of adenine hydrochloride by W. COCHRAN[7]. In COCHRAN's structure the extra hydrogen is placed on the N_1 position in agreement with the assignment suggested here for protonated DNA. Of course, one does not "see" a proton with X-rays. The X-ray F_0—F_c transform indicates a maximum in electron density at a position 0.89 Å from the center of the N nitrogen, demonstrating the presence of an N_1–H bond in the vicinity. The positive charge added by the proton appears to be shared between the N_{10}, N_1, and N_9 positions on adenine via resonance between four different structures. Although the N_9 of adenine has its N_9–H bond replaced by an N_9–C bond in DNA it is still possible to draw the same four resonating structures. Whether it is justifiable to say that cytosine also protonates on its N_1 position in DNA is less certain. However, it is possible to draw three reasonable resonating structures of cytosine with the extra positive charge shared by the N_1, N_4 and N_7 nitrogens, respectively, and on this basis the comparison is believed to be a good one. Thus it appears most likely that mild acid treatment protonates the N_1 positions of adenine and cytosine in DNA and also that this gives a better explanation of the hydrogen-bond breakage by acid.

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Studies of ribose metabolism
IV. The metabolism of D-glucuronolactone in normal and pentosuric human subjects

A pathway for ribose biosynthesis from D-glucuronic acid in the mammalian organism has been postulated[1,2] as a result of the demonstration in animal tissues of the following reactions:

1. D-glucuronic acid ⟶ L-gulonic acid[3]
2. L-gulonic acid ⟶ L-xylulose[2]
3. L-xylulose ⟶ xylitol[4]
4. xylitol ⟶ D-xylulose[4]
5. D-xylulose ⟶ D-xylulose-5-phosphate[5]
6. D-xylulose-5-phosphate ⇌ D-ribulose-5-phosphate[6]
7. D-ribulose-5-phosphate ⇌ D-ribose-5-phosphate[7]

A block in this pathway has been suggested[8] to explain the urinary excretion of L-xylulose by persons with the genetic disturbance, essential pentosuria[9].

We have recently obtained evidence in man[10] for ribose synthesis from glucose *via* the oxidative and the nonoxidative reactions of the pentose phosphate pathway[11]. Our studies were carried out with a ribose "trapping" technic, suggested by the observation of TABOR AND HAYAISHI[12] that imidazoleacetic acid (IAA) riboside appears in the urine of rats given IAA. Using this technic, we have now demonstrated ribose biosynthesis from D-glucuronolactone, uniformly labeled with ^{14}C, in a human subject with normal carbohydrate metabolism. Evidence is also provided for an impairment of this pathway in a subject with pentosuria.

A 67-year-old man with coronary artery disease but with normal carbohydrate metabolism and a 52-year-old man with pentosuria were each given IAA hydrochloride[13] by mouth, and 30 min later, intravenous D-glucuronolactone, uniformly labeled with ^{14}C. (This compound, which was obtained through the generosity of Dr. N. E. ARTZ of the Corn Products Refining Co., Argo, Ill., was dissolved in 0.9% saline and sterilized by passage through a bacteriological filter.) Urine was collected during the ensuing 10 h, and a portion was passed over Dowex-1 acetate, which

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adsorbs the IAA riboside. L-xylulose was recovered by eluting the resin with water, until the effluent was free of radioactivity. The aqueous effluent was deionized by passage through a mixed-bed resin (MB-3; Fisher Scientific Co.), and lyophilized. The residue was applied to Whatman No. 1 paper for descending chromatography in *n*-butanol–ethanol–water (52:32:16). The strip corresponding in location to authentic L-xylulose was cut out, eluted with water, and re-chromatographed in *n*-butanol–acetic acid–water (40:10:50). The strip in the position of xylulose was eluted and analyzed for ketopentose[14] and for radioactivity. The riboside was recovered from the Dowex resin and hydrolyzed by procedures described previously[15]. The resulting ribose was deionized by passage through a mixed-bed resin, further purified by paper chromatography, and analyzed for pentose[16] and for radioactivity. The results are summarized in Table I.

TABLE I

URINARY L-XYLULOSE AND RIBOSE IN NORMAL AND PENTOSURIC PATIENTS GIVEN IMIDAZOLEACETIC ACID (IAA) AND D-GLUCURONOLACTONE UNIFORMLY LABELED WITH ^{14}C

	Normal	*Pentosuric*
D-glucuronolactone administered (counts/min)	1.57×10^7	7.85×10^6
(μmoles)	370	185
IAA hydrochloride administered (μmoles)	2000	1000
Urinary L-xylulose (0–10 h) (μmoles)	0.5	3950
Relative molar activity (counts/min/μmole)		85
Total ^{14}C content (counts/min)	300*	$3.36 \cdot 10^5$
% administered ^{14}C	0.002*	4.3
Ribose from urinary IAA riboside (0–10 h)		
(μmoles)	275	208
% administered IAA	14	21
Relative molar activity (counts/min/μmole)	33	
Total ^{14}C content (counts/min)	9100	190*
% administered ^{14}C	0.058	0.002*

* Significance doubtful, because of small quantity of radioactivity.

The significant ^{14}C incorporation in the urinary ribose of the "non-pentosuric" patient is consistent with the conversion of D-glucuronolactone to ribose via reactions 1–7 above. A similar observation was made in a study of ribose excreted by a rat given IAA and ^{14}C-labeled glucuronolactone[17]. A small quantity of L-xylulose was found in the normal subject's urine, which is in accord with a previous demonstration of traces of this pentose in the urine of non-pentosuric human subjects[8]. Its ^{14}C content in the present experiment indicates that it was very likely derived from D-glucuronolactone.

The pentosuric subject excreted a large quantity of L-xylulose, the radioactivity of which demonstrates its origin from D-glucuronolactone. TOUSTER and his associaties have previously shown that D-glucuronolactone is a direct precursor of L-xylulose in a pentosuric individual[18]. Our subject also excreted ribose in an amount comparable to that excreted by the normal individual. The fact that it contained virtually no radioactivity, however, indicates that it was derived from sources other than glucuronolactone.

These observations demonstrate a mechanism for ribose biosynthesis from D-glucuronolactone in a human subject with normal carbohydrate metabolism. The pathway involved is presumably that suggested by the studies with animal tissues[2–7]. Strong support for the postulated block in this pathway in subjects with essential pentosuria is provided by the absence of significant ribose biosynthesis from D-glucuronolactone in our pentosuric subject.

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Reverse mutarotation of poly-L-proline*

The mutarotation of poly-L-proline I ($[\alpha]_D^{25} = +40°$) into poly-L-proline II ($[\alpha]_D^{25} = -540°$) has been described by KURTZ, BERGER AND KATCHALSKI[1]. Further work on this subject has been published since[2,3]. In the present note the reconversion of poly-L-proline II into poly-L-proline I is reported.

When a 5% solution of polyproline II in formic acid is diluted 10-fold with *n*-propanol, the specific rotation of the polymer changes within a week from $[\alpha]_D^{25} = -415°$ to $[\alpha]_D^{25} = -25°$. The polyproline precipitated by ether at this stage shows $[\alpha]_D^{25} = +50°$ (concn., 0.35% (w/v) in glacial acetic acid), and it mutarotates into polyproline II in this solvent. Like polyproline I, it is very sparingly soluble in water, and its infrared spectrum is identical with that of polyproline I, differing significantly from that of polyproline II[2] (see Fig. 1).

Reverse mutarotation of poly-L-proline II into poly-L-proline I occurs equally well when *n*-butanol is used instead of *n*-propanol. In the case of methanol and ethanol, reverse mutarotation

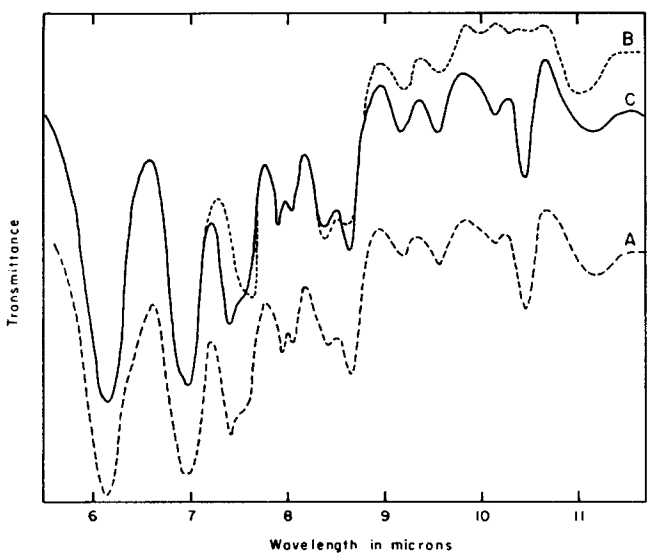

Fig. 1. The infrared spectrum of poly-L-proline I ($[\alpha]_D^{25} = +40°$), — — — (A); poly-L-proline II ($[\alpha]_D^{25} = -540°$), - - - - - - (B); and poly-L-proline obtained from poly-L-proline II after reverse mutarotation ($[\alpha]_D^{25} = +50°$) ———— (C). All spectra were taken from KBr pellets.