U. S. NAVAL SUBMARINE MEDICAL CENTER

Submarine Base, Groton, Conn.

REPORT NUMBER 561

SALIVARY THIOCYANATE SECRETION DURING A FLEET BALLISTIC MISSILE SUBMARINE PATROL

by

LT R. P. Wray, MC, USN, and CDR W. R. Shiller, DC, USN

Bureau of Medicine and Surgery, Navy Department
Research Work Unit MR011.01-5007.01

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Gerald J. Duffner, CAPT MC USN
COMMANDING OFFICER
Naval Submarine Medical Center

27 January 1969

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SUBMARINE MEDICAL RESEARCH LABORATORY
NAVAL SUBMARINE MEDICAL CENTER REPORT NO. 561

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Submitted by:

William R. Shiller
Commander, DC US Navy
Head, Dental Research Branch

Reviewed and Approved by:

Charles F. Gell, M.D., D.Sc.
Scientific Director

Reviewed and Approved by:

Joseph D. Bloom
Commander, MC US Navy
Director, SMRL

Approved and Released by:

Gerald J. Duffner
Captain, MC US Navy
Commanding Officer

SUMMARY PAGE

THE PROBLEM

The closed atmosphere of a fleet ballistic submarine contains several contaminants, some of which are tobacco smoke products. A biological examination to determine the extent of effective smoke inhalation both in smokers and non-smokers would be a useful tool in adding to environmental health knowledge in this closed atmosphere. The salivary thiocyanate level seemed promising as such a tool.

FINDINGS

Salivary thiocyanate levels showed a strong positive relationship to smoking; however, no significant changes were found in either smokers or non-smokers on patrol. No relationships were found with dietary factors or amount smoked.

APPLICATIONS

Salivary thiocyanate measurements do not appear to be sufficiently precise or the atmospheric factors are not sufficiently extreme to warrant the use of this measurement in assessing tobacco smoke intake.

ADMINISTRATIVE INFORMATION

This investigation was conducted as a part of Bureau of Medicine and Surgery Research Work Unit MR011.01-5007—Factors Influencing Salivary Thiocyanate Secretion During A Two Month Isolation Period. This report has been designated as Submarine Medical Research Laboratory Report No. 561. It is Report No. 01 on this Work Unit, and was approved for publication as of 27 January 1969.

This document has been approved for public release and sale; its distribution is unlimited.

PUBLISHED BY THE NAVAL SUBMARINE MEDICAL CENTER

ABSTRACT

Tobacco smoke products make up an undetermined amount of the atmospheric contaminants of a fleet ballistic missile submarine. A biological measurement of the degree of effective tobacco inhalation both in smokers and non-smokers would be a useful tool in environmental health studies. Salivary thiocyanate levels seemed promising as such a tool.

Thirty volunteers were selected from a submarine crew: 14 smokers and 16 non-smokers. Saliva was collected by standard methods during refit and on patrol. Thiocyanate content was measured and relationships were sought with dietary, smoking and patrol factors. Strong positive relationships were found between the thiocyanate levels and smoking, but no significant changes were found in either smokers or non-smokers on patrol. No relationships were found with dietary factors or with the amount smoked. It is concluded that the atmospheric effects are not extreme enough to be detected by this biological method.

SALIVARY THIOCYANATE SECRETION DURING A FLEET BALLISTIC MISSILE SUBMARINE PATROL

The relationship of thiocyanate to basal metabolism and thyroid function has been of some interest to physiologists (1, 2). Thiocyanate seems to exert an inhibitory effect on the iodine "pump" of the thyroid gland increasing the clearing of iodine from the gland and reducing the iodine uptake. As an end result the basal metabolic rate is lowered. Thiocyanate occurs normally in about equal amounts in all body fluids with the exception of saliva. The normal values for serum thiocyanate are generally reported to be less than 1.0 mg/100 ml while the saliva contains up to 25 mg/100 ml. The source of this normally occurring thiocyanate is generally thought to be the cyanide from the metabolism of proteins, the ingestion of some cyanide containing vegetables and from the cyanide in tobacco smoke (3).

Much concerning the physiological role of thiocyanate is unclear. It has been suggested that one role is the de-toxification of body cyanides. In the past, thiocyanate has been used therapeutically to control hypertension (3). It is still used occasionally in body fluid studies (4). The excretion of thiocyanate normally occurs in the urine and feces.

The normally high levels of thiocyanate in saliva has led to some studies of its oral significance but no definite relationship with oral health has been demonstrated (5).

A marked increase in salivary thiocyanate is seen in individuals who are heavy smokers (6, 7). An attempt has been made with some success by Densen and co-workers (7) to use salivary thiocyanate levels as an indicator for effective degree of tobacco smoke inhalation. They were able to demonstrate general expected relationship in groups as the subject individuals ceased and resumed smoking. It was impossible to control for dietary and other factors in their study.

The fleet ballistic missile submarine environment would appear as quite a practical place for a study similar to that of Densen and co-workers for two reasons: One, the nature of the environment makes it possible to analyze for dietary and atmospheric factors; and two, from a practical standpoint the thiocyanate levels may lead to a better understanding of the effects of a closed, relatively smoke laden atmosphere.

A study was therefore designed to study the salivary level of the thiocyanate ion with regard to four variables: Smoking, diurnal variation, diet, and atmosphere. By way of explanation, the "atmosphere" variable refers to a closed submarine versus an open submarine; in other words, "Will breathing tobacco smoke in a closed atmosphere result in increased salivary thiocyanates in nonsmokers?"

MATERIALS AND METHODS

Thirty volunteers were selected from the crew. There were 14 smokers and 16 nonsmokers. In all other respects the subjects were a random selection of volunteers.

The saliva was collected as follows: The subjects were given a one-half stick of pure paraffin to chew and the first two "spits" were discarded. The following three "spits" were collected in a wide mouth receptacle and a 300 ul sample was placed in a microcentrifuge tube for thiocyanate analysis. In order to allow for diurnal variation collections were made before breakfast (0600-0800) and after the evening meal (1900-2100). The actual time was recorded at each of these collections. The collections were made for seven consecutive days during refit, during the second week of patrol, and during the last week of patrol.

Each evening at bedtime during each collection period the subjects were given a mimeographed diet questionnaire. This was done to discover the foods ingested that day with particular emphasis on those foods known to contain cyanates such as cabbage, broccoli, cauliflower, turnips, radishes, garlic, horseradish, mustard, and almonds.

Analyses were performed by a micro adaptation of the method described by Barker included in Clinical Laboratory Diagnosis (8). The data were analyzed factorially. The analyses of variance were concerned with the four variables stated in the introduction.

RESULTS

Thiocyanate secretion patterns are given for the thirty subjects in Table I. The "S" preceding the subject number denotes that the individual is a smoker. All values are mean thiocyanate levels in milligrams per 100 milliliters plus or minus one standard error of the mean. Usually each mean value was based on 14 samples.

It is of note that very few individuals showed any remarkable changes between collection periods. Subject "S07" did show a significant decrease in the second and third periods compared with the first ($P < .01$). This individual quit smoking after the first collection series. Subject 18 offered the possibly opposite observation having begun smoking after the first collection period. The differences in this case were not significant. In passing, it should be noted that the salivary thiocyanate levels in this group of subjects agree with previously reported studies.

The secretion pattern as influenced by smoking and by the patrol are given in Table II. It is noted that there is a significant difference in the levels between smokers and non-smokers. No other comparisons are remarkable.

The analysis of variance was used, Table III, to compartmentalize the effects of smoking and patrol effects on the thiocyanate levels. Only in the case of the smoking variable was the f ratio great enough to be considered statistically significant ($P < .001$). In other words, there were no significant effects of the patrol nor of interactions between patrol effects and the smoking variable. The diurnal factor is included in Table IV, and again the smoking variable accounts for the significant amount of variances.

Dietary responses were used to study the relationship of cyanide containing foods and the salivary thiocyanate levels. The results are summarized in Table VI. It is apparent that no relationshp was found.

Table I

Individual salivary thiocyanate levels at the various test periods.

Subject	Prepatrol	Patrol 1st period	Patrol 2nd period
01	9.48 ± 0.80	8.80 ± 0.78	9.82 ± 1.11
S02	14.93 ± 1.96	15.01 ± 0.80	22.69 ± 2.58
03	8.02 ± 0.55	9.17 ± 0.73	10.72 ± 0.89
04	9.01 ± 1.24	8.52 ± 0.60	7.14 ± 0.90
S05	16.59 ± 1.67	14.07 ± 1.43	15.79 ± 1.06
S06	12.84 ± 0.94	14.10 ± 1.66	13.54 ± 1.29
S07	20.57 ± 1.85(NS)	11.48 ± 0.66(NS)	12.03 ± 1.49
08	10.17 ± 1.05	11.92 ± 1.78	10.96 ± 1.10
09	10.63 ± 1.27	9.68 ± 1.20	10.76 ± 0.98
S10	26.32 ± 4.22	13.98 ± 4.92	27.46 ± 2.67
S11	18.25 ± 2.77	16.27 ± 1.28	21.78 ± 1.95
12	9.00 ± 0.81	11.78 ± 1.67	10.96 ± 1.14
S13	19.03 ± 2.08	20.00 ± 1.78	23.30 ± 2.60
S14	11.51 ± 1.24	14.54 ± 2.12	14.28 ± 0.96
15	10.80 ± 1.12	9.48 ± 0.99	7.67 ± 0.78
S16	16.33 ± 1.56	18.79 ± 1.09	16.77 ± 1.17
17	16.67 ± 2.55	10.64 ± 1.01	10.85 ± 1.49
18	15.25 ± 1.40(S)	17.41 ± 1.47(S)	16.56 ± 1.20
19	13.73 ± 0.79	13.50 ± 1.36	15.89 ± 0.96
S20	21.46 ± 2.12	13.30 ± 0.77	18.67 ± 1.16
21	9.78 ± 0.80	9.06 ± 1.15	7.46 ± 0.60
S22	11.69 ± 0.91	14.16 ± 0.74	15.26 ± 1.22
23	16.30 ± 2.13	12.03 ± 2.01	10.78 ± 1.35
S24	13.55 ± 1.88	14.72 ± 1.49	13.78 ± 1.24
25	15.84 ± 1.92	10.83 ± 1.15	6.84 ± 0.81
S26	16.84 ± 1.17	13.01 ± 0.99	17.56 ± 0.71
27	8.86 ± 0.51	9.98 ± 1.15	9.63 ± 2.08
S28	16.19 ± 2.52	14.69 ± 0.71	14.08 ± 0.99
29	13.52 ± 0.99	10.25 ± 0.91	9.75 ± 0.86
30	13.45 ± 1.96	12.12 ± 0.64	12.06 ± 1.89

Table II

Thiocyanate as influenced by smoking and patrol effects.

	In port	On patrol 1	On patrol 2	Total Collections
Smokers	16.87* ± 1.0942** (N=14)	15.29 ± 0.56 (N=14)	17.97 ± 1.14 (N=14)	16.71 ± 0.57 (N=42)
Non-smokers	11.91 ± 0.75 (N=16)	10.85 ± 0.65 (N=16)	10.53 ± 0.64 (N=16)	11.10 ± 0.36 (N=48)
Combined	14.22 ± 0.78 (N=30)	12.92 ± 0.53 (N=30)	14.00 ± 0.93 (N=30)	13.72 ± 0.44 (N=90)

*Mean mg/100 ml thiocyanate

**Standard error of the mean

An attempt was made to assess the effect of the number of cigarettes smoked on the thiocyanate levels. The daily response concerning the number of cigarettes smoked was matched with the evening salivary thiocyanate level. These data are summarized in Table V. None of the mean differences are statistically significant.

DISCUSSION

A first glance at the results would indicate that nothing remarkable was found in this study. The data certainly indicate that inhaling an atmosphere contaminated by tobacco smoke does not increase the salivary thiocyanate levels. Perhaps it might be well, however, to state this interpretation slightly differently: "No relationships were found between salivary thiocyanate levels and the living in a closed environment." The emphasis here should probably be on the applicability of the thiocyanate test on evaluating the effects of smoke present in the air. Perhaps the effect is present but the test used was not sensitive enough to detect it.

The foregoing naturally leads to a consideration of the diet and smoking variables. Densen and co-workers attempted to use the salivary thiocyanate levels as a guide to evaluation of smoking changes. Their somewhat equivocal results were ascribed to possible dietary effect, but no relationship was seen between diet and the thiocyanate levels. The number of cigarettes smoked also showed little relationship. The fact that smokers showed consistently higher levels than did non-smokers would indicate that the amount smoked should also influence the level, but again perhaps the test is not sensitive enough to detect the differences.

Table III

Analysis of variance for thiocyanate levels: Effect of smoking and isolation.

Source of Variation	Sum of Squares ss	df	Mean Squares ms	f
Smokers	705.6707	1	705.67	75.72
Patrol	29.1042	2	14.55	
Interaction	38.1219	2	19.07	
Between groups SS	772.8968	5		
Within groups SS	783.2120	84	9.32	
Total SS	1556.1088	90		

Table IV

Analysis of Variance
Effect of time of day, smoking, and patrol effects.

Variable	Sum of Squares ss	Degrees of Freedom df	Mean Squares ms	f
Time of day (AM or PM)	19.97	1	19.97	
Patrol effect	19.22	2	9.61	
Smoking	1573.40	1	1573.40	136.70
Pooled interactions	147.95	5	29.59	
Between groups	1760.55	9		
Within groups	1957.35	170	11.51	
Total	3717.90	180		

Table V

Number of cigarettes smoked and the thiocyanate level.

Number of cigarettes	Number of Subjects	Thiocyanate level (mg/100 ml)
1 - 10	8	16.66 ± 1.10*
11 - 20	14	15.64 ± 0.97
21 - 30	12	16.66 ± 0.86
31 or more	6	18.60 ± 1.28

*One standard error of the mean

Table VI

Relationship of cyanogen containing foods and salivary thiocyanate levels.

	Thiocyanate level Mean (mg/100 ml)
Cyanogen foods ingested on the day of sample collection	16.04 ± 0.97*
Cyanogen foods not ingested	16.98 ± 0.79

*Standard error of the mean

CONCLUSION AND SUMMARY

Salivary thiocyanate secretion evaluated prepatrol and twice on patrol revealed little variability in individual secretion. No significant differences were noted between collection periods.

Smokers showed significantly greater thiocyanate secretion levels than did non-smokers.

No relationships were found between dietary factors and the quantity of cigarettes smoked.

It is concluded that salivary thiocyanate levels are rather individually consistent and smoking has a decided effect on these levels; however, all other factors studied in the FBM environment are without relationship. This method of physiological evaluation is therefore considered to be of little value in the submarine environment.

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UNCLASSIFIED
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*
Naval Submarine Medical Center, Submarine Medical Research Laboratory

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP

3. REPORT TITLE
Salivary Thiocyanate Secretion During A Fleet Ballistic Missile Submarine Patrol

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*
Interim Report

5. AUTHOR(S) *(First name, middle initial, last name)*
Reginald P. Wray, LT, MC, USN
William R. Shiller, CDR, DC, USN

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
27 January 1969	4	8

8a. CONTRACT OR GRANT NO.

b. PROJECT NO.
MR011.01-5007.01

c.

d.

9a. ORIGINATOR'S REPORT NUMBER(S)
Submarine Medical Research Laboratory Report No. 561

9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*

10. DISTRIBUTION STATEMENT
This document has been approved for public release and sale; its distribution is unlimited.

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY
Naval Submarine Medical Center
Box 600, Naval Submarine Base
Groton, Connecticut 06340

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Thirty volunteers were selected from a submarine crew: 14 smokers and 16 non-smokers. Saliva was collected by standard methods during refit and on patrol. Thiocyanate content was measured and relationships were sought with dietary, smoking and patrol factors. Strong positive relationships were found between the thiocyanate levels and smoking but no significant changes were found in either smokers or non-smokers on patrol. No relationships were found with dietary factors or with the amount smoked. It is concluded that the atmospheric effects are not extreme enough to be detected by this biological method.

DD FORM 1 NOV 65 1473 (PAGE 1)
S/N 0101-807-6801

UNCLASSIFIED
Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Saliva						
Thiocyanate						
Submarine						
Atmosphere						