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ANIMAL ACTIVITY PROTOCOL
University of Mississippi Medical Center

To be completed by IACUC

Protocol Number: 0801 Date: 11/16/99 Classification: 2 (D)

1. Principal Investigator Name: Roger B. Johnson, DDS, PhD
Title: Professor
Dept: Periodontics
Phone/Pager: 984-6115
E-mail: rjohnson@sod.umsmed.edu

2. A. Other personnel working with animals: None
Name: _____ Name: _____
Title: _____ Title: _____
Phone/Pager: _____ Phone/Pager: _____

B. Have all the individuals listed above submitted their completed training requirements registration form? Yes No

3. Project title: Effects of diet on the initiation of gingivitis

4. Proposal is: New
 3-yr. Full Submission
 Revision of Existing Protocol (highlight/italicize/any changes)

5. Will any "outside" contracts be used in this study that involve live animals?
 No
 Yes (if yes, provide information on the level of involvement)

6. Funding Source: Grant Title: Effects of diet of the initiation of gingivitis
Funding Source: Iams Company
Covered Dates: 10/1/99-9/30/00

Department funding
Has this proposal received any peer review? Yes No

7. Procedure Category: Acute (anesthesia/euthanasia without recovery)
 Survival

8. A. Anticipated start date of study: 12/1/99
 B. Study duration: (maximum - 3 years) #1 year 1-2 years 2-3 years

All investigators MUST adhere to a federally mandated three-year cycle of full protocol review, even if a funding period exceeds three years in duration.

Animal Husbandry & Care

9. Animal Requirements:

Species A: <u>Canine</u>	Species B: <u>Feline</u>
Strain/stock: <u>Beagle</u>	Strain/stock: _____
Sex: <u>M</u>	Sex: <u>M</u>
Source: <u>IAMS Company</u>	Source: <u>IAMS Company</u>
Total number of animals to be used: <u>21</u>	Total number of animals to be used: <u>21</u>

Animal numbers MUST be calculated for a period not to exceed three (3) years from the start of the study. See 8-B.

10. Will animals be involved in a breeding program at UMC?

No
 Yes

Note: If yes, provide a specific description of the type of breeding program to be utilized (e.g. harem/monogamous, who is to be responsible for mating, who is to be responsible for weaning, how will genetic quality be ensured?).

- | | | | |
|--|-------------------------------------|--------------------------|----------------------------------|
| 11. Potential hazards to personnel or other animals: | <u>NO</u> | <u>YES</u> | |
| A. Chemical toxins in bedding/cages/carcasses | <input checked="" type="checkbox"/> | <input type="checkbox"/> | |
| Reviewed by Risk Mgmt? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> PENDING |
| B. Radioisotopes in bedding/cages/carcasses | <input checked="" type="checkbox"/> | <input type="checkbox"/> | |
| Reviewed by Radiation Safety? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> PENDING |
| C. Infectious agents or recombinant DNA usage | <input checked="" type="checkbox"/> | <input type="checkbox"/> | |
| Reviewed by Biohazards Committee? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> PENDING |

Note: If yes, provide specific details of specialized animal husbandry, care, cleaning, or decontamination procedures, especially identifying responsible parties.

12.	Animal Husbandry:	<u>Standard</u>	<u>Nonstandard</u>
	Feeding	<input type="checkbox"/>	<input checked="" type="checkbox"/>
	Watering	<input checked="" type="checkbox"/>	<input type="checkbox"/>
	Caging/housing	<input checked="" type="checkbox"/>	<input type="checkbox"/>

Note: Provide complete explanation and justification for any **nonstandard animal husbandry**. Protocols listing non-standard cleaning/sanitation (e.g. metabolic caging, restraint chairs, transport devices) must provide complete details of the cleaning and sanitation, as well as validation methods.

Special diets will be provided by IAMS. These diets will meet or exceed the AAFCO requirements for all nutrients. In addition, IAMS will conduct laboratory analyses on all diets to ensure that they meet specifications for all nutrients.

13. Will animals be housed outside of the LAF for greater than 12 hours?
 No
 Yes Where? _____

Note: If yes, provide complete explanation and justification for any **decentralized animal housing**.

Experimental Procedures & Animal Manipulations: Items 14&15 MUST be written specifically for review by persons lacking scientific training (ie. in non-technical/lay terminology). Assistance in preparation of this federally mandated requirement may be obtained by consultation with the UMC Office of Research, ext. 5-5000.

14. In non-technical/lay terminology, what is the objective of the proposed Animal Activity Protocol?

Dogs and cats develop periodontal diseases as they age, which leads to loss of teeth. Currently, the only effective method for prevention of gingivitis/periodontal disease is daily brushing of the teeth by the pet owner. These procedures are time consuming and may not be permitted by some animals. Thus, prevention of gingivitis/periodontal disease by diet could be very beneficial. The objective of this study is to determine whether several dietary formulations could either inhibit initiation or promote resolution of gingivitis/periodontal disease in the dog and cat. If so, use of these diets could prevent initiation of periodontal diseases in these animals and future loss of teeth.

15. A. What is the rationale for using animals rather than using non-animal models?

The dog and cat have unique dietary requirements, which does not allow testing of these diets on other species, such as rodents. In addition, it seems reasonable to test these dietary formulations on the ultimate consumer of the diet, the cat and dog. There are currently no in vitro models for testing these diets.

B. What is the rationale for using the particular animal species noted in #9?

The diets, if successful, will be marketed to owners of dogs and cats. Thus, these animals must be used to test the diets. Each species has unique dietary requirements, which does not allow testing of these diets on other species, such as rodents.

16. Will surgical procedures be a component of this Animal Activity Protocol?
 NO YES

Note: If answered YES, complete Appendix A.

17. Does this proposal include any of the following procedures?
- | <u>NO</u> | <u>YES</u> | |
|-------------------------------------|--------------------------|--|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Prolonged Physical Restraint |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Multiple Major Surgical Procedures |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Food and/or Fluid Restriction |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Animal Pain and/or Distress (<i>other than momentary for injections, etc.</i>) |

Note: If answered YES, complete Appendix B.

18. Does this proposal utilize non-human primates? NO YES

Note: If answered YES, complete Appendix C.

19. Provide a complete description of the animal procedures and experimental design (exclusive of specific surgical procedures). (Detail drugs, dosages, post-procedure management, biologic sampling, nursing care of implants [catheters, acrylic, flow probes], etc. (Note, complete descriptions of surgical procedures must be provided in Appendix A.)

We propose to test three dietary formulas using three animal groups and utilizing a blinded, split-mouth design. We do not anticipate any veterinary medical problems during the experiments; however, if problems occur, they will be reported to the veterinary staff. **Baseline evaluation (Week 1-Week 4)**. Animals will be delivered to the University of Mississippi Medical Center and will be maintained on a standard diet for 4 weeks to equilibrate their metabolism. One week following initiation of the standard diet, animals will be weighed and anesthetized using medetomidine (1 mg/ml), IM (1000 µg/m²)

supplemented by 1-2% isoflurane (if required), and teeth will be cleaned of plaque and calculus by scraping. Prior to initiation of the experimental diets (week 4), animals will be weighed and anesthetized using medetomidine (1 mg/ml), IM (1000 µg/m²) supplemented by 1-2% isoflurane (if required), and baseline periodontal evaluations will be performed. This will include assessments of gingival sulcular depths at mesial and distal line angles on facial and lingual surfaces of all teeth, plaque and bleeding indices on facial and lingual surfaces of all teeth, collection of gingival crevicular fluid on mesiofacial and distofacial surfaces (using filter paper strips), collection of whole unstimulated saliva (5 minute collection by the "drool method"), and obtaining standardized periapical radiographs of all teeth. A blood sample [5 ml (cat) or 10 ml (dog) from the external jugular vein] and a biopsy of the gingiva between 1st and 2nd premolar teeth (using a skin biopsy punch) will also be obtained. A breath sample will be taken for analysis of sulfur compounds. Thermal images of the gingiva will be made in each sextant. The anesthesia will be reversed by atipamezole (5 mg/ml) in the same volume as medetomidine and the animals returned to cages. Animals will then be placed on an experimental dietary regime for 8 weeks (Week 4- Week 12) prior to initiation of gingivitis using three experimental diets furnished by IAMS. The diets will all meet the nutritional needs of the animal. The principal investigator will be blinded in regard to the specific dietary formulation of each animal.

Initiation of gingivitis. On week 12, animals will be weighed, anesthetized as previously described, and periodontal evaluations will be performed as previously described. Silk suture material placed will be placed adjacent to the gingival margin of all posterior teeth on the right side (Lindhe et al., 1973). The left side will be sham operated and will serve as an untreated control. A breath sample will be taken for analysis of sulfur compounds. Thermal images of the gingiva will be made in each sextant. A biopsy of the gingiva between the maxillary 2nd and 3rd premolar teeth (using a skin biopsy punch) will also be obtained. In addition, a blood sample [5 ml (cat) or 10 ml (dog)] will be collected. The anesthesia will be reversed as previously described and the animals returned to cages. Fourteen days later (Week 14), animals will be weighed, anesthetized and the suture materials removed from all teeth. Assessments of gingival sulcular depths and plaque and bleeding indices, collection of gingival crevicular fluid and saliva will be made and standardized periapical radiographs will be made. A blood sample [5 ml (cat) or 10 ml (dog)] will also be collected. These parameters will be compared to baseline values and to the control sites. In addition, a biopsy of the interdental papillae between premolar teeth 2 and 3 on experimental and control sides will be made using a skin biopsy punch. Plaque and calculus will be scraped from all teeth except premolar tooth 4 and molar tooth 1. The anesthesia will be reversed and the animals returned to cages. Gingival biopsies will be solubilized and assayed for inflammatory biomarkers in the laboratory.

Resolution of gingivitis. Animals will continue receiving experimental diets during the gingivitis recovery period (Weeks 14-16). At week 16, animals will be weighed, and anesthetized as previously described. Assessments of gingival sulcular depths and plaque

and bleeding indices, collection of gingival crevicular fluid and saliva and standardized periapical radiographs will be made. In addition, a biopsy of the interdental papillae between premolar tooth 4 and molar tooth 1 on resolved experimental and control sides will be made using a skin biopsy punch. A breath sample will be taken for analysis of sulfur compounds. Thermal images of the gingiva will be made in each sextant. A blood sample [5 ml (cat) or 10 ml (dog)] will also be collected. These parameters will be compared to previous data from experimental and the control sites. Remaining plaque and calculus will be scraped from premolar tooth 4 and molar tooth 1. The anesthesia will be reversed and the animals returned to cages. Gingival biopsies will be solubilized and assayed for inflammatory biomarkers in the laboratory. **At the end of the experiments, animals will be euthanized.**

20. Explain and **justify** how the number of animals requested was determined.

(Flow diagrams/tables to define animal use are encouraged)

We plan to use 7 animals per group, based on a power analysis of data derived from a recent study of periodontal disease in OVX sheep. Using a significance level of 0.05 and equally distributing the animals between 3 groups, a power analysis suggested that a sample size of 7 animals could detect a significant difference in periodontal disease parameters between the groups.

21. Indicate room(s) where animal procedures (other than surgery) will be conducted.

8th floor, Research Wing

Studies involving animal transportation to locations other than the housing area must identify the animal transport device, the nature of shrouds used to cover the transport device, and describe the route of transport.

22. **A. At what point in the proposed protocol will animals normally be euthanized, (experimental end-points)? Or at what point will any individual animal be euthanized?**

Animals will be euthanized at the end of the test period. If an animal experiences unanticipated health problems during the experiments, it will be euthanized by overdosage of sodium pentobarbital (IV).

- B. What criteria will be used to determine if an animal is to be euthanized prior to, rather than at, the anticipated end-point of an experiment? We will accept the advice of the veterinary staff regarding treatment of unanticipated health complications during the experiments and euthanize animals if appropriate.

Advice of veterinary staff.

Note: Contact the Office of Research, ext. 5-5000, for recommendations on the assessment criteria.

23. What procedures will be used to euthanize the animals? NA Note: Secondary methods are recommended to ensure death. (Consult Section VII

of the LAF Training & Procedural Manual for appropriate methods of euthanasia.)

Assurances

Have all personnel received a medical evaluation from UMC Student/Employee Health?

No

Yes

Have all personnel become familiar with the *LAF Training & Procedural Manual*?

No

Yes

Review of the available resources and previous experiments have determined that the proposed activity is not unnecessarily duplicative of previously reported activities.

No

Yes

You are required by law to provide a written narrative of the sources you consulted to determine whether or not alternatives exist to procedures that may cause pain and distress. Minimal information should include: the databases searched or other sources consulted, the date of the search and the years covered by the search, and the key words and/or search strategy used to determine that no alternatives were available to the painful or distressful procedure. Reduction and refinement should be addressed in addition to replacement. [AWA Section 13(a)(3)(b) and 9 CFR, part 2, Section 2.31 (d)(1)(ii)] (See the Office of Research web site to read the regulations.)

Sources utilized: Index Medicus Toxline other Grateful Med
 Medlar AWIC
 MEDLINE Agricola

Search date: September 27, 1999

Covered years of search: 1975-Present

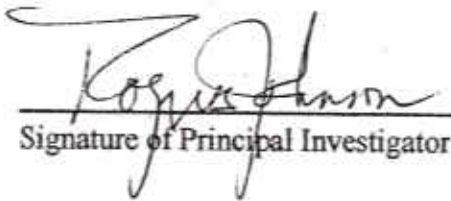
Number of "hits": 73

Key words: (To include in vitro, animal model, etc.)

Gingivitis, animal models, periodontal disease

Certification of the Principal Investigator:

Signature certifies that the Principal Investigator will conduct the project in full accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, USDA regulations, and UMC policies governing the use of live vertebrate animals for research and teaching purposes. The procedures involving animals will be conducted by trained or experienced personnel or under the direct supervision of trained or experienced persons. It is understood that IACUC approval is valid for a period of 12 months following the date of original approval and must be renewed annually for continued approval. I understand there is a 3-year requirement for full protocol rewrite. **It is further understood that should this project be submitted for external funding, the information presented on the UMC Animal Activity Protocol form accurately reflects the animal use in the full grant application.**



Signature of Principal Investigator

11/4/99
Date

Approval by the Attending Veterinarian:



Signature

Nov 10, 99
Date

Approval by the Institutional Animal Care and Use Committee:



Signature

11/16/99
Date

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

2500 North State Street
JACKSON, MISSISSIPPI 39216-4505

Institutional Animal Care
and Use Committee

Area Code 601
815-5000
Fax 601
815-5010

November 22, 1999

Dr. Roger B. Johnson
Dept. of Periodontics
Univ. of Mississippi Medical Center
2500 North State Street
Jackson, MS 39216


Dear Dr. Johnson:

Thank you for submitting the additional information requested for your initial submission protocol, *Effects of Diet on the Initiation of Gingivitis*, that was reviewed at the September 1999 meeting of the Institutional Animal Care and Use Committee. The subcommittee has completed the review process and your protocol is approved. This protocol is assigned protocol number 0801 with an approval date of November 16, 1999. Please refer to this number when ordering animals or making inquires about this protocol.

Also, please be aware that approval of your protocol does not necessarily assure timely availability of adequate animal housing space. Please contact the Laboratory Animal Facilities at extension 4-1385 concerning animal housing availability.

My telephone extension is 5-5000 if you have additional questions about your protocol. Enclosed is a copy of the protocol with signatures for your file.

Sincerely,


Chairman, IACUC

DJD/jmg

Cc: 