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Nondialyzable Manganese, Copper and Gold Levels in Saliva of Normal Adult Subjects[1]

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A review of the literature reveals a limited amount of information on the trace element concentrations in human saliva. Only three reports were noted on copper content (1-3), while none were observed for manganese or gold. de Jorge *et al* (3) present evidence that copper in saliva is present in the ionic form.

The purpose of this report is to present evidence that the copper, manganese and gold are bound to a substance or group of substances too large to be permeable through a standard cellulose membrane, and to present concentrations of these elements as determined by neutron activation technique.

METHODS AND MATERIAL

Saliva samples were obtained from 21 apparently normal, fasting adult subjects, ranging in age from 19 to 48 years. No mechanical or chemical stimulus was employed and the samples were collected into properly cleaned glass test tubes. Eight of these subjects were females (19-42 years) and thirteen were male subjects (33-48 years). All subjects did have dental repairs, including amalgam fillings. Two subjects had gold inlays.

The collected samples were centrifuged for 30 minutes at 3,000 rpm. One ml aliquots of water clear supernatent were dialyzed, neutron activated, and quantitated for copper, manganese and gold according to the procedure described previously (4). Concentrations of standards included in these assays were as follows: copper 1.11 μg/1.0 ml; manganese, 0.016 μg/1.0 ml; gold 0.97 μg/1.0 ml. Irradiations were carried out for 30 minute periods, in the Rabbit-hole of the CP-5 reactor, Argonne National Laboratories, Argonne, Illinois. The flux at 4.6 MW was 1.8×10^{13} neutrons/cm^2/sec.

The effect of buffer contribution to the nondialyzable levels of copper and manganese was also studied on saliva obtained from 16 additional subjects. These subjects were apparently in good health. The nondialyzable levels obtained for above elements were within the ranges observed in the 21 subjects listed in Table I. The results obtained indicated that the extent of buffer contribution was 0.019 μg/100 ml (6.8%) for managnese, 0.46 μg/100 ml (6.2%) for copper, and none for gold, therefore no correction was required. The details

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of the procedure used in establishing such buffer contribution will be presented in a subsequent publication.

Total protein determinations were carried out on all saliva samples collected. Only the water clear supernatent portions were used for the analysis (5).

Copper determinations were also carried out on 5.0 ml aliquots of saliva using the procedure described by Eden and Green (6).

RESULTS

Sodium—Although dialysis does remove quantities of sodium from saliva to permit gamma-ray spectroscopy, considerable amounts still remain. No attempt was made to establish quantitatively the nondialyzable level of sodium. It appears, however, that this level may be approximately 10 to 40 μg/100 ml. Buffer contained 8.7 μg Na/100 ml.

Copper—Copper content of dialyzed saliva as determined by neutron activation showed an average value of 7.53 μg, std.dev. ± 3.45 μg/100 ml. The range of concentration was between 2.93 and 15.6 μg/100 ml. (Table I). The average buffer contribution to the above average value was 0.46 μg/100 ml.

Copper content of nondialyzed saliva could not be established by the chemical procedure employed (6) because of inadequate sensitivity of the method.

Manganese—The average manganese content of dialyzed saliva was 0.28 μg, std.dev. ± 0.06 μg/100 ml. The range of values varied between 0.05 and 0.79 μg/100 ml. Average buffer contribution to the above mean value was 0.019 μg/100 ml.

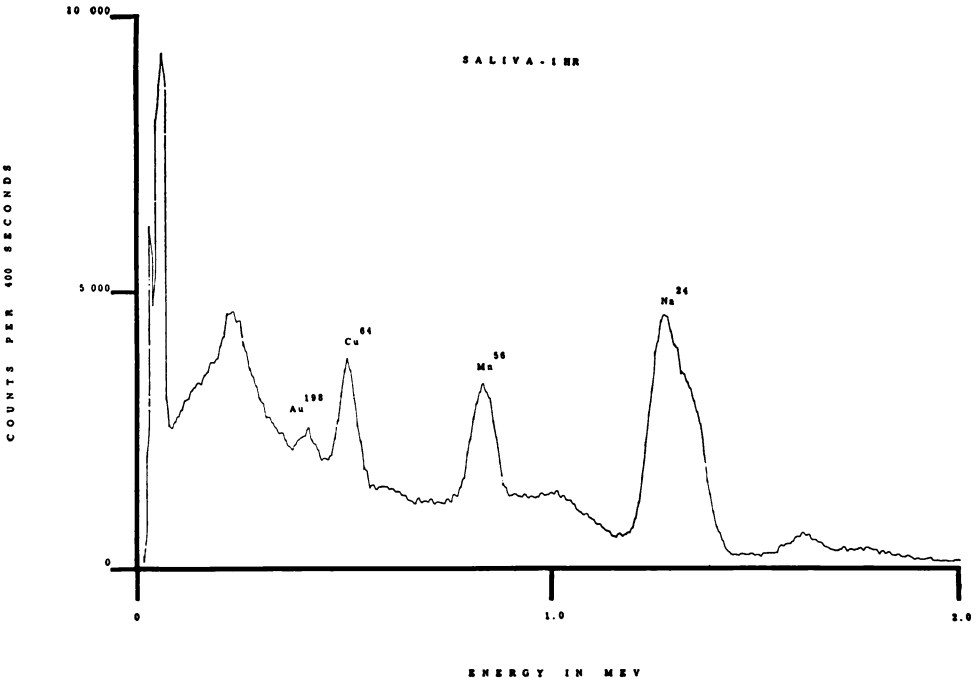

Fig. 1. Gamma-ray spectrum of dialyzed saliva, one hour after a 30 minute irradiation.

Gold—Gold was present in all saliva samples tested. No values were obtained from six samples due to accidental loss during the decay period required to reduce the background in order to distinguish more clearly the peak of ^{198}Au (Fig. 1, 2). Gold concentrations in saliva of subject 8 and 20 (Table I) were not included in the computation of the mean concentration, since both subjects had gold inlays. The mean nondialyzable gold concentration in the saliva of the remaining 13 subjects was 0.06 μg, std.dev. $\pm$ 0.02 μg/100 ml. No detectable amount of gold was noted in the buffer.

Figure 1 illustrates gamma-ray spectra for ^{24}Na, ^{56}Mn, ^{64}Cu, and ^{198}Au. At extreme left, MeV 0.077, are peaks which may represent ^{197}Hg.

Figure 2 illustrates the gamma-ray spectrum of dialyzed saliva approximately 20 hours after irradiation. Counting was done at this time in order to reduce the background and to obtain better counting statistics. Half-life of elements involved is as follows: ^{198}Au, 65 hours; ^{24}Na, 15 hours; ^{64}Cu, 12.8 hours; ^{56}Mn, 2.58 hours.

DISCUSSION

Neutron activation has proved to be a useful tool in the study of trace elements in biological material (4, 7). Its present application to the study of trace elements in saliva indicates the presence of nondialyzable manganese, copper and gold in all the samples examined. We are not aware of any report in the literature on the presence of manganese or gold in human saliva. Three reports have been made on copper concentrations in human saliva (1-3).

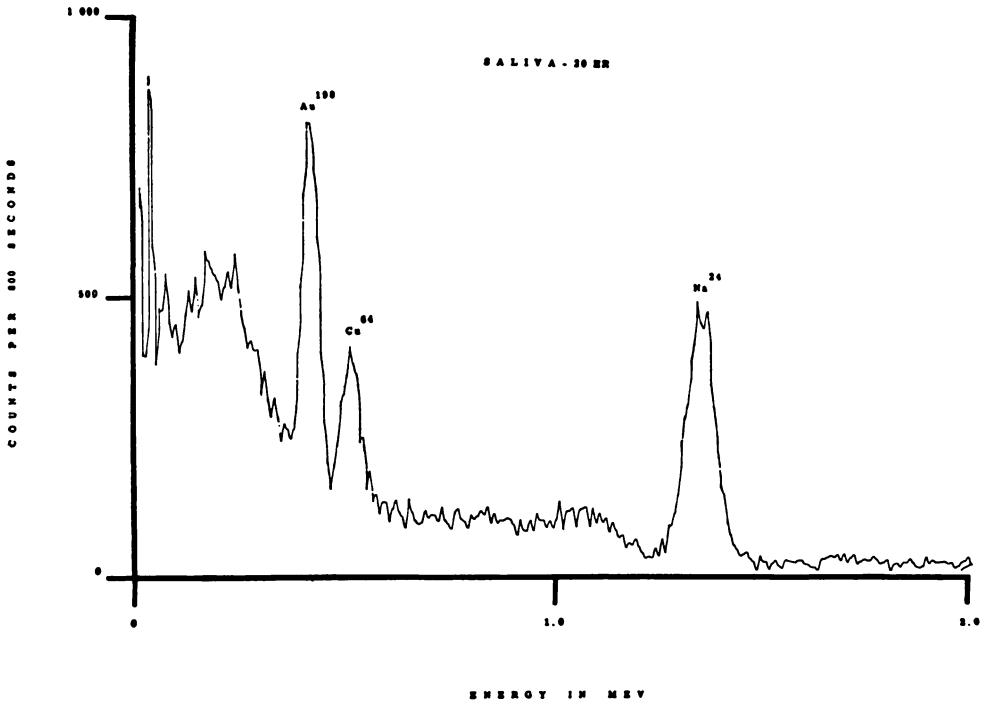

Fig. 2. Gamma-ray spectrum of dialyzed saliva approximately 20 hours after a 30 minute irradiation.

Munch-Petersen (1), using a spectrographic method for analysis, reported 6.3 μg/100 ml saliva (range 2-22 μg) as the mean copper concentrations, based on analysis of salivas obtained from 20 subjects. Collection of samples was made without the use of salivary stimulants. Dreizen *et al* (2) collected saliva with the aid of paraffin. Chemical analysis of these samples (48 subjects) indicated a mean copper concentration of 25.8 μg (range 10-48 μg) per 100 ml saliva. de Jorge (3), also using a chemical method of estimation, reported results of analysis of salivas obtained from 30 subjects. The mean copper concentration was 31.7 ± 15.1 μg/100 ml (range 5-76 μg).

The average copper concentration in the saliva of subjects in this study, 7.5 ± 3.4 μg/100 ml, is in good agreement with the concentration reported by Munch-Petersen (1). The average concentrations reported by Dreizen *et al* (2) and de Jorge *et al* (3), are considerably greater than the concentrations obtained in this study, although the range concentrations appear to overlap. It is possible

TABLE I. TOTAL PROTEIN, NONDIALYZABLE COPPER, MANGANESE AND GOLD LEVELS IN SALIVA OF NORMAL ADULT HUMAN SUBJECTS

Subject	*Age*	*Sex*	*Protein mgs %*	*Micrograms per 100 ml*		
				Copper	*Manganese*	*Gold*
1.	19	F	80	8.43	0.19	0.04
2.	21	F	60	4.44	0.05	0.13
3.	21	F	25	6.60	0.13	——
4.	25	F	30	5.95	0.36	0.06
5.	26	F	140	12.22	0.54	0.05
6.	29	F	50	5.11	0.20	——
7.	33	M	210	10.35	0.31	0.07
8.	36	M	70	9.91	0.54	0.64*
9.	37	M	35	2.93	0.19	0.07
10.	38	M	80	3.75	0.09	0.04
11.	38	M	80	3.01	0.07	0.01
12.	38	M	70	11.30	0.39	——
13.	40	F	50	3.44	0.20	——
14.	40	M	100	4.61	0.16	——
15.	41	M	90	6.03	0.57	0.05
16.	42	F	70	6.65	0.22	——
17.	42	M	100	9.01	0.18	0.05
18.	43	M	70	7.60	0.10	0.03
19.	44	M	120	8.40	0.26	0.06
20.	47	M	35	12.86	0.79	0.21*
21.	48	M	100	15.61	0.45	0.05
Mean			79	7.53	0.28	0.06
Standard deviation			± 41	± 3.45	± 0.06	± 0.02

*Subjects with gold inlays.

that the reason for these higher levels may be found in the mode of sample collection (use of stimulants). The excellent agreement of the copper results in this study with the results for total copper reported by Munch-Petersen (1) appears to indicate that the copper present in the saliva exists in a bound form. Presence of proteins (Table I) may suggest that the copper, manganese and gold are bound in part at least as metallo-protein complexes.

Several explanations may be offered on the source of copper, managnese and gold in human saliva. It is possible that these elements are normally present. Neutron activation of tooth segments (enamel and dentin) revealed a substantial concentration of ^{24}Na and ^{64}Cu. It is possible also that these elements come from food degradation, since certain foods are particularly rich in manganese and copper. Amalgam of the teeth fillings contains mercury, silver and copper, and as such may contribute to their presence in saliva, since all subjects studied did have dental repairs. Subjects with gold fillings and inlays showed greater concentrations of nondialyzable gold than did subjects without such repairs. It is possible that a combination of any or all explanations above may account for the presence of these elements in apparently normal adult subjects. Study of edentulous patients is indicated. Questions on the biological significance of mercury and gold with regard to long term effects upon the human body can only be brought about by further study.

SUMMARY

Saliva samples from 21 apparently normal adult human subjects were analyzed for trace elements using the neutron activation technique.

All samples contained nondialyzable copper, manganese and gold in the following concentrations: Cu, 7.53 ± 3.45 μg/100 ml; Mn, 0.28 ± 0.06 μg/100 ml; and Au, 0.06 ± 0.02 μg/100 ml.

Evidence indicates that copper, manganese and gold are carried in saliva as nondialyzable metallo-complexes. The level of nondialyzable copper found in this study is comparable with the concentration of total salivary copper reported in the literature.

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