The biliary-duct hyperplasia and hepatic cell alterations are similar to the effects of butter yellow(5,6,7) as well as of a number of other agents(8,9). This atypical proliferation of biliary ducts and of fibrous tissue is called cholangiofibrosis and is considered to be a precursor of biliary-duct carcinoma(7). If analogy to butter yellow is extended, it is probable that dietary alterations will cause more rapid onset of these lesions.

Such reactions to feeding an antioxidant raise questions as to mechanism and site of action. Relations worthy of investigation are those involving Vit. A, Vit. E, and choline and methionine. Further work may also be of value in elucidating lobular localization of hepatic injury and the responses of biliary-duct epithelium and hepatic cells.

Summary. *Flectol H,* a polymer of 1,2-dihydro-2,2,4-trimethylquinoline, when fed to rats produced fatty change frequently localized to the midzonal area of the hepatic lobule. Chronic administration led to alteration of hepatic cells, necrosis, and atypical biliary-duct hyperplasia.

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Extratrabecular Crystallization in Rickets; Effects of Vitamin D, Calcium and Norethandrolone.*† (26223)

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Recent observations have revealed the presence of abnormal mineral deposits adjacent to the bone trabeculae of chicks fed a diet containing 50% ground seeds of *Lathyrus odoratus*(1). Another series of experiments, yet unpublished, involving suckling rats injected with the toxic agent of the lathyrus seed, Beta-amino-propionitrile, has produced similar extratrabecullar masses. In both instances and also in a recently observed specimen of human osteogenesis imperfecta, the large round crystals were best seen in dark-phase microscopy; they were birefringent in polarized light. The bone trabeculae were immature, fibrillar; they were deficient in polysaccharides and minerals. A current series of experiments involving undemineralized sections of tibiae from rachitic chicks variously treated has revealed a similar condition.

Materials and technics. Five groups of 10 newborn chicks/group were fed the low Ca AOAC diet(2) without Vit. D. After one week the diet was supplemented in 4 groups as follows: (a) $Ca_3(PO_4)_2$ 1%; (b) Vit. D^3 1000 units %; (c) Norethandrolone (Nilevar, Searle) 0.5 g/kilo; (d) Norethandrolone as in (c) with $Ca_3(PO_4)_2$ as in (a). Group 5 was unmodified. At 2 weeks of age, the untreated birds were suffering from severe rickets as judged by tarso-metatarsal radiographs(3), bone ash, Ca^{45} intestinal absorption(4) and by the histological picture of cartilage and bone. Calcium and norethan-

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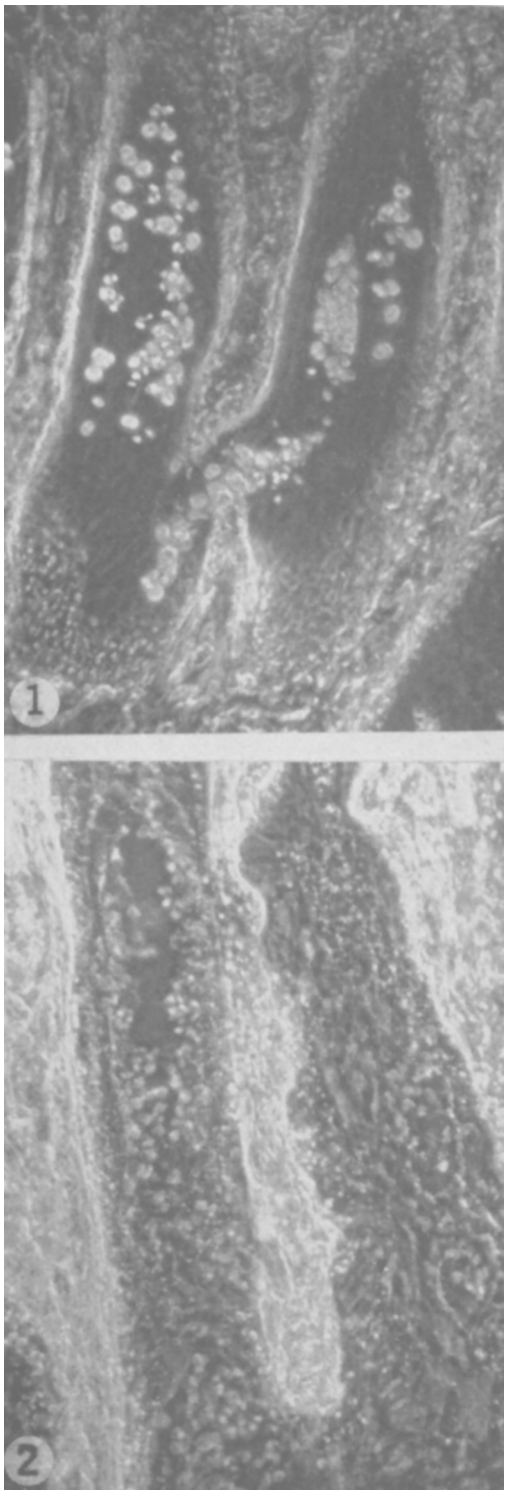

FIG. 1. New periosteal bone trabeculae and extratrabecular crystals, from 12 μ celloidin section of undemineralized rachitic chick tibia. Dark M phase contrast × 600.

FIG. 2. A comparable area from tibia of a chick treated with vit. D^3 for 1 wk. Dark M phase contrast × 600.

drolone treatments have modified the histology and histochemistry of these tissues as recently recognized(5). The intimate effects of these agents on the mineral fraction are still unknown.

After 2 weeks in the present series, the chicks were given 100 μc P^{32} in 0.5 ml distilled water intraperitoneally. Five chicks from each group were sacrificed 2 hours later. The right tibia was roughly dissected, fixed in 10% neutral aqueous formaldehyde embedded in celloidin and cut at 12 μ. For phase contrast microscopy, the sections were mounted in neutral synthetic resin (Permount, Fisher) without staining.

Observations. Unstained sections examined under negative phase contrast (Dark M, AO) have revealed the presence of round masses, 3-5 μ in diameter, shining bright except for a small dark core (Fig. 1). These were located along thick, dark newly-formed trabeculae (Fig. 1) and were present in the untreated rachitic group and also in all treated birds except those which received Vit. D. The brilliant objects were more numerous but of similar size in the sections from birds treated with calcium, norethandrolone or norethandrolone-Ca. In the chicks which received Vit. D, the large extratrabecular masses were generally not observed (Fig. 2). The bone trabeculae appeared denser and bright (Fig. 2). The objects thus seen in negative phase were rapidly dissolved in acid dyes; they were stained blue by azur *II* at pH 7.4. The Von Kossa reaction has yielded irregular concentrations of coarse precipitate between the trabeculae. These could have been interpreted as artefacts in absence of the phase contrast evidence (Fig. 1). The extratrabecular masses were birefringent in polarized light. Under the present conditions, they produced a P^{32} autoradiograph. Alpharadiography(5) has shown them as uniformly dense.

Discussion. The positive Von Kossa reaction and P^{32} autoradiography at the sites of extratrabecular globule formation, point to

the presence of calcium phosphate in these crystals which appear in the vicinity of trabeculae unable to mineralize in rickets as well as in osteolathyrism of the chick(1), BAPN poisoning of young rats and human osteogenesis imperfecta.

Calcium and norethandrolone help bone growth but do not favor maturation(6). Calcium and norethandrolone combined have apparently produced maturation of the organic matrix(6); the present experiments reveal however that extratrabecular crystallization is not reduced by that treatment as it is by Vit. D (Fig. 2). The present experiments indicate also that even in rickets, the local mineral deposit is fairly abundant. The numerous osteoclasts which inhabit the vicinity of the growth sites might be instrumental in local mobilization of salt in order to "make the interstitial fluid rich in calcium," a condition essential for mineralization as postulated by Leriche and Policard(7). The primary lesion of bone in rickets which is cured by Vit. D would then be related to the organic matrix. On the other hand, the increased Ca^{45} uptake by bone under high Ca deposit(8) may be intimately responsible to the presence of extratrabecular crystals and not to an activation of bone growth.

Summary. Large extratrabecular crystals are present in rachitic bones of chicks. They disappear after treatment with Vit. D.

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Additional Evidence for Involvement of RNA in Maintenance of ATP Levels in Homogenates.* (26224)

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Rat liver homogenates preincubated with ribonuclease (RNAse) lose the capacity to convert mevalonic acid (MVA) to non-saponifiable material (NSF) and cholesterol(1). This loss of synthetic capacity is associated with absence of ATP in these homogenates (2,3). ATP is present and conversion of MVA to NSF occurs if the homogenates are supplemented prior to or after preincubation with autoclaved liver extract, or with relatively large amounts of RNA, or with DNA, or with various anionic polymers such as polyethylene sulfonate or heparin (4,5,6). It has been concluded that a polyanion, which in the case of whole liver is RNA or a ribonucleoprotein, is essential for maintenance of ATP levels in tissues(7,8). Inactivation of biosynthetic capacity in liver homogenates with respect to MVA conversion to NSF also is observed on preincubation with various polycations such as protamine or polylysine(9,10). The effect of these agents similarly is preventable or reversible with the same group of polyanions. These findings with polycations raise the question as to whether inactivation with RNAse is attributable to its enzymatic activity or to its capacity as a basic protein to combine with or precipitate polyanions unrelated in structure to RNA. The experiments described here demonstrate that when RNAse is denatured by acid and heat under condi-

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