ble those of some of the repeating subunits along the periphery of the complex (Figs. 2*C* and 2*D*).

Electron micrographs of the isolated LRT negatively stained with phosphotungstate show arrays of tetrads (Fig. 2*F*). These tetrads have the same average dimension (130 to 150 Å) as, and closely resemble, the central tetrad of the native complex (Figs. 1 and 2). The dense core of the tetrads may be partly attributed to penetration of the phosphotungstate.

On the basis of the biochemical and electron-microscopic data, a tentative model of the pyruvate dehydrogenase complex has been constructed (Fig. 3). The component enzymes of the complex are represented as spheres, the diameters of which were calculated from the molecular weights of the enzymes and an assumed partial specific volume of 0.73 ml/g. These diameters would be 75, 40, and 64 Å for the decarboxylase, LRT subunit, and flavoprotein, respectively. The model consists of 16 molecules of pyruvate decarboxylase (large spheres) and 8 molecules of flavoprotein (medium-size spheres) arranged in two rings one above the other. These two rings surround the lipoic reductase-transacetylase aggregate which comprises 64 subunits (small spheres) arranged into four stacks. We cannot as yet specify the sequence of pyruvate decarboxylase and dihydrolipoic dehydrogenase molecules in the two rings.

The volume of PDC calculated from electron microscope measurements (minimal dimensions 300 by 200 Å), on the assumption that the shape of the particle may be approximated as either an oblate ellipsoid or a right circular cylinder, is about 9.5×10^{-18} cm^3. The anhydrous volume of the complex calculated from hydrodynamic data (*1*) is about 5.8×10^{-18} cm^3. The difference (approximately 64 percent) between the two volumes may be attributed to the open structure of the complex which is indicated by the electron micrographs and represented schematically by the model. This interpretation adequately explains the high frictional ratio (f/f_0) of the complex, which has been calculated to be 1.6.

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Fluoride: Its Effects on Two Parameters of Bone Growth in Organ Culture

Abstract. *Bones of the forepaws of young rats were subjected to varying concentrations of fluoride ions in organ culture. The formation of DNA and protein synthesis were evaluated by measurements of the uptake of tritiated thymidine and C^{14}-labeled proline. Fluoride concentrations as high as 10 to 20 parts per million had no demonstrable effect in vitro on these basic parameters of skeletal growth.*

Interest in the effects of fluoride ions on growth was originally aroused by the observation that deficient growth accompanied skeletal and dental lesions in sheep and cattle exposed to very high concentrations of fluoride (*1*). Further studies were initiated when it was recognized that small amounts of fluoride were of value in preventing human dental caries and it was consequently necessary to evaluate the potential hazards of its use. It has been shown that fluoride concentrations in the drinking water must be maintained constantly above 100 parts per million in order to inhibit growth in domestic and experimental animals (*2*). The decrease in stature and weight seen in animals exposed to large amounts of fluoride for long periods is accompanied by mottling of dental enamel and by signs of early skeletal fluorosis, but no other indications of pathologic changes are usually seen (*3*). Extensive studies of humans exposed to natural water supplies having considerably more than the currently recommended fluoride concentration of 1 ppm have revealed no evidence of effects on growth (*4*).

Data from a study of multiplication of HeLa cells in vitro were recently interpreted as indicating inhibition of growth by fluoride (*5*). In such systems of isolated cells, growth is defined solely by rate of cell division, a more limited definition than that employed in studies of intact animals or of tissues in organ culture. In view of these results, we thought that the concentration of fluoride necessary to cause depression of protein synthesis as well as cell division should be determined in organ culture. Organ culture of rapidly growing bone was chosen for such an investigation because of the obvious relation between skeletal tissues and statural growth, and because in the intact animal fluoride is accumulated by the skeleton.

For each of the three experiments in this series, eight littermate 5-day-old Sprague-Dawley rats were used. Endochondral ossification of the bones of the rat forepaw begins shortly before birth. At 5 days of age the phalanges and metacarpals each consist of a single ossifying area, with cartilaginous ends and an epiphyseal plate at which rapid cartilage proliferation is occurring. Their appearance is that of a miniature long bone.

The first, second, and third proximal phalanges and the second metacarpal from each forepaw were dissected free from soft tissue, explanted into a plasma-thrombin clot on a small Millipore filter, and then placed in tubes for culture at 37°C. The tubes were rotated slowly in a roller drum. Standard 199 medium, supplemented with 10-percent horse serum and with a penicillin-streptomycin mixture, was employed. Tritiated-thymidine, 0.5 μc/ml, was added as a tracer for DNA synthesis; C^{14}-labeled proline, 0.2 μc/ml, was used in the same medium to monitor synthesis of collagen (structural protein) (*6*). Analysis of the standard medium showed that the fluoride concentration was 0.05 to 0.10 ppm (*7*). Sodium fluoride was added to adjust the concentration of fluoride ions to desired levels between one and 500 ppm. The medium was changed every 48 hours.

In experiment A, a replicated Latin square design (4 × 4) was used so that all interactions could be evaluated. This design was slightly modified in experiments B and C to take advantage of the lack of difference in response between the littermates in the first experiment, and to allow the use of eight rather than four different concentrations of fluoride.

After 6 days in culture, the bones were removed from the plasma-thrombin clot, thoroughly washed, air-dried, and then combusted in oxygen (*8*). The carbon dioxide produced by the combustion was absorbed by phenethylamine and the H_2O was dissolved in a scintillation solution containing phenethylamine, ethanol, and toluene (*9*). The H^3 and C^{14} were counted simultaneously in a liquid scintillation counter at 0°C.

In each experiment, additional bones cultured in the presence of each different concentration of fluoride were fixed in formalin and sectioned, and stripping film autoradiographs were made (*10*). Figure 1, an autoradiograph of the epiphyseal growth center of a bone cultured in the presence of 15 ppm fluoride, shows the large number of labeled nuclei and the extracellular deposition of labeled protein which occurred in culture. This autoradiograph is indistinguishable from that of the control which was cultured in a medium with a fluoride concentration of less than 0.1 ppm. In Fig. 1, the swollen cartilage cells are arranged in parallel vertical columns in the normal manner. Disruption of this orientation, which is a sensitive indicator of growth disturbance (*11*), was seen in autoradiographs of sections from bones cultured in the presence of fluoride concentrations higher than 30 ppm. With such concentrations we also observed a decrease in the mitotic rate (shown by a drop in the number of labeled nuclei in the autoradiographs).

Data for thymidine-H^3 and proline-C^{14} uptake are shown in Figs. 2 and 3. Statistical treatment of the data revealed that thymidine uptake from fluoride concentrations of 0 to 20 ppm fluoride could be best fitted by a horizontal straight line. Analysis of variance showed that there was a 95-percent probability of detecting a 5-percent drop in thymidine incorporation over the range of 0 to 10 ppm. Proline incorporation was linear to fluoride concentrations of 10 ppm; there was a 95-percent probability of detecting a 10-percent drop over this range. A small

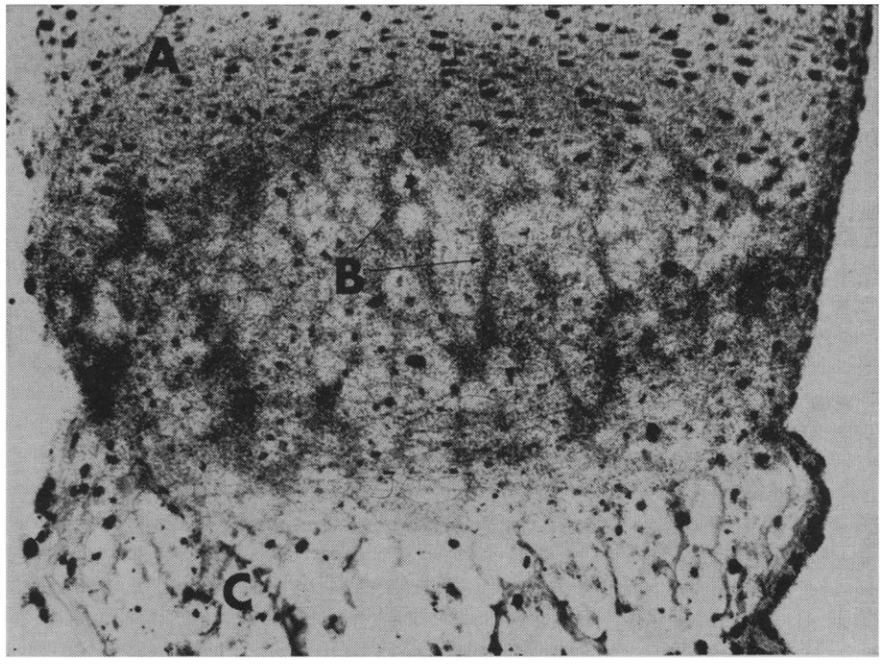

Fig. 1. Autoradiograph of epiphyseal plate of rat metacarpal bone 6 days after explantation into organ culture in the presence of 15 ppm fluoride ions. Labeled nuclei in proliferating cartilage are shown (*A*), as well as bands of labeled protein (*B*), and spicules of bone (*C*) (× 150).

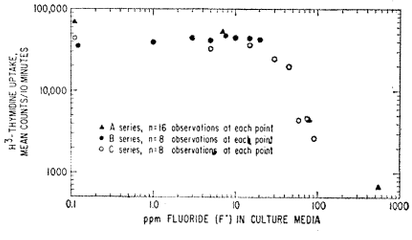

Fig. 2. Uptake of thymidine-H^3 by metacarpal bones in organ culture as a function of fluoride concentration.

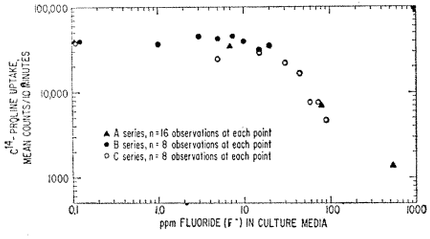

Fig. 3. Uptake of proline-C^{14} by metacarpal bones in organ culture as a function of fluoride concentration.

but statistically significant deviation from linearity occurred in proline-C^{14} incorporation when the fluoride concentration was between 10 and 20 ppm. Both the C^{14} and H^3 counts diminished at 30 to 45 ppm and were very low at 60 ppm. The relationship approximates an exponential drop in uptake beginning just beyond 20 ppm for thymidine-H^3 and between 10 and 20 ppm for proline-C^{14}, with a steeper slope for thymidine-H^3 (see log-log plots in Figs. 2 and 3).

The concentration of fluoride ions in tissue fluids never reaches levels comparable to the high concentrations used in the culture medium in our experiments. In humans, fluoride concentrations of approximately 0.2 ppm are maintained in the plasma when water containing 1.0 to 2.5 ppm is ingested (*12*). Enzyme inhibition and symptoms of acute fluoride intoxication occur when fluoride doses leading to high concentrations of fluoride ions in the tissue fluid are administered to experimental animals. This is presumably analagous to what occurred in our cultures when the fluoride concentration was more than 20 ppm. Isolated or abnormal cells in tissue culture may be more sensitive to the effects of fluoride ions than are organ systems in culture. There was no evidence in these experiments, nor is there any convincing evidence in the work of others, that inhibition of organized skeletal growth in vitro occurs when the concentration of fluoride is below the point between 10 and 20 ppm at which acute toxic responses begin to appear.

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Thymus: Role in Maturation of Fetal Lymphoid Precursors

Abstract: *Liver cells from homozygous embryonic mice were subjected to serial passage through appropriate thymectomized and sham-thymectomized F_1 hybrids. Passage through a nonthymectomized host was required for maturation and/or proliferation of the potential immunologically competent cells in the embryonic liver.*

The work of Miller (*1*, *2*) and others (*3*) has firmly established the importance of the thymus in the development of immunological competence by the newborn rodent and in the recovery of the homograft response by adult mice that have been irradiated. Two hypotheses have been advanced to explain the role played by the thymus in the development and maintenance of immunologic integrity: (i) A cellular hypothesis suggests that the thymus is an essential if not exclusive source of the immunologically competent cells which populate the peripheral lymphoid tissues (*1*, *3*). (ii) A humoral hypothesis states that the potential immunologically competent cells are derived from other tissues but are dependent to a greater or lesser degree upon a thymic hormone for their stimulus to mature or proliferate or both (*4*). These two hypotheses have not been considered mutually exclusive. Direct evidence of cellular migration from (and into) the thymus has been presented in both newborn and adult mice (*5*); however, the functional significance of these observations remains unclear. On the other hand, Osoba and Miller (*6*) and Levey *et al.* (*7*) have presented convincing evidence that the thymus produces a filterable factor which has a significant effect upon the proliferation and function of distal lymphoid tissues. Other recent work (*8*) has shown that mouse fetal liver and perhaps other nonthymic embryonic tissues contain potential immunologically competent cells. It is the purpose of this report to show that, within the context of the experimental model, potential immunologically competent cells derived from mouse fetal liver are dependent upon the thymus for their "functional maturation" or proliferation or both.

The presence of potential immunologically competent cells in fetal liver was demonstrated by a modified parental F_1 hybrid, "graft-versus-host," method. In contrast to adult lymphoid tissue, fetal liver from a homozygous donor produces no significant mortality when injected into a sublethally irradiated F_1 hybrid, one parental strain of which is identical to that of the embryo (*8*). However, if after 60 days the lymphoid tissues of this primary host are injected into a second F_1 hybrid (one parental strain identical to that of the embryo but the second parental strain differing from the second parent of the primary host) a significant number of deaths occur within 60 days. This may be summarized as follows: *A* fetal liver $\rightarrow$ (*A* $\times$ B) $F_1 \rightarrow$ (*A* $\times$ C) F_1 and, as is the case in these experiments, $\rightarrow$ (*A* $\times$ B) F_1.

Fetal liver was obtained from 13 A/Sn $\times$ A/Sn embryos (20 days old). It was gently suspended in a balanced salt solution and portions were injected into thymectomized or sham-thymectomized adult primary hosts which had just received 500 rad whole body x-radiation. Noninjected, sham-thymectomized, irradiated mice served as controls. After 60 days the survivors from each group were killed individually, the spleen and lymph nodes of each were gently disrupted, and the resultant cell suspension was injected into two sublethally irradiated secondary recipients. After 60 days the surviving secondary hosts were killed, and their lymphoid tissues were transferred as described to sublethally irradiated (500 rad) tertiary hosts which were genetically identical to the primary hosts. Only the primary hosts were thymectomized or sham-thymectomized. Completeness of thymectomy was confirmed upon subsequent autopsy when the animal died or when it was killed. Death of the primary, secondary, or tertiary hosts within 60 days was taken as evidence for the presence of mature immunologically competent parental cells in the inoculum. The mice were housed randomly, six to eight to a cage. The primary and tertiary hosts were adult male and female (A/Sn $\times$ DBA)F_1 mice and the secondary hosts were (A/Sn $\times$ CBA)F_1 mice.

There were no deaths among the primary hosts or their controls (Table 1). Seven deaths occurred among the eight secondary recipients of parental fetal liver cells passed through sham-thymectomized primary hosts. In contrast, the mortality among the secondary recipients of fetal liver cells passed through *thymectomized* primary hosts did not exceed that found in the control group (one out of eight as against two out of nine). Eight of 14 tertiary recipients of fetal liver that had been passed through thymectomized primary hosts died within 60 days, while there were no deaths in the appropriate control group.

These results again substantiate the presence of potential immunologically

Table 1. Sixty-day mortality in primary, secondary, and tertiary recipients of parental fetal liver. Only primary hosts and primary controls underwent thymectomy or sham-thymectomy. All recipients received 500 rad x-radiation (whole body) prior to injection.

Parental fetal liver (A/Sn)	Treatment of primary host	60-day mortality (No./total)		
		Primary (A/Sn $\times$ DBA)F_1	Secondary (A/Sn $\times$ CBA)F_1	Tertiary (A/Sn $\times$ DBA)F_1
+	Thymectomy	0/4	1/8	8/14
+	Sham	0/4	7/8	
−	Sham	0/5	2/9	0/6