of thymus chromosomes, while thiocyanate, reported by Herve and Bacq(3) actually to *increase* X-ray lethality, was found here to increase nucleoprotein viscosity. On the other hand, most of the agents tested did have parallel activity in their effects on nucleoprotein viscosity and *in vivo* X-ray toxicity. Among these are cysteine(4,5), cyanide(6), and glutathione(5), which respond positively in both tests, and cystine(5), thiouracil (7,8), and ascorbic acid(5), which give negative results in both tests, although ascorbic acid does show a positive effect in the nucleoprotein viscosity test after a prolonged period of time.

In addition, the parallelism holds true for the experiments on the effect of cysteine on X-ray reduction of nucleoprotein viscosity. Patt *et al.*(4) have shown that cysteine protects rats from the lethal action of X-rays only if administered shortly before irradiation. Similarly, it was found here that cysteine protects nucleoprotein solution from viscosity reduction by X-rays much more effectively if added prior to the irradiation.

The possibility must remain, therefore, that some such phenomenon as this cysteine augmentation of nucleoprotein viscosity is a partial explanation of the reduction in X-ray lethality by cysteine and some other agents. The exceptions noted may represent entirely separate phenomena. Thiourea, for example, may protect against X-rays by reducing the metabolic level of the whole organism, in line with the ideas of Blount and Smith(7) and others. No explanation is here offered for the paradoxical behavior of thiocyanate.

Summary. 1. Cysteine, glutathione, cyanide, and thiocyanate greatly increase the viscosity of alkaline solutions of nucleoprotein (thymus chromosomes), while urea, uracil, thiourea, thiouracil, cystine, and BAL do not do so. Ascorbic acid increases the viscosity only after prolonged incubation. Hydrogen peroxide is without effect on the viscosity. 2. If cysteine is added to the alkaline nucleoprotein solution before X-radiation, the expected reduction in viscosity is largely prevented. If the cysteine is added after X-radiation, it is much less effective in this respect. 3. The suggestion is made that these phenomena may be relevant to the question of the mechanism of cysteine and some other agents in increasing resistance of organisms to X-ray lethality.

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Chemical Constituents of Skeletal Muscle from Normal Subjects and Patients with Rheumatic and Non-Rheumatic Diseases.* (18585)

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In the course of an investigation(1) of the incidence of lymphocytic lesions in skeletal muscle of patients with arthritis, biopsy specimens of fresh human muscle became available for simultaneous chemical analysis. Though

* This investigation was aided by grants from the Webster Underhill Fund and the Masonic Foundation for Medical Research and Human Welfare.

1. Sokoloff, L., Wilens, S. L., Bunim, J. J., and McEwen, C., *Am. J. Med. Sc.*, 1950, v219, 174.

the protein composition of normal and atrophic rabbit muscle has been studied in detail by Fischer and Ramsey(2), and the collagen and elastin content of normal human muscle has been determined by Lowry *et al.* (3), few data are available at present on the protein composition of human skeletal muscle and its variation in disease states. In the present study, the concentration of a number of protein constituents of human muscle were determined in normal subjects and patients with certain diseases. The chemical data obtained were correlated with the results of histological examination of the samples analyzed. A brief, preliminary report of results has been made elsewhere(4).

Methods. Muscle samples from 40 individuals were selected at random from the 202 included in the report by Sokoloff, Wilens, Bunim and McEwen(1), with the exception that individuals with a marked degree of muscular atrophy were not included because of the practical difficulty of obtaining sufficient muscle for both chemical and histological study. Muscle samples from all the 13 normal healthy adult males of the larger series were included. Each of these volunteers was thoroughly examined and it was established that they were free of any demonstrable disease. Ten were white and 3 were negroes; their ages ranged from 22 to 55. The distribution of patients according to diseases is given in Table I.

TABLE I. Distribution of Patients According to Disease.

Disease	No. of patients
Rheumatoid arthritis	8
Gout	4
Degenerative joint disease	3
Tuberculous spondylitis	4
Subacute bacterial endocarditis*	2
Marie-Strumpell spondylitis*	2
Pulmonary tuberculosis*	1
Erythema nodosum*	1
Bronchial asthma*	1
Syphilis*	1
Total	27

* Grouped in Table III as "Miscellaneous diseases."

The following muscle constituents were determined: total protein, non-protein nitrogen, collagen, myosin,† adenosinetriphosphatase (ATP-ase) activity of the myosin, and water. Under local procaine anesthesia, 3 to 4 g of gastrocnemius muscle were removed and divided into 2 portions. One was fixed for histological study, the other was immediately packed in ice and finely minced. From 0.7 to 1.0 g of mince were rapidly weighed into a thick walled centrifuge tube and the myosin was extracted by a modification of the method of Greenstein and Edsall(5). The time elapsing between excision of the muscle sample and chemical extraction was kept under 25 minutes. The mince was extracted twice, for one hour each time with 8 times its volume of cold 0.5 M potassium chloride and 0.03 M sodium bicarbonate solution. The residue after extraction was kept for the collagen determination. The combined extracts, after centrifugation in the cold, were poured into 10 volumes of copper-free distilled water and the precipitate left to settle over night in the cold. The precipitated myosin was washed with water, centrifuged in a calibrated tube and redissolved in 1.0 M potassium chloride equal in volume to the packed centrifugate. This solution was centrifuged to remove insoluble impurities, the pH adjusted to 6.8, and the volume was adjusted to exactly 10 ml. Aliquots were taken for determination of ATP-ase activity and for nitrogen content by the micro-Kjeldahl procedure. Myosin concentration was calculated from the nitrogen content on the basis of the value of 16.6 percent nitrogen determined by Bailey(7). The residue remaining after the extraction of myosin was washed three times

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† Myosin as referred to in this paper is "classical myosin" as extracted by Greenstein and Edsall(5) and is equivalent to the actomyosin of Szent-Gyorgyi(6).

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TABLE II. Volumes and Concentrations of Components Used in Determination of ATP-ase Activity.

		ml				
		1	2	3	4	5
Component	Concentration	At initial conc. of myosin	At ½ conc. of myosin	ATP control	Myosin control	Reagent control
Sodium adenosinetriphosphate	.6 mg labile P/ml	.35	.35	.35	X	X
Myosin	.30 mg N/ml	.20	.10	X	.20	X
Calcium chloride	.02 M	.10	.10	.10	.10	.10
Borate buffer	.02 M	.30	.30	.30	.30	.30
Water	X	.05	.15	.25	.40	.60

with water and allowed to stand over night at room temperature in 10 times its volume of 0.1 N sodium hydroxide. The sodium hydroxide was removed and collagen was determined in the resulting residue by the method of Lowry *et al.*(3). Collagen was determined as gelatin N in the supernatant solution after autoclaving, assuming 18.0% N in collagen (8). ATP-ase activity was measured using the sodium salt of a denosinetriphosphate as substrate. This was obtained by converting the dibarium salt‡ with sodium amberlite IR-100 resin cycled with sodium carbonate as suggested by Polis and Meyerhof(9). The sodium ATP was adjusted to a concentration of 600 μg of hydrolysable phosphorus per ml. Myosin solutions to be used for ATP-ase activity were made up to a concentration of 0.30 mg of nitrogen per ml. As shown in Table II, ATP-ase activity was measured at 2 myosin concentrations. This was necessary to insure a substrate excess for myosin of high ATP-ase activity. When on occasion, the myosin extracts were particularly high in ATP-ase activity and a linear increase of ATP-ase activity with nitrogen concentration did not prevail, the myosin extract was diluted sufficiently to assure an adequate substrate excess. The determination was carried out at pH 9.0 in 0.006 M borate buffer, and in the presence of 0.002 M calcium chloride. In Table II are given quantities of the various substances with appropriate blanks. The reaction was stopped after incubation at 37°C for 6 minutes, by the addition of 0.5 ml of 10% trichloroacetic acid to each tube. The precipitate was centrifuged, and the phosphorus content of the supernatant solution was determined by the method of Fiske and Subbarow(10). After correcting for the blanks in tubes 3, 4 and 5 (Table II), ATP-ase activity was expressed as the number of micrograms of phosphorus liberated per mg of myosin nitrogen in 6 minutes.

For the determination of water content, a 0.2 to 0.3 g sample of muscle mince was dried at 105°C for 24 hours. Repeated determinations showed that constant weight was reached under these conditions. The dried residue was digested by the Kjeldahl procedure for the estimation of total nitrogen. Non-protein nitrogen was determined on a 0.3 to 0.4 g sample of muscle mince, which was homogenized in a Potter homogenizer. The protein was precipitated with 5 ml of 50% trichloroacetic acid, and the volume was made up to 50 ml. After filtering, a suitable aliquot was taken for micro-Kjeldahl determination. The total protein value was determined by subtracting the non-protein nitrogen from the total nitrogen. By means of this procedure it was possible to eliminate from the total protein value any extraneous nitrogen introduced into the muscle by the local procaine infiltration, although special care was taken to avoid infiltration of the muscle during biopsy. The evaluation of atrophy was based on the histological appearance of the muscle

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‡ Armour and Co.

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TABLE III. Chemical Constituents of Skeletal Muscle of Normal Individuals and Patients. (Mean values ± S.D.)

Group	No. in group	No. with lesions*	No. with atrophy	% water	Total protein as % dry wt	Myosin N as % protein N	Myosin as % wet wt	Collagen N as % protein N	Collagen as % wet wt	ATP-ase† units per mg myosin N
A. Normals without lesions	6	0	0	77.3 ±1.83	73.2 ± 2.61	23.9 ±4.18	3.99 ±0.76	12.1 ±3.64	2.01 ±0.27	1437 ±149
B. Normals with doubtful lesions	3	–	0	77.9 ±0.51	70.6 ± 3.31	19.0 ±4.30	3.06 ±0.66	10.1 ±3.60	1.58 ±0.41	1419 ±446
C. Normals with definite lesions	3	3	0	78.6 ±1.16	74.9 ± 3.42	14.0‡ ±0.28	2.10§ ±0.03	10.7 ±3.93	1.72 ±0.48	1500 ±391
Rheumatoid arthritis	8	7	7	76.2 ±3.67	60.6§ ± 7.98	14.9§ ±2.64	2.13§ ±0.24	9.48 ±2.08	1.19§ ±0.32	1038§ ±374
Degenerative joint disease	3	2	3	73.5‡ ±2.31	51.0§ ±14.1	17.8 ±5.02	2.35‡ ±0.49	10.5 ±1.84	1.23§ ±0.12	794§ ± 81
Gout	4	3	4	73.9‡ ±0.84	58.3§ ± 6.97	11.9§ ±0.87	1.69§ ±0.31	11.2 ±5.33	1.53 ±0.67	681§ ±272
Tuberculous spondylitis	4	1	0	78.4 ±1.31	71.4 ± 4.05	15.7§ ±2.61	2.38§ ±0.41	10.6 ±4.50	1.57 ±0.56	881§ ± 96
Miscellaneous diseases	8	2	1	76.6 ±1.29	68.6‡ ± 5.02	17.5§ ±4.26	2.77§ ±0.79	11.4 ±2.22	1.79 ±0.16	988§ ±445

* Lymphocytic infiltrate.
† Micrograms of phosphorus liberated in 6 min. at 37°C.
‡ Probability of chance occurrence of difference between means as compared with Group A is .05 to .01.
§ Probability is <.01. P values have been corrected for small sample size.

TABLE IV. Relation of Atrophy to Composition of Skeletal Muscle. (Mean values ± S.D.).

Group	Members of group	No. of individuals	% water	NPN as % dry wt	Total protein as % dry wt	Myosin N as % protein N	Myosin as % wet wt	Collagen N as % protein N	Collagen as % wet wt	ATP-ase units per mg myosin N
I	Normals	13*	77.9 ±1.68	1.31 ±0.12	74.0 ± 3.67	21.1 ±5.19	3.43 ±0.65	11.4 ±3.46	1.67 ±0.55	1475 ±256
II	Patients without atrophy	12†	76.8 ±2.27	1.30 ±0.46	67.2‡ ± 8.79	17.1‡ ±3.76	2.66§ ±0.72	11.3 ±2.96	1.61 ±0.46	1000§ ±340
III	Patients with atrophy	14	75.6‡ ±2.98	1.20 ±0.19	60.3§ ± 7.54	14.5§ ±3.14	2.09§ ±0.33	9.98 ±2.81	1.29‡ ±0.44	881§ ±324

* One individual was not included in Table III, as amount of muscle tissue available was insufficient for satisfactory search for muscle lesions.
† One patient in this group included in Table I was not included in Tables III and IV, as chemical analysis was incomplete.
‡ Probability of chance occurrence of the difference of the means is between 0.05 and 0.01.
§ Probability is 0.01 or less. P values have been corrected for small sample size.

sections.§ The criteria for the presence of atrophy were (1) an increase in the number of sarcolemmal nuclei and (2) a diminution of the mean fiber diameter. Both these changes are observed in the presence of well developed atrophy. With a lesser degree of atrophy, the mean fiber diameter may be reduced only slightly, because of a lack of uniformity in the degree of atrophy of the various muscle fasicles, despite the increase in the number of sarcolemmal nuclei in the atrophied areas. In such cases the atrophy was characterized as mild. All patients in whom the muscle sections demonstrated mild

§ The authors are indebted to Dr. L. Sokoloff for this study.

atrophy or greater are included in the group of "patients with atrophy" (see Group III, Table IV).

Results. 1. *Water.* The percentage of water varied significantly from the normal only in the patients with gout and degenerative joint disease (Table III) and those with atrophy (Table IV). The mean value in the normal subjects was 77.9%. Because of the relative constancy of the muscle water content, it is possible to use wet weight as a base line in the comparison of many of the analytical data.

II. *Total protein.* The concentrations of total protein, as expressed in Table III in per cent of dry weight, are sensitive to variation in the adipose fat content of the muscle. Significance may not be attached to the absolute values of the total protein for this reason. The concentrations of myosin and collagen, however, have been expressed below in per cent of the total protein. These values are independent of variation in the adipose fat. In the 13 normal volunteers (Group I, Table IV) the total protein was 74% of the dry weight. Myosin constituted 21.1% and collagen 11.4% of the total protein. Though the volunteers were carefully screened, and showed no clinical or microscopic evidence of atrophy, in 3 of the 13 volunteers, distinct lymphocytic infiltrates were observed, and in 3 others, smaller or "doubtful" lesions were found. The relationship between the presence of these lesions and muscle composition (Table IV) is discussed below.

III. *Myosin.* The myosin fraction of the total protein, (Table III), was significantly reduced in all categories of patients studied except the group with degenerative joint disease. It was also diminished in the 3 volunteers in whom definite muscle lymphocytic infiltrates were present. Whereas the mean value of the myosin fraction of the total protein in the 6 normals without lesions was 23.9%, in the 3 "normal" individuals with lesions, the corresponding figure was 14.0%. The lowest value among patients, 11.9%, was recorded in the group with gout. The myosin concentrations as per cent of wet weight were correspondingly reduced.

IV. *Collagen.* The per cent of the muscle protein present as collagen did not depart significantly from the normal in the various disease states (Table III). The range was 9.48 to 11.2% in the patient group as compared with an average of 11.4% in the normals. The absolute content of collagen, calculated on the basis of wet weight, however, was somewhat decreased in patients with rheumatoid arthritis and degenerative joint disease.

V. *Adenosinetriphosphatase.* The ATP-ase activity of the myosin extracts (Table III)

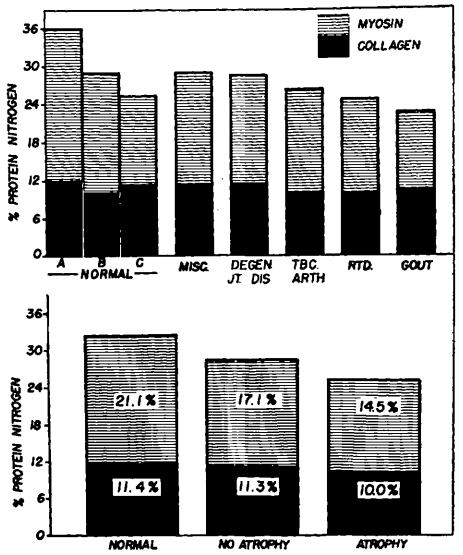

FIG. 1.

Above: Per cent of total protein nitrogen of gastrocnemius muscle as myosin and collagen. A—Subjects with no "nodules"; B—Subjects with doubtful "nodules"; C—Subjects with definite "nodules." Below: Relation between atrophy and concentration of myosin and collagen in muscle, expressed as % total protein nitrogen.

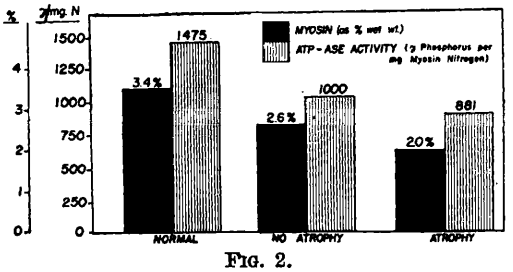

FIG. 2.

Relationship between presence of atrophy and the myosin and ATP-ase activity of gastrocnemius muscle. Myosin is expressed as % wet weight and ATP-ase activity as μg of phosphorus hydrolyzed in six minutes.

showed little variation among control subjects. It was, however, significantly reduced in all patient groups. The mean ATP-ase activity in the normals was 1437 units per mg of myosin nitrogen (Table III, Fig. 2.) In patients with rheumatoid arthritis a mean value of 1038 units was recorded. The lowest mean value, 681 units, was found in the group with gout. There was no significant statistical difference in the ATP-ase activity of the patients with miscellaneous diseases as compared with patients with rheumatic diseases. There was, however, significant difference between these two groups and the normals.

V. *Relationship of composition to muscular atrophy.* The 39 subjects were divided into 3 groups using the criteria of muscular atrophy cited above. In Group I (Table IV) were placed the 13 control subjects, in Group II the 12 patients without microscopic evidence of atrophy, and in Group III the 14 patients whose muscle sections demonstrated atrophy. Significant decreases in myosin and ATP-ase content were observed in Group II. In Group III, these changes were even more marked. Though there was a moderate fall in the wet weight per cent of collagen in the patients of Group III, the collagen fraction of the total protein was not significantly reduced in either Group II or Group III.

Discussion. It is apparent that many factors may influence the protein composition of muscle in a group of hospital patients. The age of the patient, the amount of physical activity and the nutritional status are to be considered in addition to the specific type of disease process. In selecting as experimental subjects the patients of a ward population, and comparing them with a group of active, healthy controls, it is difficult to exclude as a variable any of the above mentioned conditions. It is not surprising, therefore, that the differences in the analytical pattern noted between volunteer subjects and the patients are not specifically characteristic of any of the several incapacitating diseases studied. The changes noted, which are discussed below, appear rather to be dependent on general differences between normal individuals and the broad group of patients with musculo-skeletal disease.

Myosin, as referred to in this paper, is essentially the actomyosin of Szent-Gyorgyi (6). The determination of this protein in muscle is difficult, and the quantity extracted increases with the time of extraction. Fischer and Ramsey(2) in discussing this problem, point out that with advancement of physico chemical methods the definition of myosin has changed considerably, and that as yet "no ratio between true myosin content and myosin extractable with a certain method can be established." These authors compared the amount of myosin extracted under standard conditions with the total amount of non-collagenous protein of muscle as a base line. In the present paper, we have compared the myosin similarly extracted with the total protein content of the muscle. For the determination of myosin, we have used essentially the same extraction procedure as employed by Fischer and Ramsey, except that we have substituted potassium chloride, for lithium chloride, having found in preliminary experiments that the use of both salts gave approximately the same results. To assure more complete extraction, we have carried out two successive one-hour extractions instead of the single procedure employed by Fischer and Ramsey. The analytical figures obtained must rest in our experiments, as in those of previous investigations, essentially on an "operational definition" of the myosin. Comparison of analytical data from different individuals is based upon strictly identical procedure of extraction. The reliability of this method is indicated by the relatively good internal consistency of the analytical figures of myosin in the different groups (Table III). Comparison of these with the collagen analyses (Table III) on the same groups of individuals shows a similar spread of the analytical data in terms of standard deviation from the mean. The use of 0.4 molar alkali halide salts in the extraction of myosin has hitherto been applied only in animals and lower forms. The application of this type of extraction procedure to human muscle involves the assumption that human myosin has solubility

characteristics similar to those of animal myosin. This assumption appears to be justified by the work of Bailey(7) who observed no significant differences in the properties of myosin, extracted by the method of Greenstein and Edsall(5) from a large number of animal species.

Myosin nitrogen in the 6 normal individuals without lymphocytic lesions was 23.9% of the total protein nitrogen and myosin was 3.99% of the wet weight of the muscle. The latter figure is somewhat lower than that of 4.75% observed by Fischer and Ramsey in normal rabbit muscle. The myosin content was significantly decreased in the 3 volunteers who had lymphocytic infiltrates but were otherwise normal individuals. It is difficult at this time to explain this decrease. It is important to note, however, that the ATP-ase activity of the extractable myosin from these individuals was not less than that from the other normals. Myosin was decreased significantly in all groups of patients, both as a fraction of the total protein and also as percent of wet weight. In experiments in which atrophy has been artificially induced in rabbit muscles by such measures as tenotomy and denervation, Fischer and Ramsey (2), reported decreases in myosin similar to those which we have observed in patients.

Collagen as per cent of total protein did not vary significantly between normals and patients. Though the precision of the determination of collagen is not high, the results indicate that the fraction of the total protein present as collagen was similar in both groups. It was surprising to find no relative increase in the collagen concentration in the presence of atrophy. Since neither the proteins of skeletal muscle other than myosin and collagen nor the lipids were analyzed, we cannot present data to account for the constancy of collagen concentration in the presence of falling myosin concentration. It would appear, however, that the process of atrophy in patients with musculo-skeletal disease, at least when it is mild to moderate, is not one of collagen replacement.

Beginning with the experiments of Engelhardt and Ljubimova(11) and leading up to the recent work of Szent-Gyorgyi(6), it has been shown that classical myosin (actomyosin of Szent-Gyorgyi) is intimately bound with, though separable from the enzyme ATP-ase (9,12,13). The activity of this enzyme in muscle undergoing atrophy has not as yet received attention in animal experiments. The concentration of ATP-ase in muscle may be of particular significance in evaluating the functional status of muscle because of the important role which this enzyme plays in the transformation of chemical to mechanical energy(6,7,11,14). It was, therefore, a matter of interest to study the effect of atrophy, in the course of disease, on the concentration of this enzyme system. The degree to which the ATP-activity of the myosin extracted from patients was diminished was unexpected. These findings suggest that myosin may contain a variable amount of ATP-ase from individual to individual and lends support to the point of view cited above, though not entirely accepted(9,12,13), that myosin and ATP-ase are independent substances.

Summary. 1. Gastrocnemius muscle samples from 13 normal individuals and 27 patients with arthritis and a number of miscellaneous diseases were analyzed for total protein, non-protein nitrogen, collagen, myosin, adenosinetriphosphatase and water. The chemical data were correlated with the results of histological examination of the muscle samples analyzed. 2. In muscle from 13 volunteers, myosin constituted 21.1 per cent of the total protein and 3.99 per cent of the wet weight. Significantly lower values were found, however, in all groups of patients, as well as in 3 normal volunteers in whom lymphocytic infiltrates were demonstrated microscopically. 3. The collagen fraction of the total protein did not depart significantly from the normal in any of the diseases studied, and did not change significantly in the pres-

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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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