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Effects of Prolonged Chronic Vitamin A Deficiency in the Rat With Special Reference to Odontomas.*

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The gross and microscopic pathological changes induced by a relatively acute vitamin A deficiency have been thoroughly studied[1] and the mechanism of the process of repair described.[2, 3] On the other hand, a mild chronic deficiency of the vitamin, such as is more likely to occur in man, has received less attention. The production of such a borderline condition in animals is attended by many difficulties; chief among these is the fact that an excess of the vitamin is readily stored and external evidence of the deficiency removed whereas too small a supply results in the more acute type of the deficiency and in death. The following procedure, however, was found satisfactory for the production of a chronic deficiency of long duration.

Albino rats were specially prepared by withdrawing vitamin A from the mother's diet before the young were 13 days old. At weaning (21 days) the animals were caged individually and fed a purified, vitamin A-free ration adequate in all other respects. When either beginning decline in weight, the first stages of xerophthalmia, or continued excess cornification of the vaginal epithelium of the females appeared (at an average age of 52 days), a small but known quantity of vitamin A in the form of cod liver oil† was administered by mouth. Thereafter, the amount of vitamin A to be given each day was determined for each individual rat from its clinical state (body weight, appearance of eyes, snuffles, and vaginal smear). Thus, the quantity of the vitamin was so adjusted that the animals were maintained in a state of incipient vitamin A deficiency for periods up to one year. The average daily dose of vitamin A required varied from 0.7 to 6.0 (range 0.7-8.0) Inter-

* A contribution from the Dental Study Group, Yale University School of Medicine.

1 See current review, Robertson, E. C., *Am. J. Med. Sci.*, 1936, **192**, 409.

2 Wolbach, S. B., and Howe, P. R., *J. Exp. Med.*, 1933, **57**, 511.

3 Wolbach, S. B., and Howe, P. R., *Am. J. Path.*, 1933, **9**, 275.

† The cod liver oil was assayed in our laboratory by the method prescribed in the U. S. Pharmacopeia XI.

national Units. A general relationship existed between the dose required and the age and/or weight of the animals; the dose had to be increased progressively throughout the experiment.

The averaged growth curves were normal in shape, but the absolute values were slightly low. There was a 54% mortality among animals between the ages of 81 and 365 days, the majority of deaths resulting from tracheal obstruction by a muco-purulent plug. Otitis media, sinusitis, and congested nares were commonly observed. Large numbers of calculi were found frequently in the urinary bladder both in the animals that died earlier and in those that lived for a year.

The most striking and consistent effect of the chronic deficiency was on the incisor teeth, beginning when the rats were about 50 days of age. These teeth showed progressively a loss of the normal orange pigment, the development of opacity, a distortion of shape (crossing, twisting, transverse and longitudinal ridging), and eventual exfoliation of the erupted portion. Large solid lumps, not sensitive to the touch, developed in the maxillae.

The incisor teeth showed histological changes similar to those observed by Wolbach and Howe[3] in rats fed a diet free of vitamin A. The majority of animals surviving 365 days on the low vitamin A diet developed, in addition, tumor growths (odontomata) and, in a few instances, supernumerary incisor teeth. The tumors arose from the pulp and consisted chiefly of spindle-shaped cells similar to the embryonic cells of the pulp tissue. Some of the tumors proliferated to the point of replacement of the entire alveolar bony structures of both the upper and lower jaws and even extended to and ulcerated through the gingival margin. Small groups of squamous epithelial cells were distributed throughout the spindle cell proliferations, some of which had undergone complete keratinization. Groups of columnar cells resembling odontoblasts also occurred. Many of these aggregates of cells were adjacent to atypical dentine and osteodentinal structures. Imperfect forms of germinal tooth bud structures were found in some of the tumors. The original incisor teeth of the majority of the rats were greatly distorted or replaced by the tumor growth. The ameloblasts of the enamel organs of all of the teeth developing odontomas were atrophied and metaplasia to squamous epithelium was encountered. Tumor proliferations were not present in the molar teeth of these rats, possibly because these teeth are not of the continuously-growing type, as are the incisors, and probably because they were fully developed before the deficiency became manifest. It is possible that the inevitable repeated slight decline and recovery of the animals,

incident to the method of feeding, have emphasized the production of denticles in the incisors which has been noted by Wolbach and Howe[3] in the more acute condition.

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Nature of the Anticlotting Activity of Streptococci *in vitro*.

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While studying factors affecting the formation of the fibrinous inflammatory barrier in acute local streptococcal inflammation, Dennis and Berberian[1] confirmed the observations of Tillett and Garner[2] on streptococcal fibrinolysin, and described the presence of an anticoagulant in filtrates of certain strains of hemolytic streptococci and of virulent viridans streptococci. Although certain strains of erysipelas streptococci produced both fibrinolytic and anticoagulant factors, sound evidence was offered that we were dealing with 2 distinctly different substances. The differences were: fibrinolysin was never produced by *Strep. viridans;* fibrinolysin acted only upon human plasma clot, while the anticoagulant prevented the clotting of both human and rabbit recalcified oxalated plasma; and fibrinolysin was thermolabile while the anticoagulant was thermostable. Anticlotting factor masked the action of fibrinolysin whenever the two occurred together. Tunnicliff[3] confirmed our observations and correlated the anticlotting activity with the smooth (S) phase of greening streptococci. Neter and Witebsky,[4] apparently unaware of our earlier observations, recently reported on the anticoagulant activity of streptococcal filtrates, but failed to recognize the fundamental difference between anticoagulant and fibrinolytic phenomena.

During the past 3 years we have extended our observations on

* Observations on purification of the anticlotting were made by one of us (E. W. D.) during tenure of a Rockefeller Research Fellowship in Bacteriology, at Harvard Medical School, 1934-35.

1 Dennis, E. W., and Berberian, D. A., *J. Exp. Med.*, 1934, **60**, 581.

2 Tillett, W. S., and Garner, R. L., *ibid.*, 1933, **58**, 485.

3 Tunnicliff, R., *J. Infect. Diseases*, 1936, **58**, 92.

4 Neter, E., and Witebsky, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 549, 858.