is another major source of error in microdensitometry, not unique to the image analysis method used here. Some of this stray light can affect the apparent optical density of the measured object. Glare tends to be specimen dependent, so its effect varies from field to field. In this system a dynamic glare correction is automatically applied. The transmission light microscope is the device utilized to produce an optical image which is then projected onto the photosensitive face of the vidicon tube scanner[11].

Image Scanning—The face of the vidicon tube is made of material that produces a local potential difference proportional to the intensity of light striking it. The sampling method of the scanner then converts the optical image to an electronic signal consisting of continuously varying analog voltage. High voltage corresponds to bright (white) portions of the image, while low voltage corresponds to the darker regions.

Image Digitization—The total image is composed of ~500,000 picture points or pixels, each of which is sensitive to the light intensity incident on it and contributes to the total analog video signal. It is within the auto detector module (see Fig. 1) that the analog video signal of each pixel is assigned a "gray level" value between 0 and 63. The 63 value corresponds to the brightest area over the total image, the 0 value set to some internal standard for black. The result of this digitization procedure is a digital image consisting of a matrix of numbers each with a value between 0 and 63. This is the basic data set produced by the image analysis system.

Data Reduction Module—To allow selectivity as to what portion of the total field is to be scanned, a variable frame size option is employed. This allows restricting the measurement to some quadrilaterally defined portion of the field. A frame size measuring 600 pixels horizontally by 10 pixels vertically is used to scan each sample. The 600 horizontal pixels under the 160× magnification of the microradiograph allows a sufficiently wide scan to be made across the sample and adjacent negative.

The minicomputer interface allows further processing of the 600 × 10 matrix of pixels, each with a value between 0 and 63. In this case, it is the 10 vertical pixels that are acted upon. To minimize any error due to variable sensitivity of a particular pixel, some defect in the microradiograph itself (dust or a scratch), a single value is obtained from the 10 vertical numbers by simply taking the average. This produces what is called an "average gray level" for 600 horizontal sampling points. Using appropriate calibration methods it was determined that under these conditions each pixel has a width of 0.46 μm. The data are then transferred to magnetic tape as a pair of numbers corresponding to position in scan and the average gray level or "reading" at that position.

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Quantitative Microradiographic Study of Simultaneous Demineralization/Remineralization of Dental Enamel in Weak Acid Buffers

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Abstract □ The remineralization behavior of weak acid-treated bovine tooth enamel has been investigated using a recently developed quantitative microradiographic method. Acetate buffer solutions at pH 4.5 containing calcium, phosphate, and 10 ppm fluoride were used in this study. When the solution ion activity product ($K_{FAP} = a_{Ca}^{10} a_{PO_4}^{6} a_F^2$) was 1×10^{-108}, the remineralization of the demineralized region was relatively uniform and complete. On the other hand, when the K_{FAP} was $\sim \leq 1 \times 10^{-112}$, remineralization of the outer 10–20 μm was incomplete. In addition, for the smaller K_{FAP} solutions there was significant demineralization in the deeper recesses of the originally demineralized region. These results agree with a recent chemical kinetics study in which it was proposed that $K_{FAP} = 1 \times 10^{-112}$ demarcated the region of solution conditions in which remineralization only occurs from that in which simultaneous demineralization/remineralization takes place. A model consistent with all of the data is proposed.

Keyphrases □ Microradiography—quantitative study of dental enamel demineralization/remineralization, solution ion activity product □ Dental enamel—demineralization/remineralization, solution ion activity product, quantitative microradiography □ Demineralization—dental enamel, simultaneous remineralization, solution ion activity product, quantitative microradiography

Recently, Fox *et al.* (1) investigated the influence of the solution ion activity product ($K_{FAP} = a_{Ca}^{10} a_{PO_4}^{6} a_F^2$) on the remineralization of weak acid-treated bovine tooth enamel and hydroxyapatite pellets. Solutions containing calcium-45, phosphate, and fluoride in acetate buffers were used. The calcium-45/fluoride molar ratios determined for the remineralized enamel by perchloric acid etch biopsy indicated the predominant formation of fluorapatite [$Ca_{10}(PO_4)_6F_2$; FAP] or fluoridated hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_{2-x}F_x$], where $0 \leq x \leq 2$ when $pK_{FAP} \simeq 108$. When the pK_{FAP} of the solutions were ≥112, the calcium-45/fluoride ratios were found to be considerably <5. From these results, the authors proposed that a pK_{FAP} value of ~112 marked the demarcation between remineralization only and simultaneous demineralization/remineralization for these acidic remineralizing solutions.

The purpose of the present investigation was to study the remineralization of bovine tooth enamel under the same conditions as those of Fox *et al.* (1), employing a recently developed (2) quantitative microradiographic technique. A special point of interest was to examine the hypothesis that simultaneous demineralization/remineralization sets in when the pK_{FAP} value is ~ ≥112 for these solutions.

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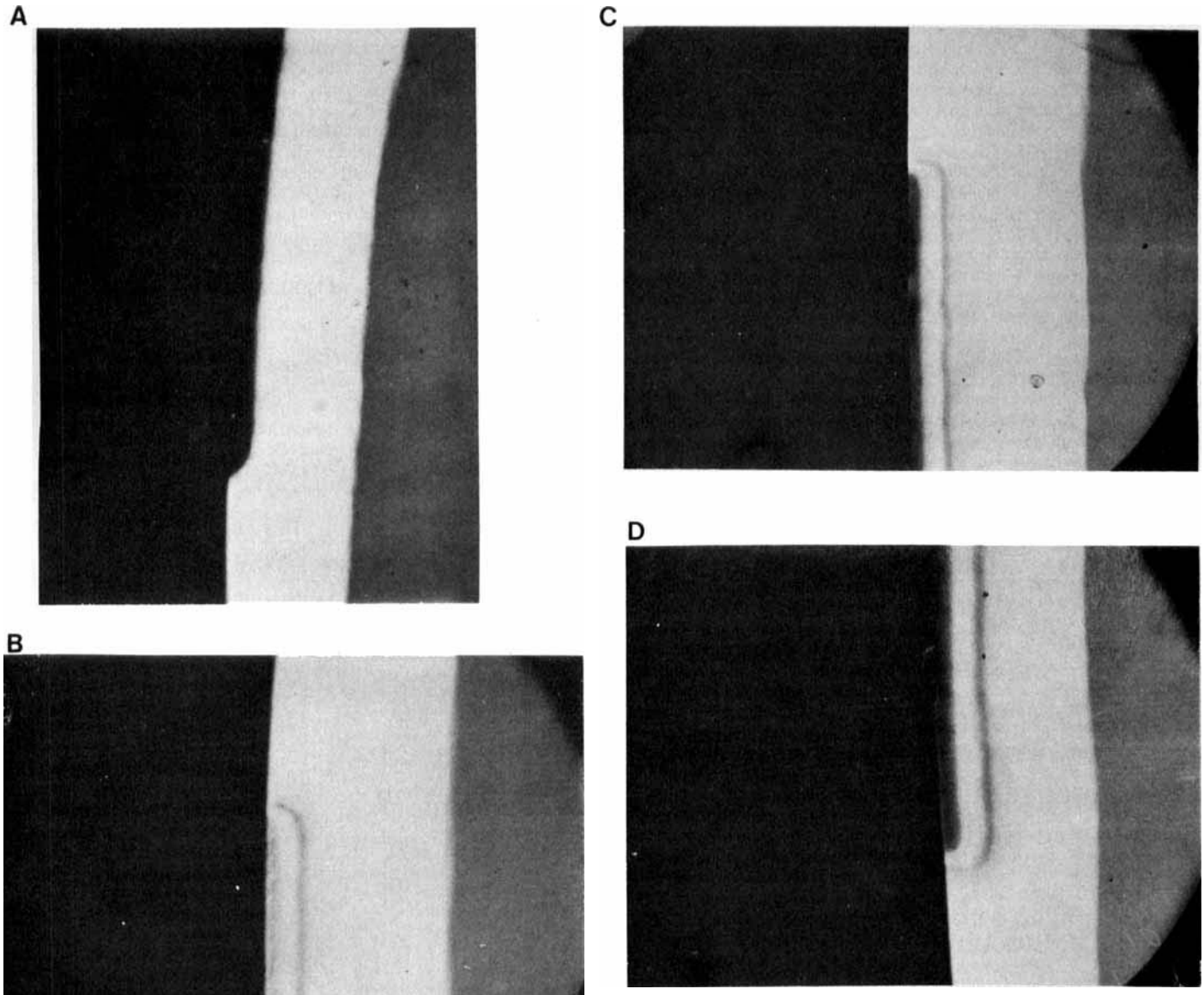


Figure 1—*Bovine tooth enamel demineralized for 6 h in 10 mL of 0.10 M acetate buffer (μ = 0.5, pH 4.5) with calcium = phosphate = 3.5 mM. Remineralization was done in different solutions (see Table I) for 24 h. Key: (A) no remineralization; (B) solution 1, pK_{FAP} = 108; (C) solution 2, pK_{FAP} = 112; (D) solution 3, pK_{FAP} = 118.*

EXPERIMENTAL

Buffer Dissolutions—*Dissolution*—A solution ~16% saturated (on a molar basis) with respect to the thermodynamic solubility of hydroxyapatite was used for demineralization. The solution was 0.1 M acetate buffer containing 3.5 mM each of total calcium and phosphate. The pH was adjusted to 4.5 with sodium hydroxide and the ionic strength to 0.5 M by the addition of sodium chloride.

Remineralization—For remineralization, 0.1 M acetate buffers (pH 4.5) containing different amounts of total calcium and phosphate depending on the desired bulk solution activity product ($K_{FAP} = a_{Ca}^{10} a_{PO_4}^{6} a_F^2$) as shown in Table I were employed. These solutions also contained 10 ppm fluoride (in the form of sodium fluoride) with sodium chloride added to adjust the ionic strength to 0.5 M. All chemicals used were of analytical grade.

Bovine Teeth—Teeth from 8-week old strictly Kosher calves were obtained from packing houses in the Chicago area. From these, only reasonably flat incisors without any visible surface defects were used experimentally. The pellicle was removed from the labial surface using 400- and 600-grit silicon carbide abrasive disks.

Demineralization and Remineralization Procedures—A selected bovine tooth was covered with nail enamel except for a 0.25-cm^2 window on the labial surface. The tooth was then placed in 10 mL of the 16% partially saturated buffer solution for 6 h. From experimental results obtained by Yonese *et al.* (3) the demineralizing solution was not saturated with respect to hydroxyapatite after 6 h of demineralization. During demineralization the solution was gently agitated with a wrist-action shaker. The temperature was maintained at 30°C.

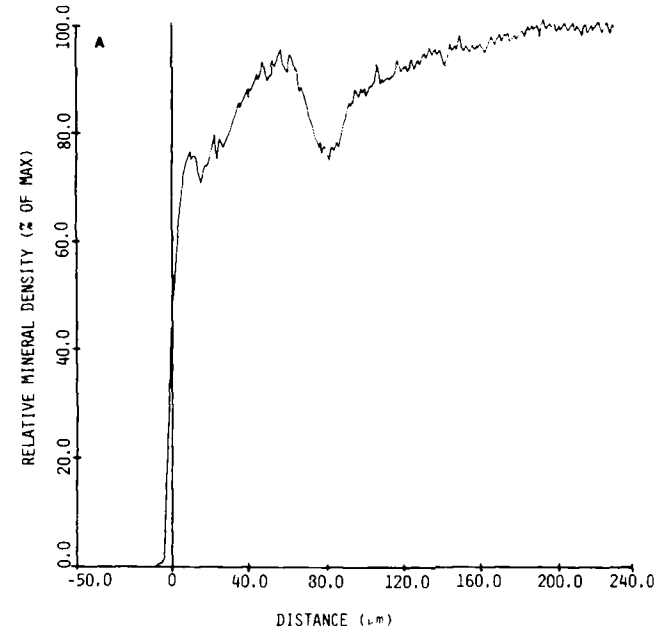
Table I—Ion Activity Products of Solutions [a] Used in this Study

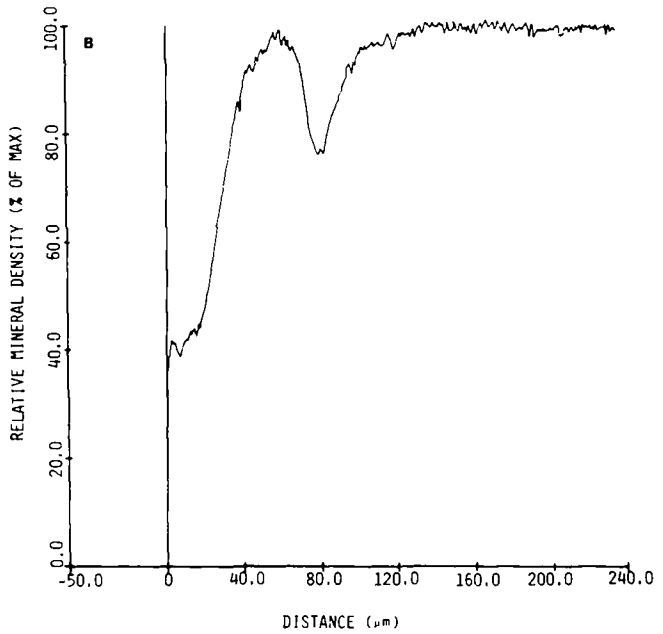
Solution	Calcium, mM	Phosphate, mM	Fluoride, ppm	pK_{FAP}	pK_{HAP}
1	12.00	12.00	10	108	120
2	6.87	6.87	10	112	124
3	2.86	2.86	10	118	130

[a] pH 4.5; μ = 0.5; 0.1 M acetate buffer.

The previously demineralized sample was immediately placed in 50 mL of the desired remineralization solution. During the remineralization the solution was again gently agitated and maintained at 30°C.

Sample Preparation—The demineralized/remineralized tooth was mounted on a circular saw fitted with a high concentration diamond wafering blade. Sections as thin as possible were cut through the exposed window perpendicular to the enamel surface. Each section was then ground plane-parallel to a thickness of exactly 100 μm as determined by a micrometer. Final polishing, using diamond paste on a rayon cloth, was done to remove any small aberra-





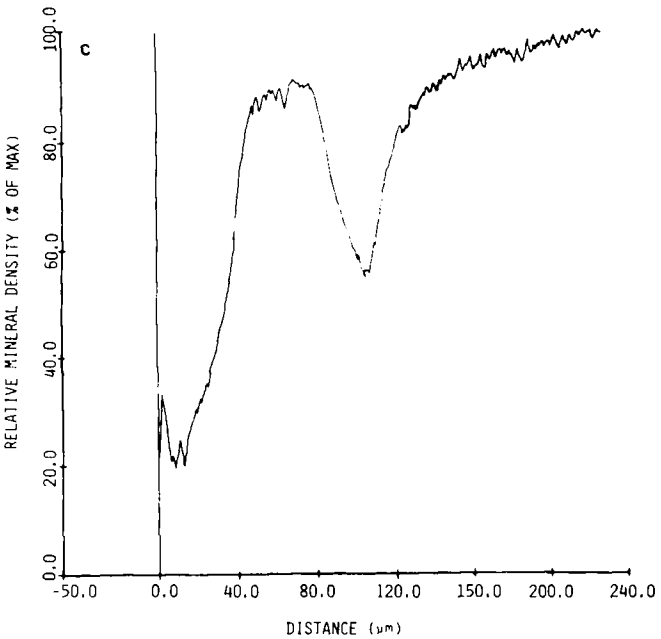


Figure 2—*Relative mineral density* versus *distance for remineralization experiments described in Fig. 1. Key: (A) $pK_{FAP} = 108$; (B) $pK_{FAP} = 112$; (C) $pK_{FAP} = 118$.*

tions resulting from the initial grinding using 600-grit silicon carbide paper.

X-Ray—A section was placed in direct contact with the emulsion on a 5 × 5 cm high-resolution glass plate[1]. Directly opposite the enamel surface to be analyzed, a wedge made of bovine enamel was placed. The wedge allowed comparison of the negatives of different samples by factoring out any deviations in the time- and temperature-dependent film development process.

The sample was placed in the center of the X-ray beam[2] for 25 min. The instrument settings were 40 kVp and 3 mA. The negative was developed in developer solution[3] according to the manufacturer's instructions.

Density Profiles—Quantitation of the demineralization/remineralization process was accomplished using the image analysis system[4]. By the procedure outlined in the previous article (2), scans were made across the sample as well as on the adjacent step wedge. The data recorded on magnetic tape were analyzed by a computer program in which the wedge data and sample data were used to calculate the mineral density *versus* position in the tooth.

[1] Eastman Kodak, Rochester, N.Y.
[2] Hewlett-Packard, McMinnville, Ore.
[3] D-19 *Developer*; Eastman Kodak, Rochester, N.Y.
[4] Cambridge Instruments, Inc., Monsey, N.Y.

RESULTS AND DISCUSSION

As shown by the photomicroradiographs (Fig. 1) and the mineral density *versus* position profiles (Fig. 2), the influence of the solution activity product on the remineralization of bovine tooth enamel is profound. Remineralization under conditions in which the solution activity product equals 10^{-108} gives a relatively uniform and essentially complete recovery of mineral density to the original surface of the previously demineralized sample (Figs. 1B and 2A). Under conditions where the pK_{FAP} of the remineralization solution is high (*i.e.*, 112) the recovery appears less complete, particularly in the outer 20 μm (Figs. 1C and 2B). There is also the appearance of a less dense band at a depth slightly greater than that produced by the demineralization treatment alone. The experimental results for conditions in which the solution activity product was varied from 10^{-114} to 10^{-118} also indicated this less dense outer 20 μm in the enamel sample. The band below the original "lesion" (Fig. 1A) depth was now significantly deeper and more diffuse (Figs. 1D and 2C).

Mineral density profiles of this type indicate that the situation is more complex than that of simple mineral deposition in the form of fluorapatite (1). Indeed, simultaneous dissolution must also be occurring in order to explain the loss of mineral deeper than the original "lesion." The presence of a more highly remineralized region under the less dense outer 20 μm found when remineralization was done in solutions with $pK_{FAP} \geq 110$ indicates that the

bulk solution activity product is not the sole determinant of the remineralization process.

The following discussion attempts to present a mechanism that is consistent with the present observations and with the chemical kinetic data obtained by Ludwig *et al.* (4), Fox *et al.* (1), and Katdare *et al.* (5). Thermodynamic calculations show (Table I) that a bulk solution with $pK_{FAP} = 108$ is supersaturated with respect to fluorapatite and saturated or supersaturated with respect to the ion activity products, K_{HAP} values, governing the relatively rapid rate of hydroxyapatite dissolution [*i.e.*, $pK_{HAP} = 120$ for site 1 and $pK_{HAP} = 128$ for site 2 described by Fox *et al.* (6)]. Ludwig *et al.* (4) have shown, however, that for apatite powder containing ~5% carbonate, the corresponding apparent solubilities may be greater by approximately four orders of magnitude in the K_{HAP} values. The carbonate level in the bovine enamel used in these experiments is ~4%. The modest band of demineralization (at ~80 μm depth) observed with the $pK_{FAP} = 108$ case is consistent with this consideration. With increasing pK_{FAP} the solutions become increasingly unsaturated with respect to hydroxyapatite (and carbonate apatite), and the deeper and wider band of demineralization observed at ~110 μm for $pK_{FAP} = 118$ would be consistent with this thinking.

The remineralization behavior in the region 0–20 μm is in agreement with the results of experiments with hydroxyapatite and carbonate containing apatite crystal suspensions reported by Katdare *et al.* (5). At $pK_{FAP} = 108$, fluorapatite suspension crystal growth rates were found to be relatively rapid but decreased significantly (*i.e.*, by a factor of ~10) at $pK_{FAP} = 112$, and the rates were negligibly slow at $pK_{FAP} = 116$. These findings by Katdare *et al.* (5) may easily explain the rather complete and uniform remineralization observed at $pK_{FAP} = 108$ in the region 0–20 μm and the much poorer extent of remineralization in this region at $pK_{FAP} = 112$ and 118. It may be concluded, therefore, that the remineralization behavior in the outer 10–20 μm may be primarily controlled by the bulk solution activity product.

The most interesting aspect of the results in Figs. 1 and 2 is that related to the highly mineralized region at intermediate depths (*e.g.*, at ~50 μm in the $pK_{FAP} = 112$ experiment). The following process is proposed to explain why remineralization occurs at intermediate depths but not at or near the surface (for the $pK_{FAP} = 112$ and 118 experiments). Ludwig *et al.* (4) have shown that, even for apatites containing ~5% carbonate, dissolution rates should be negligible when $pK_{FAP} \leq 115$. Therefore, for lesions to form at 80–90 μm in the $pK_{FAP} = 108$ and 112 experiments, it is reasonable to postulate that the microenvironment (aqueous pores) pK_{FAP} values in the deep recesses of the lesion must be >115 as a result of aqueous fluoride depletion caused by fluorapatite (or fluorhydroxyapatite) formation or fluoride adsorption in the outer layer and at intermediate depths. Fluorapatite deposition and fluoride depletion at the intermediate depths (40–50 μms) would be enhanced because mineral dissolution at 80–90 μm and the resultant outward flow of calcium and phosphate ions may significantly elevate the K_{FAP} values to far above the bulk K_{FAP} value. This simultaneous dissolution (at 80–90 μm) and remineralization (at 40–80 μm) process continues until sufficient aqueous fluoride concentrations (corresponding to $pK_{FAP} \simeq 115$) can build up in the deeper regions of the lesion.

To test the hypothesis that continued dissolution beneath the level of the original lesion is a transient phenomenon, additional studies have been conducted at different pK_{FAP} values for 3, 6, 12, 24, 48, and 96 h. At $pK_{FAP} = 116$, for example, the simultaneous dissolution and remineralization did not proceed beyond 12 h, and the lesion pattern remained essentially unchanged beyond that time period. Interestingly, Fox *et al.* (1) found that ^{45}Ca-labeled hydroxyapatite pellets dissolved only for 12–16 h in $pK_{FAP} = 116$ solutions. These are bulk-solution conditions for which simultaneous dissolution and remineralization will occur. The simultaneous demineralization/remineralization then appears to be a transient phenomenon indicating that once sufficient remineralization has occurred at the intermediate depths, dissolution no longer continues under the control of the microenvironmental K_{HAP} in the deeper regions of the enamel.

These results support the hypothesis by Fox *et al.* (1) that $pK_{FAP} \simeq 112$ marks the demarcation between mainly remineralization only and simultaneous demineralization/remineralization. For high pK_{FAP} solutions (*e.g.*, 118), fluoride deposition *via* fluorapatite or fluorhydroxyapatite formation occurs mainly at intermediate depths and involves, to a large extent, the reaction between bulk solution fluoride and the calcium and phosphate ions dissolved from the deeper regions of the lesion. For $pK_{FAP} = 108$, the remineralization is mainly controlled by the bulk solution, and the contribution to fluorapatite or fluorhydroxyapatite formation from the mineral dissolved in the lesion is minimal.

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