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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† Arizona Agri. Exp. Station Journal Article #902.

‡ Present addreess: Ray Ewing Co., Division of Hoffmann-LaRoche, Inc., Pasadena, Calif.

ant steam heat for temperature control between 65-95°F. The coolers were equipped with microfilters to minimize dust.

Silica sand (ASTM C-190)§ was used as the potting medium after treatment with 1 N hydrochloric acid, and by heating at 800°C for 8 hours in ½ inch layers to insure removal of fluorides when needed.

The water system consisted of a double distillation followed by passage through a deionizer and a millipore filter. The water purity was checked constantly with a resistance meter and averaged 15-16 million ohms resistance.

All chemicals employed were checked for fluoride and recrystallized where necessary until all salts used contained less than 0.01 ppm fluoride. The nutrient solution employed contained <0.001 ppm fluoride.

The composition of the nutrient solution used in the culture of the plants was essentially the same as that of Kurtz and Maier (8). The pH was adjusted to 6.0 for soybean and to 8.0 for grain sorghum plant culture.

The original seeds of sorghum (*Sorghum vulgare* variety DD38) and soybean (*Glycine max* variety Lee) contained approximately 4 ppm fluoride. The fluoride content of the first crop of seeds produced under the greenhouse conditions was 0.014 ppm. Part of these seeds have been used for replanting. Amino acid analyses of the soybean and sorghum were found to correspond in concentration to those grown under field conditions.

The soybeans were washed, ground and processed by autoclaving at 12% moisture for a period of 40 minutes under 15 lb. Previous studies have shown treatment to be adequate for the destruction of inhibitors present in raw soybeans. Fluoride analyses were accomplished by distillation with perchloric acid according to the method of Willard and Winter(9) using the apparatus described by Huckaday *et al*(10) and the distillates obtained by this procedure were treated by the method of Megregian(11).

All components of the basal diet were analyzed for fluoride prior to use and recrystallized where necessary. The vitamins used were in the crystalline form or purest form obtainable and in most cases needed no further treatment. The calcium source required special treatment in order to render it free of fluoride. Calcium carbonate (12.3 ppm F) was treated with excess of perchloric acid and the resulting solution heated (160°C) for 6 hours; the cooled solution was then treated with sodium hydroxide and the precipitated calcium hydroxide filtered off and dried.

The dietary composition employed was: 55% full-fat soybeans, 23.25% grain sorghum, 9.8% sucrose, 1.5% vitamin mix, 8% salts, 2% corn oil. The diet was supplemented with vitamins and minerals adequately to satisfy the requirements for these micronutrients. Since the procedures employed might also be expected to produce a low bromide level, 5 ppm of bromide as (NaBr) was added.

The 3 dietary treatments employed were as follows: (1) Minimal fluoride diet using greenhouse-grown sorghum and soybeans (fluoride content less than 0.005 ppm); (2) Minimal fluoride (as 1) plus 2.0 ppm F (same as diet one, but with addition of 2.0 ppm F as sodium fluoride) and; (3) Normal control using field-grown sorghum and soybeans, (fluoride content 2.67 ppm).

Nine weanling (20 days of age) Sprague-Dawley rats were used per treatment (3 males and 6 females). The experimental period was 10 weeks. The rats were distributed among the dietary treatments according to litter, sex and weight. The weanling rats were housed in individual stainless steel cages in a temperature controlled environment equipped with microfilters to minimize dust. The average carcass fluoride analysis of the weanling rats was 0.72 ppm F (fresh weight bases).

At the end of the growth period, all rats were sacrificed by anesthetization with ether and blood samples collected. Livers were quickly removed, rinsed with saline solution and homogenized in Krebs-Ringer-phosphate solution. The homogenates (20%) were quick-frozen and stored at 0°C until the

§ Ottawa Silica Co., Ottawa, Ill.

TABLE I. Effect of Minimal Fluoride Diet on Rat Growth, Feed Conversion and Fluoride Deposition in Bones.

Dietary treatment	Avg starting wt	Avg final wt	Feed conversion (g feed/g gain)	% Nitrogen digestability	Avg ppm fluoride in tibiae at 91 days of age
Minimal fluoride (<.005 ppm F)	36.3	267.8	5.27	95.9	2.92 (5)*
Minimal fluoride plus 2.0 ppm F	36.2	251.4	5.14	96.1	34.63 (4)
Normal control (2.67 ppm F)	36.1	277.7	4.87	95.4	12.54 (4)

* No. of determinations.

enzyme activities could be measured. The blood was kept in an ice bath for one hour and then centrifuged. Serum enzyme activities were measured the same day. Serum alkaline and acid phosphatases were measured with p-nitrophenylphosphate as the substrate at pH 9.3 and 4.8 respectively, according to the method of Bessey *et al*(12) and Andersch and Szczpinski(13). Glutamic-oxalacetic and glutamic-pyruvic transaminases were run by the Sigma-Frankel method.‖ Lactic dehydrogenase was determined by the Berger-Broida method.¶ Isocitric dehydrogenase activities were determined by the method of Taylor and Friedman(14). The method of Martland and Robinson(16) was used to prepare the humerus bone for alkaline phosphatase analysis.

Results and discussion. The feeding of a low fluoride diet, containing less than 0.005 ppm by analysis, to weanling rats for a period of 10 weeks failed to produce a significant change in final body weights or in weight gain during the experimental period (Table I). Feed conversions, calculated from feed consumption data and weight gains, were not significantly different when tested by Duncan's Multiple Range Test(17). Utilization of dietary nitrogen was in the range of 95.4-96.1% and was not affected by the presence of either 2.0 ppm added fluoride (as NaF) or the 2.67 ppm measurable fluoride present in the field-grown dietary ingredients (Table I).

Marked differences were noted in the fluoride content of tibiae from the animals fed the respective diets (Table I). The animals fed the minimal fluoride diet showed an average fluoride content of 2.92 ppm in the tibiae. Muhler(6) has reported that the feeding of a diet which contained not more than 0.1 ppm fluoride resulted in a maximum storage of 23-29 ppm in the femurs of rats at 80 days of age. These data suggest that the available fluoride content of the minimal fluoride diet was quite low. The feeding of 2 ppm fluoride as NaF resulted in a level of 34.6 ppm fluoride in the tibiae at 91 days of age. The natural fluoride which was present in the field-grown soybeans and grain sorghum was apparently much less available to the rat than NaF (Table I).

Measurements of serum alkaline and acid phosphatase levels indicated no significant differences among the respective dietary treatments in either of these enzymes (Table II). Maplesden *et al*(15) were unable to demonstrate a change in the serum alkaline phosphatase activity with the feeding of high fluoride diets. Maurer and Day(5) were also unable to obtain a significant difference in serum alkaline phosphatase activity attributable to the low fluoride diet fed.

Serum and liver levels of the glutamic-oxalacetic and glutamic-pyruvic transaminases were estimated and no statistically significant differences in the activities of these two enzyme systems from either source could be attributed to the respective dietary treatments employed (Tables II and III). Cohen (18) reported that the serum activities of the glutamic-oxalacetic system were in the order of 4 times those obtained for the glutamic-pyruvic system.

‖ Sigma Technical Bulletin, No. 505, 1961.

¶ Sigma Technical Bulletin, No. 500, 1960.

TABLE II. Effect of Dietary Fluoride Level on Serum Enzymes.

Dietary treatment	Phosphatases		Transaminases		Dehydrogenases	
	Alkaline*	Acid*	GOT†	GPT†	Lactic‡	Isocitric§
Minimal fluoride (<.005 ppm F)	7.53	1.54	132.2	36.1	891	591[a]‖
Minimal fluoride + 2 ppm F as NaF	8.19	1.61	152.8	38.0	793	384[b]
Normal control (2.67 ppm F)	7.75	1.35	126.1	30.9	764	404[b]

* Expressed as μmoles of p-nitrophenol liberated/hr/ml of serum.
† Sigma-Frankel units/ml serum/hr.
‡ Berger-Broida units/ml of serum.
§ mμmoles of alpha-ketoglutarate formed/ml of serum/hr.
‖ Means having different letter superscripts were significantly different at 0.05 level of probability.

Lactic dehydrogenase levels of serum or liver were likewise not statistically altered with the feeding of the minimal fluoride diet. These data would indicate that there was no impairment in heart, liver or kidney due to the low fluoride conditions imposed (Tables II, III).

Significant elevations in isocitric dehydrogenase activities of the serum from animals fed the minimal fluoride diet were observed (Table II). A concomitant decrease was observed in the isocitric dehydrogenase activity of livers from rats fed the minimal fluoride diet (Table IV). *In vitro* addition of fluoride (10^{-4} M) to the isocitric dehydrogenase assay system produced a significant inhibition in activities with liver homogenates from each of the dietary treatments.

TABLE III. Dietary Fluoride Content and Liver Enzyme Activity.

Dietary treatment	Lactic dehydrogenase*	Transaminases†	
		Glutamic-oxalacetic	Glutamic-pyruvic
Minimal fluoride (<.005 ppm F)	2573	25.3	37.7
Minimal fluoride plus 2.0 ppm F	2922	33.5	38.7
Normal control (2.67 ppm F)	2613	40.6	32.9

* Berger-Broida units/mg tissue.
† Sigma-Frankel units/mg tissue/hr.

TABLE IV. Effect of *in vitro* Fluoride Concentration on Isocitric Dehydrogenase Activity in Liver Homogenates.*†

Dietary treatment	Addition of fluoride *in vitro*	
	None	10^{-4} M
Minimal fluoride (<.005 ppm F)	383[e]	274[a]
Minimal fluoride + 2.0 ppm F	436[f]	297[ab]
Normal control (2.67 ppm F)	436[f]	330[bcd]

* mμmoles of alpha-ketoglutarate formed/mg tissue/20 min.
† Means having different letter superscripts were significantly different at 0.05 level of probability.

Summary. The feeding of a minimal fluoride (<0.005 ppm F) diet for a 10-week period to weanling rats demonstrated significantly lower levels of bone fluoride compared to the control group receiving the minimal diet plus 2 ppm fluorine. Serum and liver enzymes activities were determined, and the only significant differences noted were an increase in serum isocitric dehydrogenase and a decrease in activity of this enzyme in the liver.

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Relationship of Burned Area in Rats to Incidence of the Anti-Globulin Reaction (Coombs' Test).* (29670)

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In recent experiments in our laboratory(1) positive anti-globulin reactions (Coombs' tests) were observed in 51 of 56 rats 4 days after they had received full thickness 37% body surface burns. The positive test was observed to persist in some animals and disappear in others by the end of the 3 week experimental period. No correlation was found between the several parameters studied at regular postburn intervals (Coombs' tests, hematocrit, reticulocyte values, serum protein levels) and death or survival. Thus it seemed desirable to determine whether: (a) the positive Coombs' test is directly related to the percent body surface burned, (b) a decrease in percent of burn area would effect a change in the postburn time at which a positive Coombs' test could be detected, (c) the other parameters studied including survival would be significantly altered if animals were subjected to reduced area burns, and (d) such changes could be correlated with changes in the incidence of the Coombs' test. In this report 3 groups of animals receiving 26%, 20% and 14% burns, respectively, have been studied in relation to these questions.

Materials and methods. Male Sprague-Dawley rats (190-210 g) were subjected to back burns in water at 90°C for 40 seconds under deep ether anesthesia. Each animal except the normal controls received intraperitoneally in 3 injections immediately postburn (p.b.) and at 5 and 10 hours (p.b.) 2% of the body weight of a 1/6 M sodium lactate solution and 7% of the body weight of a 1.4% solution of NaCl. The average percent body surface burns were as follows: group A (18 rats) 25.5%, SD=1.72; group B (13 rats) 20.2%, SD=0.99; and group C (14 rats) 14%, SD=2.5. Six non-burned injected controls and 4 non-burned, non-injected controls were also studied. All blood determinations were performed on lateral tail vein blood. Hematocrits were done by the standard microhematocrit method and reticulocyte values were determined by the method of Brecher(2). Serum protein electrophoretic studies were performed using the Spinco Model R electrophoretic system with Veronal buffer pH 8.6, ionic strength 0.075. Anti-globulin tests were performed on thrice washed erythrocytes of each animal preburn and postburn at 4, 7, 10, 14, 21, 28 and 35 days using rabbit anti-rat gamma globulin. Sixty blood samples from animals in groups A and B and 70 samples from group C animals were streaked on blood agar plates and incubated at 37°C. At autopsy the spleens and thymi were excised and weighed to the nearest milligram.

Results. Survival. Postburn (p.b.) survival numbers were not significantly different

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