ence of atrophy. The per cent of collagen based on wet weight, however, was significantly reduced in the group of 8 patients with rheumatoid arthritis and 3 patients with degenerative joint disease. 4. The adenosinetriphosphatase activity of myosin was fairly uniform among normal subjects. It was significantly reduced, however, in all categories of patients, although no statistically significant differences were found to distinguish one disease from another. 5. Atrophy was noted microscopically in 93% of a group of patients with rheumatoid arthritis, degenerative joint disease and gout. The outstanding analytical changes in the presence of muscle atrophy were decreases in the concentration of total protein, myosin and adenosine triphosphatase. There was no evidence of collagen replacement of myosin in the course of atrophy.

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Electron Microscopy of Crystalline Plates in Rabbit Papilloma. (18586)

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Infectious papillomatosis of rabbits is characterized by warty growths of the skin (1). These lesions, of which a viral agent is considered to be the etiological agent, have been shown to have many features common to mammalian tumors(2-7). The wart arises from the basal cell layer and the growth is primarily of epithelial elements. There is extensive keratinization without accompanying desquamation. Bryan and Beard describe the lesion as consisting mostly of cutaneous horn and being a "structureless, lifeless mass of cell debris composed of pigment, keratin and insoluble, denatured protein(8)." There is a marked difference in the macroscopic appearance of the warts in domestic and wild rabbits, but Hurst has stated that there is no difference macroscopically in warts experimentally induced in domestic and wild rabbits (1a).

The present investigation reports the study of a component found in extracts of the papillomatous lesions.

Methods. The papillomatous lesions used in this study were freshly harvested from the diseased animals or were harvested previously and stored in glycerol (50%). All material, which consisted of the top half of the cutaneous cap, was carefully cleaned of hair and other superficial matter before using. The glycerol stored lesions were washed in running tap water for 2 to 6 hours to remove the excess glycerol. Homogenates of the papillomas, both from the wild and domestic rabbits, were prepared using distilled water and the Waring blendor and care was exercised to avoid heating the preparation above room temperature during this procedure. Clearing was done in a refrigerated centrifuge at 20,000 times gravity for 2 minutes. The supernatant liquid was divided into 2 portions. One portion was dialyzed against water in the cold and then examined in the electron microscope. The second portion was first examined in the electron microscope and then acetate buffer (pH 4.5) was added to bring the pH near the isoelectric point of most proteins. After another centrifugation at 20,000 times gravity for 2 minutes, a small pellet was discarded and the acetate ions

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8. Bryan, W. R. and Beard, J. W., *J. Nat. Cancer Inst.*, 1941, v1, 607.
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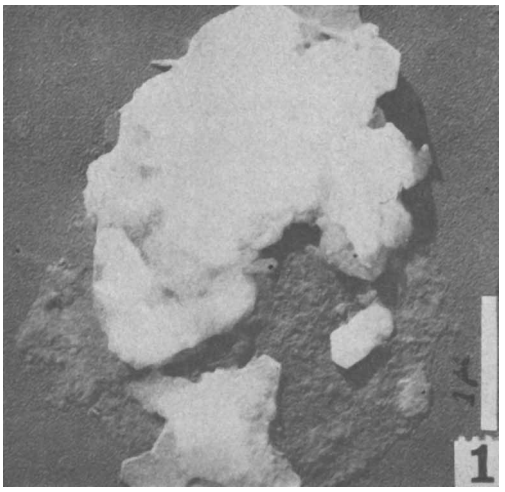

FIG. 1.
Common finding, showing the association between crystalline and non-crystalline material. Cr shadowed.

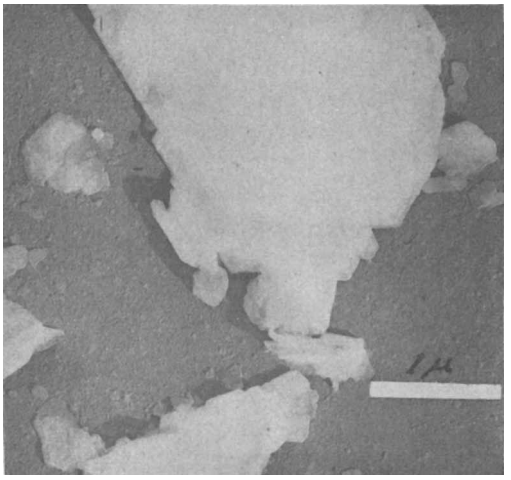

FIG. 2.
Crystals exhibiting large size range. Large opaque crystalline sheet is a crystal aggregate. Note difference in crystal thickness as shown by shadow lengths. Cr shadowed.

removed by dialyzing against water in the cold for 24 hours. The liquid was reexamined in the electron microscope. Homogenates were also prepared in an alcohol-water mixture (1:1). The supernatant fluid of these preparations was centrifuged and the sedimented pellet resuspended in water for examination in the electron microscope. Acetone and chloroform were added to the supernatant of the water homogenates and repeatedly shaken together. After separation of the water and acetone or the water and chloroform had occurred, samples were taken from the water fraction for examination.

Results. Crystalline material, like that shown in the accompanying micrographs is always found in the water extracts of the papillomatous lesions. It is present in the freshly harvested and glycerin stored warts. The close association of crystalline material and non-crystalline material, which is quite characteristic, can be seen in Fig. 1. Treatment of the preparations with acetate buffer appeared to effect a partial separation of the crystalline material and the non-crystalline substances.

The individual crystals which we have observed can be divided roughly into single and multiple crystals. The single crystals are represented by the very small, hexagonal plates seen in Fig. 2. The multiple crystals appear to be made of several of the hexagonal plates crystallized together as represented in Fig. 3 and 4. The large multiple crystals frequently have the property of being laminated structures made up of thin crystalline sheets which can be noted in Fig. 3 and 4. These crystalline sheets show a wide variation in surface area but usually exhibit a remarkable degree of thinness. The smaller crystals measure approximately 125 mμ over their shortest distance while the large crystals measure several microns. Of especial interest are the small hexagonal plates seen on and between the crystalline sheets. All measurable angles of the crystals, single or multiple, are approximately 120 degrees.

The crystals exhibit no change in shape, size or density under the very hot electron beam of the bias gun. If they are first viewed in the electron microscope (for identification) then the screen removed and washed with water for a period of time, there is no change in the morphology of the crystals. However, if a water homogenate is permitted to stand for several hours, either at room temperature or in the cold, the crystals will break up into smaller entities and ultimately disappear suggesting that they are somewhat soluble in water. After the crystals once disappear on standing, they do not reform suggesting that their occurrence is not an artifact of drying

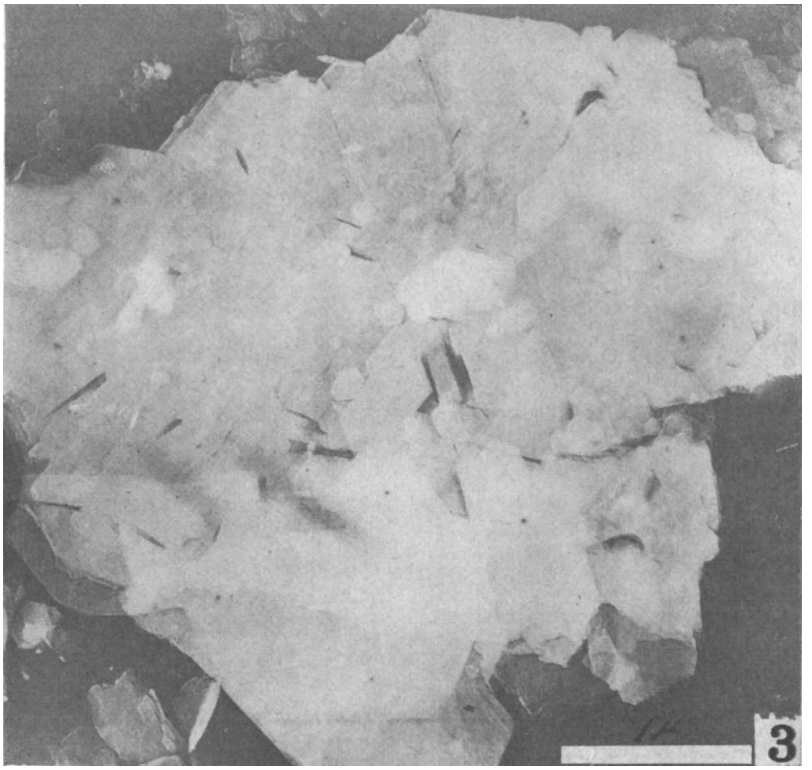

FIG. 3.
Large lamellar sheets of predominantly crystalline material. Cr shadowed.

and presumably that they form during the development of the papillomatous lesion. Examination of the water solutions containing crystals to which acetone, chloroform or alcohol had been added by the stated procedures continued to show crystals suggesting the lack of solubility in these organic solvents. We have not yet separated the crystals with any degree of purity from the contaminating matter which is always found in an extract of this kind. The extracts which had been partially cleared of protein by centrifugation in acetate buffer and then dialyzed were subjected to ultracentrifugal fractionation, light absorption studies, electron diffraction and electrophoresis. No positive identification was made.

Discussion. It appears from our studies that a component of the papillomatous lesions exists in the crystalline state. The finding of the crystals in both fresh and glycerol stored papillomas excludes the possible desiccating action of the glycerin forming the crystals during storage. They are only sparingly soluble in water thus making their presence in the water extracts indicative of their existence in the papilloma in a crystalline state. These crystals, in contrast to the recently observed virus crystals, are more like the classical crystalline aggregates and cannot be resolved into discrete macromolecular components in the electron microscope(9).

A review of the literature which discusses the histopathology of the papillomas suggests a mechanism whereby the crystals could be formed. It is known that the virus causes a lively proliferation of the basal cell layer of the epithelium. The growing epithelium first extends under the adjacent epithelium, and downwards to the fibrous corium, thereafter protruding upwards as papillae which may grow to a large size. There is definite inter-

9. Wyckoff, R. W. G., *Interscience Publishers, Inc.*, New York, pp. 151-178.

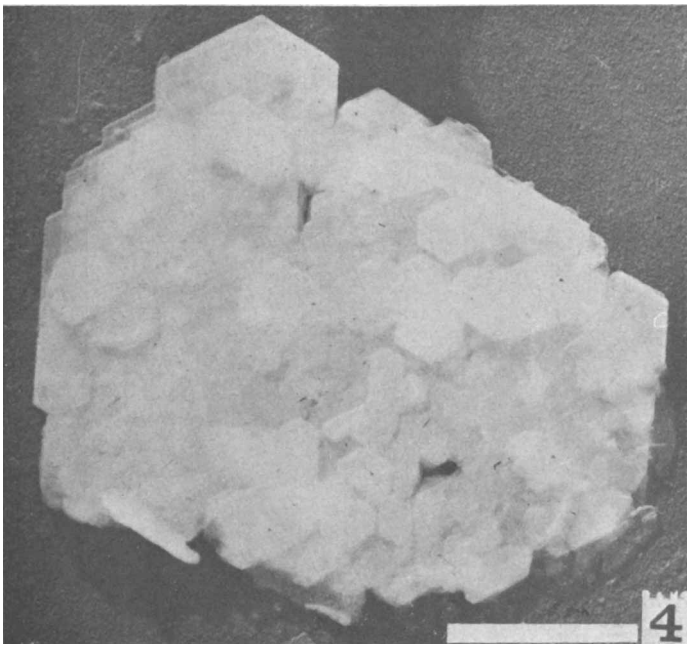

FIG. 4.
Large crystalline aggregate showing the laminated structure and many adherent smaller crystals. Note the tendency for the orientation of the crystals to be affected by their neighbors. Cr shadowed.

ference with the blood supply because of the lateral pressure with resultant drying of the tops of the papillae. Desquamation does not occur so that the growth becomes capped with a firm, dessicated cutaneous horn(3). If the conditions of drying and internal pressure are sufficient then crystallization may occur. The formation of such discrete crystals means that there must be available in a local region and in some concentration, a substance which can undergo crystallization. Whether these crystals are formed by a lytic action during degeneration or whether it is a normal substance produced during the growth process is not known.

Summary. The finding of crystalline platelets in the water extracts of papillomatous lesions of the wild and domestic rabbit is noted. A few of the elementary properties of the crystals are discussed.

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Agglutination of Red Cells by Synthetic Lysine Polypeptides.* (18587)

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It has been shown that basic high molecular weight lysine polypeptides(1), are capable of aggregating tobacco mosaic virus(2), and ag-

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1. Becker, R. R., and Stahmann, M. A., *J. Am. Chem. Soc.*, in press.