The result demonstrates a remarkable uniformity of ^{32}P incorporation into different classes of DNA, suggesting that all DNA molecules are synthesized at the same rate in the course of cell division. It should be noted, however, that a considerable degree of heterogeneous labelling of different DNA fractions has been recently observed[11] when the cell suspension of the rabbit bone marrow or isolated nuclei of the rabbit thymus are incubated *in vitro* with thymidine-^{14}C. The discrepancy might be attributed to the difference in the isotopic precursors used or merely to the difference of experimental conditions such as between those *in vivo* and *in vitro*.

In the next experiment, a preparation of DNA labelled with ^{32}P was digested with crystalline deoxyribonuclease, and the fragments were separated on a Dowex-1-formate column using formic acid and formic acid-ammonium formate mixture as eluants. The effluent was roughly classified into six fractions according to the elution concentrations. No particular effort was made to identify each fraction obtained. Here again, no heterogeneous distribution of ^{32}P in different DNA fragments was found (Table II) despite the fact that the ratio of E_{260} to E_{275} varied widely through the fractions. The result obtained here is in good agreement with that of MOLDAVE AND HEIDELBERGER[5] who reported intramolecular homogeneity in DNA synthesis as seen by the incorporation of ^{32}P and glycine-2-^{14}C. Their results together with ours would indicate that phosphorylation of DNA precursors takes place in a very short period so that non-uniform incorporation of ^{32}P is not detectable in such a time interval studied. However a definite conclusion on this matter will have to be left until a more precise fractionation of ^{32}P-labelled DNA digests is undertaken.

TABLE II

DISTRIBUTION OF ^{32}P AMONG DIFFERENT FRACTIONS OF FRAGMENTS OF RABBIT APPENDIX DNA DIGESTED WITH DEOXYRIBONUCLEASE

Fraction No.		*1*	*2*	*3*	*4*	*5*	*6*	*7*
	Unfractionated							
R.S.A.	174	157	157	149	176	170	133	199

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The conversion of 1-^{13}C-D-glucuronolactone to 5-^{13}C-L-xylulose in a pentosuric human

ENKLEWITZ AND LASKER[1] reported in 1935 that D-glucuronolactone greatly enhances the excretion of L-xylulose in individuals with essential pentosuria. More recently, we confirmed their finding and also showed that small increases in urinary xylulose levels occur when normal humans and guinea pigs are fed glucuronolactone[2]. The high yield of xylulose in pentosuric individuals suggests

that the conversion is a fairly direct one. The low yield in "non-pentosuric" humans and guinea pigs is consistent with accumulating evidence that L-xylulose is a normal metabolic intermediate[3,4]. By analogy with the biological conversion of 6-phospho-D-gluconic acid to D-ribulose-5-phosphate[5] and of D-glucuronolactone and L-gulonolactone to L-ascorbic acid[6,7], mechanisms for the transformation of D-glucuronolactone to L-xylulose have been suggested which involve the loss of the carboxyl carbon[2].

Experimental work on the mechanism of the transformation depended in part upon the availability of suitably-labeled glucuronolactone. The Corn Products Refining Company, Argo, Illinois, developed syntheses of 1-^{13}C- and of 6-^{13}C-D-glucuronolactone, and it is through the generosity of Dr. NEAL E. ARTZ of that firm that we obtained samples of these preparations for tracer experiments in a human. In addition, Dr. FRANK EISENBERG, Jr., and Dr. DEWITT STETTEN, Jr., of the National Institute of Arthritis and Metabolic Diseases, furnished us with a portion of 1-^{13}C-D-glucuronolactone they had received from Dr. ARTZ. The ^{13}C analyses of our products were made possible through the generous cooperation of Dr. SIDNEY WEINHOUSE, of the Lankenau Institute for Cancer Research, who arranged to have samples analyzed by the mass spectrograph at the Sun Oil Company, Marcus Hook, Pennsylvania. We are indebted to Mr. JOSEPH PAULSEN of that firm for these analyses.

Our experiments were carried out with the same male pentosuric subject (G.W.) who had been studied in earlier work[2]. Since previous experiments on the effect of glucuronolactone on xylulose excretion had involved the use of 5 g oral doses of test compound[1,2], it seemed advisable to continue the practice in tracer experiments, especially since we could not obtain consent to inject glucuronolactone. Each of the labeled preparations (0.140 g 6-^{13}C-D-glucuronolactone and 0.187 g 1-^{13}C-D-glucuronolactone containing 60% and 40% excess ^{13}C in the respective labeled positions) were mixed with a large amount of unlabeled compound to give the isotopic content indicated below in the description of each experiment.

5.14 g 6-^{13}C-D-glucuronolactone containing 1.63% excess ^{13}C in the terminal position was administered in one oral dose as a solution in 200 ml of water. Since previous experience had shown that the effect of glucuronolactone on xylulose excretion lasts only a few hours, the urine was collected during the next 6 h and xylulose isolated as follows. 125 ml of the urine was deionized with ion-exchange resins and chromatographed on a Dowex-1 (borate) column, fractions containing xylulose being pooled, deionized, and purified by paper chromatography[2]. The pentose was further purified *via* the crystalline *p*-bromophenylhydrazone derivative and then sent to Dr. WEINHOUSE, who converted it to barium carbonate for isotopic analysis. No excess ^{13}C was found (xylulose ^{13}C content, 1.09%; glucose standard, 1.08%).

By the time 1-^{13}C-D-glucuronolactone became available, a much simpler method for isolating xylulose from pentosuric urine was in use in this laboratory. 5.0 g glucuronolactone containing 1.52% excess ^{13}C in C-1 was administered orally in one dose. 50 ml of the urine collected during the subsequent 6 h-period was deionized over Amberlite IR-120 and Duolite A-4 and the filtrate evaporated to dryness. The residue was dissolved in a small volume of methanol for preparative chromatography (ascending) on sheets of Whatman 3 MM paper, with 80% propanol as solvent. The position of the ketopentose was determined by spraying a narrow strip of each paper with naphthoresorcinol reagent[8]. The appropriate horizontal length of paper was then cut out and eluted with water. The eluate was evaporated to dryness, the residue dissolved in 1 ml 95% ethanol, and 50 ml ether added to precipitate impurities. After standing in the refrigerator overnight, the solution was filtered, the filtrate evaporated to dryness, and the precipitation step repeated. Evaporation of the filtrate yielded 50 mg of oil which appeared completely pure by colorimetric assay by the cysteine-carbazole method[9] and by paper chromatographic analyses in 4 solvent systems: *n*-butyl alcohol pyridine–water (10:3:3), *n*-butyl alcohol saturated with 1% ammonia water, ethyl acetate-pyridine water (5:2:5), and benzene–*n*-butyl alcohol pyridine water (1:5:3:3). Naphthoresorcinol and periodate[10] spray reagents were used. No detectable amounts of glucuronic acid, glucuronolactone, or other material was present.

The xylulose, converted to barium carbonate by Dr. WEINHOUSE, assayed 0.12 and 0.13% excess ^{13}C in duplicate analyses. Since the carbon chain of the administered substance had an average excess ^{13}C of 0.25%, the result suggested that half the urinary xylulose was derived directly from the administered glucuronolactone. This conclusion is in harmony with previous non-isotopic studies. It is obvious that the high isotope content of the isolated pentose could not have resulted from the presence of a small amount of impurity.

The labeled xylulose was subjected to a degradation procedure designed to establish the ^{13}C content of C-5, the most probable location of the tracer. It is well established that periodate oxidation of ketoses is less complete than that of aldoses, both linkages adjacent to C-2 being less readily cleaved than others[11,12,13,14]. This would lead to a lower yield of formaldehyde from the hydroxymethyl group adjacent to the carbonyl than from the other terminal group. This situation seems to apply particularly to xylulose. In periodate oxidations carried out in sodium bicarbonate (pH 6.5), we, as well as REEVES[12], obtained 85 to 90% yields of formaldehyde from

fructose, whereas we have obtained between 63 and 76% with xylulose. Periodate oxidation of the labeled xylulose should therefore yield essentially all of C-5 as formaldehyde, together with a smaller percentage of C-1.

To 15 mg of the labeled xylulose dissolved in 2.3 ml water were added 2 ml 1 *N* $NaHCO_3$ and 2 ml 0.3 *M* HIO_4. After 1 h at room temperature, 3 ml 1 *N* HCl and 2 ml 1.2 *N* $NaAsO_2$ were added to terminate the reaction. 3 ml 2 *N* NaOH were added and the solution distilled to near dryness to obtain the formaldehyde. Three additions of 15 ml portions of water, each followed by distillation, were made to ensure complete recovery of the formaldehyde. The combined distillates, after addition of 100 mg $Na_2S_2O_5$ to bind the formaldehyde, were concentrated under vacuum to 2 ml and then to dryness with a current of air and gentle warming. To liberate SO_3, the residue was dissolved in 0.5 ml HCl and allowed to stand for 30 min at room temperature. KIO_3 was added and the resulting solution oxidized to convert the formaldehyde to barium carbonate[15]. The latter material was sent to Dr. Weinhouse, who reported that it contained 0.54% excess ^{13}C. This result demonstrated that most of the label was in a terminal position of the pentose. If it had been equally distributed between C-1 and C-5, the formaldehyde would have contained 0.31% excess ^{13}C ($^5/_2 \times 0.125\%$). If all the label were in only one terminal position, this carbon atom would contain 0.625% excess ^{13}C ($5 \times 0.125\%$). This labeled position could not be the one adjacent to the carbonyl, since periodate oxidation would have yielded a greater amount of formaldehyde from C-5 than from C-1, thus producing formaldehyde with less than 0.31% excess ^{13}C. On the other hand, labeling exclusively in C-5 should lead to formaldehyde with an isotope excess approaching 0.625%, this value being lowered to the extent of cleavage of C-1. The value of 0.54% indicates that a 58% yield of formaldehyde occurred (16% cleavage of C-1), a result in agreement with the trial experiments on unlabeled pentose.

These experiments demonstrate that administered D-glucuronolactone is a direct precursor of L-xylulose in the pentosuric human, that the carboxyl carbon of glucuronolactone is lost in the conversion, and that its aldehydic carbon becomes C-5 of xylulose. There is no experimental or theoretical reason to postulate cleavage of the carbon chain, and, as indicated above, feasible mechanisms for the direct conversion are available. Although elucidation of the mechanism by which D-glucuronolactone serves as a precursor of L-xylulose in the pentosuric does not necessarily yield information concerning the normal mode of synthesis of the pentose by the pentosuric or by other mammals, it is significant that recent studies have disclosed the presence of liver enzymes which act on glucuronic acid and its lactone[16,17,18]. In fact, one report[18] describes an enzyme system which specifically liberates C-6 as CO_2. It therefore seems probable that D-glucuronolactone is on the normal biosynthetic pathway of L-xylulose. Further work on L-xylulose formation in animals is in progress in this laboratory.

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