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FINAL REPORT

ENVIRONMENTAL FACTORS AND DENTAL CARIES

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## OBJECTIVES

A. To seek explanations for the high level of immunity to dental caries observed in naval recruits growing up in specific geographic areas in the United States.

B. To explain the mechanism of action by which the agent or agents bringing about the relative immunity to caries exerted their effects.

## REPORT

In seeking the reason for the low level of caries activity in the areas in Northwest Ohio, South Carolina and Florida, from which the caries-free naval recruits came, field trips were made to these locations to determine whether there was any common factor relating to sociologic makeup, occupation, race or custom, which was common to all three areas. In the absence of any clearcut characteristics which set these areas apart from others in the United States, attention was directed to possible differences in the water supplies or foodstuffs used with the thought that fluorine or other trace elements might be responsible for the observed differences. Thereafter, systematic surveys were made of the trace element content of the water supplies in the special areas, then of soil sources of trace element intake. Concomitantly, explanations for the mode of action of the special waters and trace elements on caries were sought.

Water Studies

The trace element investigation of water supplies required extensive field work to obtain water samples from taps in the homes, kitchens and schools of 36 of the caries-resistant recruits studied at the Great Lakes Naval Training Center. These samples and later samples from other sources were analyzed in the laboratories of the Geological Survey for 25 elements (aluminum, barium, beryllium, bismuth, boron, cadmium, chromium, cobalt, copper, iron, gallium, germanium, lead, lithium, manganese, molybdenum, nickel, rubidium, silver, strontium, tin, titanium, vanadium, zinc and zirconium). The indications of trace element relationships to caries coming from these analyses were then compared with the findings of water analyses on samples obtained from control areas, and finally, with published regional information on trace element content of water supplies and caries prevalence in military recruits.

To give assurance that the conditions operating ten or twenty years ago, during the period of tooth formation and active caries experience of the naval recruits studied at Great Lakes, had not changed insofar as their contribution to a low order of caries prevalence was concerned, examinations were made of 12-14-year-old school children in both Northwest Ohio and in South Carolina, and Florida. These showed low caries levels below what is found associated with fluoride drinking waters in other areas.

The chemical analyses of the water samples collected from

the homes and schools of the caries-resistant naval recruits showed that the levels of molybdenum and strontium were clearly higher than in the water supplies of 7 other communities in Ohio. High levels of boron and lithium were also present in the special waters, although not to the same level of statistical significance as existed for molybdenum and strontium. High levels of boron, lithium, molybdenum and strontium were also found in low-caries areas in South Carolina.

Another correlation of the trace element content of the water supplies to caries prevalence in military recruits was made by comparing the levels of boron, strontium, lithium and molybdenum in the water supplies used by the major population groups in the States with the least caries with those of the States with the worst caries pictures. This comparison revealed a valid correlation between high levels of boron, lithium, molybdenum and strontium in the water and lower caries, and also gave some indications that copper in the water had an adverse effect upon dental caries. The foregoing findings are summarized in the following Table:

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Median Concentrations in Micrograms per liter for which  
Significant Differences were Established

Element	NW Ohio	Best States	7 Ohio Cities	Worst States	N. E. S. Carolina	Others S. Carolina
Boron	150	80	28	13	1,450	45
Lithium	19	14	3	0	7	1
Molybdenum	17	4	3	0	6	0
Strontium	6,100	320	160	20	105	30

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To indicate further whether the trace elements boron, lithium, molybdenum and strontium in the Northwest Ohio water were adding additional protection against caries to that given by the presence of 1.2 ppm of fluoride in the drinking water, a comparison of caries prevalence was made between 12-14-year-old children in the special low-caries area (Fort Recovery - Delphos) and those in Portsmouth, where there is 1.0 ppm of fluorine in the water. The lower (35.7%) caries (DMFS) in the Fort Recovery - Delphos children than in the Portsmouth children was more than could be accounted for by difference in the fluoride content of the water and would seem to be related to the higher contents of boron (350 versus 40 ppb), lithium (38 vs. 6 ppb), molybdenum (25 vs. 2 ppb) and strontium (5,300 vs. 200 ppb) respectively in the Fort Recovery - Delphos water than that of Portsmouth.

#### Trace Elements in Soil and Vegetation

Since it is obvious that trace elements can be ingested by man in food as well as water, the trace element content of the food consumed in the special low caries areas was investigated. For this purpose, vegetables were purchased from stores, roadside stands, or grown specially for the purpose. Analyses were made at the Ohio Agricultural Research and Development Center, in Wooster, for aluminum, barium, boron, calcium, copper, iron, magnesium, manganese, molybdenum, phosphorus, potassium, silica, sodium, strontium and zinc.

In relation to the trace element content of cooked foods, it should be pointed out that there can be a change of trace element

content during the process of cooking, and so the amount of trace elements ingested with the food is not necessarily that which was initially in the food, but may be a combination of its original trace element content as influenced by the trace element content of the food and the water in which it was cooked. For instance, we were able to show that the fluorine, lithium, molybdenum, and strontium content of beans was increased when they were cooked in the Northwest Ohio water, containing high quantities of these elements, and, on the other hand, the content of iron remained unchanged, and that of aluminum, barium, boron, copper, manganese, nickel and rubidium, were lost from the beans during the cooking process. This observation is of interest because it seems to be the first finding of an increase, rather than a loss, of elements during the cooking process.

That the trace element content of the vegetables can contribute to trace element intake is indicated by our finding that vegetables grown in the caries-resistant area in Northwest Ohio had higher amounts of molybdenum and strontium than those grown in Eastern or Central Ohio. However, there was no significant difference in the level of boron and there were lower levels of manganese. In the low-caries area in South Carolina it seems that the lower levels of the key trace elements in the water may be compensated for by intake from vegetables, particularly dark green leafy vegetables which are consumed in greater quantities in that area. Our trace element analyses showed that beet greens, turnip greens, mustard greens, dandelion greens and collards accumulate trace elements, including strontium and molybdenum,

to give levels twice as high as those found in other vegetables from Ohio.

To answer the question of whether, in face of the widespread transportation of foodstuffs in the United States, the trace element content of the soil and vegetation could reflect itself in the trace element intake in man, trace element analyses were made of cows' milk and human blood in areas where special trace element situations existed in the soil and vegetation. A definite association was found between the selenium and molybdenum concentrations in milk in association with higher levels in the pastures. In man, significantly more selenium was present in the blood from Rapid City, S.D. and Cheyenne, Wyo., and Fargo, S.D., than was found in Lima, O., or Muncie, Ind. Variations in the blood levels of cadmium, copper, lead and molybdenum in the blood could not, however, be associated with known sources of these elements.

#### Mode of Action

Animal feeding experiments and bacteriological studies were carried out and cast light on the mechanisms by which trace elements could modify the caries activity. That constituents of the Northwest Ohio water can exert a caries inhibitory effect in rats was demonstrated by the finding that the addition of 1 per cent by weight of the ash of beans cooked in the Northwest Ohio water to a cariogenic diet resulted in a significant reduction in the number and severity of carious lesions when compared with a supplement of bean ash cooked in distilled water, or distilled water containing the same amount of fluorine as the Ohio water.

An exploratory comparison of teeth from Northwest Ohio and Rochester, New York, using X-ray emission spectrographic methods shows a much greater content of strontium in the teeth from the low-carries area than in the Rochester teeth. This is in keeping with our finding of a tenfold greater content of strontium in the femurs of rats fed Northwest Ohio bean ash and water than in animals fed control diets.

That strontium could modify morphology of teeth was demonstrated by Castillo's work at the Eastman Dental Center, which showed that when this element was fed during amelogenesis there was thickening of the dentin, widening of the tooth sulci and lessening of their depth towards a form which is associated with lower caries susceptibility.

The possibility that the lower cariogenicity may be the result of the trace elements modifying bacterial growth or metabolism was investigated by studies on material collected from the surface of the molars of the rats used in the caries experiment. No differences were found in the numbers of intracellular or extracellular polysaccharide-forming organisms. There was, however, a difference in the number of acid-forming organisms that were identified by zoning in hydroxyapatite-tryptone-sucrose-agar plates. This acidogenic count was reduced by 61 per cent in the rats using the Ohio bean ash and water, as compared with a reduction of 36 per cent in animals receiving a 1.5 ppm fluoride supplement.

To further examine the apparent inhibition of acidogenic organisms by the Northwest Ohio water bacteriological media were made

with this water and with Rochester water, and with water with added buffer components, and tested against 14 strains of acidogenic streptococci. Again, it was clear that there were less zones of apatite clearance when the Northwest Ohio water was a constituent of the media. This finding of quantitative differences in the dissolution of radioactive enamel by bacteria in the presence of the Northwest Ohio water indicates that the water influences the solubility of tooth apatite. This finding is in keeping with demonstration by Dr. Shrestha, of the Eastman laboratories, that strontium fluoride is more effective in reducing enamel solubility of intact enamel than is sodium fluoride.

#### CONCLUSION

The findings to date indicate that the explanation for the disproportionate numbers of caries-resistant recruits from Northwest Ohio and Northeast South Carolina may be the elevated dietary intake of at least boron, lithium, molybdenum and strontium, along with optimal fluoride water supplies.

Investigations ~~to date~~ indicate the desirability of investigating further the effects of combinations of trace elements upon tooth morphology, its destructibility by acid and also effects upon bacterial metabolism. It would seem wise to pay particular attention to the role of strontium in these trace element effects.

(The details of the studies upon which this Report is based are given in full in the various Progress Reports which have been submitted during the progress of this research and in the publications listed herein.)

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