reported in experimental Vit. B_6 deprivation in man(9).

The failure to demonstrate increased XA in urine after tryptophane loading does not exclude lack of pyridoxine group of vitamins. Another metabolite of tryptophane metabolism, such as 3-hydroxykynurenine, acetyl kynurenine or kynurenine, may have been the accumulated products in these cases(10). It is possible, as suggested by Roberts and Baxter(11), that the ultimate compound deficient in Vit. B_6 shortage is gamma amino butyric acid (GABA) which is formed by decarboxylation of glutamic acid, a reaction activated by pyridoxal-5-phosphate as coenzyme.

Three of the 5 rum fitters subsequently recovered through a stormy course with associated delirium tremens. One rum fitter presented as *status epilepticus*. The others had 1-2 isolated seizures. EEG's were normal in patients with rum fits. One of the 2 patients with alcoholism and associated epilepsy had an abnormal EEG (No. 7). All rum fitters showed multiple signs suggestive of deficiency of members of the Vit. B complex group of vitamins. the most common was depapillation and reddening of tongue, diffusely reddened oral mucous membranes, and injected conjunctivae. None showed evidence of scurvy.

Convulsions in alcoholics have been related to alcohol withdrawal and to idiopathic or post-traumatic epilepsy. It has been possible to separate a group of these patients who exhibited Vit. B_6 deficiency and who had no evidence of previous epilepsy, or of abnormal EEG's.

Summary. 1. Increased xanthurenic acid excretion after 10 g dl-tryptophane was demonstrated in 3 of 5 patients with rum fits. This metabolic defect was corrected by Vit. B_6 in a second tryptophane load test. 2. Patients with alcoholism and associated epilepsy, acute and chronic alcoholism, cirrhosis, acute hallucinosis-tremulousness, acute peripheral neuropathy, Wernicke-Korsakoff syndrome, and a non-alcoholic, healthy individual did not demonstrate significant Vit. B_6 deficiency by this test. 3. It is postulated that Vit. B_6 deficiency is etiologically related to rum fits.

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Experimental Lathyrism in the Rat. Nature of Defect in Epiphysial Cartilage. (24204)

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Profound alterations have been described in the cartilages, bones and aortas of animals fed the seeds of *lathyrus odoratus* or treated with certain chemical compounds such as beta-aminopropinonitrile (BAPN), aminoacetonitrile (AAN)(1), and most recently semicarbazide(2). No agreement has been reached concerning the basic metabolic defect

TABLE I. Hydroxyproline (Collagen) Content of Epiphysial Cartilage from AAN-Treated and Control Animals.

AAN-treated				Control			
Wt dry cartilage (mg)	Hydroxy-proline content (μg)	Collagen content (μg)	% collagen	Wt dry cartilage (mg)	Hydroxy-proline content (μg)	Collagen content (μg)	% collagen
1.3	3.2	240	18.6	3.0	6.6	494	16.5
1.45	3.8	282	19.5	1.5	3.4	257	17.1
1.2	2.5	186	15.5	1.25	2.8	218	17.4
2.0	4.4	328	16.4	1.3	2.6	194	15.0
3.65	8.0	597	16.6	1.65	3.6	267	16.3
2.4	5.9	440	18.3	.85	2.3	171	20.1
		Avg	17.5			Avg	17.1

responsible for the lesions. Both mucopolysaccharide and collagen components of the tissues in question have been implicated(1). Several years ago one of us(3) called attention to an extraordinary physical transformation of the epiphysial cartilage of animals fed sweet pea seeds, stating that "the epiphysial cartilage has become a sort of pulpy mass which appears in the fresh state, under the microscope, so lacking in cohesion that individual cells are found floating about by themselves." Subsequent observations of cartilage from animals which had received BAPN, AAN, and semicarbazide have confirmed this observation. The conclusion has seemed inescapable that, in comparison with fresh normal epiphysial cartilage which has a certain physical stability largely due to presence of collagen fibrils in the matrix, cartilage from treated rats might be deficient in this important fibrous protein. The experiments described herein, which were undertaken to demonstrate such a defect, have led us to postulate an entirely different concept concerning the change in epiphysial cartilage injured by lathrogenic materials.

Materials and methods. Young albino rats, approximately 40 g in weight, were given 20 mg AAN* by daily gavage. Although alteration in epiphysial cartilage can be detected as early as 24 hours, the optimal interval for maximum change is about 48 hours. The epiphysial cartilage, which by this time has become a mushy mass, was dissected away from the bone and perichondrial tissues under a binocular microscope, dried over phosphorus pentoxide and weighed on a torsion balance. For chemical determinations, samples were hydrolyzed in 6N HCl at 138°C for 3 hours. A modified(5) Neuman and Logan(4) procedure for hydroxyproline was then carried out. Collagen is expressed in terms of hydroxyproline by multiplying the amount of the latter by the factor, 7.46(4). For the centrifugation and electron microscopic studies known amounts of tissue were placed with 2 cc of distilled water in a plastic tube and disintegrated at 1°C with a microhomogenizer (Lourdes). The homogenate was then placed in tubes of the SW-39 rotor of the preparative model "L" Spinco centrifuge and spun at 5,000 rpm for 10 minutes, 10,000 rpm for 30 minutes, and finally 30,000 rpm for 30 minutes. The sediments of each centrifugation were resuspended in distilled water; drops of these and the final supernate were placed on grids coated with formvar or parlodion films, dried and shadowed with uranium at angles of arc tan 1/10. Electron microscopic observations and micrography were carried out with an RCA-electron microscope EMU-3B. Epiphysial cartilage from normal control animals was treated in similar fashion for chemical and electron microscopical studies.

Results. Chemical analysis of cartilage, freed of bone and perichondrial elements, from treated and control animals is shown in Table I. The normal collagen content, based on hydroxyproline determination of this tissue, is about 16-18% on a dry weight basis. It will be noted that little difference from this is found in animals receiving the lathyrogenic compound. This might lead one to assume

* Kindly supplied by Dr. I. V. Ponseti.

that the collagen content of the cartilage from AAN-treated rats in unaffected. Such an assumption is contrary to the gross appearance of the cartilage which has been described

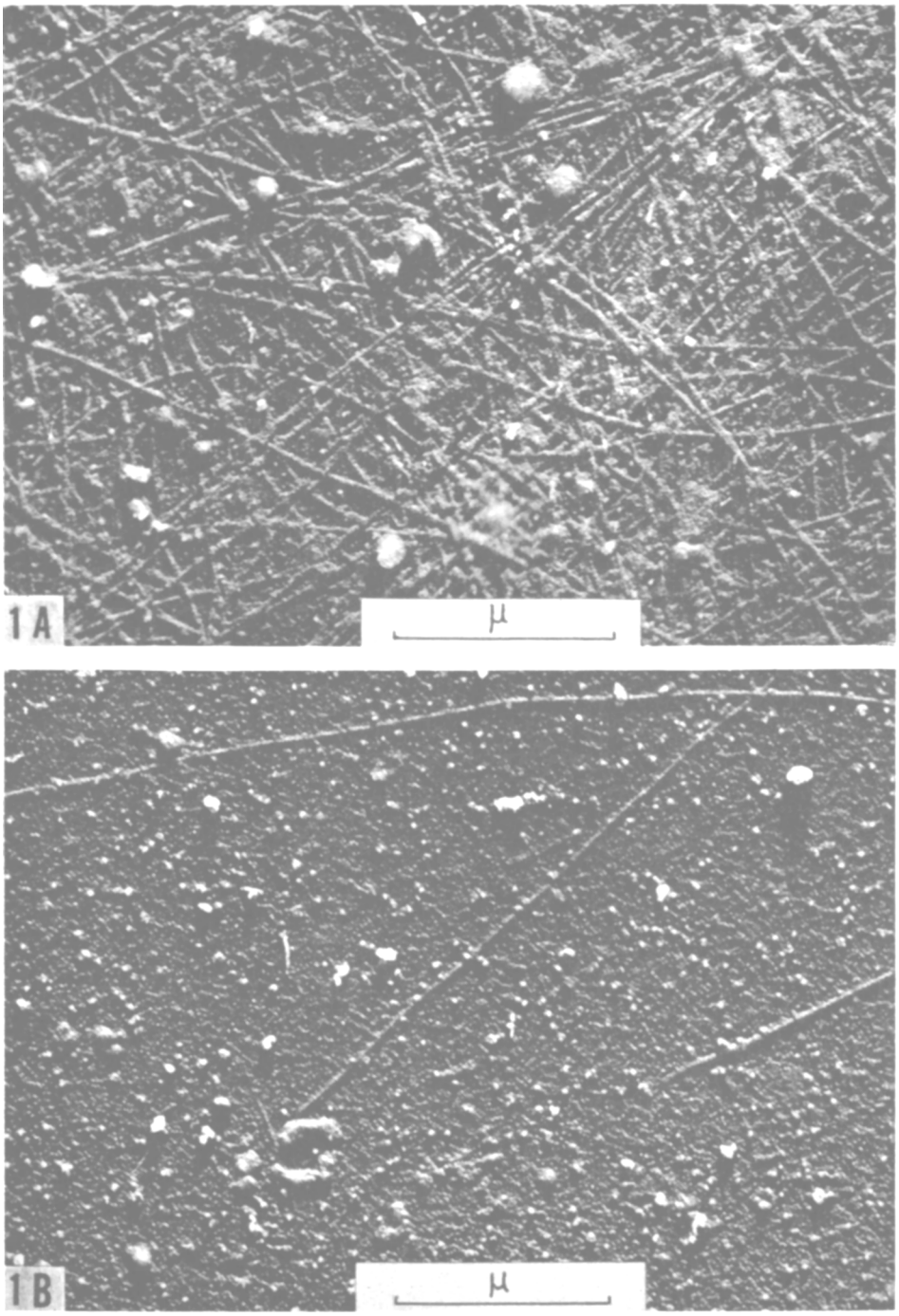

FIG. 1. Electron micrographs of rat epiphysial cartilage matrix. Sediment of 30,000 rpm suspended in water, air-dried on parlodion film, shadowed with uranium ($\tan^{-1}$ 1/10). (A) normal control and (B) AAN-treated animal, ×31,200.

above. The following studies of centrifuged fractions of homogenized cartilage are pertinent.

Electron microscopical observations of the sediments of 5, 10, and 30 thousand rpm from normal animals revealed fibrils ranging in width from 180A to 220A with varying lengths. Many of these fibrils were more than 80 μ long. Some of the fibrils showed periodicities of about 100-135A and 180-200A, while others did not suggest any periodicity in these airdried unstained shadowed specimens.

Fig. 1A shows the dense network of filaments found in the sediment of 30,000 rpm from the normal controls. In its supernate, Fig. 2A, fibrils are virtually absent, though a high concentration of particles is seen. The majority of these particles have widths of 100-125A or less; others vary from 250A to as high as 1500A. The appearance of the larger particles suggests that they are aggregates of the small ones since the majority of this size would have been sedimented at this centrifugal field, assuming a 1.2 g/cc particle density.

In contrast to these observations the number of fibrils in the sedimentation fractions from the cartilage of the AAN-treated animals is considerably reduced. Fig. 1B shows filaments from the sediment of the 30,000 rpm fraction of AAN treated animals with widths in the range of 100A to 200A and particles with widths of about 120A or more. In contrast to the normal controls the 30,000 rpm supernate of the treated rats in the majority of experiments appeared to contain a larger number of particles of about 40-90A in width and others in the range of 200-500A as seen in Fig. 2B. Many of the smaller particles appear to aggregate uniformly as seen throughout this electron micrograph.

Discussion. Examination of epiphysial cartilage in the fresh state clearly shows the physical deterioration which can be induced in a few days by feeding sweet pea seeds or by administration of lathyrogenic agents. This alteration appears to be associated with normal growth of cartilage, which we (unpublished observations) and others(1) have noted. Since the growth cycle of the cells of the epiphysial cartilage of rats at this age is about 48 to 60 hours(6), it is clear that 2 days of treatment would be sufficient for formation of new, diseased matrix which then might be expected to exhibit the alterations described above. The few fibers which are present doubtless date from the period before treatment was begun. It is also possible that some new fibers are formed, though not to the extent seen in the normals.

The pertinent literature on the pathogenesis of the changes in cartilage and other tissues has been reviewed(1). Several recent contributions are of interest in light of the observations presented herein. When subcutaneous abscesses are produced in rats, there is a delay in appearance of mature collagen and an abnormal persistence of amorphous intercellular material in AAN-treated animals as compared with controls(7). When incisions are made in the skin of rats, there is decreased formation of collagenous fibers in animals treated with lathyrogenic agents(8). Finally, in bone and cartilage from chicks treated with BAPN no alterations in hydroxyproline, hence collagen, content have been detected, though an increase in solubility of components of cartilage in 1M sodium chloride has been noted (Levene, C. I., Fed. Proc., 1958 v17, 445, abstract). All of these observations are in keeping with the findings we have described in epiphysial cartilage.

In order to have collagen fiber formation several conditions must be fulfilled. First, the constituent amino acids must be in optimal concentrations; second, they must be in proper alignment and linkage to produce the basic collagen molecule (tropocollagen)(9). These molecules then aggregate to form mature collagen fibers. Fiber formation *in vitro* has been described to result from the action of certain physiochemical procedures on the tropocollagen molecule(9). The *in vivo* mechanism of fiber formation is not as yet understood. The observations presented herein would appear to implicate the mechanism responsible for transformation of tropocollagen into mature collagen molecules. The basic defect, whether physical or enzymatic,

remains to be elucidated. The possible role of mucopolysaccharide in this phenomenon must be kept in mind(10).

Summary. The hydroxyproline, and hence

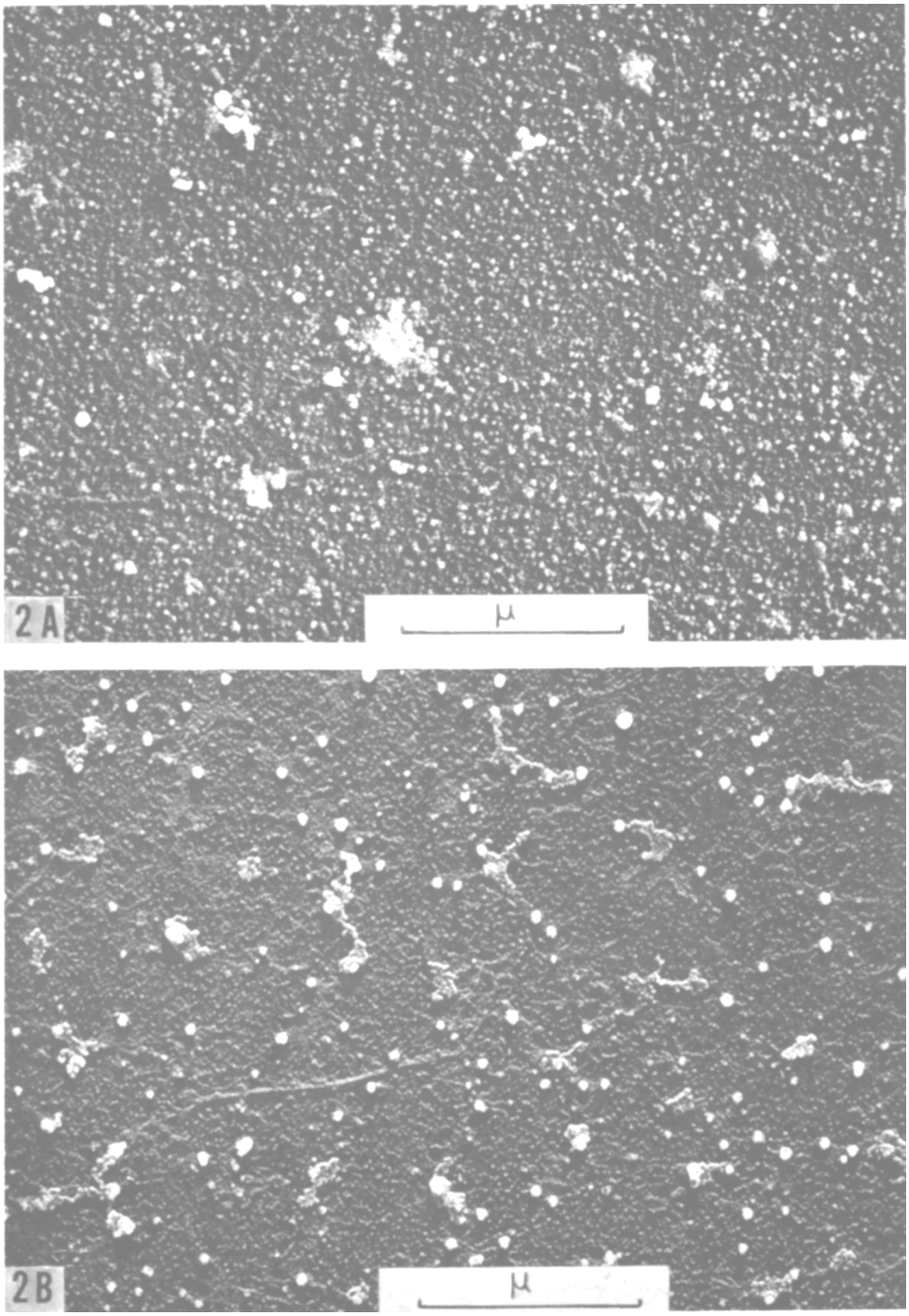

FIG. 2. Electron micrographs of rat epiphysial cartilage matrix supernate of 30,000 rpm fraction No. 4 in distilled water. Air-dried on parlodion film, shadowed with uranium ($\tan^{-1}$ 1/10). (A) from normal control and (B) AAN-treated rat, ×31,200.

collagen, content of epiphysial cartilage from rats treated with AAN is not reduced from the normal. Homogenized fractions of cartilage from rats which have received AAN are virtually devoid of collagen fibrils when examined with electron microscope. The defect appears to be a failure of the tropocollagen molecule to form collagen fibers.

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Cytopathogenicity in Tissue Cultures by a Tumor Virus from Mice. (24205)

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A virus, which we shall refer to as SE polyoma virus, was recovered from tissue cultures inoculated with tumor material from mice and was shown to induce multiple tumors in mice(1,2,3) and hamsters(4). The present report describes cytopathic effects which appear to be produced by the SE polyoma virus in tissue cultures. The results so far obtained show that these effects parallel the capacity of tissue culture fluids to induce tumors in hamsters.

Methods. Tissue cultures from embryos of Swiss mice were propagated in 2 ounce prescription bottles by modification of the trypsin digest method of Youngner(5). Synthetic medium 199(6) containing 2% inactivated calf serum was used to initiate growth, and medium 199 with 1% inactivated rabbit serum was used as maintenance medium. For quantitative titration or serum-virus neutralization tests the cells were retrypsinized with 0.25% trypsin in Earle's balanced salt solution(7) at 35°C for 15 to 20 minutes, centrifuged, washed, resuspended in initial medium and dispensed in roller tubes. The cells in the retrypsinized cultures were clear and uniform. The strain of the agent used, originated from liver and spleen of a single AKR mouse with spontaneous leukemia and was passed serially into 3 newborn mice, with intervening passages in tissue cultures, followed by 2 to 4 passages in newborn hamsters with intervening tissue culture passages. Each passage animal had developed neoplasms. Tumor tissue from only one animal from each litter was used for passage. Titration of virus at several passage numbers was made in roller tube cultures of retrypsinized mouse embryo cells by preparing decimal dilutions and inoculating 4 tubes/dilution with 0.5 ml each. The cultures were refed at weekly intervals with 2 ml of nutrient fluid. Serum-virus neutralization tests were also carried out in roller tube cultures. Mixtures of equal volumes of 10-fold dilutions of the polyoma virus and 1% antipolyoma virus rabbit serum and mixtures of similar dilutions of virus and normal rabbit serum, were held at 36°C for 4 hours before being added to the roller tubes. The antiserum was made by hyperimmunizing rabbits with fluids from tissue cultures infected with the virus. The tubes were refed after one week with medium containing 1% immune and normal rabbit serum respectively. The final microscopic examinations were made at 3 weeks. To demonstrate induction of tumors, hamsters 1 to 3 days of age were injected with 0.2 ml doses of tissue culture fluid