turnover was obtained from the changing rates with time in removal of radiocalcium from the skeleton and from comparisons of calcium specific activities of the skeleton and plasma of the animals sacrificed at increasing times after administration of the radiocalcium. In this study the effect of skeletal accretion was minimized. The results confirm those previously obtained when skeletal growth was a possible factor influencing the results. These findings and interpretations are consistent with the concept that the bone salt exchanges the calcium of crystal surfaces rapidly and that of the interior of crystals more slowly by recrystallization. The specific activities of different bones and the change in specific activity of the bones with time indicate that anatomical location of bone influences its calcium turnover.

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A Rapid Method for Distinguishing D-Glucosamine from Galactosamine in Biological Preparations. (19718)

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In the course of a study of the constituents of a polysaccharide isolated from *Shigella flexneri*(1), it became necessary to be able to distinguish between the 2 naturally-occurring aminosugars, glucosamine and galactosamine. These substances have very similar R_f values and do not separate well in a number of solvent systems(2). The N-2,4 dinitrophenyl derivatives separate only slightly better(3) and methods for isolating derivatives involve more laborious procedures with relatively large amounts of aminosugars(4,5).

Brown reported the phosphorylation of D-glucosamine by hexokinase, and adenosinetriphosphate (ATP), the product being identified as D-glucosamine-6-phosphate(6). As is shown below, galactosamine does not function as a substrate for hexokinase. It is therefore, possible to differentiate between these aminosugars; and, in cases where these are the only 2 aminosugars present, the relative amounts of each may be determined.

Materials. The aminosugars used in the experiment described by Fig. 1 were partially purified from crude hydrolysates of the polysaccharide of *Sh. flexneri*(1) and bovine tracheae(7,8) by passage over a cation exchange resin and elution with HCl. The yeast hexokinase was also only partially purified(9).

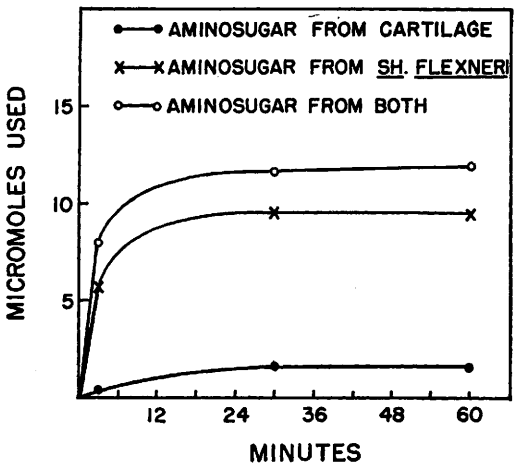

FIG. 1. Presence of D-glucosamine in hydrolysates of the polysaccharide of *Sh. flexneri*. The reaction mixture contained 33 micromoles ATP, 13 micromoles galactosamine from chondroitin sulfuric acid or 11 micromoles aminosugar from the polysaccharide of *Sh. flexneri*, or both, .006 M $MgCl_2$ and yeast hexokinase in .06 M veronal buffer, pH 8. Incubated at 30°C. The utilization of aminosugar corresponds to the D-glucosamine content of the reaction mixture.

Method. D-glucosamine is phosphorylated in the presence of ATP and hexokinase. Any unreactive aminosugar is determined after removal of glucosamine-6-phosphate by treatment of the reaction mixture with $ZnSO_4$ and $Ba(OH)_2$. This is necessary since the phosphorylated aminosugar also gives the color reaction of the free aminosugars.

The reaction mixture of the data in Fig. 1 contained the following:

ml	
4	.1 M veronal buffer, pH 8
.4	.1 M $MgCl_2$
.9	aminosugars (Fig. 1)
.3	.11 M ATP

The initial concentration of aminosugar was determined in the supernatant fluid obtained after adding a 1 ml aliquot of the above mixture to 1 ml of $ZnSO_4$ and then adding 1 ml $Ba(OH)_2$(10). 0.2 ml of hexokinase was added to 4 ml of the remaining mixture to start the reaction which was incubated at 30°C. One ml samples were fixed with $ZnSO_4$ and $Ba(OH)_2$ at timed intervals. The supernatant fluid which is free of protein and phosphorylated compounds was used for the determination of free aminosugars by the colorimetric method of Elson and Morgan(11).

Results. The galactosamine preparation showed a utilization amounting to only 10-15% of the total aminosugar added, whereas 85% of the aminosugar obtained from the specific polysaccharide of *Sh. flexneri* was phosphorylated. The limited disappearance of aminosugar from the galactosamine preparation indicates that some D-glucosamine may have been present in the chondroitin sulfuric acid from which the galactosamine was isolated. It may also be seen that the presence of galactosamine did not prevent the phosphorylation of glucosamine obtained from the hydrolysate of the polysaccharide.

Discussion. It is possible to carry out determinations on crude hydrolysates of biological materials provided an excess of ATP is added so that it does not become limiting by the phosphorylation of glucosamine or of other sugar residues such as glucose in the hydrolysates. In cases where acid hydrolysis is not desirable, ATP-destroying enzymes, if present in amounts sufficient to prevent the maintenance of an excess of ATP during incubation with hexokinase, may be eliminated by deproteinization and neutralization of the sample before adding it to the test system.

The disappearance of as little as 0.1 micromole of glucosamine per color test may be detected easily when it is the only aminosugar present. With galactosamine as the only aminosugar, no disappearance of color equivalents occurs during incubation with hexokinase and ATP. Mixtures of the 2 aminosugars may be accurately analyzed for both components provided at least 10% of the total aminosugar is D-glucosamine. Because of the non-utilization of galactosamine during treatment with hexokinase, it is easier to detect small amounts of this aminosugar in the presence of large amounts of D-glucosamine than it is to detect a small decrease in color due to the presence of a trace of D-glucosamine in the presence of a large amount of galactosamine.

Summary. An enzymatic method is described for distinguishing D-glucosamine from galactosamine. The method is based on the fact that glucosamine is phosphorylated by ATP in the presence of hexokinase, whereas galactosamine is not. Application of the procedure for the assay of biological preparations is illustrated by the demonstration of D-glucosamine in hydrolysates of a polysaccharide isolated from *Shigella flexneri.*

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