STUDY TITLE:

MUCOUS MEMBRANE IRRITATION STUDY IN THE HAMSTER

(ADA Method)

TEST ARTICLE:

Nylon Denture Material

IDENTIFICATION NO.:

Not Supplied

SPONSOR:

DR. RITA FOLEY
FOLEY DENTAL PROFESSIONALS
1214 ERIC LANE
LAKE ZURICH, IL 60047

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TABLE OF CONTENTS

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SUMMARY

A cheek pouch mucosal irritation study was conducted in the hamster. The test article, Nylon Denture Material, was secured by suture to the right cheek mucosa of ten hamsters; a flattened USP negative control disc was secured to the left cheek mucosa of all ten animals and served as the comparative control. After 2 weeks of exposure, the hamsters were euthanatized and their oral mucosa recovered in toto for microscopic tissue evaluation.

Cheek mucosa was grossly normal in appearance when observed for irritation at day 14. Samples of the implant sites were processed and found to be within normal histomorphological limits.

Under the conditions of this study, the test article would not be considered irritating to the hamster cheek mucosa. As anticipated, the USP reference control was not an irritant.

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7-23-97
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INTRODUCTION

A cheek mucosal irritation study of the test article identified below was conducted in the hamster. The oral mucosa of the hamster, lined with stratified squamous epithelium, has numerous subepithelial blood vessels present that become dilated and easily observed when irritation has occurred. The purpose of this study was to evaluate the irritation potential of the test article in contact with the oral mucosal tissue of the hamster. The test article was received on June 19, 1997. The animals were implanted on June 24, 1997, and the oral mucosal tissue was excised on July 8, 1997.

MATERIALS

The sample provided by the sponsor was identified and handled as follows:

Test Article:	Nylon Denture Material
Identification No.:	Not Supplied
Storage Condition:	Room temperature
Preparation:	The test article was tested as received.
Control Article:	USP polyethylene negative control reference sheet purchased by NAmSA from the offices of the US Pharmacopeial convention were cut into disc form (approximately 8 mm x 0.2 mm).

METHODS

Test System:

Species:	Hamster (Mesocricetus auratus)
Strain:	Lak:LVG(SYR)BR
Source:	Charles River Laboratories
Sex:	Male
Body Weight Range:	86 to 108 grams at implantation
Age:	No particular age was prescribed for this test
Acclimation Period:	Minimum 5 days
Number of Animals:	Ten
Identification Method:	Ear punch

Justification of Test System:

The highly vascularized buccal mucosa of the hamster cheek has been used traditionally to evaluate materials for mucosal irritation potential. The American Dental Association has recommended the hamster as an appropriate model for the oral mucosal test.

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Animal Management:

Husbandry:	Conditions conformed to Standard Operating Procedures which are based on the "Guide for the Care and Use of Laboratory Animals."
Food:	PROLAB® R-M-H 1000 Rodent Diet was provided daily.
Water:	Freely available, municipal (Irvine, CA) water was delivered through an automatic watering system.
Contaminants:	Reasonably expected contaminants in feed or water supplies did not have the potential to influence the outcome of this test.
Housing:	Animals were individually housed in stainless steel suspended cages identified by a card indicating the lab number, animal number, test code, sex, animal code and date implanted.
Environmental:	The room temperature was monitored daily. The temperature range for the hamster was 66-76°F. The room humidity was monitored daily. The humidity range for the hamster was 30-70%. The light cycle was controlled using an automatic timer (12 hours light, 12 hours dark).
Facility:	NAmSA is an AAALAC accredited facility.
Personnel:	Associates involved were appropriately qualified and trained.
Selection:	Only healthy, previously unused animals were selected.

Experimental Procedure:

Each hamster was weighed and anesthetized by intraperitoneal injection of a combination of ketamine hydrochloride/xylazine (133 mg/kg + 13.3 mg/kg) at a dose of approximately 0.2 ml/100 g of body weight. Each cheek was examined for signs of preexisting irritation. Any animal exhibiting irritation was excluded from the study.

Sterile 23 gauge needles were introduced from the outside to the inside of the cheek. The placement of needles corresponded to the approximate position as the holes in the material. Individual samples with prethreaded sutures were secured to the oral mucosal tissue by feeding the suture ends through the appropriate needles. The needles were withdrawn and the suture was tied snugly to the external surface of the cheek. The sutures were tightened enough to keep the article in place against the oral tissue. The right cheek was implanted with two test samples; one near the upper buccogingival junction and one in proximity to the lower buccogingival junction. The control articles were similarly placed in the left cheek. Animals were observed frequently in their cages as they recovered from anesthesia.

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Hamsters were checked visually on a daily basis for general health. The presence of sutures was checked daily to verify the presence of test and control articles. At termination, all sites were processed.

Termination:

At 14 days, the animals were euthanatized by carbon dioxide inhalation and weighed. The skin was cut along the upper and lower jawline and the cheek immediately anterior to the pouch opening was removed. The articles were carefully lifted away from the mucosa and the mucosal layer was examined for signs of irritation. The exposure sites were excised and the articles were removed. The sites were placed in 10% Neutral Buffered Formalin (NBF).

Mucosal tissue sites from each animal were routinely oriented and embedded in paraffin, cut, and stained in Hematoxylin and Eosin (H&E). Microscopic evaluation was conducted by a board certified veterinary pathologist.

RESULTS

Clinical Observations: All animals appeared clinically normal throughout the study. Three animals were noted with loss of test article during the 14 day observation period. Animals #8 and #10 had a loss of both implants on Day 9 and Day 10, respectively. Animal #1 had a loss of one implant on Day 10.

Body Weight and Macroscopic Examination:

Body weight data were considered acceptable. Individual body weights and macroscopic findings are presented below:

Hamster	Weight (g)		Macroscopic Observation of Cheek Mucosa			
	Day 0	Day 14	Test		Control	
			Upper	Lower	Upper	Lower
1	86	132	*, **	*	MN	MN
2	100	148	MN	MN	MN	MN
3	98	146	MN	MN	MN	MN
4	100	137	MN	MN	MN	MN
5	99	132	MN	MN	MN	MN
6	108	119	MN	MN	MN	MN
7	101	118	MN	MN	MN	MN
8	88	129	MN**	MN**	MN	MN
9	90	126	MN	MN	MN	MN
10	95	135	MN**	MN**	MN	MN
Mean:	96.5	132.2				

MN = Macroscopically Normal
* Mild congestion observed
**Test article not present

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Microscopic Evaluation: Results of postmortem tissue evaluation are presented in the histopathology report, pages 8 through 14 of this report. There were no significant findings related to the test or control articles. However, observed lesions at both test and control sites were noted by the pathologist, due to the method used to secure the implants to the oral mucosal tissue by penetration of the cheek with a 23 gauge needle and fixation with suture. Macroscopic changes noted at the time of gross necropsy for animal #1 showed no correlated changes microscopically.

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAmSA. Any extrapolation of these data to other samples is the responsibility of the sponsor.

CONCLUSION

Under the conditions of this study, the cheeks implanted with the test article were similar to cheeks implanted with the reference control material. Microscopic evaluation of the cheek mucosa conformed to normal histomorphological limits. The test article would not be considered an irritant to the hamster cheek mucosa.

RECORD STORAGE

All raw data pertaining to this study and a copy of the final report are to be retained in designated NAmSA archive files.

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NORTH AMERICAN SCIENCE ASSOCIATES
SPONSOR STUDY NO. 97C-12302-00

CVD/IDEXX VS, Inc. Study No. X7003499

Histopathology Report

A De Paoli

Pathologist
ALEXANDER De PAOLI, DVM, Ph.D.
Diplomate, American College of
Veterinary Pathologists

7/17/97
Report Date

ADP:cem

Received by CVD/IDEXX VS, Inc. were formalin fixed test and control buccal pouch samples from ten hamsters on NAmSA buccal irritation study 97C-12302-00, CVD/IDEXX VS, Inc. Study No. X7003499.

Tissues were trimmed, processed, blocked, sectioned, stained with H&E, examined microscopically and narrative descriptions of lesions provided as requested.

RESULTS/DISCUSSION

Results of microscopic evaluation for each site are given in the attached Individual Animal Histopathology Reports.

Observed lesions in both treated and control sites suggest these changes were induced by injection. However, a lack of information as relates to study protocol precludes further interpretation of said changes.

Individual Animal Histopathology Report

Animal No. 1

Test -

Two sections of buccal pouch examined. A focal area of fibrosis and chronic inflammation is present in the wall of the pouch in one section. The affected area extends from the buccal mucosal epithelium to the epidermis of the overlying skin. Several hair follicles in affected dermis are moderately distended with keratin while others are missing destroyed by the inflammatory process. The underlying muscular coat has been replaced by fibroblasts while a few fibers show ballooning degeneration. The epidermis overlying the area of inflammation is mildly acanthotic.

Control -

Two sections of buccal pouch are examined. A focal area of fibrosis is present in the wall of the pouch with local disruption of muscular coat. The epidermis overlying the area of the fibrosis is moderately acanthotic while the mucosal epithelium overlying this layer is mildly thickened.

Animal No. 2

Test -

Two sections of buccal pouch are examined. A focal area of fibrosis and mild chronic cell infiltration is present in the buccal wall disrupting the muscular coat and extending from the mucosal epithelium to the opposing epidermis. The epidermis and mucosal epithelium over the area of fibrosis are thickened (acanthotic) and hyperkeratotic.

Control -

Two sections of buccal pouch are examined. A focal abscess is present in the wall of one section. The abscess disrupts the muscular coat and is surrounded by fibrosis. The mucosal epithelium overlying the abscess is thickened.

Individual Animal Histopathology Report

Animal No. 3

Test -

Two section of buccal pouch are examined. A mild focal area of fibrosis disrupts the submucosal muscular coat in both sections. Mild thickening is noted in overlying mucosal epithelium.

Control -

Two sections of buccal pouch are examined. A focal are of minimal fibrosis is present in the muscular coat and submucosa of one of the sections.

Animal No. 4

Test -

Two sections of buccal pouch are examined. A squamous epithelium inclusion cyst is present in one section. The cyst is surrounded by a collar of neutrophils which in turn are surrounded by an area of fibrosis. The epidermis overlying this inflammatory lesion is mildly thickened, acanthotic and hyperkeratotic as is the opposing mucosal epithelium. Adnexal structures in the affected area of the skin are missing.

Control -

Two sections of buccal pouch are examined. A focal area of fibrosis, muscle degenerate and mild chronic inflammation is present in the submucosal area of one section. The mucosal epithelium overlying the affected area is moderately thickened and hyperkeratotic.

Individual Animal Histopathology Report

Animal No. 5

Test -

Two sections of buccal pouch are examined. An area of fibrosis extending from the mucosa to the skin is present in both sections. The area of fibrosis disrupts and replaces the regional muscle coat as well as skin adnexal structures. The mucosal epithelium overlying this area as well as the opposing epidermis are thickened and hyperkeratotic.

Control -

Two sections of buccal pouch are examined. A focus of chronic inflammation is present in the submucosa of both sections. The mild inflammatory process in one of the sections is associated with several hair shafts.

Animal No. 6

Test -

Two sections of buccal pouch are examined. A focal area of fibrosis is present in the wall of one section. The fibrosis extends from the mucosa to the opposing areas of the skin and disrupts local areas of the muscular coat. The mucosal epithelium overlying the area of fibrosis is mildly thickened. A focal area of mild chronic inflammation is present in the submucosa of the second section.

Control -

Two sections of buccal mucosa are examined. An epithelial inclusion is present in the wall of one section. The epithelium is surrounded by chronic active inflammatory cells and fibroblasts. The inflammatory process disrupts and has replaced regional muscular coat. The mucosal epithelium overlying this lesion is markedly thickened.

Individual Animal Histopathology Report

Animal No. 7

Test -

Two sections of buccal pouch are examined. A focal area of fibrosis and mild inflammation is present in the wall of one section extending from the mucosa to the opposing epidermis. The fibrosis disrupts and replaces the regional muscular coat as well as regional skin adnexa. The mucosal epithelium overlying this lesion and the opposing epidermis are moderately thickened. A mild focus of chronic inflammation is present in the submucosa of the second section.

Control -

Two sections of buccal pouch are examined. A focal area of chronic inflammation and fibrosis disrupts the buccal wall of one section. The overlying mucosal epithelium and opposing epidermis are mildly thickened.

Animal No. 8

Test -

Two section of buccal pouch are examined. A focal area of fibrosis and mild chronic inflammation disrupts the buccal wall in one section replacing regional muscle coat and skin adnexal structures. The epidermis and mucosal epidermis overlying the affected are are mildly thickened and hyperkeratotic.

Control -

Two sections of buccal pouch are examined. Both sections have a focal area of fibrosis and chronic active inflammation in submucosal area with some disruption of local muscle coat. The overlying mucosal epithelium is moderately thickened.

Individual Animal Histopathology Report

Animal No. 9

Test -

Two sections of buccal pouch are examined. A focal area of fibrosis and chronic inflammation is present in the wall of one section. The fibrosis extends from the mucosa to the opposing skin and disrupts and replaces the regional muscle coat and skin adnexa. The overlying mucosal epithelium and epidermis are markedly thickened and hyperkeratotic. A hair shaft is present at one margin of the lesion.

Control -

Two sections of buccal pouch are examined. A focal area of mild fibrosis is present in the dermis of both sections.

Animal No. 10

Test -

Two sections of buccal pouch are examined. A focal area of mild fibrosis is present in the wall of one section. Mild degeneration and atrophy of regional muscle coat is associated with the fibrosis.

Control -

Two sections of buccal pouch are examined. A focal area of fibrosis and chronic inflammation is present in the buccal wall of one section. The fibrosis extends from the mucosa to the opposing skin disrupting and replacing the regional muscle coat and skin adnexa. The mucosal epithelium and epidermis overlying this area are thickened and hyperkeratotic.