similar increase in "take" and growth of tumor occurs which likewise may be related to hepatic trauma. From these observations it would seem unlikely that the susceptibility of the liver to metastases, when compared with other organs, might be due, as has been proposed, to its relatively poor oxygen supply.

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Effect of a Low Calcium Diet on Bone Crystallinity and Skeletal Uptake of Ca^{45} in Rats.* (27970)

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It has been suggested that primary osteoporosis is due to increased bone resorption which produces a negative calcium balance (1-4). Radiotracer kinetic studies in humans indicate that in this condition the rate of bone formation is not impaired(5-7). In rats made osteoporotic through administration of a calcium deficient diet containing an adequate amount of vitamin D, the skeletal uptake of injected stable strontium was found to be increased and it was concluded that bone formation was abnormally rapid (8).

Several studies have shown that the calcium balance in osteoporosis can be improved by increasing calcium intake(2,9,10). A similar effect was observed following administration of sodium fluoride to osteoporotic patients(11,12). There is also an indication that the prolonged ingestion of 8 ppm fluoride in drinking water may have a beneficial effect in counteracting osteoporotic changes in humans(13). Since it was shown that the crystallinity of bone apatite improves with an increase of fluoride content(14,15,16), it is possible that the kinetics of bone resorption is affected by fluoride administration.

The purpose of the present study was to investigate the effect of a low calcium diet on calcium turnover in relation to bone crystallinity. The effect of fluoride on the bone changes produced by the calcium deficient diet was also examined.

Methods. Male Sprague-Dawley rats were assigned at 35 days of age to 2 groups and offered *ad libitum* a low fluoride (<1 ppm) prepared diet(17,18) and drinking water containing either 0 ppm F (Group I) or 50 ppm F (Group II) for 320 days. All were then given 0.5 ml of a solution containing 20 mc of Ca^{45} and 1.5 μg of Ca by intraperitoneal injection and killed after one hour. At sacrifice approximately 5.0 ml of blood were obtained by cardiac puncture from each rat and the plasma was analyzed for radiocalcium by methods previously described(19). One tibia and one femur were removed.

Each tibia was arbitrarily sectioned to divide the ends from the cylindrical portion of the shaft, the ends were pooled and with the

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TABLE I. Fluoride, Calcium and Phosphorus Content of Rat Femur and Comparison of X-ray Diffraction Line Broadening in Relation to Fluoride and Calcium Intake.

Group*	Ca in diet, %	F in H_2O, ppm	Ash,† %	F,† % ash	Ca,† % ash	P,† % ash	Ca/P†	β_t‡	β_{002}§
I	.1	0	65.02 ± .44	.04 ±.02	37.83 ±.23	17.69 ±.08	2.14 ±.02	1.16	.463
II	.1	50	64.53 ±1.44	1.42 ±.12	37.91 ±.29	17.48 ±.23	2.17 ±.04	.90	.495
III	.8	0	64.58 ±1.50	.05 ±.03	38.81 ±.06	17.75 ±.16	2.19 ±.02	1.13	.465
IV	.8	50	64.03 ± .61	1.12 ±.08	38.49 ±.11	17.51 ±.16	2.20 ±.02	.87	.442

* Group I: 9 animals, II: 8 animals, III: 3 animals, IV: 6 animals.
† Mean ± standard error calculated for each sample.
‡ Average values calculated from individual template β values; avg width at half maximum of the 211, 112, and 202 reflections. Precision of these values is calculated to be ± 5%.
§ The width at half-maximum of the 002 diffraction reflection corrected for instrument broadening, expressed in degrees 2 θ. Precision of these values is calculated to be ± 5%.

shaft, defatted, ashed, and analyzed for Ca^{45} (19). The femurs were defatted and powdered to pass a 200 mesh sieve; after X-ray diffraction analysis the powder samples were ashed and analyzed for F(20,21), Ca(22) and P(23).

X-ray diffraction patterns (copper K-alpha radiation) were obtained from the individual powder samples of the whole femur using a commercial geiger-counter diffractometer equipped with a high resolution slit system. The degree of resolution of the 4 principal reflections of the apatite X-ray patterns (Miller indices 211, 112, 300, 202) was used as a measure of the "crystallinity" of each sample.

To give the diffraction patterns "crystallinity" ratings, a series of 10 templates were 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 2 Θ of each of the 4 Gaussian maxima summed to form the template. Each X-ray pattern was assigned a "crystallinity" value β_t, by matching it with one of the templates. As the value of β_t 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(24) and designated β_{002}.

The experimental conditions in this study and in the previous study(11) were identical except for the different calcium content of the diet; that is, 0.1% and 0.8%, respectively.§ The femurs of the rats which had received the high calcium diet were removed and analyzed as described above.

Results and discussion. The F content of the femurs of the fluoride treated rats fed a low calcium diet was slightly higher than of those who received a high calcium diet (Table I(25). Neither fluoride nor calcium intake affected Ca and P content of the bone.

Bone crystallinity increased with the fluoride intake as evidenced by a decrease in β_t value (Table I). There was no significant change in β_{002} values indicating that fluoride treatment did not affect the hydroxyapatite crystals in the direction of the *c* axis. These findings are in agreement with previous reports(11). The degree of improvement in bone crystallinity attributable to fluoride was the same for the high and low calcium groups.

The level of calcium intake did not affect bone crystallinity, and no significant changes of β_t and β_{002} values were observed (Table I). This finding offers further support for the concept that in osteoporosis it is the total bone mass that is affected and not the properties of the individual crystals in the mineral phase.

The calculated ash weight of the whole tibia was significantly lower in the rats fed the calcium deficient diet (Table II). This decrease is mainly attributable to the reduced ash weight of the shafts and amounts to 19.4% and 18.8% for the non-fluoride and fluoride treated animals, respectively. There is some suggestion that the tibia ash weight was increased slightly by fluoride administration. Fluoride treatment on the other

§ A trace amount of carrier-free Sr^{89} was injected with the Ca^{45} in the first experiment.

TABLE II. Uptake of Ca^{45} Ash Weights and Contents of Rat Bone in Relation to Changes Produced by Different Levels of Calcium and Fluoride Intake.

Group	Ca in diet, %	F in H_2O, ppm	Ash wt of tibia ends,* g	Ash,* %	Ash wt of tibia shaft,* g	Ash,* %	Ash wt whole tibia,* g	Ca^{45}; % of dose/mg of ash*	
								Ends of tibia	Shafts of tibia
I	.1	0	.150 ±.005	53.96 ± .81	.203 ±.009	66.85 ± .70	.353 ±.012	.061 ±.003	.024 ±.001
II	.1	50	.172 ±.003	56.85 ± .57	.180 ±.007	65.05 ± .57	.373 ±.011	.048 ±.004	.021 ±.001
III	.8	0	.140 ±.009	57.57 ±1.06	.298 ±.016	70.71 ±1.68	.438 ±.023	.004 ±.004	.001 ±.0001
IV	.8	50	.145 ±.018	58.05 ± .99	.317 ±.011	69.53 ± .72	.462 ±.015	.003 ±.0009	.0009±.0005

* Mean ± standard error calculated for each sample.

hand had no effect on bone ash concentration except in the case of the tibia ends where the percent of ash was increased in the rats fed the low calcium diet.

The calcium content of the diet had no effect on the ash content of the whole femur (Table I). This was not the case with the tibia where the percent of ash in both the ends and shafts was higher in rats which had received the high calcium diet (Table II). The reason for this different response is not apparent.

The most striking finding was the markedly different skeletal uptake of Ca^{45} on the 2 dietary calcium levels. Both the tibia ends and shafts of rats fed the low calcium diet contained one hour after injection significantly more radiocalcium than their corresponding controls (Table II). In the case of the tibia ends there was a 15-fold increase, and for the shaft approximately a 25-fold increase. While concentration of Ca^{45} was consistently lower in the bone samples of the fluoride treated animals, this decrease was significant ($p<0.05$) only in the case of the tibia ends of rats receiving the low calcium diet.

These results clearly show that the metabolism of calcium at the skeletal level is profoundly altered by long term ingestion of a calcium deficient diet. That differences in absorption are not responsible for the dissimilar uptake of radiocalcium is apparent from the plasma radiocalcium values. Expressed as percent of injected dose per milliliter of plasma these were: Group I, 0.36 ± 0.03 (S.E.); Group II, 0.35 ± 0.01; Group III, 0.47 ± 0.02; Group IV, 0.37 ± 0.02. It is also improbable that the trace amount of carrier calcium which was injected would affect the radiocalcium uptake.

It has been reported that in contrast to existing concepts of the nature of human osteoporosis, the bone formation rate is normal (7). Additional evidence for this has also been obtained in a study of experimentally induced osteoporosis where the skeletal uptake of injected stable strontium was found to be increased in rats fed a calcium deficient diet. Indeed it was concluded that the rate of bone formation was increased(8).

It is generally agreed that bone radiocalcium uptake is the result of both incorporation in newly formed crystals (including recrystallization) and surface exchange on existing crystals. The proportional contributions of each process cannot be determined with certainty from the present data. However, it seems unlikely that the 20-fold increase in Ca^{45} incorporation in calcium deficient rats can be attributed to bone accretion, especially since the rate of bone formation at this age is probably low. Moreover, in one hour it would be expected that exchange rather than new crystal formation would play a dominant role.

It is pertinent that the X-ray diffraction measurements show no difference in the crystal size and/or perfection of the bone apatites derived from the 2 calcium levels. It thus appears that the increased uptake of Ca^{45} in bone of rats fed a low calcium diet is not due to changes in crystallinity. Vascularity and the permeability of the bone apatite may also affect the rate of exchange.

It has been postulated that the apatite phase in hard tissue is some form of calcium deficient hydroxyapatite where calcium ions have been shown to be missing randomly from certain structural positions(26,27). Subsequent work has shown that synthetic hydroxyapatite and dental enamel take up less Ca^{45} from solution after raising their Ca/P ratios by calcium acetate treatment(28). It is possible that the bone apatite produced by a low calcium diet is more calcium deficient than the normal bone apatite. In support of this is the trend toward a somewhat higher Ca/P ratio on the high calcium diet. However more definitive studies are required to determine if such a relationship does exist.

Summary. Chemical and X-ray diffraction analyses were performed on femurs of rats receiving a low (0.1%) and high (0.8%) calcium diet with (50 ppm) and without (0 ppm) fluoride supplementaton in the water. In addition, uptake of Ca^{45} by the ends and shafts of tibia was determined. Bone crystallinity (*i.e.*, crystal size and/or perfection) was not affected by calcium intake, and the improvement in crystal texture produced by fluoride was the same for the high and low calcium groups. Skeletal uptake of radiocalcium was approximately 20 times higher in calcium deficient animals. Fluoride decreased slightly the uptake of radiocalcium.

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Complete Spermatogenesis in Intratesticular Homotransplants of Fetal and Neonatal Testes in the Rat. (27971)

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It is well known that mammalian testes from fetal and neonatal donors, in contrast to the adult organ, are readily transplantable and that they generally are subnormal with respect to hormone production and germ cell proliferation(1-4). If rejection by the host does not occur, subcutaneous, intramuscular and intraperitoneal transplants may persist for long periods but, being subjected to temperatures higher than those of the scrotum, they are equivalent to cryptorchid testes. Completion of spermiogenesis, with the accumulation of mature spermatozoa, has been reported only in grafts residing in sites providing temperatures approximating those of the scrotum: *viz.*, in postnatal testes sutured to the lining of the scrotum(3,5,6) and in neonatal testes persisting in the anterior chamber of the eye(7). Certain transplantation sites, including the testis, brain, anterior eye chamber, and cheek pouch of the hamster, have been designated "immunologically privileged" environments. Some factor seems to operate in these areas which reduces the capacity of the host for immunological response, permitting prolonged survival of the grafts, and it is possible that the peculiar anatomy of the lymphatic drainage from these areas may be an important consideration(8, 9).

Experiments were designed to test the interior of the testis as a transplantation site for gonads, and to compare the onset and incidence of immunological rejection of transplants from the same donor residing simultaneously in kidneys and testes of the recipient.

Material and methods. Randomly bred rats of the Sprague-Dawley strain were obtained from Hormone Assay Laboratories, Chicago. The 494 grafts recovered for this study are part of the controls being used in another project on experimental induction of actively acquired tolerance. A total of 222 *pairs* of testes from 15- and 16-day fetuses, and 34 *pairs* from neonatal animals were transplanted to the kidneys and testes of normal and unilaterally orchiectomized hosts. As a control procedure, one gonad from each donor was inserted beneath the kidney capsule and the contralateral one was placed under the tunica albuginea of the corresponding testis of the same host. The recipients were healthy adult males ranging in weight from 182 to 376 g.

Fetuses were removed from the pregnant mother under light nembutal anesthesia. Gonads were quickly excised and placed in a Ringer-phosphate solution and carefully freed from all adnexa by means of a fine needle-knife. The host testis was delivered through an abdominal incision, and the donor tissue inserted near the cranial pole by means of a cannula introduced through the opposite pole. Care was taken to prevent extensive hemorrhage within the testis or damage to the rete testis and ductuli efferentes. It was found to be unnecessary to suture the tunica albuginea. Transplants were introduced immediately below the tunica since this facilitated recovery. To prevent epididymitis and other infections, a standard procedure of administering 5000 units of penicillin and 5 mg of streptomycin daily for one week following operation was adopted.

Results. Table I indicates that all trans-