D <sub>3</sub>	Na <sub>2</sub> Glykocholsäure	10	+	10	4	1	+
E <sub>1</sub>	O Taurocholsäure	1	+	-	-	12	-
E <sub>2</sub>	Na <sub>2</sub> Taurocholsäure	5	+	-	4	9	-
E <sub>3</sub>	Na <sub>2</sub> Taurocholsäure	10	+	-	6	7	-
F <sub>1</sub>	P Lithocholsäure	1	Suspension	-	2	11	-
F <sub>2</sub>	Lithocholsäure	5	Suspension	-	4	9	-
F <sub>3</sub>	Lithocholsäure	12	Suspension	-	15	3	-
G	Q NaCl	0,9	+	-	12	7	-
O	-	-	+	6	2	15	-

\*) bei 5%iger Irrtumswahrscheinlichkeit gegenüber der Kontrollgruppe.

R

**Key**

- A Group
- B Substrate
- C Soluble in % NaCl (0.9%)
- D Ulcerations
- E Erosions
- F Per Group
- G Point-Shaped
- H Band-Shaped
- I No Finding
- J Statistically Significant
- K Oxen Bile
- L Crystalline Cholic Acid
- M Na-Decyloleic Acid
- N Na-Glycocholic Acid
- O Na-Taurocholic Acid
- P Lithocholic Acid
- Q NaCl
- R \* at 5% Error Probability Compared to Control Group

RESEARCH  
BOOKS

In only 2 of 246 animals there were 3 ulcers, in 4 other animals 1542 there were 2 ulcers per stomach.

Unusual mucosal lesions in the form of wide, deep mucosal bleeding, especially along the limit fold, originated by the application of 10% Na-glycocholic acid. Because of lesion intensity these were rated as ulcerations.

ORIGINAL PAGE IS  
OF POOR QUALITY

Figure 2: Band-Shaped Bleeding Along the Limit Fold in the Glandular Stomach of the Rat. Condition After the Application of 10% Na-glycocholic Acid and After 4 Hours of Immobilization Stress.

An increase in frequency of ulcers was only proven in the groups D<sub>3</sub>, A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>. A statistically based difference, however, occurred in the  $\chi^2$  test only between the 0 groups and groups A<sub>3</sub> and D<sub>3</sub>.

### Discussion

The present studies of rat stomachs in the case of direct application of bile and bile acids in connection with a 4 hour immobilization stress should serve for examining the effect of the duodenal reflux in connection with the pathogenesis of the gastric ulcer.

The combination of 2 methods for generating peptic mucosal lesions in the animal experiment, in the present studies the combination of immobilization stress with the application of bile and bile acids in varying concentrations, permits more precise statements on the effect of certain materials on the gastric mucosa compared to published



experiments.

With respect to the complex composition of bile, in addition to oxen bile the following bile acids in 1%, 5%, and 10% concentrations were applied in each case: cholic acid, sodium deoxycholic acid, sodium glycocholic acid, sodium taurocholic acid and lithocholic acid.

/543

Immobilization stress of rats has been tested for a long period of time as an animal experimental model for generation of gastric mucosal lesions and has been described in various variants ([3]-[8], [22], [24], and others). Several discussions of the pathogenetic course of these stress models have been published in connection with other experiments ([6], [17] and others).

The observation that bile does not cause any phlogistic changes in gastric mucosa was made in the case of transplantation of gastric mucosa to the gallbladder [9]. Instead a cholecystitis was determined in all trial animals.

On the other hand, an increase in mucosal lesions was determined due to a chronic gastritis and attributed to the duodenal reflux of bile [15]. Watkinson [32] already found bile often in his examinations of digestive juice content of empty stomachs in patients with gastric ulcers. A connection between duodenal-pancreatic reflux of bile and gastritis has also been mentioned by other authors [15]. In 1956 Siurala and Tawest [31] described so-called "Gmelinpositive agents" in 46% of extreme cases of atrophy of gastric mucosa and in 31% cases of less severe mucosal atrophy. Capper et al. [10] were able to prove an increased duodenal reflux in the case of gastric ulcers using X-ray methods, Rhodes [30] discovered significantly higher bile acid concentrations in patients with stomach ulcers as opposed to healthy patients. Due to lipophilic and hydrophilic characteristics bile and bile acids are attributed the capability of destroying the gastric mucosal barrier. Ball et al. [2] found a loss in viscosity of gastric mucosal barrier where digestive juices were neutralized. Due to reduced resistance of mucosal barrier the  $H^+$  ions directly affect

the mucosa, in which  $\text{Na}^+$  ions are released from the mucosal cells ([12], [13], [23]). Ivy [23] discovered only 15 minutes after application of bile or bile salts destruction of the functional mucosal barrier with alterations of the described ion flow, Werther [33] observed this result after 30 minutes. Via rediffused  $\text{H}^+$  ions into the mucosa an increased HCl and pepsin secretion results in the mucosa [29]. Lawson [26] determined a chronic gastritis in the case of duodenal reflux especially in connection with bile and pancreatic juice when experimenting with dogs. In the case of experiments with monkeys all degrees of a reflux-esophagitis was generated, as well as an esophageal ulcer after cholecystic ingestion [18].

The present studies with oxen bile in varying concentrations (1%, 5% and 10%) as well as varying bile acids resulted only in the animals given the 10% oxen bile (group A<sub>3</sub>) and given 10% sodium glycocholic acid (D<sub>3</sub>) a statistically significant increase in frequency of mucosal lesions in the stomach as compared to the control group (Table 2).

The important morphologically and statistically significant alterations in the animal group (D<sub>3</sub>) given 10% sodium glycocholic acid was striking.

The animals demonstrated as a whole a large degree of atony with a considerable dilation of the stomach as well as extended band-shaped areas of mucosal bleeding. The high degree of stomach atony, in connection with a stasis of the stomach as described by Dragstedt must be considered as the reasons for an increased vulnerability of mucosa. To what degree the sodium glycocholic acid has a toxic effect - Cross and Wangenstein reported in 1951 on 8 cats with necrosis of the esophagus wall after perfusion with sodium-taurocholic acid and sodium glycocholic acid [1] - remains to be examined.

Table 2: Summary of the Individual Main Groups Designated by a Letter and Composed of 3 Sub-groups in Each Case and a Comparison of the Animals with Ulcers or Erosions (in this case pre-ulcerations) and Those Where None Were Found. An increased lesion frequency results only in the groups A and D, statistically significant only in A<sub>3</sub> and D<sub>3</sub>.

	A	B	C	D	E
Gruppe	ulc.	prae. ulc.	u. B.	Zahl d. Tiere	
A	12	9	15	36	
B	2	10	24	36	
C	3	2	31	36	
D	10	3	23	36	
E	—	6	28	34	
F	—	8	28	36	
G	—	5	7	12	
O	4	1	15	20	

Key

A Group                      C Pre-ulcerations                      E # of Animals  
 B Ulcer                         D Without Findings

Sodium taurocholic acid, however, showed no visible effect in our experiments compared to the control group.

Except for the important and probably toxic effects of Na-glycocholic acid in a 10% solution only the animal group given oxen bile in a 10% solution showed a statistically significant ulcer frequency compared to the control group. As far as the oxen bile is concerned, the results are in agreement with those published, especially with clinical observations. With the exception of the 10% Na-glycocholic acid, however, the same statement may not be made about the individual bile acids tested, or their natrium salts. The effect of bile in the pathogenesis of ulcerous gastric mucosal lesions may be considered pioneering, taking the experimental results into consideration and is especially apparent in combination with other ulcerogenic factors, represented in the present experiments by immobilization stress.

## Summary

The present work studies the effect of oxen bile and individual bile acids, or their natrium salts in differing concentrations on the gastric mucosa of rats in combination with immobilization stress. A statistically significant higher frequency of ulcers was only determined in the application of 10% oxen bile. Dosages of 10% sodium glycocholic acid demonstrated strong toxic damage with atonic dilation of the stomach and extensive mucosal bleeding.

The results gained are explained in connection with published clinical and animal experiments.

## REFERENCES

- [1] Ball, P.A.J. and A.H. James, "The histological background to gastric ulcer," Lancet 1, p. 1365 (1961).
- [2] Ball, P.A.J., "The secretory background to gastric ulcer," Lancet 1, p. 1363 (1961).
- [3] Bonfils, S., G. Liefvoege, G. Rossi, and A. Lambling, "L'ulcere de contrainte du rat blanc, Modification de la frequence lesionelle par differents procedes techniques et pharmacodynamique," Cte. rend Soc. Biol., 151, Paris, p. 1149 (1957).
- [4] Bonfils, S. and A. Lambling, Psychological factors and psychopharmacological actions in the restraint induced gastric ulcer, S.C. Skoryna ed., University Press Montreal, 1963, p. 153.
- [5] Bonfils, S., J.-P. Ferrier and Chr. Caulin, "L'ulcere de contrainte du rat blanc. Methode de pathologic experimentale et test pharmacologique," Rev. Franc. Et. Clin. Et. Biol. XI, p. 343 (1966).
- [6] Brodie, D.A., R.W. Marshall and O.M. Moreno, "Effect of restraint on gastric acidity in the rat," Am. J. Physiol. 202, p. 812 (1962).
- [7] Brodie, D.A., "Ulceration of the stomach produced by restraint in rats," Gastroenterology 43, p. 107 (1962).
- [8] Brodie, D.A. and H.M. Hanson, "A study of the factors involved in the production of gastric ulcers by the restraint technique," Gastroenterology 38, p. 353 (1960).
- [9] Byers, J.M. and P.H. Jordan, "Effect of bile upon gastric mucosa," Proc. Soc. exp. Biol. N.Y. 110, p. 864 (1962).
- [10] Capper, W.M., G.R. Airth and J.O. Kilby, "A test for pyloric regurgitation," Lancet II, p. 621 (1966).
- [11] Cross, F.S. and O.H. Wangensteen, "Role of bile and pancreatic juice in production of esophageal erosions and anemia," Proc. Soc. exp. Biol. Med. 77, p. 862 (1951).
- [12] Davenport, H.W., "Destruction of the gastric mucosal barrier by detergents and urea," Gastroenterology 54, p. 175 (1968).
- [13] Davenport, H.W., "The gastric mucosal barrier," Digestion 5, p. 162 (1972).

- [14] Dragstedt, L.R., E.R. Woodward, C.A. Lineares and C. de la Rosa, "The pathogenesis of gastric ulcer," Ann. of Surg. 160, p. 497 (1964).
- [15] Du Plessis, D.J., "Pathogenesis of gastric ulceration," Lancet I, p. 974 (1965).
- [16] Evert, G., F. Niedobitek and E. Schmid, "Ulcerogenic combination effects of emotional stress and caffeine, that is coffee in animal trials," Z. Gastroenterol. 9, p. 94 (1971).
- [17] Frommolt, A., F. Niedobitek and E. Schmid, "On the influence of coffee in healing an ulcer caused by stress," Z. Gastroenterol. 10, p. 107 (1972).
- [18] Gillison, E.W., V.A.M. de Castro, L.J. Nybus, K. Kusakari and C.T. Bombeck, "The significance of bile reflux-esophagitis," Surg. Gyn. Obst. 134, p. 419 (1972).
- [19] Goldman, H. and Ch. G. Rosoff, "Pathogenesis of acute gastric stress ulcers," Gastroenterology 52, p. 227 (1968).
- [20] Guth, P.H. and P. Hall, "Microcirculatory and mast cell changes in restraint-induced gastric ulcer," Gastroenterology 50, p. 562 (1966).
- [21] Guth, P.H., X. Kozbur and P. Hall, "Microcirculatory and mast cell changes in stress ulcer; role of gastric acid," Gastroenterology 50, p. 847 (1966).
- [22] Huebner, G. H.J. Klein and M. Eder, "Studies of the fine structures in rat stomachs during acute stress generated by immobilization," Verh. Deutsch. Pathologie 54, p. 386 (1970).
- [23] Ivey, K.J., L. Den Besten and J.A. Clifden, "Effects of bile salts on ionic movement in the human gastric mucosa," Gastroenterology 59, p. 683 (1970).
- [24] Klein, H.J., Th. Geroghin, G. Huebner and M. Eder, "On the pathogenesis of stomach ulcers due to stress: morphological and pathophysiological studies using restrained rats," Virch. Arch. Abt. A Path. Anat. 352, p. 195 (1971).
- [25] Lambling, A. and J.R. Grosset, "Le reflux des secretions alcalines duodeno-pancreatico-biliaires en physio-pathologie gastrique," Arch. mal. app. 36, p. 533 (1947).
- [26] Lawson, H.H., "Gastritis and gastric ulceration," Brit. J. Surg. 33, p. 493 (1966).

- [27] Lawson, H.H., "Effect of duodenal contents on the gastric mucosa under experimental conditions," Lancet I, p. 469 (1964).
- [28] Menguy, R., "Effects of restraint stress on gastric secretion in the rat," Am. J. Dig. Dis. 5, p. 911 (1960).
- [29] Ottenjann, R., "New pathophysiological aspects of ulcers," Muench. Med. Wschr. 116, p. 181 (1974).
- [30] Rhodes, J., D.E. Bernado, S.F. Philips, R.A. Rovelstad and A.F. Hofmann, "Increased reflux of bile into the stomach in patients with gastric ulcera," Gastroenterology 57, p. 241 (1969).
- [31] Siruala, W. and M. Tawest, "Duodenal regurgitation and the state of gastric mucosa with special reference to the occurrence of surface lowering factors in gastric contents of cases with chronic gastritis," Acta med. Scand. 153, p. 451 (1956).
- [32] Watkinson, G., "A study of the changes in pH of gastric contents in peptic ulcer using the twenty-four hour test-meal," Gastroenterology 18, p. 377 (1951).
- [33] Werther, J.L., H.D. Janowitz, W.P. Dyck, M.L. Chapman and J. Rudick, "The effect of bile on electrolyte movement across canine gastric antral and fundic mucosa," Gastroenterology 59, p. 691 (1970).

Author's Address: Dr. med. J. Wiesener, Pathologisches Institut der Freien Universitat Berlin im Klinikum Westend, D-1000 Berlin 19, Spandauer Damm 130.