bryo, when grown *in vitro* for several days, became altered from a simple respiratory membrane into a multilayered keratogenic tissue. The precise identity of the stimuli directly involved in causing this metaplasia remains to be examined. One possible assumption is presence in the culture environment of metabolic requirements, not available to the CE *in ovo* but essential for manifestation of the keratogenic properties of the CE cells. Alternatively, the existence *in ovo* of inhibitory conditions, affecting the CE so as to prevent it from expressing its keratogenic potentialities, should be considered.

Clarification of these and other possibilities, might also relate to the general problem of keratinization. The CE, in being a normally non-keratogenic tissue, appears to be a particularly suitable material for studying processes and events by which cells may acquire capacities for rapid and voluminous production of keratin; it thus provides a useful source of comparison with the properties of skin. In view of the mucogenic metaplasia of embryonic skin demonstrated by Fell and Mellanby(2) and the role of Vit. A in this process(3), the reaction of the CE to this vitamin might be of interest. In this connection, one should also like to know if conditions might be found which would cause the CE to undergo *in ovo* keratogenic changes similar to those displayed in culture. These questions are being presently investigated.

Summary. A keratogenic metaplasia *in vitro* of chorionic epithelium of the chick embryo is described. Explants of chorionic epithelium from 8-day embryos, cultured for 8 days on a plasma clot, became altered from an attenuated, one-cell-thick respiratory membrane into a multilayered, squamous epithelium in advanced phases of keratinization.

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Osteolathyrogenic Action of Mercaptoethylamine and of Cystamine.* (24176)

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Beta-mercaptoethylamine, when fed to weanling rats, was found to produce gross osteolathyric lesions similar to those produced by diets containing sweet peas or β-aminopropionitrile (BAPN)(1). Ponseti *et al.*(2) were unable to confirm this finding. Results of studies on wound healing(3) and of studies using pregnant rats(4) appear to be in agreement with the negative report of Ponseti *et al.* We have now repeatedly produced osteolathyric-like lesions not only with different lots of mercaptoethylamine but with closely-related cystamine as well.

Methods. Beta-mercaptoethylamine was fed as the hydrochloride; cystamine as the dihydrochloride. Three different lots of β-mercaptoethylamine · HCl were purchased from California Fn. for Biochemical Research over a period of 18 months and were stored in vacuum desiccator. Iodometric titrations for –SH as well as chloride determinations indicated a purity of 96% or more for each of the lots. Cystamine 2HCl was purchased from Nutritional Biochemicals Corp. Iodometric determination of –S–S– following reduction(5) and chloride determination indicated a purity of 99%. These supplements were incorporated into finely milled Rockland Rat Stock

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Diet and fed to male albino rats of Sprague-Dawley strain weighing 50-62 g. Selected rats were sacrificed after 3 weeks or more on these diets. The entire aorta and one hind extremity were fixed in 10% buffered formalin. The knee regions (distal femur, joint proper, proximal tibia and fibula) were decalcified in equal parts of 10% formic acid and 20% sodium citrate. Aortas were cut into 8 segments of equal length, dehydrated, cleared and embedded in paraffin. Six to 8 μ sections were stained with hematoxylin and eosin, and with Hornowski's combined elastic and Van Gieson stains.

Results. Because of the severe growth-depressing effect of *mercaptoethylamine · HCl*, it was found advisable not to use rats too light in weight. Best results were obtained with rats weighing about 60 g and fed diets containing about 0.45% of the hydrochloride. Development of osseous lesions in different rats was not as uniform as when salts of BAPN were fed, but all rats which survived sufficiently long showed skeletal changes. Gross skeletal effects are shown in Fig. 1. No gross lesions of the aorta were seen in any of these animals. Perhaps because of its greater stability, osteolathyrogenic effects were somewhat easier to demonstrate with *cystamine · 2HCl*. Small differences in dietary levels, however, gave rise to marked differences in response. On a diet containing 0.2% cystamine · 2HCl, rats weighing 50-60 g gained weight at a rate only slightly less than that of control rats receiving the unsupplemented diet. The only gross symptom observed over a period of 7 weeks was a slight kyphosis detectable by careful palpation or by roentgenological examination. Rats receiving 0.5% cystamine · 2HCl exhibited an immediate loss in weight and some died within one week on the diet. Those that survived 12-14 days or longer had spinal curvatures, prominent, palpable exostoses on the mandibles, and showed marked skeletal lesions both grossly (Fig. 1) and microscopically. Only 1 of 8 rats survived beyond 19 days on this level. On a 0.4% level, 1 rat in 6 survived beyond 24 days. At 0.3%, all rats survived beyond 5 weeks at which time some of animals were sacrificed. All rats on 0.3% and 0.4% levels developed gross skeletal changes typical of osteolathyrism, palpable skeletal lesions becoming detectable in about 3 weeks and 2 weeks respectively at these levels.

Histopathologically, similar aortic and skeletal lesions were observed in both groups of animals. Although no gross aneurysms were seen in aortas of any rats fed mercaptoethylamine or cystamine, aortas of animals in both groups showed swelling and degeneration of tissues between elastic fibers of the media. In most animals the elastic fibers remained intact. Some showed multiple small areas of destruction involving the outer ⅔ of the media and sparing the inner portion. All elastic fibers were destroyed in these areas with considerable replacement by collagen. These changes (Fig. 2) were similar to elastolytic changes observed in the aortic media of rats and mice receiving sweet peas or BAPN(6,7,8,9). In the aorta of one rat receiving mercaptoethylamine · HCl, calcium deposition had taken place in areas of medial destruction. No evidence of dissecting hemorrhage or aneurysm was seen in any of the animals.

Periosteal lesions were observed in all specimens from both groups. These showed: 1, fibroblasts with collagen formation, 2, osteoblasts with osteoid formation, 3, chondroblasts with cartilage formation, 4, basophilic granules or globules in new bone and cartilage matrix (Fig. 3). Similar changes have been produced by sweet peas, BAPN, and aminoacetonitrile(10,11,12,13). Extensive recent hemorrhage in the proliferating periosteum was seen in one animal receiving cystamine · HCl. Periosteal hemorrhage has been reported in rats fed sweet peas(14).

Changes observed in the epiphyseal cartilage were: 1) irregular thickening and broadening of the plate, 2) balloon degeneration of cartilage cells, 3) abnormal proliferation of cartilage cells, 4) disruption of parallel rows of cartilage cells to form irregular groups or masses, 5) fibrillar degeneration of cartilage matrix, 6) areas of complete destruction of both cells and matrix with space or cleft for-

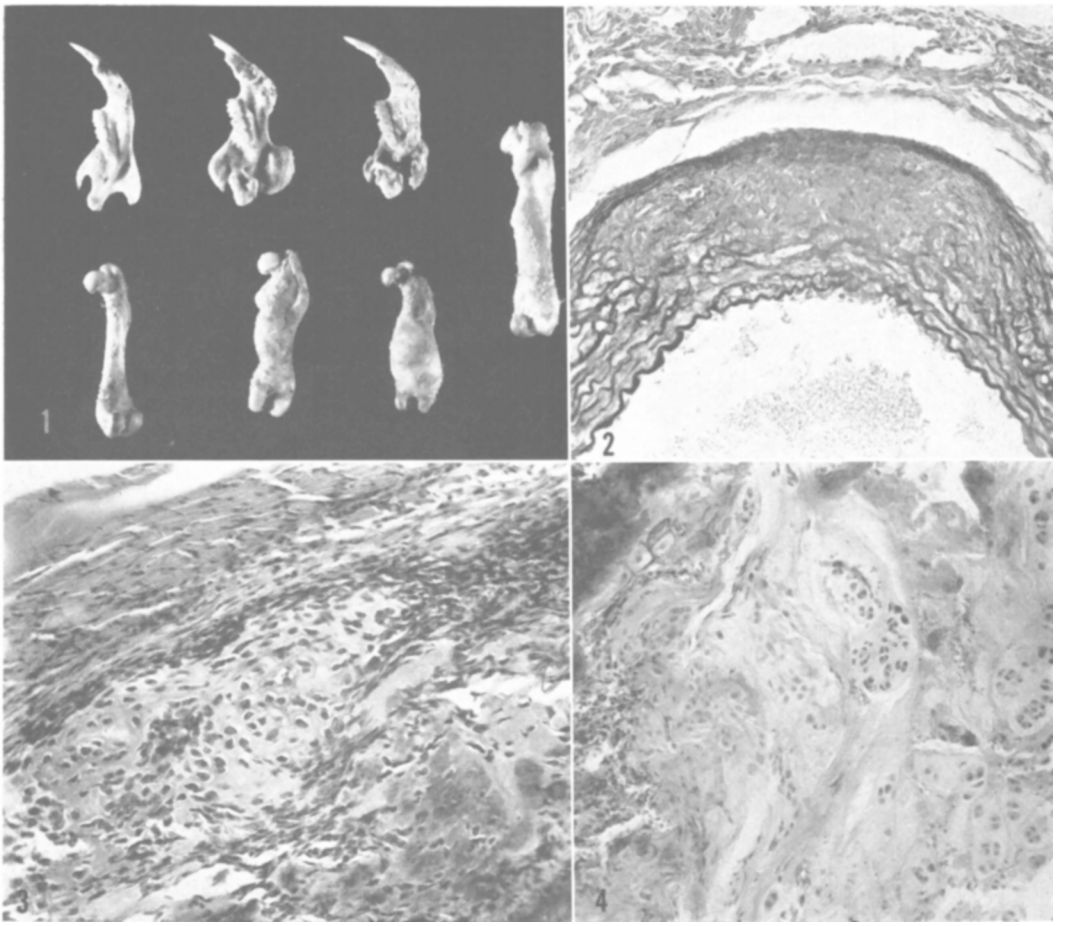

FIG. 1. Rat femurs and mandibles, left to right: normal; .45% β-mercaptoethylamine • HCl, 22 days; .5% cystamine • 2HCl, 22 days; .45% β-mercaptoethylamine, 84 days.
FIG. 2. Aorta of rat receiving .4% mercaptoethylamine • HCl for 37 days. × 200.
FIG. 3. Periosteal proliferation in same rat as Fig. 2. × 300.
FIG. 4. Epiphyseal cartilage in rat receiving .5% cystamine • 2HCl for 22 days. × 300.

mation (Fig. 4). Sweet peas, BAPN and aminoacetonitrile produce similar changes (11,12,15).

Specimens from all experimental rats examined showed fatty transformation of bone marrow. This was generally more evident in the epiphysis than the metaphysis. Fatty transformation of bone marrow has been described as an occasional finding in osteolathyrism induced by feeding sweet peas(14). In osteolathyrism resulting from ingestion of semicarbazide, fatty transformation of bone marrow is even more marked than when mercaptoethylamine or cystamine is fed (unpublished).

In the joint proper, no definite changes in synovial membranes or articular cartilage were observed.

No hernias were seen. However, the incidence of hernias in hundreds of rats receiving sweet peas or BAPN has been well under 1% in contrast to its high incidence in other laboratories(6,16).

Discussion. The fact that gross skeletal lesions and histopathological changes in periosteum, epiphyseal cartilage, and aortic media of rats fed β-mercaptoethylamine · HCl or cystamine · 2HCl seem identical to lesions observed in rats fed sweet pea toxin (BAPN) appears to indicate that the syndromes are identical. The use of the term *osteolathyrism* (13) for these conditions therefore seems jus-

tified. Whether these different substances affect the same specific metabolic reaction or act at different points in the same metabolic sequence remains to be determined. There are, however, differences between manifestations of mercaptoethylamine or cystamine toxicity and those produced by osteolathyrogenic nitriles. The former have a much narrower range of osteolathyrogenic activity between lethal and totally ineffective levels. Aortic lesions were also much less severe when compared on the basis of severity of bone lesions. In fact, no evidence of dissection or aneurysm formation was observed in any animals.

Since β-mercaptoethylamine (cysteamine) and cystamine are readily interconvertible by oxidation and reduction respectively, it is likely that the *in vivo* toxic compound is the same in both instances. The more consistent results obtained with cystamine are probably due to its greater stability in the diet.

In view of our findings, the results of toxicity experiments reported by Bacq(17) are entirely inexplicable. He fed cystamine · 2HCl at levels up to 1% for periods up to 2 months. His rats grew well and showed no symptoms of lathyrism. Although his rats were somewhat larger than ours, our experience with cystamine would lead us to expect a high mortality and 100% incidence of osteolathyrism in rats surviving as long as 2 weeks.

Summary. The osteolathyrogenic action of β-mercaptoethylamine (cysteamine), when fed to rats as the hydrochloride, was confirmed. Cystamine was found to have similar properties. Gross and microscopic skeletal changes were similar to those produced by osteolathyrogenic nitriles. Aortic lesions were much less severe than when β-aminopropionitrile is fed, but medial swelling and lysis of elastic fibers were observed. These substances showed a narrow range of osteolathyrogenic activity, a dietary level of 0.2% cystamine · 2HCl causing only very slight spinal curvatures, while a level of 0.5% was rapidly lethal to most animals.

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