than of dimethoxyphenyl penicillin to give the same degree of protection in these tests.

Discussion. It is interesting that as the side chain of the α-phenoxyalkyl penicillins becomes larger, activity against the penicillin-sensitive strain of *S. aureus* decreases. At the same time, in the case of some of the members of the series, there is an increase in activity against a penicillinase-producing *S. aureus*. This is especially seen with the compound α-phenoxyisobutyl penicillin. Of the members in the series examined, this compound shows the greatest *in vitro* and *in vivo* activity against a penicillinase-producing resistant staphylococcus. However, the activity is considerably less than that seen with dimethoxyphenyl penicillin. In the cases examined, the activity against the resistant staphylococcus paralleled the penicillinase resistance and it would thus appear that such compounds are active against these organisms by virtue of their ability to withstand degradation by the staphylococcal penicillinase.

The penicillins in this series have an asymmetric carbon in the side chain and therefore exist as diastereoisomeric mixtures. The method of preparation was such that approximately equal amounts of each diastereoisomer would be present. The data presented therefore represent the combined activities of both diastereoisomers.

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Studies on Glucose Metabolism in Cartilage *in vitro*.* (26656)

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Survey of the literature reveals a paucity of *in vitro* metabolic studies on carbohydrate metabolism in cartilaginous tissue, particularly in the presence of added hormones. However, the *in vivo* effects of growth hormone and insulin on mucopolysaccharide metabolism have been intensively investigated. Dorfman and Schiller(1) reported decreased uptake of S^{35} from injected $Na_2S^{35}O_4$ and C^{14} from acetate-1-C^{14} or glucose-U-C^{14} into chondroitin sulfate isolated from skin of diabetic rats. Insulin treatment returned these values toward normal. In hypophysectomized rats, there was a rapid decline in incorporation of these labeled compounds into chondroitin sulfate and hyaluronic acid. Growth hormone given to these hypophysectomized rats restored only incorporation into chondroitin sulfate without any apparent ef-

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fect on incorporation into hyaluronic acid. Roden and Dorfman(2) found in experiments *in vivo* that glucose-6-C^{14} was equally distributed between the hexosamine and uronic acid moieties of mucopolysaccharides isolated from rat skin, and with the finding that the labeled carbon was still in the 6 position concluded that glucose is incorporated into mucopolysaccharide without splitting and reassembling of the carbon chain.

Since the rachitic weanling rat develops tibial epiphyseal plates approximately one-half centimeter thick containing a plentiful population of chondrocytes, this tissue was selected as suitable for *in vitro* experiments. In addition, the ease in preparing these animals and the rapidity whereby tissues can be excised, sliced and incubated are further factors contributing to the adequacy of this tissue for the experiments proposed.

Experimental. Male weanling rats of the Charles River strain (Sprague-Dawley descendants) were fed a high calcium rachitogenic diet (Nutritional Biochemicals Rachitogenic Diet #2, U.S.P.). For each experiment, 24 of these animals were killed after 3 to 4 weeks on the diet at which time gross examination revealed florid rickets. The hind legs were severed and all soft tissue rapidly dissected from the knee joint. Using the tibia as support, all adventitious tissue was scraped away from the epiphyses; the femur and its epiphysis were removed; the tibial epiphysis was sliced 4 to 5 times longitudinally with a razor blade and then transversely between the epiphysis and tibial shaft. From each epiphysis approximately 4-6 slices, each about ½ mm thick, were therefore prepared and randomly distributed into 6 piles on an iced glass plate until all animals had been killed. The 6 random piles each contained 175-250 mg of cartilage slices, and each pile was then placed in a 50 ml incubating flask containing 3 ml Krebs-Ringer bicarbonate buffer with 5 mM specifically labeled glucose-C^{14}. In different experiments, the order of the flasks was reversed to eliminate experimental selection. The flasks were then sealed with serum stoppers, placed on a Dubnoff apparatus and shaken 100 oscillations/minute for 3 hours at 38°C. For the first 10 minutes, the flasks were flushed with 95% O_2 : 5% CO_2 to re-equilibrate the buffer at pH 7.4.

At the end of incubation, the slices were removed and frozen for later analysis of polysaccharide content and radioactivity. To the medium was added 2 ml of 2 N H_2SO_4 and the liberated CO_2 trapped in 0.5 ml 1 N NaOH, converted to $BaCO_3$, plated on stainless steel planchets, weighed and counted in a thin window proportional flow counter(3, 4). Initial glucose specific activity was determined by preparation of the phenylosazone, recrystallization and counting as above.

Tissue mucopolysaccharide was isolated by a modification of Astrup's procedure where the frozen cartilage was pulverized in a Micro-Waring blendor into a smooth watery homogenate(5,6). This was diluted to 50 ml with distilled water and 25 ml of saturated benzidine hydrochloride added. After stirring, 2 ml of 5 N HCl was added, the mixture centrifuged and the supernatant fluid discarded. To the precipitate was added 10 ml of 10% NH_3 and 50 ml of ether, and the mixture transfererd to a separatory funnel. The aqueous layer was filtered and the filter paper rinsed with 10% NH_3. The filtrate was collected and solid NaCl added to a concentration of approximately 0.5%. Four volumes of absolute ethanol were added, the solution cooled and the white flocculent precipitate centrifuged. The precipitate was dissolved in 10 to 15 ml of water and dialyzed in a Visking 24/40 membrane against 20 to 30 volumes of water overnight at 4°C. It was then lyophilized, the residue weighed and dissolved in distilled water. An aliquot was dried on a steel planchet, weighed, and counted.

Glucose-1-C^{14} and glucose-6-C^{14} were purchased from New England Nuclear Corp. Crystalline insulin was kindly provided by Dr. W. R. Kirtley of Eli Lilly and Co. and bovine growth hormone, lot #R50109, was obtained through the courtesy of the Endocrinology Study Section of Nat. Inst. of Health.

Results. In Table I are summarized the results of 7 experiments comparing the metabolism of glucose-1-C^{14} and glucose-6-C^{14}

TABLE I. Effect of Insulin and Growth Hormone on the Oxidation of Glucose-1-C^{14} or Glucose-6-C^{14} to CO_2 in Rachitic Rat Cartilage *In Vitro*. Glucose, 5 mM; insulin, 0.1 unit/ml; growth hormone, 0.33 mg/ml. Values in μmoles of specifically-labeled glucose carbon recovered in total medium and gaseous CO_2 per g wet cartilage per 3 hr incubation. Each experiment contains pooled tissues from 24 animals with stand. errors of the mean.

	Control		Insulin		Growth hormone	
Exp.	C-1	C-6	C-1	C-6	C-1	C-6
1	1.54	.88	3.53	1.35	1.75	.98
2	2.81	1.40	3.90	1.59	2.80	1.40
3	2.12	1.43	4.04	1.54	2.25	.88
4	2.60	1.23	--	—	2.24	1.16
5	1.98	1.01	2.63	1.16	2.22	1.08
6	2.98	1.40	3.58	1.53	2.63	1.61
7	2.18	1.10	3.59	1.37	2.62	1.60
Mean ± S.E. of mean	2.32 ± .19	1.21 ± .08	3.52 ± .20	1.42 ± .06	2.33 ± .13	1.24 ± .11

to CO_2 in control tissues and in tissues incubated with growth hormone. In 6 of these experiments, the effects of insulin were also studied. From the results it is clear that in both control and hormone treated tissues there is a significantly greater oxidation of glucose carbon-1 compared to carbon-6, suggesting operation of the direct oxidative pathway. Insulin significantly increases oxidation of both carbon-1 ($P<0.01$) and carbon-6 ($P<0.01$) to CO_2, but there was no apparent alteration in presence of the growth hormone preparation.

Isolation of tissue polysaccharide and determination of specific activity resulted in an overall mean value of 15.3 $\mu\mu$M of specifically-labeled glucose incorporated into 1 mg of polysaccharide. In control tissues, mean glucose incorporation was 12.4 $\mu\mu$M/mg and in insulin treated tissues 16.1 $\mu\mu$M/mg polysaccharide. However, analysis of paired incubations showed an increase of 19 ± 12% in presence of insulin which was only suggestive of significance ($P<0.2$). On the other hand, mean polysaccharide incorporation in presence of growth hormone was 18.3 $\mu\mu$M/mg and paired data showed a more significant increase of 61 ± 19% ($P<0.05$) compared to the paired control tissues.

More surprising, mean incorporation of carbon-1 into polysaccharide was 18.2 $\mu\mu$M and that of carbon-6 12.1 $\mu\mu$M. Again, analysis of paired tissues showed this difference to be significant, namely a 60 ± 17% ($P<0.05$) greater incorporation of glucose carbon-1 compared to carbon-6. No significant alteration in this ratio was effected by either insulin or growth hormone.

Discussion. The data show several definite metabolic characteristics of cartilage cells derived from rachitic rats. One characteristic is a significantly greater oxidation of glucose carbon-1 compared to carbon-6, suggesting operation of the direct oxidative pathway. However, recovery of specifically-labeled carbon in tissue polysaccharide does not support this selective loss of carbon-1; in fact, more carbon-1 than carbon-6 is recovered in polysaccharide. This discrepancy cannot be resolved unless other pathways of asymmetric metabolism are operative, such as failure of triose isomerase effectively to equilibrate triose phosphates or another pathway, such as that involving formation of uridine diphosphoglucuronic acid and eventual cleavage of carbon-6 to CO_2 (the glucuronic acid pathway). That the latter pathway is operative in rachitic rat cartilage is given support by the recent isolation of uridine diphosphate and several of its glycosides, namely glucose, glucuronic acid and acetyl galactosamine (unpublished observations).

The stimulatory effects of the 2 hormones are clearly separable, insulin increases glucose oxidation to CO_2 with only a suggestive increase in polysaccharide synthesis whereas growth hormone does not increase glucose oxidation but significantly increases polysaccharide synthesis. Extrapolation of these results to *in vivo* metabolism is dangerous for many reasons including the extremely

unphysiological dose of hormones used in these experiments, the lack of homogeneity of the growth hormone preparation, and most important, the overall unphysiological milieu of *in vitro* incubation.

Summary. Cartilage slices obtained from weanling rachitic rats oxidize glucose carbon-1 to CO_2 more rapidly than carbon-6. Insulin increases oxidation of both carbons whereas growth hormone has no effect. Growth hormone significantly increases glucose carbon incorporation into tissue polysaccharide.

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