susceptible cell sites. An alternate hypothesis is that the cells undergo a change under the influence of the dye which makes them resistant.

The discrepancy between my findings and those of Gochenour and Baron(3), who observed photodynamic resistance in continuous cell cultures, is as yet unexplained; but may be due to the differences in the method used for testing resistance. These workers used a lower light intensity and cell cultures grown in 2-ounce bottles under agar overlay.

Summary. Photodynamic destruction of monkey kidney cells by irradiation with visible light from a fluorescent light source at .20 watt/cm^2 for 15 minutes occurs 4 hours after staining with acridine orange, neutral red, proflavine, and toluidine blue. Resistance to this photodynamic effect occurs when cells stained with the same dyes are incubated in the dark for 24-48 hours prior to exposure. Lowering the temperature of incubation to 4°C inhibited the resistance forming process. A resistance-producing factor could not be obtained from resistant cells which would convert sensitive cells. Aeration of the cells or addition of fresh media does not reverse their photodynamic resistance. Thus, the depletion of a media constituent is not the cause of photodynamic resistance. Cell lines vary as to their susceptibility to photodynamic action and their ability to develop photodynamic resistance. However, under the experimental conditions outlined in this paper, primary cell cultures display resistance, whereas, continuous cell lines do not.

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Comparative Fixation of Sr^{89} and Ca^{45} by Calcified Tissues as Related to Fluoride Induced Changes in Crystallinity. (27594)

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It was previously reported(1,2) that discrimination against strontium as compared to calcium, in formation of synthetic hydroxyapatite was greater in large, more slowly grown crystals than in small, more rapidly precipitated crystals. In a subsequent study of the fixation of Sr^{89} and Ca^{45} by several different tissues of the rat(3) it was shown that this discrimination increased with an increase in crystallinity as determined by X-ray diffraction line broadening analysis. Crystallinity is a measure of degree of structural perfection and size of the crystals, where more perfect and/or larger crystals are said to be more crystalline. Recent studies of rat and human bone apatite indicate that crystallinity improves with increasing incorporation of fluoride(4,5). The purpose of the present study was to investigate the crystallinity changes resulting from the long-term ingestion of fluoride in relation to the comparative metabolism of Sr^{89} and Ca^{45} in the rat.

Experimental procedure. Male Sprague-Dawley rats 35 days old were divided into 5 groups. All received the same prepared diet (6,7) containing 0.8 ppm (parts per million) fluoride. Groups were assigned drinking water containing fluoride (as NaF) as fol-

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lows: I, 0 ppm; II, 10 ppm; III, 25 ppm; IV, 50 ppm; V, 100 ppm.

The animals were maintained on an *ad libitum* feeding and watering regimen for 320 days at which time each was treated by intraperitoneal injection of 0.5 ml of a solution containing trace amounts of Ca^{45} and carrier-free Sr^{89}, plus 0.05 mg of calcium. At the end of one hour the rats were killed and the tibiae and incisor teeth were removed. Immediately prior to sacrifice approximately 5.0 ml of blood was obtained from each rat by cardiac puncture. The incisors were cut transversely at a point approximately one-fifth of the distance from the base to the incisal tip; the basal segment was reserved for analysis without attempt to separate the enamel from dentin. All samples were defatted by extraction in alcohol for 8 hours and in ether for 4 hours.

For radiochemical analysis, the basal segment from one upper incisor was combined with the corresponding segment of a lower incisor. These samples together with the pooled ends and associated shaft of one tibia from each rat were weighed after drying at 105°C and after incineration overnight at 600°C. The ash of each was dissolved in a slight excess of hydrochloric acid and analyzed for Sr^{89} and Ca^{45} by methods previously described(1).

The X-ray diffraction powder patterns of hard tissue apatites are generally poorly resolved compared to the patterns of well-crystallized minerals. This lack of resolution results from the broadening of the individual diffraction maxima because of the small size and imperfection of the crystals of these biological apatites. For X-ray diffraction analysis, the segments of the remaining tibiae and incisor teeth were pooled according to type and experimental group and ground to pass a 200 mesh sieve. X-ray diffraction patterns (Copper K-alpha radiation) were obtained on the powdered samples using a commercial geiger counter diffractometer with a high resolution slit system. The degree of resolution of the 4 principal reflections of the apatitic X-ray patterns (Miller indices 211, 112, 300, 202) was used as a measure of the crystallinity of each specimen.

To give each diffraction pattern a crystallinity rating, a series of 10 templates was constructed to represent the hydroxyapatite X-ray diffraction powder patterns with 10 different amounts of line broadening. Each template was designated by a β_t value, the width at half maximum, in degrees 2θ, of each of the 4 Gaussian maxima summed to form the template. The X-ray patterns were then assigned crystallinity, *i.e.*, β_t, values by matching them with the proper template. As the β_t value decreases, the crystal size and/or crystal perfection increases.

The width at half maximum of the 002 reflection for each of the patterns was corrected for instrument broadening by the method of Warren(8) and designated as β_{002}.

The powder samples of tibia ends and shaft used in the X-ray study were ashed and analyzed for fluoride(9,10).

Results and discussion. Table I gives the ash content, fluoride content, and X-ray diffraction data for the 5 animal groups. As expected, the fluoride content of the bones and incisors increased with a rise in fluoride concentration in the drinking water. The percent ash of the tibiae was not changed, while the percent ash of the incisor bases decreased with fluoride treatment.

The β_t values of both the shaft and the end samples decreased with increasing fluoride content. This observation, indicative of an increase in crystal size and/or crystal perfection, agrees with previous results on human bone(5). There were no significant changes in β_{002} values of any of the bone samples. The β_{002} is a measure of crystallinity in the c axis direction only, while β_t is related to the crystallinity in both the a and c directions. Thus fluoride treatment did not change the crystals in the c axis direction but did cause growth and/or crystal perfection in the direction of the a axis. Similar unpublished results have been obtained on human bone by one of us (ASP).

In contrast to bone, incisor apatite crystallinity did not change with an increase in fluoride ingestion as indicated by the lack of change in β_{002} or β_t values. The reasons for this different response to fluoride are not immediately apparent. While the incisors con-

TABLE I. Comparison of X-Ray Diffraction Line Broadening and Fluoride Content of Rat Tibia and Incisors.

Group*	F content of H_2O (ppm)	Sample	Ash, %	F, % ash	β_t‡	β_{002}§
I	.0	Ends of tibia	57.57 ± 1.06	.030	1.15	.545
		Shaft of tibia	70.71 ± 1.68	.020	1.10	.464
		Base of incisor†	65.05 ± 1.07	.038	.70	.408
II	10.0	Ends of tibia	57.13 ± .55	.165	1.10	.481
		Shaft of tibia	73.25 ± 2.70	.139	.90	.446
		Base of incisor	63.64 ± 1.34	.097	.70	.351
III	25.0	Ends of tibia	58.77 ± .66	.471	1.00	.523
		Shaft of tibia	70.60 ± .55	.291	.85	.467
		Base of incisor	62.03 ± 2.67	.156	.70	.354
IV	50.0	Ends of tibia	58.05 ± .99	.684	.95	.513
		Shaft of tibia	69.53 ± .72	.523	.80	.467
		Base of incisor	62.09 ± 2.26	.350	.70	.408
V	100.0	Ends of tibia	57.23 ± .60	1.070	.85	.452
		Shaft of tibia	69.66 ± .65	.796	.75	.459
		Base of incisor	58.46 ± .53	.460	.70	.406

* Group I: 3 animals; II, III, IV and V: 6 animals.
† Proximal fifth of upper and lower incisors.
‡ The template β value; avg width at half maximum of the 211, 112, 300, and 202 reflections. Precision of these values is calculated to be ± 5%.
§ Width at half maximum of the 002 X-ray diffraction reflection corrected for instrument broadening, expressed in degrees 2θ. Precision of these values is calculated to be ± 5%.

tained less fluoride than the tibia ends and shaft at each level of fluoride supplementation, improvement in bone apatite crystallinity occurred at fluoride concentrations below those found in some of the incisor samples (Table I). On the other hand the incisor samples were initially more crystalline than either of the tibia samples and therefore the incisor apatite may be less susceptible than bone apatite to further improvement in crystallinity through fluoride incorporation. It is also possible that equilibrium with fluoride has been more nearly realized by bone apatite than by incisor apatite, since, in rats of this age the more mature bone contains proportionately less new mineral than the rapidly growing incisor base, as illustrated by the differential radioisotope uptake (Table III).

Serum levels of Sr^{89} and Ca^{45} in the experimental animals are shown in Table II. Uptake of these isotopes by the tibiae and incisors is summarized in Table III. An interesting comparison with respect to metabolic activity can be made between these results and previously published data on young rats (3). Despite the fact that serum radioisotope concentrations in the earlier experiment were essentially the same as in this study, uptake of Sr^{89} and Ca^{45} in the 25-day-old rats was 40 to 50 times higher for the tibia ends and shafts, respectively, than for the same tissue in the one-year-old rats. In addition, a reversal takes place in the relationship between radioisotope uptake by the tibia end and uptake by the incisor base. In the young rat the tibia ends are more metabolically active than the incisors, while the opposite is true in the older rat.

TABLE II. Serum Levels of Sr^{89} and Ca^{45} in Control and Fluoride Treated Groups of Rats.

Group	F content of H_2O (ppm)	Sr^{89}*	Ca^{45}*	Sr^{89}:Ca^{45}*
		% of dose/ml of serum		
I	.0	.476 ± .0113	.467 ± .0206	.99 ± .05
II	10.0	.451 ± .0067	.385 ± .0170	1.18 ± .05
III	25.0	.419 ± .0226	.406 ± .0305	1.04 ± .04
IV	50.0	.433 ± .0109	.375 ± .0214	1.15 ± .03
V	100.0	.458 ± .0158	.459 ± .0190	.99 ± .02

* Mean ± stand. error calculated for each sample.

TABLE III. Uptake of Sr^{89} and Ca^{45} by Rat Bone and Incisors in Relation to Fluoride Induced Changes in Crystallinity.

Group	F content of H_2O (ppm)	Sample	Sr^{89}*	Ca^{45}*	Sr^{89}:Ca^{45}†
			% of dose/mg of ash		
I	.0	Ends of tibia	.00349 ± .00020	.00371 ± .00038	.91 ± .03
		Shaft of tibia	.00111 ± .00056	.00111 ± .00	.91 ± .08
		Base of incisor‡	.00840 ± .00084	.00839 ± .00155	1.02 ± .11
II	10.0	Ends of tibia	.00337 ± .00012	.00311 ± .00024	1.10 ± .05
		Shaft of tibia	.00111 ± .00006	.00111 ± .00007	1.12 ± .09
		Base of incisor	.00838 ± .00064	.00925 ± .00034	.93 ± .06
III	25.0	Ends of tibia	.00298 ± .00014	.00276 ± .00013	1.09 ± .08
		Shaft of tibia	.00096 ± .00021	.00089 ± .00002	1.08 ± .01
		Base of incisor	.00883 ± .00251	.00962 ± .00263	.91 ± .08
IV	50.0	Ends of tibia	.00291 ± .00018	.00279 ± .00009	1.04 ± .047
		Shaft of tibia	.00097 ± .00044	.00087 ± .00046	1.08 ± .052
		Base of incisor	.00730 ± .00237	.00766 ± .00207	.94 ± .010
V	100.0	Ends of tibia	.00327 ± .00018	.00305 ± .00020	1.15 ± .07
		Shaft of tibia	.00107 ± .00003	.00110 ± .00080	1.00 ± .08
		Base of incisor	.01046 ± .00134	.01218 ± .00195	.88 ± .05

* Mean ± stand. error calculated for each sample.
† Mean ± stand. errors for each sample calculated from individual ratios.
‡ Proximal fifth of upper and lower incisors.

In general the bones which increased in crystallinity due to fluoride treatment showed a decreased radioisotope uptake. In the bones of the 100 ppm fluoride treated animals an increased crystallinity was associated with a higher uptake than that found in the 25 and 50 ppm animals. It has been reported that high levels of fluoride produce in animals histologic and metabolic changes in soft tissue, bone, and other organs such as kidney(11,12,13). In rats receiving 100 ppm fluoride such changes could affect radioisotope retention.

The Sr^{89}/Ca^{45} ratios of the tibia samples were generally equivalent to the ratios of corresponding serum specimens. On the other hand the Sr^{89}/Ca^{45} ratios of the incisor samples were lower than the serum ratios, indicative of a discrimination against Sr relative to Ca. Past studies showed that new crystal formation and not surface exchange is primarily responsible for the differential uptake of Sr and Ca(3). The better crystallized, more slowly formed apatite retained relatively less Sr than Ca, while the poorly crystallized, more quickly precipitated material retained equal quantities of the ions(1,2). These results would suggest that the discrimination against Sr in the incisor is due to the fact that this material, relative to bone, is better crystallized and contains relatively more new crystals as evidenced by its higher radioactivity. The lack of discrimination in fluoride treated bone despite the increased crystallinity is probably due to the small amount of new crystal formation in these tissues.

Summary. X-ray diffraction analyses, and Sr^{89} and Ca^{45} uptake studies in tibiae and incisors of control and fluoride treated one-year-old rats were carried out. In the tibia ends and shafts an improvement of bone apatite crystallinity in the direction of the *a* axis and not the *c* axis was produced by fluoride ingestion. No changes in incisor apatite crystallinity were observed. Uptake of Ca^{45} and Sr^{89} was decreased in the tibia ends of the 25 and 50 ppm F treated animals. Sr^{89}/Ca^{45} ratios in the tibia samples were the same as in the serum specimens. There was a discrimination against Sr in relation to Ca in the incisor bases. Radioactivity in the tibia of one-year-old animals was 1/40 to 1/50 of that found in a group of young animals treated similarly in an earlier experiment. In the young rat the tibia ends were found to incorporate more Sr^{89} and Ca^{45} than the incisor, while the opposite was found in the older rats.

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Effect of Ouabain on Acid Secretion and Electrolyte Content of Frog Gastric Mucosa.* (27595)

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We have described some aspects of the electrolyte composition of frog gastric mucosa as the first step in a study of the relation between cell composition and secretory ability (1). In this paper we show that the concentration of ouabain (G-strophanthin) which inhibits acid secretion also causes K in the tissue to fall and Na to rise. If the mucosa is incubated in a solution in which part of the Na is replaced by K, tissue concentrations are returned toward normal, and acid secretion is restored.

Methods. Our methods for measuring acid secretion by gastric mucosal sacs of *Rana pipiens* have been described(2). The only change in technic is that the tissues are now always incubated under a Po_2 of approximately 2,000 mm Hg which reduces aerobic glycolysis to a negligible rate. Likewise, our methods for incubating mucosas for determination of volumes of distribution of radioiodinated serum albumin (RISA), inulin and mannitol and our analytical methods for tissue electrolytes have been described, together with evidence showing that the 4-hour volume of distribution of RISA is probably a correct estimate of the extracellular space(1).

Results. The inhibitory effect of ouabain on acid secretion by frog gastric mucosa is shown in Fig. 1.

The effect of 10^{-4} M ouabain on electrolytes and spaces of RISA and inulin in frog gastric mucosa is shown in Fig. 2. $[Na]_o$ was 117 mM, $[K]_o$ 9 mM and $[Cl]_o$ 104 mM. The control values are the cumulative ones collected in our laboratory over a 2-year period, and include observations made in parallel with those in which ouabain was used. Consequently, the differences can be attributed to ouabain and not to seasonal variations or changes in methods. The climbing curves for RISA and inulin show diffusion of the substances into the tissue over the 4-hour period. The curves for H_2O, Na, K and Cl show the amounts in the tissues at time of analysis. Ouabain produces no change in total H_2O, but reduces both RISA and inulin spaces. Four-hour mannitol spaces, not shown in the figure, were for control mucosas 545 $\pm$ SEM 17 ($n = 30$) and for ouabain-treated mucosas 410 $\pm$ SEM 10 ($n = 20$). The parallel reduction in these 3 spaces without increase in total H_2O shows that the cells swelled at the expense of extracellular fluid space. During incubation tissue Na rose and K fell, doubtless reflecting similar changes in intracellular concentrations. On the assumption that the 4-hour RISA space is true extracellular volume the intracellular concentrations can be calculated. However, it seems likely that there are at least 2 cell types in

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