measurements of inhibition of Mg-activated ATPase in cardiac myofibrils under similar conditions is not a model for relaxation as suggested in the literature. Cardiac myofibrils may therefore contain a contaminating Mg-activated ATPase which is not Ca dependent and not associated with contraction.

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Regulation of Plasma Fluoride in Rats.* (29668)

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The constancy of fluoride content of plasma of humans using communal waters with a wide range of fluoride concentration has been reported(1). The mean plasma fluoride concentration of hospitalized patients was not different from that of normal individuals. The only circumstance in which there was a suggestion of higher than normal plasma fluoride values was in 3 cases of demineralizing bone disease. Brzezinski, Gedalia, Danon, and Sulman(2) reported no difference between the fluoride contents of the blood of pregnant and non-pregnant rats. Their data also suggest that large variations in aqueous fluoride intake did not appreciably affect the blood fluoride concentration.

The present investigation was designed to examine the effects of dietary and physiological variables which might influence the plasma fluoride concentration. The factors of fluoride intake, skeletal fluoride content, starvation and parathormone administration have been studied to determine the ability of the homeostatic mechanisms controlling the plasma fluoride concentration to cope with these stresses.

Experimental. Male Sprague-Dawley rats were used. All animals were fed *ad libitum* Purina Laboratory Chow which contained 60-85 ppm of fluoride and received varying intakes of fluoride *via* the drinking water. The animals were bled by heart puncture using specially washed equipment to eliminate fluoride contamination(1), and heparin was used as an anticoagulant. The bones were cleaned of soft tissues, freed of marrow, dried, and fat-extracted with an equal-part mixture of absolute alcohol and ethyl ether for 48 hours before being ashed at 500°C. The blood plasma samples and the ash of the humeri were analyzed for fluoride by the microdistillation method of Singer and Armstrong(3). Analyses for plasma calcium were carried out by the method of Buchner and Shively(4).

Study I. Relation between skeletal and plasma fluoride contents. In this study 93 of a total of 106 rats, approximately 60 days of age, were divided into 4 sub-groups (Table I, Part 1). They were fed laboratory chow containing 60-70 ppm of fluoride and were given distilled water containing either 0, 10, 30, or 50 ppm of fluoride. This regimen was continued for 60 days and the animals were then given distilled water for 30 days before being sacrificed. The other 13 animals of the study (Table I, Part 2) were given laboratory chow with a slightly higher fluoride content (85 ppm) than that received by the animals described above and were maintained on either distilled water or distilled water plus 50 ppm of fluoride until time of sacrifice. These animals were killed after 60 days on the dietary regimen.

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TABLE I. Fluoride Concentrations of Plasma and Bones of Rats Maintained on Various Levels of Aqueous Fluoride Intake.

(Study I)

Fluoride concentration in water (ppm)	Body wt (g ± S.E.)		Serum		Humeri	
	Initial	Sacrifice	No. animals	F (ppm ± S.E.)	No. animals	F in ash (% ± S.E.)
Part 1						
0	183* ± 1	379 ± 7	29	.24 ± .008	30	.10 ± .003
10		375 ± 12	19	.25 ± .031	21	.13 ± .006
30		396 ± 7	18	.26 ± .028	21	.21 ± .004
50		386 ± 7	19	.26 ± .026	21	.29 ± .005
Age (days)	60	150				
Part 2						
0		343 ± 11	5	.26 ± .026	5	.12 ± .016
50		346 ± 9	8	.30 ± .016	8	.25 ± .010
Age (days)	60	120				

* Applicable to all animals of Parts 1 and 2.

Study II. Effect of parathyroid extract administration on plasma fluoride levels. Twenty rats (initial age 60 days; 180-200 g body weight) were fed laboratory chow and distilled water containing 20 ppm of fluoride for 64 days before being transferred to a regimen of the same food but with distilled water for 56 days. This treatment is known to produce an elevated skeletal fluoride content (see also Table I). The experimental group of 12 animals received daily subcutaneous injections of 100 USP units of parathyroid extract (Eli Lilly and Co.) over the last 4 days. Eight of the animals served as controls and were given over the same period daily injections of 1 ml of isotonic saline. Four hours after the last injection all animals were bled by heart puncture and plasma calcium and fluoride contents were determined.

Study III. Effect of starvation on plasma fluoride levels. Fluoride is widely present in natural foods and waters and the only assured procedure by which fluoride intake can be reduced to near zero is by starvation. To determine the effects of starvation on plasma fluoride content 43 animals (initial age 48 days; 60-70 g body weight) were given laboratory chow containing 60-80 ppm fluoride and distilled water with 50 ppm of added fluoride for 72 days. The laboratory food and fluoride containing water was then removed from their cages and lump refined sugar, a bottle of distilled water and a bottle of 0.1% sodium chloride in distilled water were provided. Sugar and saline were given to obtund starvation acidosis and to support the extracellular fluid compartments. Seventeen animals were sacrificed immediately, 12 animals at 3 days and 14 animals at 7 days after being deprived of their normal food and water supply. Analyses for the fluoride contents of the plasma and humeri were made. These and other results are given in Table II.

Results and discussion. Even though up to a 3-fold increment in concentration of fluoride in the humeri of animals of Study I was produced by increasing the fluoride content of the drinking water from 0.0 to 50 ppm, plasma fluoride concentrations, among the 4 groups of animals, were not different (Table I, Part 1). Weight gains of the animals were not influenced by level of fluoride intake. The possibility that the final 30-day period of distilled water intake allowed adjustment of the plasma fluoride levels of animals of Part 1 to common values by uptake of fluoride by the skeleton is disproved by the results obtained with the animals used in Part 2 of this study. These animals received laboratory chow with a slightly higher fluoride content than those of Part 1 and continued, up to time of sacrifice, to ingest water containing 50 ppm fluoride. The plasma fluoride contents of the 2 groups of animals also were not different. These results support our observation(1) showing that plasma fluoride content of humans receiving 0.10 to 2.5 ppm fluoride in their communal water are effi-

TABLE II. Fluoride Content of Plasma and Bones of Rats Maintained on High Fluoride Intake for 72 Days Prior to Starvation.
(Study III)

Days starved	Body wt at sacrifice (g ± S.E.)	Age in days	Plasma No.	Plasma F (ppm ± S.E.)	Humeri* No.	Humeri* Dry wt (g ± S.E.)	Humeri* Ash wt (g ± S.E.)	Humeri* F dry wt (% ± S.E.)	Humeri* F ash wt (% ± S.E.)
0	373 ± 6	120	17†	.34 ± .024	13	.5473 ±.0076	.3844 ±.0049	.23 ±.006	.33 ±.008
3	357 ± 7	123	8‡	.28 ± .026	12	.5718 ±.0150	.3828 ±.0092	.22 ±.005	.32 ±.006
7	328 ± 4	127	14	.37 ± .023	14	.5607 ±.0086	.3913 ±.0066	.20 ±.003	.29 ±.005

* Dry weights and ash weights are for 2 humeri.
† Plasma values for fluoride include 4 animals given the same treatment but not used for analyses of humeri.
‡ Inadequate plasma sample obtained from 4 animals.

ciently adjusted by homeostatic mechanisms which operate over a considerable variation of environmental exposure to fluoride.

The observations made with the animals in Study II receiving parathyroid extract also demonstrate the efficiency of regulation of body fluid fluoride concentration. Each of the 12 animals in the experimental group exhibited elevated plasma calcium levels, whereas no increase in plasma calcium was noted in the control group. Plasma obtained from the 20 animals prior to injection of parathyroid extract or saline had a mean calcium content of 9.8 ± 0.18 (S.E.) mg%. At time of sacrifice the 8 control animals had a mean plasma calcium content of 9.7 ± 0.29 (S.E.) mg% and the 12 animals which received the injections of parathyroid extract had a mean plasma calcium content of 12.6 ± 0.33 (S.E.) mg%.

A mean plasma fluoride concentration of 0.24 ± 0.0024 (S.E.) ppm was obtained with the experimental animals and 0.27 ± 0.0013 (S.E.) ppm with the control animals. These plasma fluoride contents are not different from those of rats used in the other studies. Even though the turnover of the skeleton was increased by the parathyroid hormone as evidenced by the elevated plasma calcium, it appears that fluoride mobilized from the skeleton as a result of this process was either rapidly absorbed at sites of new bone formation or was excreted into the urine.

Table II summarizes the findings and analytical results obtained from an investigation of the effect of starvation on fluoride content of tissues of rats raised on high fluoride diet. There was a decline, statistically significant ($P<0.005$) at the 7-day period, in the humeral fluoride contents on both the dry and ash basis without any apparent decrease in ash or fresh bone weights. There was, however, no significant difference in serum fluoride levels of animals sacrificed as controls (at zero day) or after 3 or 7 days of starvation. These results indicate that the fluoride content of the body fluids can be supported by the skeletal pool when the intake of fluoride is markedly reduced.

The plasma fluoride contents exhibited a considerable variation as is indicated by the values for standard error of the means. Nevertheless, the results are sufficient to demonstrate that the mean plasma fluoride contents did not vary outside of control values under conditions in which the amount of fluoride ingested daily by the rats was markedly increased or reduced to zero. These observations indicate the effects of operation of mechanisms which prevent sustained large excursions of plasma fluoride concentrations. Two organs involved in regulation of plasma fluoride concentration have been identified. As shown here and in other studies, the skeleton rapidly accumulates fluoride(5) and in relation to the fluoride intake(6). The kidneys participate effectively in control of plas-

ma fluoride concentration since the renal clearance of fluoride by dogs(7) and the human(5) was always much higher than clearance.

Summary. The plasma fluoride concentration in rats was not altered by widely varying fluoride intakes which were sufficiently different to produce a 3-fold variation in skeletal fluoride load. Parathyroid extract administered to animals resulted in a significant increase in plasma calcium without altering plasma fluoride concentration. Food deprivation of rats for a period of 7 days, with access to distilled water, saline solution and sugar did not significantly alter the mean serum fluoride level of the experimental animals. The results indicate that the fluoride content of body fluids is adjusted during periods of high fluoride intake or long periods of food deprivation by the skeleton. Additional studies are required to delineate the role of the kidney in fluoride homeostasis in conditions of parathyroid extract mobilization of the skeleton and in those of deprivation of fluoride intake.

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Effect of a Minimal Fluoride Diet on Rats.*† (29669)

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Several workers have attempted to demonstrate an essential function for fluoride. Sharpless and McCollum(1), Phillips, Hart and Bohstedt(2), Evans and Phillips(3), Lawrenz(4), Maurer and Day(5) and Muhler(6) were some of the early workers who directly approached this problem. In some cases, the minimal diets employed contained fluoride as judged by carcass fluoride analyses or in others the purified diets employed were inadequate for support of normal reproductive performance. In no case has an indication of an essential function for fluoride been noted.

The only report which does not support the conclusion that fluoride is a non-essential element is that of McClendon and Gershon-Cohen(7), who obtained a marked reduction in growth rate and extensive caries of the molars, due apparently to the lack of dietary fluoride. However, no data were presented on the fluoride content of the tissues of the animals at the beginning or end of the experimental period and the specially prepared diet was not fed in combination with fluoride.

We have undertaken to study the effect of feeding natural foodstuffs of minimal fluoride content on growth and other biochemical processes in the rat.

Materials and methods. A hydroponic technique for culture and production of sorghum and soybean of minimal fluoride content was employed in a greenhouse constructed for this purpose. The building was equipped with 4 evaporative coolers and radi-

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