ate use of a defined selecting environment. The difficulty in isolating an inositol-independent culture through deliberate selection using an inositol-free medium tends to support this hypothesis. It is conceivable that unsuspected changes in the nutritional requirement of a cell culture could occur and thereby contribute toward further conflicting results. The role played by the serum protein component of the minimal growth medium is not known. A large number of substances, biologically active in low concentration are known to be present in dialyzed serum. Some of these substances are the enzymes, vitamins, hormones and trace metals. Thus, medium containing serum proteins should be considered non-chemically defined. Sera from various sources are known to influence cell propagation in a number of ways(4,10). The failure to demonstrate inositol as an essential for fresh explants of human amnion cell under the described experimental condition is also of interest. It suggests that caution is desirable in the generalization of data obtained through study with stable laboratory strains of human cells.

Summary. Under the described experimental conditions, qualitative differences in the inositol requirement of the following human cells have been found: 1) between several wild strains of HeLa cell obtained from several laboratories; 2) between a wild strain of conjunctival cell and a subculture propagating in a low glucose medium, and 3) between a stable laboratory strain and fresh explants of human amnion cells. The significance of these findings was discussed.

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Deposition of Fluoride in Soft Tissues Following Skeletal Saturation.* (24261)

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The significant reductions in dental caries as a result of communal fluoridation can no longer be questioned. However, the biological effect of fluoride ingested at the level used to fortify fluoride-deficient water on tissues other than the dental structures has received little attention because: (1) determination of these micro-quantities of fluoride requires specialized analytical technics; and, (2) the almost immeasurably small quantities of fluoride found in tissues other than the teeth and skeleton had until recently been considered negligible. In addition, fluoride ingested at the level of 1 μg/ml is thought to be detoxified rapidly by excretion in the urine and incorporation into the skeleton. Recently, however, Muhler(1) and Buttner and Muhler(2) have shown that certain dietary factors (ascorbic acid and fats) increase fluoride retention both in the skeleton and in certain specialized soft tissues. It is important, then, to

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determine both distribution of fluoride in the skeleton and in the soft tissues when rats are given fluoride over prolonged periods of time, and to determine if fluoride is retained in soft tissues at a greater rate following skeletal saturation. This study was designed to investigate distribution of fluoride in the heart, liver, kidney, femur, sternum, and carcass, when rats are given amounts of fluoride considered optimal for children using water from communal fluoridation sources.

Materials and methods. A total of 93 weanling Sprague-Dawley strain rats were divided according to weight into 2 groups. Group I (52 animals) received exactly 1.0 mg F (as NaF) daily by stomach tube. Group II (41 animals) served as control and were not stomach-tubed. All animals were housed in large wire-screen cages, and were given fluorine-free drinking water and a low-fluoride (F = 0.5 μg/g) stock corn diet *ad libitum*.

At intervals of 15 days, 6 animals from each group were sacrificed. Both kidneys, the liver, heart, femur, and sternum were removed from each animal and analysed for fluorine by methods previously described(3). In preparation for analysis the tissues were placed in small silica dishes and dried in vacuum dessicators to constant weight. An accurately weighed 0.30 g sample of low-fluoride CaO was then added to each dish, the contents moistened with fluorine-free water, dried under infared lamps, and ashed in a muffle furnace for 6 hours at 600°C. Femurs were freed of soft tissue, charred under infared lamps, and similarly ashed at 600° for 6 hours.

After the desired tissues had been removed, the skinned carcasses were placed in silica dishes, charred over Meker burners, and ashed for 6 hours. For analysis, a homogeneous, powdered aliquot of femur, sternum, or carcass ash was placed directly in the perchloric acid distillation flask.

Results. Fig. 1 presents the results of fluoride analysis of the various tissues studied.‡ All of the soft tissues contained less than 3 μg total fluoride, even after ingestion of over 90,000 μg of fluoride. More significant is the fact that there were no trends suggesting that fluoride content would increase measurably with ingestion of additional fluoride.

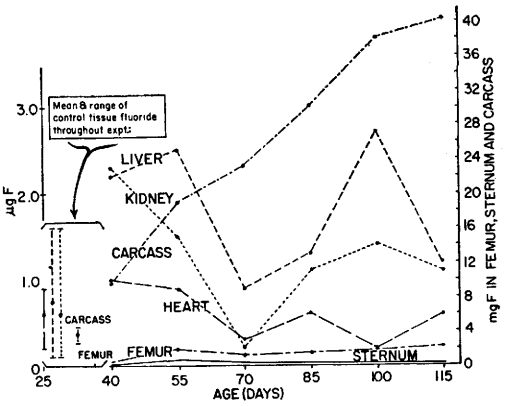


FIG. 1. Fluoride content of tissues of animals given 0 or 1 mg F daily and determined at 15-day intervals. (Each point represents mean of 6 animals.)

Fluoride content of the carcass increased markedly throughout the experiment, reaching a value of over 40 mg after 90 days of fluoride ingestion. The proportion retained, expressed as % of fluoride given, decreased from a maximum of 65% after 15 days of fluoride ingestion to an average 45% retention for the entire 90-day period.

The method of preparation of tissues for analysis was adopted after an extensive investigation was made of several procedures designed to (1 provide adequate fixation of fluoride during ashing; and (2 give a reproducible basis for calculation of fluoride concentration. Methods included (a) vacuum drying, (b) oven-drying, (c) homogenizing, and (d) freezing, all of which were followed by addition of calcium oxide and ashing. Procedure (a), used in this study, was found to be the most accurate and convenient for these purposes, as well as for satisfying the other requirements of the analytical procedure.

Some authorities(4,5) have advocated an elaboration of the general fluorine analysis procedure when undertaking analysis of tissues or blood which contain significant amounts of iron and other interfering substances. A standardization curve obtained on blood samples by adding known amounts of fluoride to small quantities of blood and sub-

‡ The numerical figures for fluoride content will be sent to anyone interested upon request.

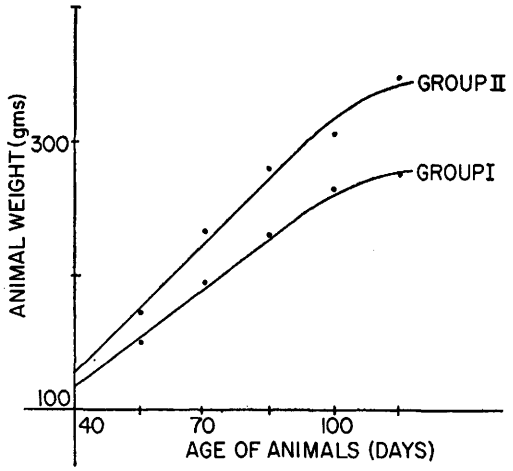


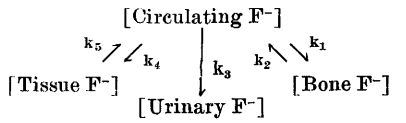
FIG. 2. Comparison of wt gain of animals given 1 mg F daily (Group I) with that of control animals (Group II).

jecting the ashed samples to analysis gave a curve which did not differ significantly from the curve obtained for analysis of pure fluoride solutions. The difference between the two curves was well within the experimental error of the general analysis curve for these amounts of fluoride. These results suggest that if extreme care is taken in determination of tissues with a low fluoride content, the standard analysis procedure can be used, particularly if other variables in the experiment are well-controlled.

Comparison of growth rates of the 2 groups of animals used in this study is presented in Fig. 2. The effect of fluoride at this concentration on weight gain is unmistakable. Decrease in weight gain in the fluoride group, however, has occurred with no significant increase in soft tissue fluoride content.

Discussion. Retention of fluoride in the carcass decreased noticeably after the period of rapid skeletal growth, at about 70 days of age, indicating that one factor in skeletal retention of fluoride is rapid turnover of the skeletal tissues during growth. Savchuck and Armstrong(6), and Miller and Phillips(7), reported that there was a greater uptake of fluoride by the femurs of young animals than with mature rats. Accretion of fluoride during rapid skeletal growth results from two mechanisms: (a) incorporation of fluoride into the tissues as they grow and increase in size; and (b) greater metabolic activity of the constituents of newly-formed bone, with accompanying greater deposition of fluoride. Perhaps a third factor, *i.e.*, absence of appreciable amounts of previously deposited fluoride, should also be considered.

Closely allied to this age-growth factor in its influence on fluoride retention is the intrinsic metabolic turnover rate of different tissues, as illustrated in the results of femur and sternum analysis. Concentration of fluoride in the femur was always greater than in the sternum, when based on ash weight. Fluoride deposition in bone of the same age may be dependent on vascularity of the bone, with consequent greater retention in bones having the higher blood supply. It has been assumed in the past that if soft tissues were to retain fluoride, this would begin to occur as the primary deposition site, the skeleton, approached fluoride saturation with the result that fluoride would not be as readily retained in the skeleton and would be deposited in the soft tissues. During the last 15 days of this 90 day study period only 14% of the ingested fluoride was retained by the skeleton compared to 65% retained initially. This suggests that saturation was being approached asymtotically. The final tissue fluoride values under these conditions were, however, well within the range of controls not receiving fluorine. Evidently fluoride not retained in the skeletal tissues was excreted rather than being secondarily deposited in vital tissues. This suggests that in the normal animal organism a situation favorable to the animal exists with regard to relative magnitudes of the equilibrium constants in the following equilibria:



Thus, when the organism has had little previous exposure to fluoride, resulting in a small Bone F^-, $k_1 > k_3 >> k_2$. However, as saturation of the skeleton is approached, k_2 may assume some magnitude, permitting a trend toward higher levels of circulating plasma fluoride. From these studies it would appear

that $k_3 > k_4$, so that labile fluoride is rapidly excreted. Nothing is known at present of the relative magnitudes of k_4 and k_5 or of the toxic consequences of a rapid turnover of fluoride through tissues in which only a trace amount may be found at any one time. Conceivably these equilibria could be altered by pathological conditions, particularly by renal disorders, which would decrease the constant k_3 in relation to k_4, or by endocrine dysfunction, causing increased turnover of skeletal constituents, an increase in k_2, with corresponding rise in level of circulating plasma fluoride. Investigation of these dynamic aspects of fluoride metabolism clearly is needed.

Summary. Administration of 1 mg F daily for 90 days to weanling rats failed to demonstrate any increase in fluoride content or concentration of heart, liver, or kidney, even as fluoride saturation of the skeleton was approached. Fluoride content of the carcass, femur, and sternum increased markedly with fluoride ingestion but rate of retention decreased throughout the experiment, particularly after attainment of skeletal maturity. Although no increase in soft tissue fluoride was demonstrated, the toxic effect of this level of fluoride (1 mg per rat per day) was demonstrated by the decreased weight gain of the fluoride animals when compared to the control group.

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Serum Gamma Globulin and *Trypanosoma cruzi* Agglutinin in Embryonic, "Normal" and Germ-Free Chickens.* (24262)

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The transmission of maternal serum proteins and antibodies to chick embryos *via* the yolk has been established by various investigators(1-3). The serum proteins of the developing embryos show progressive changes with age; Marschall and Deutsch(2) found low levels of gamma globulin in the serum of the 11-day embryo; later this serum fraction appeared in increasing amounts until after hatching. Brandly *et al.*(1) reported the absence of Newcastle virus antibody in the sera of 11-day embryos; they showed significant amounts of this antibody in the sera of 15-day-old, and high titers in the sera of 18-day-old embryos. Similar developmental patterns of *Trypanosoma cruzi* agglutinin in the sera of developing chick embryos was demonstrated by Borsos and Warren(4). It was also shown that germ-free chicks 86 days of age or older were devoid of detectable *T. cruzi* antibody. In contrast, all "normal" adult controls contained this factor in high titer. Recently, it was reported that the sera of 8 to 17-week-old germ-free chickens contained less gamma globulin than the "normal" controls; however, no difference was found between "normal" and germfree birds 4 to 8 weeks of age (5).

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