Studies on the Biosynthesis of Glucosamine in the Intact Rat

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Little is known about the biosynthesis of glucosamine in mammalian organisms. Previous studies have indicated the presence of enzymes in several mammalian tissues capable of synthesizing glucosamine (1, 2). Moreover, there is evidence that glucose, without scission of its carbon chain, may serve as the precursor of glucosamine in the intact animal (3, 4), as in bacteria (5, 6). However, except for studies on the mucopolysaccharides of skin (7, 8), no information is available on the rate of turnover of glucosamine in mammalian tissues. It would be of special interest to have information concerning the rate of turnover and the site of synthesis of the serum glucosamine, which is present in large concentrations and is apparently entirely protein-bound. As a major carbohydrate component of the serum glycoproteins, it undergoes wide fluctuations in concentration in a variety of physiological and pathological states (9).

In the present study a comparison was made of the incorporation of radioactivity from glucose-C\(^{14}\) into the protein-bound glucosamine of various tissues of the intact rat. Of the tissues studied, liver and serum showed the most rapid synthesis of glucosamine from glucose, and a detailed analysis of the turnover of glucosamine in both of these tissues was made. Evidence is also presented that the liver is the primary site of synthesis of the serum glucosamine.

**EXPERIMENTAL**

*Animals*—Male albino rats of the Wistar strain, weighing between 235 and 300 gm., were used. All animals were fed Purina chow *ad libitum* before as well as during the experiment, up to the time of death. Each animal received a single intraperitoneal injection of 10 to 15 \(\mu\)c. of uniformly labeled glucose-C\(^{14}\) (2.08 \(\mu\)c. per mg.) in 2 ml. of 0.85 per cent sodium chloride. At various time intervals before death 0.2 ml. of blood was taken from the tail vein of each rat without anesthesia for the purpose of determining the blood glucose specific activity. The animals were killed at varying time intervals ranging from 45 minutes to 48 hours after the injection of the glucose-C\(^{14}\). They were then excised and placed on ice. The liver was divided into portions weighing a maximum of 5 gm., and each portion was treated separately throughout the following procedure.

The tissue was minced with scissors and then homogenized for about 4 minutes in 7 per cent ice-cold trichloroacetic acid (5 ml per gm. of tissue). The homogenate was then centrifuged and the precipitate re-extracted twice more with trichloroacetic acid in a similar manner. The liver extracts were pooled and saved for the isolation of glycogen. The trichloroacetic acid precipitate was then suspended in 0.3 X trichloroacetic acid (3 ml. per gm. of tissue), heated in a capped centrifuge tube in a boiling water bath for exactly 8 minutes, and then rapidly cooled in an ice bath. Under these conditions, no glucosamine was released from the protein precipitate, although about 8 amoles of anthrone-positive material per gm. of liver, which probably represent residual glycogen as well as weakly bound hexoses, were released. After this, the residue was separated by centrifugation, washed once more with cold 0.3 X trichloroacetic acid, and then washed with 95 per cent ethanol (6 ml. per gm. of tissue) in order to remove the trichloroacetic acid. The residual ethanol was removed from the precipitate over a steam bath. The dry material was then transferred to a 25-ml. glass-stoppered Pyrex volumetric flask and hydrolyzed for 10 hours in 3 N HCl (3 to 4 ml. per gm. of tissue) in a boiling water bath in order to release the glucosamine. After the hydrolysis the flask was made up to volume with water and the hydrolysate was filtered. A measured amount of the filtrate was then concentrated to dryness three times in a vacuum at 45° in order to remove most of the HCl. Columns of Dowex 50-X4 cation exchange resin (200 to 400 mesh) in the hydrogen form were prepared. The resin was washed according to Boas (10), and 20 ml. of a 1:1 volume suspension of the resin in water was placed into a glass column of 1.6-cm. internal diameter. The residue obtained upon concentration of the hydrolysate was taken up in about 15 ml. of water and quantitatively transferred to one of these columns in order to separate the remaining neutral sugars from the glucosamine. The column was then washed with about 1300 ml. of distilled water, after which the glucosamine was eluted with 2.0 N HCl. The first 7 ml. of the eluate contained no amino sugar and were discarded. All of the amino sugar was recovered in the next 10 ml. of eluate. The hydrolysate was completely decolorized by passage through the Dowex column. With the above procedure less than 8 per cent of the tissue amino acids was present in the collected eluate.

In order to separate the glucosamine from the remaining amino acids and to form a derivative suitable for determining the radioactivity, the glucosamine was converted to the glucose phenylsulzone (11). In the case of liver, which had been divided into 5-gm. portions up to this point, the Dowex 50 eluates were...
combined and concentrated in a vacuum and made up to a volume of 10 ml. The glucosamine of the other tissues was present in the 10-ml. of eluate collected from a single column. An aliquot was taken for the determination of the glucosamine, and a maximum of 20 μmoles of carrier glucosamine was added to the remainder. In the case of the liver, since there were already about 20 μmoles of glucosamine present in the eluates, there was only very slight dilution of radioactivity by the carrier. The phenyl-
osazone was formed in 12 ml. of an acetate buffered solution (1.2 M sodium acetate; 0.4 M acetic acid) containing 1 mmole of phen-
ylhydrazine hydrochloride and about 10 μmoles of glucosamine in capped centrifuge tubes, by heating for 11 hours in a boiling wa-
ter bath. The osazone was then allowed to crystallize in the cold. It was washed four times with water and recrystallized from 50 per cent ethanol. Further recrystallization resulted in no change in specific activity. With this procedure a yield of 50 to 55 per cent was obtained after washing and recrystallization. Shorter periods of heating such as have been used for the forma-
tion of the phenylasozone from glucose directly (12) re-
sulted in very poor yields, e.g. 20 per cent in 2.5 hours. The osazones formed from the tissue eluates decomposed at about 204°, as did the osazones formed from pure glucose or glucosa-
mine.

The liver glycogen was precipitated from the pooled cold trichloroacetic acid extracts by the addition of 1.2 volumes of 95 per cent ethanol. It was washed with 70 per cent ethanol and then hydrolyzed for 2.5 hours in 1 N H₂SO₄ in a boiling wa-
ter bath. The hydrolysate was neutralized and an aliquot taken for the determination of the glucose released. To determine the specific activity, the remainder was used for the preparation of the glucose phenylasozone under the same conditions as for the glucosamine, but with heating for a period of only 2.5 hours.

**Isolation of Glucosamine and Glucose from Blood**—The blood collected from the animal at death was permitted to clot and about 3 to 4 ml. of serum were obtained. The serum proteins were precipitated by the addition of 15 volumes of 95 per cent ethanol. It was washed with 70 per cent ethanol and then hydrolyzed for 2.5 hours in 1 N H₂SO₄ in a boiling wa-
ter bath. The hydrolysate was neutralized and an aliquot taken for the determination of the glucose released. To determine the specific activity, the remainder was used for the preparation of the glucose phenylasozone under the same conditions as for the glucosamine, but with heating for a period of only 2.5 hours.

**Analyzis**—Glucose from glycogen and blood was determined by the Nelson-Somogyi method (14, 15). Total hexoses, except amino sugars, were determined with the anthrone reagent of Roe (16). The total hexosamines were determined on the Dowex 50 eluates by the Boas modification of the Elson-Morgan method (10). The percentage of glucosamine and galactosamine in a mixture of the amino sugars was determined according to the method of Rosman and Daffner (17), except for the following modifications. Because of the large concentration of nonhexos-
amine nitrogen in the Dowex eluates, the formation of the N-acetyl derivatives of the amino sugars was carried out by increasing the amount of acetic anhydride added to each tube to 0.15 ml. of a 12.5 per cent solution (volume for volume). The pH was maintained by the addition of 0.1 ml. of 4 N Na₂CO₃ into the saturated Na₂CO₃ used in the original method. The heating period following acetylation was increased to 5 minutes. Because of the enhanced color formation obtained in the presence of borate buffer compared to carbonate buffer, chromogenic formation was accomplished in the presence of 0.1 ml. of 0.8 M borate buffer, pH 9.1 (18), with a heating period of 7 minutes on a vigorously boiling water bath.

**Paper Chromatography**—Descending chromatograms were run in several solvent systems on Whatman No. 1 paper in order to identify the sugars present in the eluates from the Dowex 50 columns. In order better to identify the nature and proportion of the hexosamines present, they were converted to the cor-
responding pentoses as well as to their N-acetyl derivatives, which were subsequently chromatographed. The amino sugars were converted to pentoses by reaction with ninhydrin as de-
scribed by Stoffyn and Jeanloz (19). Because of the presence of relatively large amounts of amino acids, the ninhydrin was added in large excess as a 4 per cent ninhydrin solution in 2 per cent pyridine to the sugar sample dissolved in 0.1 M citrate buffer (pH 4.7). This reaction mixture was deionized by passage through a mixed bed ion exchange column (Amberlite MB-3), concentrated in a vacuum, and the pentoses chromatographed.

The N-acetyl derivatives of the amino sugars were prepared for chromatography in the same manner as for the colorimetric determination. Salts and amino acids were removed by passage through a mixed bed ion exchange column, followed by concentra-
tion in a vacuum, and chromatography of the N-acetyl deriv-
atives on borate-treated paper as described by Cabib et al. (20).

All sugars were located on the paper chromatograms by the silver nitrate method of Trevelyan et al. (21). In addition, the aniline phthalate reagent (22) was used for the location of pen-
toses as well as hexoses, and amino sugars and their N-acetyl derivatives were specifically located with the Elson-Morgan method (23).

**Measurement of Radioactivity**—The radioactivity of the glu-
osamine, as well as of the glucose from either blood or glycogen, was determined on the glucose phenylasozone derivatives plated on stainless steel planchets in a windowless gas flow proportional counter (24). The activity of the injected glucose was also determined on a sample converted to the glucose phenylasozone. All counts were adjusted to 5.0 × 10⁶ c.p.m. (about 10 μc.) injected into a 250-gm. rat.

Extensive studies were undertaken in order to exclude the possibility that the osazone formed from the glucosamine was contaminated with radioactivity from neutral hexoses, especially serum glucose and liver glycogen, or amino acids. As will be shown later, neutral hexoses were present in the Dowex eluates by chromatographic analysis. However, in order to test this in a more sensitive manner, highly labeled glucose was added at varying steps of the isolation of the glucosamine from liver and serum. In the case of serum, no activity was present in the glucosamine derivative when radioactive glucose was added either to the serum before precipitation to simulate the free glucose or to the protein precipitate before hydrolysis to simulate protein bound neutral hexose. For liver, the amount of neutral
hexose remaining in the tissue after the hot trichloroacetic acid extraction was determined to be 5 to 7 amoles per gm., either by the anthrone reaction directly on the protein precipitate or from the Dowex 50 effluent and wash after a relatively mild hydrolysis (0.8 N HCl for 4 hours at 100°). Glucose in that quantity and with a specific activity much higher than that of the glycogen from any of the experiments was added to the protein precipitate before hydrolysis. Under these conditions, no activity was present in the osazone from glucosamine. It must be pointed out, however, that if radioactive glucose in an amount equal to that present in the unextracted liver (about 250 amoles per gm.) was added to the precipitate before hydrolysis, some radioactive contamination resulted despite passage through a Dowex 50 column. The removal of the glycogen before hydrolysis is therefore essential. The results of the experiments to be reported confirm these contamination studies, as the blood glucose and blood glucosamine activity changed in an inverse manner, and there was no correlation between liver glycogen and liver glucosamine activity. A mixture of highly radioactive alanine and serine was added to the protein precipitate before hydrolysis and also directly to the Dowex eluate before osazone formation with no resulting activity appearing in the glucosamine derivative.

RESULTS

Identification of Amino Sugars—In order to identify the amino sugars and to determine whether any other sugars were present, the Dowex 50 eluates were chromatographed in a variety of systems. When the eluates from liver and serum were run in n-butanol-ethanol-water, 4:1:1, (40 hours) (19), only a single spot was found, corresponding to either glucosamine or galactosamine. After treatment with ninhydrin, the two amino sugars could be well resolved as their pentose derivatives: arabinose, Rglucose 1.37; lyxose, Rglucose 1.98. In the chromatograms of either serum or liver, only a single spot was present, corresponding to the arabinose standard or the glucosamine standard treated similarly with ninhydrin. Kidney and serum were chromatographed in a pyridine-ethyl acetate-water-glacial acetic acid system, 5:5:3:1, as described by Fischer and Nebel (25), in which the glucosamine standard migrated 24.7 cm. and the N-acetylgalactosamine migrated 25.3 cm. in 30 hours. For both liver and serum glucosamine, which is quite constant at any one time, the glycogen specific activity, on the other hand, reaches its peak by 3.75 hours. From the ratio of liver glucosamine specific activity to serum glucosamine specific activity (Table II), it may be seen that the serum is at first much less active than the liver, has about equal activity at 3.75 hours, and is more active than the liver at all times thereafter. This is graphically demonstrated in Fig. 1. It may be noted that there is little variation in the amount of glucosamine of the different livers. The serum protein-bound glucosamine pool was calculated from the glucosamine level and an assumed serum volume of 4 per cent of body weight.

Incorporation of Radioactivity into Liver Glycogen—In order to be able to compare the activity in liver glucosamine with a more familiar substance, the activity of liver glycogen at various times is shown in Table II. Contrary to the activity of the glucosamine, which is quite constant at any one time, the glycogen activity varies widely among animals even at the same time and shows no definite course with time. It may be noted that there is no correlation between the activity of the glycogen and that of the glucosamine. This is well demonstrated in Rat 15 (Table II) killed at 18 hours, which had a highly labeled glycogen, and yet showed the expected decline in the glucosamine activity.

Incorporation of Radioactivity into Glucosamine of Other Tissues—The activity of the protein-bound glucosamine of several other organs at various times was also determined (Table III). It may be seen that none of these reaches the early high activity present in liver glucosamine. The spleen has the highest specific activity of these organs, possibly because of its large blood content.

Incorporation of Radioactivity into Glucosamine after Injection of Uniformly Labeled Fructose-C14—An attempt was made to study further the relationship of the liver and serum glucosamine by injecting with C14-labeled fructose, which in the intact animal.
Radioactivity in liver glucosamine, serum glucosamine, and liver glycogen after glucose-C\textsuperscript{14} injection

All counts adjusted to $5.0 \times 10^{4}$ c.p.m. injected into a 250-gm. rat.

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Time (hrs)</th>
<th>Liver glucosamine</th>
<th>Liver glucosamine*</th>
<th>Liver glycogen</th>
<th>Liver glucosamine equivalents</th>
<th>Total activity</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Specific activity</td>
<td>Total activity</td>
<td>Specific activity</td>
<td>Total activity</td>
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<td></td>
<td>c.p.m. / µmole</td>
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<td>c.p.m.</td>
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<td>21.3</td>
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<td>9.4</td>
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<td>22.4</td>
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<td>70.3</td>
<td>22.3</td>
<td>1,570</td>
<td>72.2</td>
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</table>

* Mean serum glucosamine level: 4.99 ± 0.20 µmoles per ml. Total activity calculated from a mean pool of 50 µmoles per 250-gm. rat, with the volume assumed to be 4 per cent of body weight.

† Quantity refers to the total µmoles in the liver of a 250-gm. rat.

† Fructose-C\textsuperscript{14} injected instead of glucose-C\textsuperscript{14} under the same conditions.

Radioactivity in glucosamine of rat tissues after glucose-C\textsuperscript{14} injection

All counts adjusted to $5.0 \times 10^{4}$ c.p.m. injected into a 250-gm. rat. Total activity refers to the total c.p.m. in the glucosamine of the tissues of a 250-gm. rat. The mean ± the standard error of the glucosamine content of the tissues in µmoles per 250-gm rat was kidney, 10.8 ± 0.4; testes, 4.48 ± 0.19; lung, 5.74 ± 0.47; spleen, 1.36 ± 0.00.

From Fig. 1 it may be noted that the serum glucose curve crosses the liver glucosamine curve to the right of its maximum. This would suggest that a simple product-precursor relation such as that described by Zilversmit et al. (28) is not adequate to explain the relationship between the serum glucose and the liver glucosamine. However, the curves for the serum glucose, liver glucosamine, and serum glucosamine appear to fit a model described by Russell (29), as well as by Bornman and Huseby (30).
In such a system the specific activity of the liver glucosamine would be determined not only by synthesis from the serum glucose, but also by a return of molecules from the serum glucosamine pool. This system could be described as follows:

Serum glucose \( \xrightarrow{v_1} \) Liver glucosamine \( \xrightarrow{v_1} \) Serum glucosamine

This model requires that the maximal specific activity of the liver glucosamine be reached before its curve crosses that of the serum glucose, which is considered its precursor, and that the maximal specific activity of the serum glucosamine occur only after both the liver and serum glucosamine have crossed the curve of the serum glucose (29). It may be seen from Fig. 1 that the data are consistent with this scheme, and moreover, such a system is physiologically quite credible.

By formulating a set of equations, an attempt has been made to calculate the rates of the above reactions from the curves shown in Fig. 1. In the equations to be used in this discussion, the following symbols will be employed: \( s_A, s_B, s_C \), the specific activities of the serum glucose, liver glucosamine, and serum glucosamine, respectively; \( B \) and \( C \), the amounts of glucosamine present in liver and serum respectively; \( B^* \) and \( C^* \), the amounts of radioactive glucosamine present in liver and serum; \( t_1 \) and \( t_2 \), time and times, between which the calculations were made. The rate of conversion of liver glucosamine to serum glucosamine, \( v_1 \), may be calculated from the formula of Zilversmit et al. (28), which describes a simple product-precursor relationship.

\[
\frac{dB}{dt} = v_1 s_A + s_B - (v_1 + v_2) s_B
\]

In a steady state \( v_2 + v_4 \) equals \( v_1 + v_3 \). By making this substitution and replacing \( B^* \) equals \( s_B \), the following equation is obtained.

\[
\frac{dB}{dt} = v_1 (s_A - s_B) - v_3 (s_B - s_C)
\]

Integration of this equation gives

\[
B(s_B^2 - s_B) = v_1 \left[ \int_{t_1}^{t_2} s_A dt - \int_{t_1}^{t_2} s_B dt \right] - v_3 \left[ \int_{t_1}^{t_2} s_B dt - \int_{t_1}^{t_2} s_C dt \right] dt
\]

The integrals in this equation may be obtained from the areas between the serum glucose and liver glucosamine curves from \( t_1 \) to \( t_2 \) and between the liver glucosamine and serum glucosamine curves from \( t_1 \) to \( t_2 \), respectively.

At the maximum of the liver glucosamine curve, \( \frac{dB}{dt} = 0 \), and, as may be seen from Equation 3, the following relationship must exist.

\[
\frac{v_1}{v_3} = \frac{B(s_B - s_C)}{s_B - s_C}
\]

where \( m \) refers to the time at which the liver glucosamine reaches its maximal specific activity. From these two equations in two unknowns it should theoretically be possible to calculate both \( v_1 \) and \( v_3 \).

When \( v_2 \) is calculated from the curves between 45 and 90 minutes, with use of the mean serum glucosamine value of 50 \( \mu \)moles, a rate of 25.6 \( \mu \)moles per hour is obtained. When \( v_1 \) and \( v_3 \) were similarly calculated from the curves between 45 and 90 minutes, with the 90-minute value used as the maximum of the liver glucosamine specific activity, a rate of 0.68 \( \mu \)mole per hour was obtained for \( v_1 \) and 16.8 \( \mu \)moles per hour for \( v_3 \). These values for \( v_1 \) and \( v_3 \) are approximations, since it is not possible to localize the maximum precisely. However, values of the same order of magnitude are obtained when other times in the vicinity of 90 minutes are chosen for the maximum. Since the values obtained for \( v_1 \) are always very much smaller than those of \( v_2 \) or \( v_3 \), indicating that the rate of interchange of glucosamine molecules between liver and serum is much more rapid than the synthesis of new glucosamine molecules from glucose, \( v_1 \) may be calculated in an alternate manner by considering liver and serum glucosamine to be essentially part of the same pool. Fig. 2 shows the relationship between the serum glucose curve and the calculated specific activity curve for this common pool. It may be noticed that the serum glucose curve crosses this common pool curve near its maximum, indicating that the steps between liver and serum glucosamine are rapid enough to consider the relationship between the serum glucose and this common glucosamine pool as a simple product-precursor one. \( v_1 \) may therefore be calculated from the curves in Fig. 2 with the same equation as has been employed for the liver and serum glucosamine relationship (Equation 1). Such a calculation carried out between 45 and 90 minutes gives a value for \( v_1 \) of 0.89 \( \mu \)mole per
When this value for \( v_1 \) is substituted into Equation 4, a value of \( 26.3 \) \( \mu \)moles per hour is obtained for \( v_2 \). This value for \( v_2 \) is probably more exact than that obtained from Equations 4 and 5, since it does not depend on the exact localization of the maximum of the liver glucosamine. When this value for \( v_2 \) is compared with \( v_3 \), it may be seen that they are almost identical, indicating that \( v_3 \) is either small or nonexistent. The turnover time for serum glucosamine, \( \frac{C}{v_3} \), is 1.95 hours. The turnover time for liver glucosamine, \( \frac{B}{v_1 + v_2} \), with the 0.80 and 26.3 used for \( v_1 \) and \( v_2 \), is 0.79 hours. The time required to replace the liver glucosamine by synthesis of new molecules from glucose, \( \frac{B}{v_1} \), is 24.2 hours, whereas the time required to replace the serum glucosamine by synthesis from glucose, \( \frac{C}{v_1} \), is 56.2 hours. These values are summarized in Table IV.

From these calculations it may be seen that the turnover time of both serum and liver glucosamine is extremely rapid and many fold faster than the time required for synthesis of new molecules from glucose. It remains open to speculation to what extent this rapid interchange of protein-bound glucosamine between liver and serum represents a breakdown and resynthesis of the glycoprotein molecules with a utilization of most of the glucosamine, or merely a transfer back and forth between liver and serum of the same glycoprotein molecules.

Many values cited in the literature for turnover time of biological compounds are based on calculations made from radioactive decay of the compound plotted as the logarithm of the specific activity versus time. It is obvious from the above considerations that such a calculation would be quite erroneous in the present system because of the large extent of recycling of glucosamine molecules between liver and serum. However, in order to compare the turnover time of liver and serum glucosamine to turnover times of compounds calculated in this manner, the logarithm of the specific activity was assumed to be linear up to 12 hours and was plotted against time by means of the method of least squares. Turnover time was calculated from the half-time by multiplying it by 1.44 (31). The half-times obtained from these decay plots were 8.9 hours for liver glucosamine and 14.0 hours for serum glucosamine; the turnover times were calculated as 12.8 hours and 20.1 hours for the liver and serum glucosamine, respectively. These values may be compared with a half-time of 1.6 to 2.3 days determined from the decay of the glucosamine of hen egg ovomucoid (4) and with a half-time of 3.4 days determined for the glucosamine of the hyaluronic acid of the skin of rabbits (8).

The rapid turnover of glucosamine noted from the calculations of the present study is consistent with the work on the \( \alpha_1 \) acid glycoprotein in guinea pigs by Boström et al. (32), who found that the peak of the glucosamine specific activity had occurred some time before 12 hours, when they made their first determination. They estimated from the decay of the specific activity of the glycoprotein that its half-time is about 1 to 2 days, which would be considerably longer than the half-time of the total serum protein-bound glucosamine determined in the present study.

The site of formation of the serum glycoproteins has been a subject of some interest, and it has been proposed by some that depolymerization of ground substance of connective tissue may give rise to these glycoproteins (9). Since the glycoproteins are a heterogeneous group, it is not necessary that there be a single site of synthesis. As judged from the data of the present study, dealing with the relationship between the liver and serum protein-bound glucosamine, it appears that the bulk of the serum glycoproteins is synthesized in the liver. This is consistent with the study of Werner (33), who concluded that the liver was the site of glycoprotein formation on the basis of experiments showing that the increase in protein-bound glucosamine after bleeding in rabbits no longer occurred if the liver had been damaged previously with phosphorus or benzene. The subnormal levels of seromucoid seen in human subjects with parenchymatous liver disease are also in agreement with such a view (34).

The rapid transfer of glucosamine between liver and serum would suggest that alterations in that compound measured in the blood would reflect changes in the metabolism of glucosamine by the liver itself.

SUMMARY

The metabolism of protein-bound glucosamine has been studied in various tissues of the intact rat with the aid of tracer doses of uniformly labeled glucose-\(^{14}\). Specific activity-time curves for serum glucose, liver glucosamine, and serum glucosamine were drawn. From these it appeared that the liver is the primary site of synthesis of the serum glucosamine. Moreover, a very rapid interchange of the glucosamine between liver and serum was calculated to take place, resulting in a turnover time of 0.8 hour for the liver and 2 hours for the serum glucosamine. The rate of synthesis of glucosamine molecules in liver from glucose was calculated to be about 0.9 \( \mu \) mole per hour, in a 250-gm. rat.

The synthesis of the protein-bound glucosamine from glucose in the other organs studied, namely, kidney, lung, testes, and spleen, was shown to be well below that of the liver.

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