From the Department of Physiological Chemistry of the University of Minnesota, Minneapolis, Minnesota.

The uptake of radioactive calcium and phosphorus by intact and carious enamel surfaces.[1]

By

YNGVE ERICSSON.[2]

There is much evidence for the normal occurrence of an ionic exchange between the surface layer of the dental enamel and the saliva. Observations in vivo of the disappearance of chalky spots of incipient caries indicate enamel changes which have been interpreted as a remineralization by some authors earlier on in the century (HEAD 1912, PICKERILL 1914, ANDRESEN 1925). Microscopic studies in vivo with a replica technique have shown that enamel surfaces which have been roughened by etching or polishing are largely smoothed out in the course of some few days, evidently by a microprecipitate of calcium salts from the saliva (WOLF & NEUWIRT 1941, NEUWIRT & WOLF 1944, RHEINWALD & STAEHLE 1949). The exchange of various ions in the enamel as a whole has been extensively investigated using radioactive tracer elements: Ca^{45}, P^{32}, Na^{22}, I^{131}, F^{18}. The authors almost unanimously state that the greatest exchange of the enamel in vivo occurs in the surface, *i. e.* an exchange with the saliva (reviews: BERGGREN 1947, LEICESTER 1949, COPP 1950). These findings are evidently of the greatest importance in the evaluation of the possibilities of

[1] This investigation was supported in part by a grant from the U. S. Public Health Service.

[2] Public Health Service Research Fellow of the National Institute of Dental Research. Present address: Royal School of Dentistry, Stockholm, Sweden.

active or passive changes, *e. g.* by fluoride ions, of the resistance of the erupted tooth to chemical agents.

Some pictures obtained by radioautography of ground sections indicate a greater uptake by carious than by intact enamel surfaces (WAINWRIGHT 1951). This gave the idea of the present investigation: to study, on a more quantitative basis, the uptake of radiocalcium and radiophosphorus by enamel surfaces with incipient caries, compared with intact surfaces of the same teeth.

Material.

The experiments were performed in vitro with human teeth showing incipient caries as chalky or discolored spots or fields without interruption of the normal surface contour. These surfaces were glossy in some cases, indicating an arrested process, and the perikymata were often intact over the whole carious area. In other cases they appeared dull and rough, indicating acute superficial dissolution. Freshly extracted vital teeth were used in all tests except two where the teeth had been stored for several months. On extraction the age and sex of the patient was recorded, with a brief statement on the caries and calculus status.

Method.

Immediately on extraction the tooth was cleaned with scaler and brush and then rinsed 5 minutes in tap water and 1 minute in distilled water. After drying, the enamel surfaces to be exposed were chosen under a magnifying-glass. Thin aluminium discs, 4 mm diam., were pressed on these surfaces. The rest of the tooth was covered with wax which was carefully melted on along the borders of the discs. These were removed, and the tooth placed in a wax mould fitted to the wax-covered crown and containing enough of the radioactive solution to cover the exposed enamel surfaces. The whole procedure required no longer than one hour. In tests Nos. 1—3 the tooth was covered with Duco cement instead of wax; the time required for the procedure was 4—5 hours in these cases.

The radioactive elements used were Ca^{45} and P^{32} as calcium

chloride and sodium phosphate, respectively. Two batches of $Ca^{45}Cl_2$ with different activities were used, one for tests Nos. 1—8 (VIII), and the other for tests Nos. 9—12 (X).

Data for Isotopes Used.

Batch VIII. $Ca^{45}Cl_2$ in weak HCl. Assay on March 13, 1952: 0.0526 mc/ml ± 10 %. Contamination less than 0.1 %. Heavy metals less than 10 p. p. m., Nitrate neg., Ca = 101.5 mg/ml.

Tests performed Oct. 29—Nov. 26 with a dilution of 1 ml of the above stock solution to 25 ml with a $CaCl_2$ standard containing 2 mg Ca/ml. 1 ml was neutralized with about 0.05 ml normal NaOH, using phenolphtalein as indicator.

Batch X. $Ca^{45}Cl_2$ in weak HCl. Assay on Aug. 21, 1952: 0.775 mc/ml ± 10 %. Ca approx. 26.0 mg/ml. Radiochemical purity = 99.9 %. Heavy metals less than 10 p. p. m.

Tests performed Nov. 26—Dec. 6 with a dilution 1 : 10 with distilled water. 1 ml was neutralized with 0.01 ml normal NaOH, using phenolphtalein as indicator.

P^{32}. Shipment Sept. 15, 1952. At that time, according to specification, 0.025 mg carrier P per mc, pH 1—4, Fe, Ni, Al content giving no precipitate within 24 hours at pH 7—9. Dec. 3 activity calculated as 61 μc/ml.

Tests performed Dec. 4—17 with 0.8 ml of above solution, neutralized with 0.05 ml normal NaOH + 0.11 ml 0.1-normal NaOH.

The exposure times varied between 16 and 28 hours. After removal from the active solution the tooth was rinsed 5 minutes in running tap water and one-half minute in distilled water, the outlines of the exposed surfaces were marked with a fine-pointed lead pencil, the wax was removed and the whole crown covered with a layer of Duco cement to protect it from contaminations during the following stages. As soon as the cement had dried, the crown was separated from the root at the enamel-cementum junction and all the crown dentine was carefully drilled off from the inside.

Enamel fragments containing the exposed surfaces were separated with carborundum discs and a non-exposed fragment of about the same size was also split off to serve as a control. After mechanical removal of the Duco layer followed by washing in xylene and acetone and drying in air the fragments were ready for the first radioactivity assay.

This was a surface assay, arranged in the following way. A brass funnel (ARMSTRONG & SCHUBERT 1948) was fitted with a

lid having a circular opening at the center, the size of the exposed enamel surfaces. The enamel fragment was placed on a pat of wax in the funnel so that the exposed surface coincided with the opening of the lid. When the lid was pressed down the fragment was fixed in the wax with the exposed area in a standard position, the geometry of which was only slightly modified by the varying curvature of the enamel surface (Fig. 1).

Fig. 1. Cross section of enamel fragment fixed with wax in brass funnel, the exposed area coinciding with lid opening.

The lid was removed and the activity of the enamel surface determined with the brass dish in a standard position under the mica window of a GM tube.

In most tests a second determination was made after prolonged washing of the enamel fragments, in order to give an idea of the degree of fixation of the labelled atoms. The washing was made in running tap water overnight, and after drying in air at room temperature a new assay was made without changing the positions of the enamel fragments in the brass funnels.

The surface assay could be assumed to be only partial on account of self-absorption in the enamel, especially of the low-energy Ca^{45} radiation. A total assay was also made therefore, using an oxalate precipitate technique for Ca^{45}, and the ordinary dipping counter technique for P^{32}.

The following procedure was used for total Ca^{45} assay. The enamel fragments were removed from the funnels, cleaned from wax with xylene and acetone, dried at 105° C, and weighed. The calcium content was calculated as 35.5 % of the dry weight (LEICESTER 1949). Each fragment was dissolved in about 1 ml 0.5 N HCl for every 25 mg enamel. To the solutions were added sufficient volumes of 0.1 molar $CaCl_2$ solution to give a total calcium content of 80 mg. The calcium was precipitated as the oxalate monohydrate (KOLTHOFF & SANDELL 1949). The precipitates were washed with dilute ammonia, and the tubes centrifuged and the contents decanted again. The precipitates were then dried at 105° C overnight and weighed.

The weights of the precipitates were 300 mg $\pm$ 2 %. The purpose of the procedure, to "dilute" each exposed surface to the same weight of oxalate precipitate, was thus attained within these limits. The precipitate was transferred to a brass funnel as referred

to above where it formed a layer of "infinite thickness" (SINGER & ARMSTRONG 1951). The activity measurements were made with the same Geiger counter and the brass funnel in the same position as used for the surface assay.

In cases of activities exceeding 15,000 counts per minute, the determinations were made with the samples placed on a lower shelf under the GM tube, and all counts from the same test specimen were recalculated to the same geometry, using shelf factors obtained by surface and precipitate assay on both levels.

The total P^{32} assay of the enamel fragments was obtained in the following way. Each dried fragment was dissolved in 6.5 ml normal HCl and the activity of the solutions determined in a dipping counter taking about 5.8 ml. In some cases of very high activity, the determination was made after dilution 1 : 10.

In addition to the corrections for geometry and dilution, all measurements were corrected for background and resolving time losses, and the P^{32} measurements also for radioactive decay.

Results.

Condensated data on the material, and results of the radioactivity measurements, as net counts per minute, are given in Tab. 1 and 2 for the Ca^{45} and P^{32} experiments, respectively. Values which are less than three times their standard errors or less than ten per cent of the background count are given in parenthesis. The uptake by intact enamel in relation to that of carious enamel is given in Table 3 for the total assay values and the immediately preceding surface determinations. The averages of this table are illustrated in Fig. 2.

Survey and discussion of the results.

In every single test, carious enamel has taken up much more of the isotope than intact enamel. This is apparent from both surface and total assays. In most cases the greater part of the active element has remained in the enamel throughout the applied washing. There are no indications of any difference in uptake between surfaces judged as "arrested" or "acute" caries, nor between the general clinical conditions recorded.

For Ca^{45} the ratio between the intact and carious surface up-

Table 1.

Uptake and retention of Ca^{45}.

No.	Patient	Tooth	Surfaces	Surface counts		Oxalate assay
				Direct	After washing	
1		Molar (Stored)	Acid etched			55.8 ± 1.2
			Incip. caries			82.1 ± 1.3
			Intact			4.3 ± 0.8
			» , not exposed			(0.3 ± 0.6)
2		Molar (Stored)	Incip. caries, arrested	336.8 ± 3.4		130.3 ± 1.6
			» » »	449.5 ± 4.6		199.0 ± 2.1
			Intact	41.4 ± 1.0		3.4 ± 0.7
			» , not exposed	6.7 ± 0.6		(0.5 ± 0.6)
3	♀, 43. High caries. Low calculus.	+ 3	B, Incip. caries, active	638.5 ± 6.4		195.2 ± 1.9
			P, Intact	145.3 ± 1.7		15.1 ± 0.8
4	♀, 30. High caries. Low calculus.	3 —	D, Incip. caries spot	29.5 ± 0.9		6.2 ± 0.9
			M, » » »	50.5 ± 1.1		14.6 ± 1.0
			B, Intact	(2.2 ± 0.7)		(0.9 ± 0.9)
5	♀, 33. High caries. No calculus.	3 +	M, Incip. caries, arrested	91.8 ± 1.4		60.4 ± 1.3
			P, Intact	31.0 ± 1.0		6.4 ± 0.9
			B, » , not exposed	16.5 ± 0.9		4.3 ± 0.9

6	(Same as 5.)	+ 3	M, Incip. caries, arrested	220.2 ± 2.4	52.3 ± 1.3	31.9 ± 1.1
			D, » » »	75.7 ± 1.9	32.5 ± 1.1	15.0 ± 1.0
			P, Intact	64.1 ± 1.4	3.5 ± 0.9	(— 0.2 ± 0.8)
			B, » , not exposed	44.5 ± 1.3	11.7 ± 0.8	(2.4 ± 0.9)
7	♀, 25. High caries. No calculus.	3 —	D, Incip. caries (small)	106.7 ± 1.6	30.7 ± 0.9	13.2 ± 1.0
			B, Intact	59.7 ± 1.3	6.5 ± 0.9	(0.4 ± 0.9)
			LM, » , not exposed	146.5 ± 1.8	(2.3 ± 0.9)	(2.2 ± 0.9)
8	♀, 34. High caries. Low calculus.	+ 6	D, Incip. caries, arrested	104.3 ± 1.6	71.5 ± 1.4	33.9 ± 1.1
			B, Intact	59.3 ± 1.3	50.9 ± 1.2	9.1 ± 0.9
			MP, » , not exposed	2.5 ± 0.9	(1.4 ± 0.8)	(0.5 ± 0.9)
9	♀, 66. Av. caries. Low calculus.	+ 7	B, Slight chalky caries	3,900 ± 36	1,770 ± 17.3	214.4 ± 20.8
			P, Intact	770 ± 7.3	419 ± 4.1	51.5 ± 1.2
			D, Caries mark, not exp.	190 ± 2.2	58 ± 1.3	7.3 ± 0.7
10	♀, 21. High caries. No calculus.	8 —	M, Chalky caries	2,021 ± 20.2	1,541 ± 14.9	1,100 ± 10.6
			D, Intact	111 ± 1.0	84 ± 0.5	12.3 ± 0.2
			B, Ch. caries, not exp.	236 ± 2.2	168 ± 1.0	78.7 ± 0.4
11	♀, 19. Low caries. No calculus.	8 +	B, Chalky caries	4,773 ± 39.6	3,441 ± 33.8	1,278 ± 12.7
			P, Intact	158 ± 1.5	115 ± 1.1	18 ± 0.2
			D, » , not exp.	(0.6 ± 0.1)	(0.3 ± 0.1)	(1 ± 0.1)
12	(Same as 11.)	8 —	M, Chalky caries	3,424 ± 33.8	2,725 ± 26.1	1,920 ± 17.9
			D, Intact	323.5 ± 3.0	237.5 ± 2.2	39.3 ± 0.3
			B, » , not exp.	92.4 ± 0.1	3.7 ± 0.1	3.3 ± 0.1

Table 2.

Uptake and retention of P^{32}.

No.	Patient	Tooth	Surfaces	Surface counts		Solution counts
				Direct	After washing	
13	♂, 31. High caries. Low calculus.	6 +	D, Caries mark	10,098 ± 70.4	9,162 ± 68.9	21,115 ± 176.3
			B, Intact	396 ± 3.6	309 ± 2.9	892 ± 9.5
			M, Caries mark, not exp.	(2 ± 0.1)	3 ± 0.1	5 ± 2.2
			P, Intact, not exp.	(0.8 ± 0.1)	(1.8 ± 0.1)	(0 ± 2.1)
14		+ 3	M, Small caries mark	1,795 ± 17.4	1,437 ± 13.9	3,200 ± 21.7
			B, Intact	415 ± 3.7	331 ± 3.5	766 ± 7.0
			D, Caries mark, not exp.	43.8 ± 0.5	31.7 ± 0.5	50.7 ± 2.8
			P, Intact, not exposed	4.1 ± 0.1	(1.1 ± 0.1)	4.7 ± 2.4
15	♀, 38. Low caries. Much calculus.	+ 7	M, Caries mark	1,073 ± 9.5	855 ± 7.5	1,799 ± 15.6
			P, Intact	83 ± 0.8	72 ± 0.7	136.8 ± 3.2
			B, » , not exposed	(2.3 ± 0.1)	(2.0 ± 0.1)	(3.4 ± 1.8)
16		+ 5	D, Caries mark	1,198 ± 1.2	983 ± 9.4	2,236 ± 17.5
			P, Intact	378 ± 3.5	317 ± 3.0	789 ± 1.8
			B, » , not exposed	3.5 ± 0.1	(2.5 ± 0.1)	(3.0 ± 3.2)
17	♀, 53. Av. caries. Some calculus.	1 +	D, Small caries mark	845 ± 7.4	724 ± 6.9	1,844 ± 16.2
			B, Intact	359 ± 0.3	305 ± 2.8	655 ± 6.5
			P, » , not exposed	(0.6 ± 0.1)	(0.3 ± 0.1)	(0 ± 1.9)
18		+ 1	D, Small caries mark	1,837 ± 17.6	1,346 ± 13.3	3,033 ± 20.6
			B, Intact	39 ± 0.4	31.6 ± 0.3	62 ± 7.9
			P, » , not exposed	(0.5 ± 0.1)	(0.0 ± 0.1)	(2.6 ± 1.9)
19	♂, 37. High caries. Some calculus.	— 5	D, Caries mark	6,463 ± 56.5		14,908 ± 143.3
			B, Intact	1,010 ± 8.1		2,584 ± 18.7
			L, » , not exposed	(1.4 ± 0.1)		(0.5 ± 2.0)
20	♀, 17. High caries. No calculus.	+ 3	M, Incip. caries	2,207 ± 21.1		4,858 ± 24.9
			P, Intact	179 ± 1.1		411.5 ± 4.9
			B, » , not exposed	5.6 ± 0.2		17 ± 2.0
21		+ 1	M, Caries mark	1,470 ± 12.2		2,984 ± 19.6
			B, Intact	55.7 ± 0.6		116 ± 3.0
			P, » , not exposed	(0.7 ± 0.1)		(2.2 ± 1.8)

Table 8.

Uptake by intact surface, as percentage of the uptake by carious surface of the same tooth.

Ca^{45}			
Expt. no.	Surface counts		Total assay
	Direct	After washing	
3	22.8	—	7.7
4	5.5	—	0
5	33.8	—	10.6
6		7.9	0
7		21.2	0
8		71.0	26.8
9		23.6	24.0
10		5.5	1.1
11		3.3	1.4
12		8.7	2.0
Average	20.3		7.4

P^{32}			
Expt. no.	Surface counts		Total assay
	Direct	After washing	
13		3.4	4.2
14		23.5	24.0
15		8.4	7.6
16		32.0	35.5
17		42.0	35.5
18		2.4	2.0
19	15.5	—	17.3
20	8.1	—	8.5
21	3.8	—	3.9
Average	15.5		15.4

takes is much greater with surface determination than with total assay in every single case except one (No. 9). The average figures are 20.3 and 7.4 per cent, respectively. This should mean that a greater proportion of the Ca^{45} taken up by the intact enamel is located within the depth of infinite thickness than in the case of carious enamel; in other words, the carious enamel has been penetrated to a greater depth by the isotope. (Infinite thickness for Ca^{45} radiation from normal enamel has been calculated as 0.17 mm.)

In the P^{32} experiments, there are not the corresponding differences. This is probably due to the greater energy of the P^{32} radiation: even if the isotope has penetrated deeper into the carious enamel, its radiation will not be appreciably absorbed in the surface assay. (Infinite thickness for P^{32} radiation from normal enamel has been calculated as about 2.5 mm.)

In several tests, non-exposed surfaces have taken up significant amounts of the radioactive material. This has been the case especially when the control surfaces have shown incipient caries. To judge from radioautographs (WAINWRIGHT 1951) the isotopes may have reached these covered areas through cracks, lamellae or other specially diffusible formations.

The probable explanation of the greater uptake by carious enamel is the enlarged total surface of the corroded crystallites

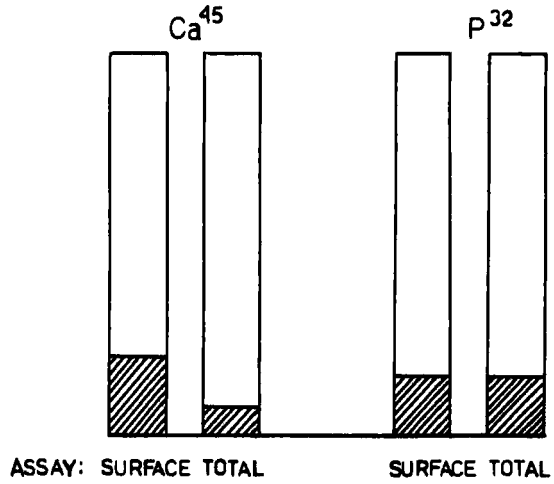

Fig. 2. Uptake by intact enamel surfaces (lined columns) in relation to carious enamel surfaces of the same teeth (full columns).

and the enhanced diffusion in these sections. A correspondingly greater uptake of fluorine and other solubility-decreasing ions is to be expected; during the course of this work it has in fact been reported (MYERS & collab. 1952) that radioautographs of tooth crowns treated with F^{18} show a greater uptake of the fluorine by enamel with slight surface defects. Altogether, these data have a very important clinical consequence: the effect of topical application of fluorides can be expected to be specially great on tooth surfaces which have already been subject to incipient carious dissolution.

Summary.

The uptake in vitro of Ca^{45} and P^{32} by intact and carious enamel surfaces has been studied. Carious enamel has been shown to take up several times more of these isotopes than intact enamel of the same tooth. The probable clinical significance of the data is discussed.

Zusammenfassung.

Das Vermögen intakter Schmelzoberflächen und solcher mit beginnender Karies Ca^{45} und P^{32} aufzunehmen wurde in vitro studiert. Es wurde gefunden, dass kariöser Schmelz vielfach mehr

von diesen Isotopen aufnimmt als intakter Schmelz desselben Zahnes. Die klinische Bedeutung dieser Befunde wird erörtert.

Résumé.

L'absorption de Ca^{45} et P^{32} par des surfaces d'émail intactes et légèrement cariées a été étudiée in vitro. On a constaté que l'émail carié ramasse une quantité plusieurs fois plus grande de ces isotopes que ne le fait l'émail intact de la même dent. La signification clinique probable de ces observations est discutée.

The author wishes to express his profound gratitude to Dr. WALLACE D. ARMSTRONG, Professor and Head of the Department of Physiological Chemistry at the University of Minnesota, for his encouragement, advice and support which made this work possible. Thanks are also due to the staff of Professor ARMSTRONG's laboratory, especially to Dr. LEON SINGER, for great helpfulness during the investigation.

References.

ANDRÉSEN, V.: The physiological and artificial mineralization of the enamel. Oslo 1925.

ARMSTRONG, W. D., and SCHUBERT, J.: Determination of radioactive carbon in solid samples. Anal. Chem. 20: 270, 1948.

BERGGREN, H.: Permeability of enamel and dentine. Sv. Tandl. Tidskr. 40: Suppl. 1947.

COPP, D. H.: Implications of atomic energy in medicine and dentistry. III. Applications in the study of bones and teeth. Oral Surg., Med., Pathol. 3: 613, 1950.

HEAD, J.: A study of saliva and its action on tooth enamel in reference to its hardening and softening. J. A. M. A. 59: 2118, 1912.

KOLTHOFF, I. M., and SANDELL, E. B.: Textbook of quantitative inorganic analysis. New York 1949.

LEICESTER, H. M.: Biochemistry of the teeth. St. Louis 1949.

MYERS, H. M., HAMILTON, J. G., and BECKS, H.: A tracer study of the transfer of F^{18} to teeth by topical application. J. Dent. Res. 31: 743, 1952.

NEUWIRT, F., and WOLF, J.: Histopathologie kazu skloviny a jeho mikroreliefovy' obraz. Česka Stomatologie 44: 427, 1944.

PICKERILL, H.: The prevention of dental caries and oral sepsis. London 1914.

RHEINWALD, U., and STAEHLE, G.: Untersuchungen zur Frage der Remineralisation des Zahnschmelzes. Stoma 2: 259, 1949.

SINGER, L., and ARMSTRONG, W. D.: Retention and turnover of radiocalcium by the skeleton of large rats. Proc. Soc. Exp. Biol. Med. 76: 229, 1951.

WAINWRIGHT, W. W.: Enamel penetration by radioactive salts of zinc, calcium, silver, plutonium, palladium and copper. J. A. D. A. 43: 664, 1951.

WOLF, J., and NEUWIRTH, F.: Reparative Vorgänge an der Oberfläche natürlicher und künstlich hervorgerufener Schmelzdefekte. D. Zahn-, Mund-, Kieferheilk. 8: 222, 349, 1941.

Address: Tandläkarhögskolan,
Stockholm C,
Sweden.