The septa are the thin hematoxylin-stained lines that can be seen crossing the inclusion body in hematoxylin-eosin preparations. Rake and Blank(2) have considered these septa to contain ribonucleic acid, basing this conclusion upon staining with pyronin-methyl green. Kurnick(10) has demonstrated that depolymerized desoxyribonucleic acid stains with pyronin similarly to ribonucleic acid. The locules between the septa, where the mature elementary bodies lie, contain desoxyribonucleic acid since these regions are stained by the Fuelgen method(2). It seems apparent, then, that the site of formation of the elementary bodies is in the material which is selectively stained with pyronin.

In the early infected cells, many of the elementary bodies are thinner than most of those found in the mature inclusions and in addition lancet shaped forms are seen. There is also an apparent absence of segmented matrix in the cytoplasm of these early infected cells which leaves the origin and fate of the elementary bodies at the early stage of infection purely speculative. It should be noted that these latter irregularly shaped bodies, which have been considered here to be elementary bodies, differ in appearance from structures that have been interpreted as mitochondria in epidermal cells and further that they have never been seen in thin sections from control material from the epidermis examined with the electron microscope.

Summary. Thin sections of *Molluscum contagiosum* lesions from man have been examined with the electron microscope. Cells were seen in the prickle cell layer of the epidermis which contained only a very few cytoplasmic elementary bodies. These are considered to be early infected cells. Cells were seen with varying numbers of elementary bodies scattered through the cytoplasm while others were entirely filled with elementary bodies forming a mature inclusion. The elementary bodies in the early infected cells differed somewhat in shape from most of the elementary bodies seen in the larger inclusions. The mature inclusion bodies were divided into locules by septa. The locules contained mature elementary bodies. The elementary bodies appear to form from the material composing the matrix of the septa by a process of segmentation and condensation. The elementary bodies themselves were sectioned and appeared in some instances to have a formed cortex with a less dense interior.

10. Kurnick, N. B., *Cold Spring Harbor Symposia on Quantitative Biol.*, 1947, v12, 141.

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Chemical Differentiation of Cartilage and Bone. II. Sulfatase Activity.* (18945)

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The presence of the polysaccharide, chondroitin sulfuric acid, has been recognized in cartilage for many years. From time to time the possible role of this material in the calcification mechanism of cartilage has been raised. That calcium might be selectively bound to the organic portion of cartilage was suggested a number of years ago by Freudenberg and György(1). More recently the hypothesis(2-4) has been brought forward

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1. Freudenberg, E., and Gyorgy, P., *Biochem. Z.*, 1921, v121, 142.

2. Levine, M. D., Rubin, P. S., Follis, R. H., Jr., and Howard, J. E., *Trans. First Conf. on Metabolic Interrelations,* Josiah Macy Jr. Found., 1949, p41.

3. Rubin, P. S. and Howard, J. E., *Trans. Second Conf. on Metabolic Interrelations,* Josiah Macy Jr. Found., 1950, p155.

that certain changes, *i.e.* depolymerization of chondroitin sulfuric acid, might take place in the calcification zone; as a result certain groupings would be made available to bind calcium. So too, liberation of sulfate might lead to local changes in pH which might affect the solubility of calcium phosphate. In addition, since there is a pronounced reduction in the sulfate content of cartilage as it is transformed into bone(5), we have felt it worthwhile to investigate costal cartilage, particularly the calcifying area, to determine whether any sulfatase activity could be demonstrated. The method is based on the use of p-nitrophenyl sulfate, a colorless compound, which in the presence of sulfatase is converted into yellow p-nitrophenol(6).

Material and methods. P-nitrophenyl sulfate was prepared according to the methods of Burkhardt and Lapworth(7) and Huggins and Smith(6). I am indebted to Dr. Norman Weisman for this synthesis. Tissue was obtained at autopsy from infants and young children, dying of a variety of causes, and from puppies. Slices of costal cartilage were made beginning in the most undifferentiated region and continuing to the area of calcification by the method previously described(8). Slices of bone with and without marrow elements, the latter being removed by a high pressure jet of water, and strips of periosteum were also assayed. The substrate consisted of p-nitrophenyl sulfate, .005 M, 1 cc and .5 N acetate buffer, pH 5.8, 2 cc. The moist tissue slices or strips were weighed on a torsion balance and placed in the substrate to which a small crystal of thymol was added. The tubes were incubated for 24 hours at 37°C. At this time 2 cc I N NaOH were added. Controls were carried through the procedure as described by Huggins and Smith (6). The intensity of color was measured at 420 mμ in a Coleman Junior Spectrophotometer using a standard calibration curve prepared from recrystallized p-nitrophenol. The results are expressed in units, 1 phenolsulfatase unit being defined as the amount of enzyme which produces the color equivalent to 10 γ of p-nitrophenol in a volume of 5 cc in 24 hours at 37°C in .5 N acetate buffer, pH 5.8, the substrate concentration being .005 M. The activity of the system was demonstrated by takadiastase which, of course, exhibits sulfatase activity(6).

TABLE I. Phenolsulfatase Activity of Cartilage, Bone and Periosteum.

Tissue	No. subjects	Phenol-sulfatase units*
Costal cartilage,† human	10	0
" " † dog	3	0
Rib shaft & marrow elements, human	10	1.2
Rib shaft & marrow elements, dog	3	.8
Rib shaft, alone, human	10	0
" " " dog	3	0
Periosteum, human	10	.2
" dog	3	.05

* Per mg wet wt; see text for definition of unit.
† All stages of differentiation(8).

Results. In Table I are shown the results of determinations performed on the various tissues already mentioned. No activity is found in cartilage until the zone of hypertrophic cells being invaded by capillaries is reached. However, when the blood elements in this area are removed activity disappears. So too, bone devoid of marrow elements exhibits no activity. Periosteum is more difficult to free of blood; this doubtless explains the presence of slight activity in washed specimens.

Discussion. It is apparent from the foregoing that there is no demonstrable sulfatase activity in cartilage and in bone freed of marrow elements for the substrate which has been employed. From studies reported in the literature which have been carried out with other tissues and substrates it would appear reasonable to consider "sulfatase" a nonspecific enzyme which is able to hydrolyze sulfate from a variety of organic compounds,

4. Gersh, I., and Catchpole, H. R., *Am. J. Anat.*, 1949, v85, 457.

5. Logan, M. A., *J. Biol. Chem.*, 1935, v110, 375.

6. Huggins, C. and Smith, D. R., *J. Biol. Chem.*, 1947, v170, 391.

7. Burkhardt, G. N., and Lapworth, A., *J. Chem. Soc.*, 1926, v1, 684.

8. Follis, R. H., Jr., *Bull. Johns Hopkins Hosp.*, 1949, v85, 360.

one of which is chondroitin sulfuric acid(9). The absence of activity in cartilage is of further interest because of the recent demonstration of the uptake of S^{35} by epiphyseal cartilage(10) and its incorporation into chondroitin sulfate(11).

Summary. Using p-nitrophenyl sulfate as substrate no sulfatase activity could be demonstrated in costal cartilage at varying stages of differentiation or in bone providing no red blood cells or marrow elements were present.

9. Soda, T., *Die Methoden der Fermentforschung*, Academic Press, N. Y., 1945, v2, 1695.

10. Dziewiatkowski, D. D., Benesch, R. E., and Benesch, R., *J. Biol. Chem.*, 1949, v178, 931.

11. Dziewiatkowski, D. D., *J. Biol. Chem.*, 1951, v189, 187.

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Preferential Incorporation of Formate Carbon into Leukemic Blood Cells as Indicated by Autoradiography.* (18946)

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Demonstration of the incorporation of C^{14} into individual blood cells was first accomplished by Boyd, *et al.*(1) who showed by means of autoradiograms that the alpha carbon atom of glycine is taken up most rapidly by rat lymphocytes. The polymorphonuclear leukocytes incorporated C^{14} from glycine less rapidly than the lymphocytes and the circulating erythrocytes were considerably less avid in the utilization of the glycine carbon. The most obvious explanation for this difference in C^{14} content of the different cell types at 25 hours after injection appears to be the difference in their rate of formation (synthesis employing the alpha carbon of glycine as a precursor) and disappearance from the peripheral blood.

In the light of these results, it appeared of interest to study hematopoiesis in leukemic animals using the autoradiographic technic. If we assume that precursors of the various types of blood cells and the mature blood cells are exposed to the same order of concentration of a labeled compound, preferential incorporation by neoplastic cells suggests (1) a difference in the rate of synthesis (if the labeled compound or a degradation product is a metabolite) or (2) qualitative metabolic differences between the formed normal or neoplastic cells.

As a first approach to the study of hematopoiesis in experimental leukemia utilizing the autoradiographic technic, C^{14}-labeled sodium formate has been employed. Formate has been reported to be a precursor of the 2- and 8-carbon atoms of desoxyribose nucleic acid (DNA) and ribose nucleic acid (RNA) guanine and adenine and the 5-methyl carbon of DNA thymine(2). Also, formate carbon has been shown to appear in the alpha carbon of serine and in glycogen(3), in the methyl group of methionine, and the methyl groups of choline(4).

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1. Boyd, G. A., Casarett, G. W., Altman, K. I., Noonan, T. R., and Salomon, K., *Science*, 1948, v108, 529.

2. Totter, J. R., Volkin, E., and Carter, C. E., *J. Am. Chem. Soc.*, 1951, v73, 1521.

3. Sakami, W. J., *J. Biol. Chem.*, 1948, v176, 995.

4. Welch, A. D., and Sakami, W. J., *Fed. Proc.*, 1950, v9, 245.