

APPLICATION FOR RESEARCH CONTRACT - PART 1

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NEW RENEWAL

CONTRACT NUMBER

1. TITLE OF PROJECT

The Effect of Local Irritants on Oral Tissues

2. DATE RESEARCH CONTRACT TO BEGIN

1 December 1955

3. NAME AND OFFICIAL POSITION OF RESPONSIBLE INVESTIGATOR

Seymour J. Kreshover, Professor of Oral Pathology and Director of Dental Research and Graduate Study.

4. DATE OF APPLICATION

15 July 1955

5. PRINCIPAL PROFESSIONAL ASSISTANT (s)

John J. Salley, Assistant Professor of Pathology and Dentistry (Co-investigator)

6. OTHER PROJECTS IN WHICH YOU ARE PARTICIPATING AND SOURCE OF SUPPORT (Other government contracts or funds from civilian foundations, etc.)

Prenatal Influences on the Development of Teeth and Supporting Structures Supported by U.S.P.H. Service Grant D-93

7. NAME AND LOCATION OF INSTITUTION WHERE WORK WILL BE PERFORMED

Medical College of Virginia School of Dentistry, Richmond 19, Virginia

SIGNATURE OF RESPONSIBLE INVESTIGATOR

8. APPLICATION APPROVED BY OFFICIAL AUTHORIZED TO SIGN FOR INSTITUTION

NAME PRINTED OR TYPED

Dr. William T. Sanger

SIGNATURE

OFFICIAL TITLE

President

INSTITUTION

Medical College of Virginia

9. APPLICATION APPROVED BY HEAD OF DEPARTMENT WHERE WORK IS TO BE PERFORMED

NAME PRINTED OR TYPED

Seymour J. Kreshover

SIGNATURE

/s/ Seymour J. Kreshover

OFFICIAL TITLE

Director of Dental Research

APPLICATION FOR RESEARCH CONTRACT - PART II

TITLE OF PROJECT		
The Effect of Local Irritants on Oral Tissues		
FUNDS REQUESTED (One year only)		
REQUIREMENTS	BUDGET	
	REQUESTED (From Office of The Surgeon General)	OTHER SOURCES *
1. PERSONNEL (List positions, salaries, and names of professional personnel, if known)		
Mrs. Peggy McNamara - Laboratory Assistant	2304.00	None
Mr. Lloyd Lahmon - Laboratory Assistant	2304.00	None
2. EQUIPMENT (Itemize)		
Animal cages	480.00	
3. CONSUMABLE SUPPLIES (Itemize)		
Experimental animals, chemicals, glassware, histologic materials, animal feed, photomicrographs, etc.	950.00	500.00
4. TRAVEL (State Purpose)		
To attend professional meetings for presentation of findings relative to this study	300.00	
5. SUB-TOTAL	6338.00 5858.00	
6. OVERHEAD (Established by official auditors with concurrence of institution or research agency and contracting officer, and may be based upon percentage of total salaries and wages, or percentage of total cost of the project. Indicate below.)	1254.76 1264.76	Error in Computation M. H.
<input checked="" type="checkbox"/> PERCENT OF SALARIES AND WAGES 27.23% <input type="checkbox"/> PERCENT TOTAL COST	7692.76 7692.76	
7. TOTAL BUDGET	\$ 7692.76	
8. ESTIMATE OF FUTURE REQUIREMENTS (To be filled out only if type of project indicates that it will continue for more than a year)		
FIRST ADDITIONAL YEAR	\$ 7000.00	
SECOND ADDITIONAL YEAR	\$	
* Other Sources - from the school, other contracts, other government agencies, foundations, etc.		

APPLICATION FOR RESEARCH CONTRACT - PART III

TITLE OF PROJECT

The Effect of Local Irritants on Oral Tissues

RESEARCH PLAN

(Include background, specific aims, methods of procedure in detail, significance of this research. Use additional pages, if necessary.)

and mucous glands in the palate, etc.

Although previous interest in tobacco as a tissue irritant has been manifested for a number of years, relatively little attention has been given to the mouth. Numerous experimental studies, utilizing tobacco in the form of extracted tars, have been concerned with the reaction of the skin of rabbits and mice to such procedures as painting. In general, these studies have resulted in a lack of agreement; the findings ranging from no effect to inflammatory response to occasional production of "benign" and "malignant" tumors. In the reviewed literature, only Roffo (Rev. Sudamer. de med. et de chir. 1:321; 1930) undertook to demonstrate experimentally the effect of tobacco on oral tissues. His findings in rabbits receiving daily whole smoke applications to the gingivae, showed "leukokeratotic lesions" after but 25 days. Although a difference in animal species precludes any valid comparison of findings with those reported in the present study, there is appreciable evidence that oral tissues, at least in mice and hamsters, have a high degree of resistance to tobacco in the form of whole smoke.

II. Specific Aims

1. Continuation of present study. As indicated in the progress report, the initiation dates of the various experimental groups have varied. For example, hamsters have been under observation for 8 to 16 months and mice for 7 to 12 months. In light of the perhaps significant finding of Wynder, Graham and Croninger (Cancer Research 13:855;1953) that CAF₁ mice require an exposure to cigarette tar for about one-half their life span (average of 71 weeks) before skin carcinomas develop, it would seem advisable to continue the period of observation in the present study to 18 months. Such a request, if approved, would entail a 6 month period of support beyond the termination date of the currently active grant.

2. Inasmuch as the hamster pouch has been demonstrated to be quite susceptible to such irritants as croton oil, as well as to various carcinogenic hydrocarbons, it may be of considerable significance to determine whether the response of this segment of intra-oral tissue to whole tobacco smoke and other irritants, is comparable to that manifested by the palate. Such a study of relatively adjacent oral tissues (one containing no glandular elements and quite removed from the environmental conditions of the mouth proper) could provide some definitive information on the subject of oral tissue response to irritants, and the role of the tongue and saliva as protective factors. (As discussed in the progress report, complete extirpation of the major salivary glands does not appreciably diminish oral secretions, possibly due to increased activity of minor accessory glands). Conversely, the demonstrated production of carcinoma in the hamster pouch, following application of various carcinogenic hydrocarbons, raises the question whether other oral tissue sites, such as the palate, have a similar degree of susceptibility to known carcinogens or follow the same pattern of resistance shown for whole tobacco smoke. No studies have been concerned with these questions.

3. Inasmuch as tobacco has been implicated by some investigators as a carcinogenic agent, and demonstrated to produce tumors in the skin of mice when applied as a tar condensate, question may be raised whether oral tissues would be similarly reactive to the tar, or manifest a resistance comparable to that shown against tobacco in the form of whole smoke. No study of this question has been reported. In this regard, it may be of some significance to note that although considerable difference of opinion prevails on the subject of the possible relationship of increased use of tobacco to a real or

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apparent increased incidence of lung cancer, there is little or no indication of a like relationship to oral tissues.

III. Method of Procedure

1. Application of whole tobacco smoke to the hamster pouch. Daily treatment of 40 hamsters will be accomplished by means of a smoking machine specifically designed to provide ready access to tissue sites and a standardized and controllable volume of smoke. Animals will be equally divided by sex and strain (golden and albino), and be of uniform age (10 to 12 weeks) at the start of the experiment. Inasmuch as studies to date (as indicated in the Progress Report) show a rather complete resistance of the palatal mucosa to whole tobacco smoke applications, and since the hamster pouch is unique in being an oral structure quite removed from the immediate environmental conditions of the mouth proper as well as devoid of glandular elements, a comparison of the response by each tissue site may provide significant information.

2. Application of croton oil (5% solution) to the oral and cutaneous tissues of hamsters and mice. This daily procedure on 20 hamsters and 30 mice, equally divided by sex and strain (as listed below), will accomplish the purpose of establishing a control to evaluate the response of the designated tissues to a known irritant. It is anticipated that this might also provide information relative to the comparable degree of response manifested by different tissue sites to an irritant other than tobacco.

3. Application of tobacco tar condensate to the oral and cutaneous tissues of hamsters and mice. Forty hamsters of uniform age (10 to 12 weeks) and equally divided by sex and strain (golden and albino) will receive daily applications, by painting, to the pouch, palate and ear. Similar applications will be made to the lips and ears of 60 male and female mice representing Swiss, C₅₇ and CAF₁ strains. The method of tar extraction will be similar to that described by Wynder et al (Cancer Research 13:855; 1953). This phase of the study may provide pertinent information relative to the response of oral tissues to tobacco in the form of tar as compared with whole smoke. Should a comparable resistance be demonstrated, there would be some experimental confirmation, as well as explanation, of the generally held impression that cancer of the mouth is not statistically related to the consumption of tobacco as may be the case with lung cancer (Krantz, J. and Carr, C.: Pharmacologic Principles of Medical Practice, 1951).

4. Application of a known carcinogenic hydrocarbon to the oral and cutaneous tissues of hamsters and mice. Twenty hamsters and 30 mice equally divided by sex and strain (as in the previous groups) will receive daily applications of 3,4-benzpyrene in acetone to the pouch, palate, lip and ear. This procedure will serve the purpose of establishing a control at the opposite extreme to contrast with that provided by the croton oil irritant. Furthermore, pertinent information, may be provided relative to oral versus cutaneous tissue response to carcinogenic agents and the comparable susceptibility of pouch mucosa, palate, lip and cutaneous tissue.

As noted above, a total of 120 hamsters and 120 mice will be treated daily. Experience, to date, has shown that this number is the maximum that can be conveniently handled. With reference to personnel, studies to date have required the services of two laboratory aides to carry out the daily experimental procedures of smoking machine

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operation and applications to the designated tissue sites. One of these persons is also charged with the responsibility of preparation of histologic material.

IV. Significance of this Research

As indicated in this application, previous and current studies have demonstrated rather pronounced resistance of oral tissues to such irritants as oil of mustard and tobacco in the form of whole smoke. It is anticipated that further study, in accordance with the suggested methods of procedure, may clarify the nature of oral tissue response to local irritants from the standpoint of susceptibility and protective mechanisms.

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1. Background

Preliminary studies on the subject of tissue response of Swiss and C₅₇ strain mice to tobacco indicated that the muco-cutaneous lip region is markedly resistant to repeated daily applications of whole smoke covering a three month experimental period. It was further demonstrated that such factors as gonadectomy and vitamin A and B complex deficiency had no demonstrable influence on tissue susceptibility. In striking contrast to these findings was the significant susceptibility of the mouse ear to identical procedures of tobacco application. The predominating change was a proliferation characterized by hyperkeratosis, hyperplasia, acanthosis and chronic inflammation of the corium. Of interest is that the degree of cutaneous tissue response was most severe in vitamin A and B complex deficient animals and appreciably less in those maintained on a normal, adequate diet. There was also the observation that gonadectomy imparts a rather marked degree of tissue resistance to the irritant in question. An evaluation of the various components of the vitamin B complex as predisposing factors in tissue response to tobacco, led to the suggestion that riboflavin, pyridoxine and pantothenic acid are of most significance. On the other hand, thiamine and nicotinic acid showed no greater influence on tissue susceptibility to tobacco than did animals on a normal, adequate diet. These findings are explainable, in part, by the demonstrated resistance of mice to niacin deficiency and by the fact that thiamine deficiency is manifested principally in non-cutaneous tissues.

Further studies extending over a more prolonged period were carried out on Swiss and CAF₁ strain mice. Although not yet completed, the nature of the findings to date, indicates that tobacco has no demonstrable effect on the oral tissues (lips) of mice regardless of sex, strain, vitamin B deficiency, gonadectomy or duration of exposure up to one year. In contrast to these observations, the cutaneous tissues (ears) of mice were effected quite readily by identical applications of tobacco; the characteristic tissue response being one of ulceration, inflammation and proliferation. No carcinomatous changes were noted. Also confirmed in this study was the influence of B avitaminosis on tissue response. However, appreciably less significant than previously demonstrated was the factor of gonadectomy in modifying the degree of cellular change. Possibly the cumulative effect of daily applied tobacco was sufficient to negate any protective influence of castration or ovariectomy.

As in the case of the mice, groups of hamsters under long range study also showed no demonstrable reaction of their intra-oral palatal tissues to whole tobacco smoke applications regardless of sex, strain (golden and albino), desalivation, vitamin B deficiency or duration of exposure up to 16 months. Although this would indicate that the oral tissues of hamsters are just as resistant to the effects of tobacco as are mice, it should be emphasized that the cutaneous tissues showed an almost comparable lack of response. Whereas this would ordinarily suggest a rather generalized type of resistance of this animal species to the irritant in question, it is of interest to note the reported findings of a high susceptibility of the oral pouch of the hamster to both irritants (eg. croton oil) and carcinogenic hydrocarbons (Salley, J.:J.D.Res.33:253;1954). Inasmuch as the hamster pouch is devoid of glandular elements and in other ways not comparable to such intra-oral structures as the palatal mucosa, the significance of the salivary flow and tongue activity as protective mechanisms remains an important consideration. In this regard, it is pertinent to note that the hamster mouth remains well lubricated despite desalivation; possibly due to hyperactive accessory salivary

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A. Background.

For the past three years, the applicant has been engaged in studies related to the effect of local irritants on oral tissues with particular reference to tobacco. In the preliminary phase of this study, it was reported (J.A.D.A. 45: 528; Nov. 1952) that whole tobacco smoke applications to the lips of mice caused little or no tissue change. These animals had been grouped previously so that one series was maintained on a normal adequate diet, another was rendered deficient in vitamin A, and a third deficient in the vitamin B complex. Two additional groups were comprised of gonadectomized mice maintained, respectively, on normal and B complex deficient diets. In striking contrast to the negative lip findings in all of these groups, was the evidence of appreciable susceptibility of the ears of certain of the same animals following identical procedures of tobacco smoke application. These changes were most marked in the vitamin B deficient mice, less so in the vitamin A deficient and normal diet groups, and least in the gonadectomized animals regardless of their diets. On the basis of the observed difference in susceptibility of oral and cutaneous tissues, it was suggested that a significant protective effect is exerted by the tongue and saliva readily removing deposited tars from the oral mucosa. It was further concluded that whereas vitamin B complex deficiency causes a markedly increased susceptibility of cutaneous tissues to tobacco irritants, orchietomy and ovariectomy establish a certain degree of resistance.

Following the above report, additional studies were carried out on mice to the end of further evaluating the various factors that seemingly contribute or predispose to altered tissue susceptibility. The first of these, concerned with the subject of vitamin B deficiency, has been submitted recently for publication in the Journal of Dental Research. Inasmuch as an appreciable portion of this work was made possible by the current grant from the Surgeon General of the Army (Contract No. DA-49-007-MD-506), due acknowledgement is made. Experimental results may be summarized as follows:

1. Vitamin B complex deficiency in mice renders their cutaneous tissues more susceptible to the irritating effects of whole tobacco smoke.
2. Riboflavin, pyridoxine and pantothenic acid are the B complex components primarily responsible for such alterations in tissue response.
3. Animals maintained on diets deficient in biotin, thiamine and nicotinic acid show no greater tissue susceptibility to tobacco smoke applications than do mice on a normal adequate diet. These observations are explainable, in part, by the demonstrated resistance of mice to niacin deficiency and by the fact that thiamine deficiency is manifested principally in non-cutaneous tissues.
4. Gonadectomy renders cutaneous tissues less susceptible to the irritating effects of whole tobacco smoke.
5. Tissue response, under the conditions of study, is not influenced by sex.

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6. The described cutaneous lesions are in striking contrast to the relative absence of pathologic change in oral tissues subjected to similar applications of whole tobacco smoke. This suggests either a basic difference in response by different tissues or a protective effect by the tongue and saliva in readily removing deposited tars.
7. The predominating feature of tissue response to whole tobacco smoke is a proliferative change characterized by hyperkeratosis, hyperplasia, acanthosis and chronic inflammation of the corium. Cellular alterations in size, shape and staining qualities as well as loss of basal cell polarity and increased mitotic activity indicate the abnormal nature of the tissue change.
8. There is no evidence of an acquired capacity of the tissues for continued proliferative change after terminating exposures to the tobacco smoke.

B. Specific aims:

The current phase of study is being supported entirely by Grant No. DA-49--007-MD-506. Although the ultimate objective is to evaluate the effect of a variety of local irritants on oral tissues, initial consideration is being given to tobacco in order to further explore and more adequately evaluate these findings discussed in the section above.

C. Methods of procedure:

Eighteen hamsters, equally divided by sex, had complete bilateral extirpations of their major salivary glands. These animals and an equal number of non-desalivated males and females were divided into groups maintained on normal adequate and vitamin B complex deficient diets. An additional 4 males, 2 of which were desalivated, were gonadectomized and included in the B deficient group. Four other identically operated animals were included in the normal diet group.

Following postoperative recovery and the development of clinically demonstrable avitaminosis, the palatal mucosa and ears of the animals in the various experimental categories were subjected to daily applications of whole tobacco smoke; method of application being by means of an apparatus specifically designed to provide a standardized and controllable volume and exposure time of smoke. Various control procedures also were established in other hamsters in order to (1) determine the possible influence of suction and pressure alone incident to the operation of the smoking apparatus; (2) evaluate the factor of heat that might be created at the site of smoke application; (3) compare findings with tissue response to another, more or less standardized, irritant such as oil of mustard.

Since the experimental procedure on hamsters is planned for an appreciably longer period of observation than were the preliminary pilot studies on mice, it would be premature to report any findings at this time other than the interesting observation that after receiving twice as many smoke applications as did the mice, none of the hamsters showed any significant tissue change. This perhaps indicates the questionable validity of making general conclusions on the basis of experiments with a single species of laboratory animal, and the specu-

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lation involved in applying such findings to man.

In order to better evaluate the long term study on hamsters and the influence of various conditioning or predisposing factors on tissue response to tobacco irritants, an additional series of CAF₁ mice are included in the general experimental plan. Forty of these, divided into castrated and non-castrated males and females on both normal and vitamin B deficient diets, are receiving daily smoke applications to their lips and ears; the duration of the experiment to parallel that of the hamster series.

Suggesting that different tissues as well as local environmental factors peculiar to such tissues may appreciably influence their response to tobacco, a third series of animals is being subjected to whole smoke inhalation. It is anticipated that this procedure will permit a comparative study of pulmonary tissue response to tobacco with those responses manifested by oral mucosa and cutaneous tissues. Method of smoke application to the lungs is by means of a face mask adapted to the smoking apparatus.

D. Significance of research:

The nature of oral tissue response to local irritants may be further clarified from the standpoints of local and general conditioning or predisposing factors.