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GRADUATE COLLEGE

THE ABSORPTION AND METABOLISM OF N-ACETYL-D-GLUCOSAMINE AND D-GLUCOSAMINE IN THE INTACT RAT

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THE ABSORPTION AND METABOLISM OF N-ACETYL-D-GLUCOSAMINE

AND D-GLUCOSAMINE IN THE INTACT RAT

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THE ABSORPTION AND METABOLISM OF N-ACETYL-D-GLUCOSAMINE AND D-GLUCOSAMINE IN THE INTACT RAT

CHAPTER I

INTRODUCTION

Cori (1), in the introduction to his classic paper, "The Fate of Sugar in the Animal Body", summarizes very concisely the problems involved in obtaining information on the overall metabolism of a sugar in the intact animal. He states: "Without an accurate knowledge of the laws of intestinal absorption it would seem impossible to follow the fate of sugar in the animal organism on a quantitative basis. For convenience, the fate of sugar in the animal body may be divided into four steps: first, the absorption from the intestinal tract; second, the passage through the blood and occasionally elimination in the urine; third, the penetration into the tissues; and fourth, the disposal in the tissues".

In the studies reported here, the investigation involves the absorption of N-acetyl-D-glucosamine and D-glucosamine, their incorporation into serum and tissue glycoproteins, their conversion into other compounds, and their elimination from the body. All studies were carried out on intact albino rats.

CHAPTER II

REVIEW OF THE LITERATURE

General Chemistry of Glucosamine (2)

The chemical properties of glucosamine are those typical of both hexose monosaccharides and primary amines. Glucosamine is a reducing sugar and behaves as do neutral hexoses towards alkaline copper and silver reagents. Oxidation of the reducing group leads to the formation of the corresponding 2-amino gluconic acid. More vigorous oxidation results in the loss of the amino group and the formation of a mixture of dicarboxylic acids such as oxalic, maleic, etc. Glucosamine is susceptible to oxidation by glycol splitting reagents, such as sodium metaperiodate and lead tetra acetate. O- and N- derivatives are formed with acetylating reagents, and methyl ethers are formed under conditions similar to those applied to other hexoses; pyranosides and furanosides are known.

Glucosamine reacts readily with carbonyl compounds to give Schiffs bases. N-acyl derivatives are formed when the amino sugar reacts with molar proportions of acid chlorides and acid anhydrides in organic solvents. Secondary and tertiary bases and quaternary ammonium derivatives result from the alkylation of the amino group. 1-fluoro-2,4-dinitrobenzene condenses with glucosamine to form the DNB

derivative. This reaction is particularly useful in the separation of glucosamine from galactosamine and from other sugars by chromatography or paper electrophoresis. Oxidative degradation with ninhydrin or chloramine-T results in the loss of the amino group and decarboxylation at carbon number one with the formation of D-arabinose.

A number of relatively specific color reactions are used in the quantitation of glucosamine. The two of major importance are the Elson-Morgan and Dische-Borenfreund tests.

In the Dische-Borenfreund method (3) the 2-aminohexoses are deaminated by nitrous acid which leads to the formation of 2,5-anhydrosugars. These sugars give a color reaction with indole in the presence of HCl. Other sugars also appear to react with indole and give values for the amino sugars that are higher than those obtained in the Elson-Morgan method. The basis for the Elson-Morgan reaction (4) is the condensation of the 2-amino hexose molecule with acetyl-acetone in alkaline solution to give pyrroles. The presence of amino acids and reducing sugars interfere with the color production. A number of modifications of the method (5), (6), (7) have been described for the estimation of glucosamine in the presence of proteins and protein hydrolysates.

Glucosamine does not give a positive Molisch reaction for carbohydrate, nor does it react with any of the acid phenol reagents for aldoses.

In aqueous alkaline conditions, glucosamine undergoes a series of complex changes, including deamination. It is readily oxidized in the presence of air. Deamination is also brought about by condensation

with phenylhydrazine and by reaction with nitrous acid.

Distribution of Glucosamine

Glucosamine is found in a wide range of animal species as a constituent of the structural substances and biological fluids.

Heparin, a sulfated mucopolysaccharide with marked anticoagulative properties, was first isolated in 1916 by McLean (8). Analysis of highly purified heparin (from lung and liver) has shown that the principle monosaccharide units are D-glucosamine and D-glucuronic acid (9),(10). Hoffman and Meyer (11) have designated the structure of the heparin disaccharide as O-(2-acetamido-2-deoxy- -D-glucopyranosyl)-(1⟶4) D-glucuronic acid.

Chitin, an important constituent of the exoskeleton of invertebrates, yields equimolar portions of D-glucosamine and acetic acid when completely hydrolyzed under acidic conditions (12). Chitin is a relatively common constituent of fungi (2). In 1934 Meyer and Palmer (13) isolated a mucosubstance from bovine vitreous humor which they named hyaluronic acid. Hyaluronic acid is now considered to be composed of repeating units of hyalbiuronic acid (N-acetyl-glucosamine and D-glucuronic acid). The hexosaminidic linkages are β(1⟶4) and the glucuronidic linkages are β(1⟶3) (11). Substances with properties similar to hyaluronic acid have subsequently been isolated from umbilical cord (14), tumor fluids (15), synovial fluid (16), ocular fluids, skin, and group A and C hemolytic streptococci (2). Blood group substances are mucopolysaccharides of high molecular weight and are composed of carbohydrates and peptide constituents. Acidic hydrolysis

of the purified A and O substances of hog gastric mucin results in the liberation of L-fucose, D-glucosamine, D-galactosamine and D-galactose, along with about eleven amino acids (2).

Other mucosubstances reported to contain glucosamine are: Submaxillary mucin, gastric mucin, urinary mucin, cervical mucins, respiratory mucins, hen egg mucosubstances and frog spawn mucin (2). Craig and Uzman reported in 1957 (17) the isolation of a lipopolysaccharide composed of glucose, galactose, glucosamine, fatty acids, and neuraminic acid from human liver. Glucosamine and its derivatives are also found in components of a wide variety of bacterial and fungal products (2).

Proteins containing carbohydrate (glycoproteins) have as their sugar constituents, in addition to the neutral sugars mannose, galactose, fucose and various derivatives of neuraminic acid, the amino sugars glucosamine and galactosamine, usually present in their N-acetyl form. N-acetylglucosamine is a major carbohydrate constituent of the following glycoproteins: Orosomucoid, fetuin, hapto-globin, ceruloplasmin, transferrin, bovine prothrombin, bovine fibrinogen, 7s gamma globulins, 19s gamma globulins, Tam and Horsfall urinary glycoprotein, human follicle-stimulating hormone, human chorionic gonadotropin, human thryroglubulin, ovine submaxillary glycoprotein, and ovalbumin (18).

The protein bound hexosamine in the serum is elevated in many disease states. Boas (19) has demonstrated an increase in serum hexosamine levels following myocardial infarctions, surgical operations,

and an acute attack of gout. In gout, an increase in the alpha-globulin hexosamines was shown to account for the observed increase in total serum hexosamine. Elevated serum hexosamine levels were also noted in rheumatic fever, rheumatoid arthritis, and lupus erythematosus. In diseases of the central nervous system, an increase of total hexosamine content and protein was found in the cerebrospinal fluid of patients with meningitis, progressive paralysis, and with brain tumors. Similar increases of hexosamine and protein were not found in degenerative vascular and senile conditions, or in treated epileptics (20). An increase in serum hexosamine has also been demonstrated in patients with diabetes mellitus (21). Winzler (22) reports the level of protein bound hexosamine (both glucosamine and galactosamine) in normal sera to be 83 ± 9 mg. %. Shetlar, et al. (23) report a somewhat lower value of 71 ± 4 mg. %.

Metabolism of Glucosamine

In efforts to determine whether glucosamine can be utilized directly by animals, various feeding experiments have been carried out. In 1929, Ariyama and Takahasi (24) fed glucosamine-HCl to rats and found an assimilation of 0.30 gm. per 100 gm. of body weight. Catchart and Fablan (2) have reported a slow rate of absorption of glucosamine from the intestine. Using sacs of hamster intestine, Wilson and Crane (25) were able to show that D-glucosamine is not transported across the intestinal wall.

Rats fed a diet supplemented with dried beef fibers which had been heated with 15% D-glucosamine showed a marked decrease of growth

and a mortality of 50% (26). The first deaths occurred at 19 days and the livers were abnormal. The addition of 20% D-glucosamine to the diet after heating had no effect.

Kent and Whitehouse (2), in their review on amino sugars, state that in man glucosamine can apparently be metabolized in amounts up to 100 mg./kg., and that larger doses fail to produce hyperglycemia or glucosuria even in insulin-treated patients.

Becker and Day (27) fed glucose-1-C^{14} and glucosone-1-C^{14} to rats and found that in those animals fed the labeled glucosone, the labeled glucosamine level in the mucoproteins was higher than in animals which had been fed glucose. Glucosamine labeled in the number one position was isolated from the ovomucoid of eggs after feeding chickens glucose-1-C^{14} (28).

Spiro (29) injected rats with glucose-1-C^{14} and found considerable incorporation of the label into the bound glucosamine of the liver and serum. There appeared to be very rapid interchange between the liver and serum glucosamine. The calculated turnover time for glucosamine was 0.8 hour for liver and 2 hours for serum. The liver appeared to be the primary site of synthesis of serum glucosamine from glucose. 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 than of the liver.

Other studies (30),(31) have also indicated the presence of enzymes in several mammalian tissues which are capable of synthesizing glucosamine. Similar enzyme systems have been found in bacteria (32),(33) and in fungi (34).

A number of observations indicate the existence of an alternative pathway (other than through glucose) utilizing preformed glucosamine in the biosynthesis of hexosamine moieties. Dorfman et al. (35) report the direct incorporation of glucosamine labeled with C^{14} and N^{15} into hyaluronic acid by hemolytic streptococci without previous deamination.

Boas (36) administered D-glucosamine HCl intravenously to rats and concluded that it was rapidly distributed in all tissues of the body, and that the greatest concentration increase was in the kidneys, liver, and intestines.

Shetlar et al. (37) have shown that the radioactivity from D-glucosamine-1-C^{14} administered intraperitoneally to rats is found in high concentrations in the kidney, liver, spleen, lung, brain, digestive system and blood serum. Over 30% of the administered glucosamine was metabolized and converted to carbon dioxide, and 15% was excreted in the urine. Examination of tissues which had incorporated radioactivity from administered D-glucosamine-1-C^{14} had shown that the labeled sugar is incorporated without appreciable degradation. Such studies (38), (39) have been made in serum glycoproteins of rats and rabbits. In the serum studies, glucosamine was isolated from serum protein hydrolysates with the radioactivity still in the number one position, and only negligible amounts of radioactivity were found in the neutral sugars. Labeled sialic acid, however, was found. In rabbits administered D-glucosamine-1-C^{14} intraperitoneally, labeled glucosamine was found in liver glycoproteins (40) and liver mucopolysaccharides (41). No radioactivity was detected in liver glycogen,

amino acids, extractable lipids or neutral sugars associated with protein. Kohn et al. (42) report similar findings on the incorporation of labeled glucosamine into glycoprotein of rat liver. They also found that administered N-acetyl-1-C^{14}-D-glucosamine was not as readily incorporated in glycoproteins as glucosamine and that it was rapidly deacetylated.

In in vitro studies of plasma glycoprotein metabolism with perfused rat liver, Richmond (43) found that glucosamine was converted to sialic acid derivatives at a slightly faster rate initially than to glycoprotein N-acetylglucosamine, and that N-acetylglucosamine was a poorer precursor of macromolecular N-acetylglucosamine than glucosamine.

Enzymatic Studies on the Metabolism of Glucosamine

Numerous studies on the metabolism in vitro of D-glucosamine have shown that the initial step in its utilization is the conversion to D-glucosamine-6-phosphate. This is apparently accomplished by a nonspecific hexokinase. Hexokinases, found in extracts of brain, liver, kidney, and yeast, are capable of phosphorylating not only glucose, but also preformed glucosamine (31),(44). Glucosamine-6-phosphate has a low reactivity with phosphoglucomutase (45), and it is doubtful if the 6-phosphate is converted to the 1-phosphate by this enzyme. The acetylation of glucosamine-6-phosphate has been studied in Neurospora crassa (46) and yeast (47). Davidson et al. have shown that the enzyme concerned is specific for glucosamine-6-phosphate (48). The resulting N-acetylglucosamine-6-phosphate can then be converted to N-acetylglucosamine-1-phosphate by a mutase (49).

The reaction of N-acetylglucosamine-1-phosphate with uridine triphosphate in the presence of a pyrophosphorylase to form UDP-N-acetylglucosamine has been described by Maley and Lardy (50). Glucosamine-6-phosphate can also be deaminated to D-fructose-6-phosphate and ammonia (51); however, Maley and McGarrahan (52) have shown in <u>in vivo</u> experiments with rat liver that the sequence of reactions from glucosamine to N-acetylglucosamine-6-phosphate, fructose-6-phosphate and ammonia is greatly influenced by the free D-glucose concentration of the liver, and because of its potent inhibitory effect on the phosphorylation of glucosamine, they have proposed another pathway: that of glucosamine $\longrightarrow$ N-acetylglucosamine $\longrightarrow$ N-acetylglucosamine-6-phosphate. They support this hypothesis by the finding in rat liver, after the intraportal injection of glucosamine-1-C^{14} and N-acetylglucosamine-1-C^{14}, the primary products N-acetylglucosamine, N-acetylglucosamine-6-phosphate, and a mixture of uridine diphosphate N-acetylglucosamine and uridine diphosphate N-acetylgalactosamine. Little or no radioactivity was detected in the hexoses, hexose phosphates, or uridine diphosphate glucose. The glucose content of liver was great enough to inhibit the phosphorylation of glucosamine in most instances. No information is available on the mechanism of incorporation of UDP-N-acetylglucosamine into glycoprotein fractions; nor has it been established that the UDP derivative is essental for incorporation. In the case of acid mucopolysaccharides, the picture is a little clearer, and uridine derivatives of the amino sugars appear to be obligatory for their incorporation into the polysaccharide polymers (53). Stanley <u>et al.</u> (54) have reported the synthesis of N-acetylglucosaminylribitol linkages

in teicholic acid by a particulate enzyme preparation from Staphylococcus aureus.

Besides incorporation into glycoproteins and acid mucopolysaccharides, UDP-N-acetylglucosamine has been observed to undergo an epimerase reaction to UDP-N-acetylmannosamine and then to sialic acids (55), (56), (57). Cardini and Leloir (58) have described the enzymatic epimerization of UDP-N-acetylglucosamine by rat liver to UDP-N-acetylgalactosamine.

The Effect of Glucosamine and N-Acetylglucosamine on Metabolism

The inhibition of glucose metabolism by glucosamine and N-acetylglucosamine has been reported by various investigators. Spiro noted a pronounced competitive inhibition of glucose conversion to glycogen and carbon dioxide, as well as a decrease in fatty acid synthesis and in the calculated glucose phosphorylation by these two amino sugars(59). Glucosamine in the presence of insulin produced a significant inhibition of glucose-1-C^{14} oxidation when it was incubated with segments of rat epididymal fat pad (60). N-acetylglucosamine was ineffective in this respect. Voss et al. (61) report that glucosamine inhibits glycolysis in both Yoshida rat and Ehrlich mouse ascites tumors. In contrast to the inhibition of glycolysis by glucosamine and N-acetylglucosamine, Kushida (62) reports that N-acetyl-D-glucosamine has no effect on the respiration of rat liver slices and that N-acetyl-D-galactosamine stimulated the respiration by about 60%.

CHAPTER III

MATERIALS AND METHODS

Radioisotopes

N-acetyl-D-glucosamine-1-C^{14} was provided through the courtesy of Dr. Domenic Iezzoni, Chas. Pfizer and Co., Brooklyn, New York. D-glucosamine-1-C^{14}, N-acetyl-1-C^{14}-D-glucosamine and D-glucose-1-C^{14} were purchased from the New England Nuclear Corporation, Boston, Massachusetts. The radiopurity of these compounds was checked by a paper chromatography technique. Aqueous samples of the isotopes were applied to Whatman No. 1 chromatography paper and developed by the descending technique with a n-butanol, pyridine, and 0.1 N HCl solvent (5:3:2) (63).

The D-glucosamine-1-C^{14} and the N-acetyl-1-C^{14}-D-glucosamine were found to be pure; but the N-acetyl-D-glucosamine-1-C^{14} was contaminated with glucosamine. This impurity was removed by passing a solution of the mixture through a Dowex-50W-X12 resin column. The pure N-acetyl-D-glucosamine-1-C^{14} was washed from the column with water.

Preparation of Feeding Solutions

One molar solutions of D-glucose (Mallinckrodt, USP), N-acetyl-D-glucosamine (Pfizer, USP), and D-glucosamine-HCl (Calbiochem, A grade) were prepared in distilled water. Although the hydrochloride form of

glucosamine was used, the solution was prepared so as to be one molar with respect to glucosamine. These solutions were checked by the paper chromatographic technique described for the labeled compounds and no impurities were found.

On the day of the experiment, 15 ml. of the appropriate one-molar sugar solution were added to a weighed aliquot of the tracer to be used. The specific activities of the tracers were high enough so that the molarity of the final solutions was not appreciably changed. The maximum amount of tracer added was 15 mg. in 15 ml. The final solutions contained 5 microcuries of isotope per ml. for the N-acetyl-1-C^{14}-D-glucosamine, D-glucose-1-C^{14}, and D-glucosamine-1-C^{14} preparations. The N-acetyl-D-glucosamine-1-C^{14} solution contained 2.5 microcuries of isotope per ml.

Experimental Animals

Young Holtzman male rats weighing approximately 100 gm. were used. Twenty-four hours prior to the experiment, the animals were fasted and provided with water only. The fasting period was generally long enough to provide a relatively empty intestinal tract.

Neomycin Sulfate Treatment

In some cases the rats were treated with neomycin sulfate (Nutritional Biochemical Corp.) for two days. The antibiotic was supplied in the drinking water at a concentration of 0.5%. The animals were allowed food on the first day and then fasted the final 24 hours prior to the experiment. At the end of the neomycin treatment period, rectal swabs were made and streaked on desoxycholate agar.

At the end of 72 hours, only an occasional colony of E. coli. could be found on the cultures from the treated rats. In the case of the untreated animals, a heavy growth of E. coli was obtained.

Insulin Treatment

Some of the animals were treated with insulin prior to the experiment. The fasted animals were injected subcutaneouly with 1.0 unit/kilogram body weight of insulin (Lilly, USP Iletin) 30 minutes before the beginning of the experiment. Although this dosage caused the animals to appear sluggish and drowsy, it did not produce a condition of shock.

Phlorizidin Treatment

Another group of animals was poisoned with phlorizidin by the method of Bogdanova and Barker (64). Prior to the experiment, the animals were given five 0.25 ml. subcutaneous injections of 10% phlorizidin dihydrate (Calbiochem) in sesame oil at intervals of 12 hours. This treatment was in addition to the routine 24 hour fasting period. At the end of the phlorizidin treatment all animals exhibited a copious glycosuria.

In some groups of animals there was a combination of neomycin and insulin treatment or neomycin and phlorizidin treatments.

Feeding of Animals

After the pretreatment and/or fasting period, the animals were weighed and divided into groups of 9 each. Each group was then fed by stomach tube 1 ml. of the 1 M sugar solution to be investigated.

The device used to feed the animals was fashioned from a tuberculin syringe fitted with a blunt nosed 20 gauge needle over the hub of which had been slipped a No. 8 urinary rubber catheter. The inside diameter of the catheter was small enough so that capillary action prevented the solution from draining after the device was filled. This assembly was fitted into a lock device which insured a constant filling of the syringe and catheter when the plunger was retracted to its maximum preset limit. The constancy of delivery of this feeding device was determined and the average error was found to be 0.82 per cent in a series of 9 on a 1 ml. delivery.

One ml. of each feeding solution was emptied from the feeding device into a volumetric flask and diluted to 200 ml. with water. The radioactivity found in this solution was used as the base line for the calculations of absorption.

Immediately after feeding, the animals were divided into groups of three and placed in Roth metabolism cages (65) where they had free access to water. They were maintained in the cages for 3 hours during which time urine was collected and expired carbon dioxide was trapped in a gas washing tower containing either 130 ml of 3M NaOH or 100 ml. of a mixture of monoethanolamine and ethylene glycol monoethyl ether (1:2 v/v) (66). NaOH was used to trap the carbon dioxide for the untreated animals fed D-glucosamine-1-C^{14}; the organic mixture was used for all other experiments. Both methods are equally efficient in trapping carbon dioxide, but the subsequent determination of radioactive carbon dioxide is much easier with the organic solvent mixture.

Sacrifice of the Animals and Recovery of the Intestinal Contents

After the three-hour period in the metabolism cages, the animals were anesthesized with ether and exsanguinated through the abdominal aorta. The gastrointestinal tract was clamped off with hemostats at its upper and lower extremities, gently pulled loose from the mesentary and placed in a beaker where it was slit longitudinally and thoroughly washed with water. The washings, intestinal contents, and instrument rinsings were transferred to a Waring blendor and homogenized. The homogenate was made up to 200 ml. with water and layered with toluene. An aliquot of this mixture was then centrifuged at 35,000 x g for 1 hour at -5° C. An aliquot of the resulting clear supernatant was then processed immediately for radioactivity determinations and the remainder stored at -20° C.

The liver was perfused with ice cold 0.85% saline and lyophilized. The washed gastrointestinal tract (stomach, small intestine, and large intestine) was lyophilized.

Studies of Serum Proteins

Blood collected from the animals was allowed to clot at room temperature, and the serum was obtained by centrifugation.

Total serum radioactivity was determined after incubating 0.050 ml. of serum with 1.0 ml. of Hyamine Hydroxide (diisobutyl cresocyethoxy-ethyl dimethylbenzylammonium hydroxide, Pilot Chemicals, Inc.) in capped scintillation vials (Nuclear Chicago Corp.) in an oven at 40°-50° C for 4 hours. Fifteen ml. of standard scintillation solution

were added (4 g. 2,5-diphenyloxazole and 50 mg. 1,4-bis-2-(5-phenyl-oxazolyl)-benzene, Nuclear Chicago Corp.) per 1000 ml. spectro quality toluene, (Matheson, Coleman and Bell). To effect solution of the Hyamine-serum complex in the toluene, 1.0 ml. absolute ethanol was added to the scintillation solution. This mixture was then counted in a Nuclear Chicago Corp. 725 room temperature liquid scintillation system. Results were expressed as c/m per mg. protein. Protein was determined by the biuret method.

Protein bound radioactivity was determined by dialyzing 0.20 ml. of serum in Visking casing against running tap water at 8° C for 72 hours. The dialyzed serum was diluted to 5.0 ml. and 1.0 ml. of this solution was pipetted into scintillation vials, taken to dryness, and then heated with 1.0 ml. Hyamine as before. The determination of radioactivity was as previously described. The results were also expressed as c/m per mg. protein.

Tissue Studies

The dried tissues (liver and small intestine) were ground in a Wiley mill to pass through a 60-mesh screen. Solutions of tissue for total radioactivity were made by heating aliquots (4 to 30 mg.) of the ground, dried tissue with 1.0 ml. Hyamine and 0.1 ml. water in capped scintillation vials at 40°-50° C for 8 hours. Fifteen ml. of the standard scintillation solution and 1.0 ml. ethanol was added to the samples and they were then counted as previously described. The activity was expressed as c/m per mg. dry tissue.

Bound radioactivity of the tissues was determined after dialysis

of homogenates of ground dry tissue in tap water. Two ml. of the homogenates were pipetted into Visking casings and dialyzed against running tap water at 8° C for 72 hours. Two ml. aliquots of the dialyzed material were pipetted into weighed scintillation vials and taken to dryness. The vials and their contents were then weighed and the weight of the tissue determined by difference. The procedure for counting the dialyzed tissues was the same as described for the non-dialyzed tissue. The radioactivity was expressed as c/m per mg. dry tissue.

Determination of Radioactivity in Intestinal Contents, Feeding Solutions, and Expired CO_2

0.020 ml. of the diluted feeding solutions and intestinal contents were pipetted into scintillation vials, 3.0 ml. absolute ethanol and 15 ml. of standard scintillation solution were added, and the radioactivity measured. Radioactivity was expressed as c/m per ml. for the feeding solutions and as total c/m washed from the gastrointestinal tract for the intestinal washings.

Radioactivity in the expired carbon dioxide, in the cases where the organic solvent mixture was used as the trapping agent, was determined by pipetting 3.0 ml. of the CO_2-amine complex into scintillation vials and adding 15 ml. of scintillation solution composed of toluene, ethylene glycolmonomethyl ether (2:1 v/v), and 5.5 gm. 2,5-diphenyloxazole per 1000 ml. of solution. Samples were counted as previously described and the radioactivity was expressed as total c/m expired in 3 hours.

In the experiment in which NaOH was used to trap the CO_2,

aliquots of the NaOH solution were mixed with an excess of saturated $BaCl_2$. The precipitated $BaCO_3$ was washed with water and suspended in absolute ethanol. An aliquot of the suspension was plated on concentric stainless steel planchets (Atomic Accessories), dried, and the radioactivity determined in a Nuclear Chicago gas flow counting system. Corrections were made for self-absorption of the sample and machine efficiency so that the determined c/m expired could be adjusted to absolute counts. This data may then be compared directly with that obtained with the liquid scintillation system.

Identification of Radioactive Compounds

Samples of the dialyzed tissues (serum, liver and small intestine) were prepared by pooling aliquots from within each experimental group. These samples were hydrolyzed in two systems, one designed to liberate amino acid and amino sugars, and a less rigorous one which liberates neutral sugars. For the amino acid and amino sugar studies of the tissues and serum, samples (10-20 mg.) were hydrolyzed in 4 ml. of 6 N HCl at 100° C in closed tubes for 20 hours. For neutral sugar studies, the samples were hydrolyzed in 2 N HCl at 100° C in closed tubes for 18 hours. The hydrolysates were then decolorized with Norite A and taken to dryness under vacuum over $CaSO_4$ or silica gel and NaOH pellets.

To effect the separation of neutral sugars, amino sugars and amino acids, the dried hydrolysate residues were dissolved in 0.5 ml. of water and chromatographed on a 1 x 6.5 cm. column of Dowex 50X12, 100-200 mesh resin in the hydrogen form. Neutral sugars were washed

from the column with 20 ml. of water, hexosamines were eluted with 30 ml. of 0.3 N HCl and the amino acids with 20 ml. of 4 N HCl. These fractions were taken to dryness by the same procedure used for the original hydrolysates and the residues dissolved in 0.5 ml. water. 0.20 ml. of these solutions were pipetted into scintillation vials, mixed with 30 ml. absolute ethanol and 15 ml. of the standard scintillation solution and counted. 0.03 ml. of the remaining solution were applied to Whatman No. 1 chromatography papers and the chromatograms developed in an n-butanol, pyridine, 0.1 N HCl (5:3:2) system. Amino acids were detected by dipping the chromatograms in 0.2% ninhydrin in a 1% solution of glacial acetic in acetone. Hexoses were detected by dipping the chromatograms in a solution of O-aminobiphenyl (3% aminobiphenyl in a 1.3% solution of phosphoric acid in glacial acetic acid), and then heating at 100-110° C for 2-5 minutes. The amino sugars were detected with both color developing reagents.

The intestinal contents were not hydrolyzed prior to application to the column because even mild acid hydrolysis destroys N-acetylglucosamine. In addition to hexoses, N-acetylglucosamine is present in the water eluant.

Radioactive sugars in the urine were detected after applying 0.005 ml. aliquots to Whatman No. 1 paper and developing the chromatogram in the butanol-pyridine-HCl solvent. The sugars were located with the aminobiphenyl reagent. The radioactivity of these spots was detected by cutting them out, placing them in scintillation vials with 7 ml. of the standard scintillation solution and counting them for 20 minutes.

Correction of Observed Counts for Quenching

All counts obtained with the liquid scintillation counter, with the exception of those from the paper chromatogram spots, were corrected for quenching by the channels ratio method (67), (68). This method was found to give results identical to those obtained by internal standardization for all samples with the exception of the liver tissues. For these tissues a special efficiency to ratio curve was constructed from nonradioactive liver and added C^{14}.

Statistical Methods

With the exception of the paper chromatogram spots, all samples were counted until a total count of at least 10,000 was obtained. The per cent error is 2.00 at the 96% confidence level for this total number of counts.

Data were compared statistically by the "t" test as applied to the means of small samples (69) using the formula

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\left(\frac{N_1 + N_2}{N_1 + N_2 - 2}\right)^{\frac{1}{2}} \left(\frac{N_1 S^2_1 + N_2 S^2_2}{N_1 N_2}\right)^{\frac{1}{2}}}$$

where $\bar{x}_1$ and $\bar{x}_2$ represent the means of two groups, N_1 and N_2, the number in each group, and S_1 and S_2, the respective standard deviation.

In the following discussion, differences are considered to be significant only where the calculated probability values are equal to or less than 0.01.

CHAPTER IV

RESULTS AND DISCUSSION

Absorption of D-Glucosamine and N-Acetyl-D-Glucosamine

In 1925, Carl F. Cori (1) published a method for the quantitative study of absorption from the whole intestinal tract of the rat. The animals were fed a known amount of the substance under investigation by a stomach tube. After a given time, the rats were killed and the amount of substance remaining in the intestines was determined quantitatively. The difference between the amount fed and the amount recovered from the whole intestinal tract was considered as the amount of substance absorbed. He proposed that the amount of a given substance that is absorbed should be proportional to the absorbing surface of the intestinal tract, and that, since the surface of the intestine will vary with body weight, larger animals will absorb more than smaller ones. He showed that the ratio of the amount absorbed to body weight was a constant. He was careful to point out, however, that this proportionality between the amount absorbed and the body weight was found in rats that were still in a period of active growth, and that it might not be valid for fully grown rats where variations in depot fat and other factors might play a role. In addition to the relationship between absorption and body weight, he was able to show

that, when the amount of sugar that is absorbed per 100 gm. of body weight is plotted against time, a straight line relationship is obtained. On the basis of these experiments utilizing the sugars glucose, galactose, fructose, mannose, xylose and arabinose, he formulated the following equation for calculating absorption coefficients (C), the amount of a given substance absorbed per 100 gm. of body weight in one hour:

$$C = \frac{A \times 100}{W \times T}$$

where A is the total amount of substance absorbed in a given period, W is the weight in grams of the rat after fasting for 48 hours and T is the length of the period in hours.

Cori found that the rate of absorption was not affected by the concentration or amount of sugar fed; however, a hypertonic sugar solution was diluted in the stomach. Other workers, using Cori's technique for studying sugar absorption, have published results which are at variance with those of Cori. Cori's conclusion that the rate of absorption of glucose was not influenced by variations in the concentration of the administered solution has been questioned. Macleod *et al.* (70) concluded that absorption was greatest from a 13.5 per cent glucose solution, and MacKay and Bergman (71) have reported that the absorption rate is influenced by the concentrations of the administered solutions. The conclusion that the rate of absorption is constant has been objected to by Pierce *et al.* (72) and by Feyder and Pierce (73), who have shown a decreased rate of absorption during the second and third hours of the absorption period. In

similar investigations, Fenton (74) was led to conclude that absorption from the intestine of the intact animal increased significantly as the concentration of the solution which was fed increased, and that the rate of absorption, as well as the absorption coefficient, decreased with time. Fenton also reported a nonlinear relationship between body weight and absorption; however, he also found that the absorption was not entirely independent of body weight.

In the investigations reported here, it was of interest not only to obtain information concerning the absorption of D-glucosamine and N-acetyl-D-glucosamine in the intact rat, but also to determine the fate of these amino sugars after they were absorbed. For these reasons, the basic technique as described by Cori (1), even though it appears not to give the best estimates of absorption rates, was chosen.

An attempt has been made to limit as many variables as possible in these studies. All animals were approximately the same age and body weight and were males of the Holtzman strain. The sugar solutions fed were the same molar concentrations. The majoritity of other studies of absorption have been made with sugar solutions whose concentrations have been expressed as percentages. It is felt that, regardless of the mechanism of movement of the sugars from the mucosal to the serosal side of the intestine, i.e., whether an active transport mechanism or a simple diffusion process is in operation, a more realistic approach to the problem of sugar concentration would be to make comparisons of absorption only when the sugars are present at

initially equal molar concentrations. The period of absorption was 3 hours in every case. This time period was chosen not only for ease in handling several animals over a staggered period of time, but also because, at intervals longer than 3 hours, the animals fed the amino sugars exhibited diarrhea.

Cori and other workers have used colorimetric copper reduction methods to determine the amount of sugar remaining in the intestine after the absorption period. These methods are not only tedious, but also have the disadvantage that substances may be present in the intestinal contents which interfere with the colorimetric determinations. In the absorption studies reported here, the amount of sugar absorbed was determined by the isotope dilution technique. From the amount of radioactivity associated with the initial sugar concentration of the feeding solution and the amount of radioactivity recovered in the intestinal washings, the amount of sugar absorbed can be calculated by simple ratio and proportion. This technique is valid only if there is no degradation of the labeled sugar while it is in the lumen of the intestine. To investigate the possibility of such degradation, aliquots of the intestinal contents from the animals fed D-glucosamine-1-C^{14} and N-acetyl-D-glucosamine-1-C^{14}, and also aliquots of intestinal contents from animals fed the same labeled sugars but treated with the antibiotic neomycin, were concentrated and chromatographed on Whatman No. 1 paper in a n-butanol, pyridine, 0.1 N HCl solvent system (5:3:2). The radioactivity associated with the chromatographically isolated compounds is given in Table 1. The only appreciable radioactivity was found associated with the original

radioactive sugar. This would appear to indicate that there is no destruction of the sugars tested in the intestine. However, this conclusion is not entirely valid since a conversion of glucosamine to a more readily metabolizable compound, such as fructose, might provide a hexose which could easily pass through the intestinal wall and would not be detected by the procedure. Data will be presented later which indicate that there is probably some destruction of the glucosamine molecule by the intestinal flora. It is significant, however, that no glucosamine was found in the intestinal contents of the animals that were fed N-acetylglucosamine. This provides direct evidence that the acetylglucosamine molecule is not appreciably deacetylated before leaving the lumen. Cori (1) investigated the possibility of loss of glucose from the intestine due to bacterial action, and found that the loss was so small that it could be neglected.

Using a combination of Cori's technique and the radioisotope dilution technique, the absorption coefficients for D-glucosamine and N-acetyl-D-glucosamine were determined. These results (Table 2) indicate that both glucosamine and N-acetylglucosamine are absorbed at approximately the same rate, and that this rate of absorption is much slower than the absorption rate for glucose. These results are in agreement with the hypothesis of Crane (25) which predicts the characteristics a sugar molecule must have in order to be actively absorbed by the intestine, i.e., the compound must possess a D-pyranose structure, a methyl or substituted methyl group at carbon five of this structure, and a hydroxyl group in the glucose configu-

ration at carbon two. It is readily apparent that both glucosamine and N-acetylglucosamine meet this criteria with the exception of the hydroxyl group in the glucose configuration at carbon two. It is not the purpose of this investigation to determine the mechanism of transport of the amino sugars across the intestinal wall.

It should be mentioned that the data given for the absorption of glucose is misleading in that it indicates that only one millimole of glucose is absorbed in a 3-hour period. Almost identical values (Table 3) were obtained for 1-hour absorption periods. It is indicated then that an animal fed 1 ml. of 1 M glucose (0.18 gm. glucose) is able to completely absorb this amount in at least one hour. This is in agreement with Cori (1) who gives a value of 0.178 gm. glucose absorbed per 100 gm. body weight per hour. The absorption data for glucose (Table 2) is included only for the purpose of contrasting the absorption rate of an actively transported sugar and the amino sugars.

There is a significant difference in the absorption of glucosamine in animals which have had their intestinal flora diminished by neomycin treatment and in untreated animals (Table 2). The decrease in the absorption rate in the treated animals could possibly be due to an inhibition by the neomycin.

It is to be noted that there is no difference in the absorption rate of N-acetyl-D-glucosamine as determined by isotope dilution with the acetylhexosamine molecule labeled in either the acetyl moiety or the glucosamine moiety (Table 2). This data, together with that from the chromatograms of the intestinal contents of rats fed N-acetyl-D-

glucosamine-1-C^{14} (Table 1), lead to the conclusion that N-acetyl-glucosamine is absorbed from the lumen without degradation. This is perhaps significant in that the majority of the naturally occurring forms of glucosamine, as found in tissue glycoproteins and mucopolysaccharides, is N-acetylglucosamine. It is possible, therefore, that an animal may utilize a dietary source of N-acetyl-D-glucosamine to supplement the de novo synthesis of acetylglucosamine compounds in the body.

Metabolism of Absorbed D-Glucosamine and N-Acetyl-D-Glucosamine

Having established that D-glucosamine and N-acetyl-D-glucosamine are absorbed from the intestine, it was of interest to follow the metabolism of these compounds in the living animal.

At the end of the 3-hour period, 10% of the absorbed radioactivity had been expired as CO_2 in the animals treated with neomycin and fed N-acetyl-D-glucosamine-1-C^{14} (Table 7). Essentially similar results were found in the untreated animals fed N-acetyl-D-glucosamine-1-C^{14}. There was a large difference in the per cent absorbed radioactivity expired as CO_2 in the treated and untreated animals fed glucosamine-1-C^{14} (4.5% for the neomycin treated animals and 17.9% for the untreated animals). This is a further indication that in animals not treated with neomycin glucosamine is degraded in the lumen of the intestine by microorganisms.

An appreciable amount of radioactivity from absorbed D-glucosamine-1-C^{14} and N-acetyl-D-glucosamine-1-C^{14} was found in the body tissues.

Radioactivity was also found in tissues of animals fed glucose-1-C^{14}. In the tables referred to below which show the distribution of radioactivity in the tissues, the data in the columns headed "Percentage Absorbed Radioactivity in ---" (serum, liver or small intestine) are the percentage of the radioactivity that was absorbed from the intestine and found to be present in the entire tissue under investigation. This value then is based upon the total radioactivity in the particular tissue and represents both "bound" and "unbound" radioactivity. The data in the columns headed "Percentage Non-Dialyzable Radioactivity in ---" (serum, liver or small intestine) is the percentage of the radioactivity in a tissue (c/m per mg.) which remains after dialysis of the tissue against running tap water for 72 hours.

In the animals treated with neomycin and then fed D-glucosamine-1-C^{14}, 2.0% of the absorbed radioactivity was in the serum (Table 4), 7.1% of the absorbed radioactivity was in the liver (Table 5), and 12.4% was in the walls of the small intestine (Table 6). The relatively high values for the percentage absorbed radioactivity in the liver and the percentage nondialyzable radioactivity in the liver of the animals fed D-glucose-1-C^{14} as compared to those fed D-glucosamine-1-C^{14} or N-acetyl-D-glucosamine-1-C^{14} probably reflects the conversion of the absorbed glucose to glycogen. Other investigators have reported (39), (40), (42) that intraperitoneally injected glucosamine is not converted to glycogen, and the data in Table 5 indicate that glucosamine or N-acetylglucosamine which is absorbed from the intestine is also not readily converted to glycogen. Results in

Tables 4, 5, and 6 also show that in the animals fed D-glucosamine-1-C^{14}, 33.2% of the radioactivity in the serum was nondialyzable or "bound" (presumably to serum protein); 16.2% of the radioactivity in the liver was nondialyzable and 23.2% of the radioactivity in the small intestine tissue was nondialyzable. These results are in agreement with those of others (37), (38), (39), (42), (43), who have reported D-glucosamine-1-C^{14} to be efficiently incorporated into plasma proteins and tissues.

In animals treated with neomycin and then fed N-acetyl-D-glucosamine-1-C^{14}, 1.4% of the absorbed radioactivity was found in the serum at the end of the 3 hour absorption period, and 19.7% of the radioactivity there was nondialyzable (Table 4); 2.2% of the radioactivity was in the liver, and 37.3% of the liver radioactivity was nondialyzable (Table 5). The small intestine tissue contained 8.7% of the radioactivity absorbed from the intestine, and 28.6% of its radioactivity was nondialyzable.

In the animals treated with neomycin and then fed glucosamine, the radioactivity which was absorbed from the intestine and present in the serum was significantly higher than similarly treated animals fed N-acetyl-D-glucosamine-1-C^{14}. The data for the percentage of the radioactivity in the serum and tissues which was nondialyzable indicate that there also was significantly greater binding of the label in the serum of the neomycin treated and glucosamine-1-C^{14} fed animals. However, the amount of label bound in the liver was significantly less than in those animals treated similarly and fed N-acetyl-D-glucosamine-1-C^{14}. There appeared to be no difference in the percentage of the

absorbed radioactivity found in the small intestine tissue of the animals fed 1-C^{14} labeled glucosamine or N-acetylglucosamine, or in the percentages of the nondialyzable radioactivity there.

It was demonstrated that there was no difference in the rate of absorption of N-acetyl-D-glucosamine-1-C^{14} and N-acetyl-1-C^{14}-D-glucosamine in the animals treated with neomycin and in those which were not. This indicates that no deacetylation of N-acetylglucosamine occurred in the lumen of the intestine. There was the possibility, however, that N-acetylglucosamine was deacetylated after leaving the lumen of the intestine. If this were to occur to any appreciable extent, it might be expected that the glucosamine formed would spill over into the urine; however, no glucosamine was found in the urine of animals fed N-acetylglucosamine. Glucosamine was detected in the urine in the experiments in which an equivalent amount of glucosamine was absorbed (Table 14). The kidney has been reported to have a very low threshold for glucosamine (75), and radioactive glucosamine has been reported to be present in the urine of rats after parental injections of tracer amounts of glucosamine-1-C^{14} (42). Glucosamine was not detected on paper chromatograms of the urine from animals fed N-acetyl-1-C^{14}-glucosamine or N-acetylglucosamine-1-C^{14}, nor was any radioactivity detected in the area of glucosamine mobility (Table 13). The data in Table 7 indicate an increase in the percentage absorbed radioactivity expired as CO_2 for the animals fed the N-acetyl-labeled glucosamine (29.9%) as compared to the N-acetylglucosamine-1-C^{14} fed animals (10.1%). This would mean that approximately 0.08 millimoles of acetylglucosamine had been deacetylated or, stated

another way, 0.08 millimoles of glucosamine (14.4 mg.) had been produced. This amount was in excess of the 4.5 mg. of glucosamine-1-C^{14} tracer injected by Shetler et al. (37) in the experiment in which they reported finding radioactivity (glucosamine) in the urine. There was no apparent explanation for the absence of labeled glucosamine in the urine of animals fed N-acetylglucosamine-1-C^{14}; however, N-acetylglucosamine is absorbed at a slow rate from the intestine and it is possible that the free glucosamine formed would be metabolized before accumulating to a serum level high enough to spill over into the urine.

If deacetylation of the acetyl-labeled hexosamine molecule does occur in the animal body, it might be expected that the distribution of the label in the tissues would differ from that obtained with the hexosamine-labeled molecule. The only significant difference detected was in the percentage of nondialyzable radioactivity in the small intestine tissue (Tables 4, 5, and 6). The acetyl-labeled hexosamine produced a greater percentage of bound radioactivity in the small intestine and this is perhaps a reflection of the incorporation of the acetate moiety into lipid material.

The data presented do not give a clear picture as to the fate of the acetyl moiety of absorbed N-acetylglucosamine. There was evidence that the molecule was deacetylated as indicated by the increased percentage of the label in the expired CO_2 as compared to the percentage expired when N-acetylglucosamine-1-C^{14} was fed. However, the deacetylation reaction does not appear to be extensive since no glucosamine was found in the urine. Kohn et al. (42), in studies made with parenterally

injected (3 microcuries) N-acetyl-1-C^{14}-glucosamine and sodium acetate-1-C^{14}, reported the recovery of 38.3% of the isotope in CO_2 in the case of administered N-acetyl-1-C^{14}-glucosamine, and 54.5% in CO_2 from administered sodium acetate. There was a small percentage of labeling, from both compounds, of liver lipids. They interpreted these results as indicating a rapid deacetylation process for N-acetylglucosamine. However, no information was given concerning the amount of sodium acetate or acetylglucosamine injected, and no consideration was made of the effect of dilution on the injected isotopes by the acetate and acetylglucosamine pools.

It has been established by short term experiments in vivo (39), (40) and in vitro (30), (43) with glucosamine-1-C^{14} that this compound in addition to being readily incorporated into tissue glycoproteins and muco-substances, was not appreciably degraded in the animal. To investigate the possibility that enzymic reactions in the tissues of the small intestine could degrade absorbed glucosamine and N-acetyl-glucosamine, samples of dialyzed serum, and liver and small intestine tissue were hydrolyzed in 6 N HCl, and the hydrolysates fractionated on Dowex 50 columns into hexosamine and amino acid components. The radioactivity of each fraction was determined. Under ideal conditions only hexosamines are eluted with 0.3 N HCl and amino acids are eluted with 4 N HCl. However, it was demonstrated by paper chromatography that the 0.3 N HCl fraction was contaminated by traces of amino acids. No contamination of the 4 N HCl fraction by hexosamines was detected. Although the vigorous hydrolysis in 6 N HCl destroys almost completely

the neutral sugars and a portion of the hexosamines, this extreme condition was necessary in order to effect a nearly complete hydrolysis of the tissues so that contamination of the fractions by peptides was minimal. For this reason, the radioactivity detected in the hexosamine fraction from the column was lower than would have been detected if the hexosamine moieties in the tissues had been liberated without partial destruction. A more gentle hydrolysis of the tissues in 2 N HCl made it possible to identify the neutral sugar components by paper chromatography. Glucose, mannose, and galactose were detected in the hydrolysates of liver and the small intestine, and mannose and galactose were detected in the serum hydrolysates. If these sugars contained carbon-14, their specific activity was not high enough to be detected by the usual procedures.

Although not strictly quantitative because of the partial destruction of hexosamine during hydrolysis, the data presented in Table 15 indicate that a majority of the glucosamine absorbed from the intestine was incorporated without degradation of the carbon chain into hexosamine-containing substances of the tissues investigated. A greater percentage of the radioactivity in the hydrolysates of tissues from animals not treated with neomycin was in the amino acid fraction than in those animals which were treated with neomycin. These results are a further indication that glucosamine was degraded by the intestinal flora. Neomycin treatment does not seem to be a factor in the incorporation into serum and tissue amino acids of carbon-14 from N-acetylglucosamine-1-C^{14} fed animals. As in the

case of the glucosamine-1-C^{14} fed animals, both hexosamines and amino acids appear to be labeled.

The Effect of Phlorizidin and Insulin on the Absorption and Metabolism of D-Glucosamine and N-Acetyl-D-Glucosamine

It has been demonstrated that under the conditions described for these investigations absorbed glucosamine was more efficiently incorporated into the hexosamine-containing components of the body than was absorbed N-acetylglucosamine. It is possible that N-acetylglucosamine does not penetrate the cell membranes and, as a result, is not available to the enzymatic mechanisms responsible for the incorporation of hexosamine into glycoproteins and mucosubstances. It may also be that preformed N-acetylglucosamine is not readily phosphorylated in the cell, although McGarrahan and Maley (52) have demonstrated that N-acetylglucosamine is readily phosphorylated, in the absence of glucose, by rat liver.

The mechanisms of action of insulin are still somewhat obscure, but it appears that insulin is involved in: the transfer of certain sugars across cell membranes, the formation of hexose-6-phosphate and the regeneration of adenosine triphosphate (76). Phlorizidin has been demonstrated to produce glycosuria, inhibit the phosphorylation of hexose and the absorption of glucose, mannose, and galactose from the intestine (76). The action of phlorizidin and insulin on the absorption and metabolism of glucosamine and N-acetylglucosamine was investigated. Since it has been shown that glucosamine was degraded by the intestinal flora, only animals that had been treated

with neomycin were used.

Treatment of the animals with insulin produced no change in the rate of absorption when glucosamine-1-C^{14} was fed; however, the amount of carbon-14 in the CO_2 expired almost doubled with insulin treatment (Table 11). A slight increase was also detected in the amount of radioactivity expired in the CO_2 from the N-acetylglucosamine-1-C^{14} fed animals; however, no change in absorption was detected. Phlorizidin increased significantly the rate of absorption of glucosamine and decreased slightly the radioactivity expired as CO_2. The effect of phlorizidin on the absorption of N-acetylglucosamine was quite pronounced. The absorption coefficient decreased from 0.068 to 0.026 after treatment with phlorizidin, and a slight increase in the percentage of absorbed radioactivity expired as CO_2 was found (Table 11).

The effects of insulin and phlorizidin treatment on the incorporation of radioactivity from absorbed glucosamine-1-C^{14} and N-acetylglucosamine-1-C^{14} into the liver, serum and small intestine tissue as compared to incorporation in untreated animals are summarized in Tables 8, 9, and 10. Insulin produced no significant effect on the incorporation into tissues of radioactivity from D-glucosamine-1-C^{14} fed animals. Insulin was also ineffective on the incorporation of radioactivity into the tissues of N-acetyl-D-glucosamine-1-C^{14} fed animals.

The effect of insulin in increasing oxidation of the carbon-14 labeled sugars was similar to its effect on glucose oxidation (Table 11),

and suggests that insulin facilitates the entry of these amino sugars into the cell. There was a slight increase in the percentage of non-dialyzable radioactivity in the serum, liver and small intestine tissues, but the differences were not significant at the 1% probability level. Wick et al. (77) have reported that insulin greatly enhances the entrance of glucosamine into the muscle cells of eviscerated rabbits. Their experimental conditions differed from those described in this investigation in that they gave the animals a maximal amount of insulin (20 units per kilogram), which necessitated the maintainence of the animal by glucose infusions, and they made a single injection of glucosamine. The findings that insulin had no significant effect on either the total amount of glucosamine in the tissues or on the amount bound in the tissues, but did increase the oxidation of glucosamine to carbon dioxide (and perhaps accounts for the lack of increase in the total amount in the tissues), suggests that accessibility to the interior of the cell is not a factor in the incorporation of glucosamine into hexosamine-containing substances of the tissues. The lack of effect of insulin on the absorption coefficient for glucosamine, in view of its enhancement of cell permeability to glucosamine, suggests that the same mechanisms are not involved in moving glucosamine across the intestinal walls as are involved in moving glucosamine into the cells. A similar argument may be proposed for the action of insulin on the metabolism of absorbed N-acetylglucosamine. The lack of effect on the rates of absorption of glucosamine and N-acetylglucosamine by insulin suggests that these sugars were not transported across the intestinal wall by a mechanism

similar to that for glucose and galactose.

The effect of phlorizidin treatment on the incorporation of radioactivity from D-glucosamine-1-C^{14} into tissues was to significantly decrease the total radioactivity in the serum and liver and to significantly increase the bound activity in the serum.

The increase in absorption of glucosamine and the increase in bound activity in the serum of animals treated with phlorizidin and fed glucosamine-1-C^{14} could possibly be explained by the following. Hexokinases have been described which phosphorylate both glucose and glucosamine (31), (32), (33), (34); it is, however, possible that a different enzyme is responsible for the phosphorylation of these two sugars and that they both compete for a source of high energy phosphate. McGarrahan and Maley (30) observed that glucose inhibits the phosphorylation of glucosamine. If the glucose-hexokinase system were phlorizidin sensitive and the glucosamine-hexokinase system were not, then the action of phlorizidin on an organism could favor the phosphorylation of glucosamine and possibly account for the observed increase in absorption rate and the increased binding of glucosamine in the serum. Phlorizidin treatment of the animals fed N-acetyl-D-glucosamine-1-C^{14} significantly increased the total radioactivity in the serum and the total radioactivity in the small intestine tissue, and significantly decreased the percentage of bound carbon-14 in the small intestine. Phlorizidin is known to inhibit the phosphorylation of hexoses. Since phlorizidin was found to decrease the percentage of bound radioactivity from absorbed N-acetylglucosamine in the small intestine

tissue, while causing an increase in the total radioactivity in the serum and small intestine tissue, it would appear that phlorizidin was inhibiting some process which is essential for the incorporation of N-acetylglucosamine into tissue bound hexosamine (possibly the phosphorylation of N-acetylglucosamine) and, as a result, the free N-acetylglucosamine level increased. The decrease in the absorption coefficient for N-acetylglucosamine could possibly also be explained on the basis of phlorizidin inhibition of N-acetylglucosamine phosphorylation.

In animals treated with phlorizidin and fed either glucosamine or N-acetylglucosamine, there was incorporation of the amino sugars into tissue bound components (irrespective of degree of incorporation). It is significant that, although the urine of the phlorizidin-treated animals contained glucosamine, galactose, glucose and mannose, no appreciable radioactivity was found associated with these sugars except in the animals that had been fed the specific sugar (Tables 12, 13, and 14). It is known that phlorizidin inhibits the resorption of glucose, galactose and mannose in the renal tubles. It follows, therefore, that if glucosamine-1-C^{14} or N-acetylglucosamine were converted to any appreciable extent into either glucose, galactose or mannose, they would be excreted in the urine.

It may be proposed from the results of the insulin and phlorizidin experiments that: (1) permeability of the cell to D-glucosamine and N-acetyl-D-glucosamine is not a limiting factor in the <u>in vivo</u> metabolism of D-glucosamine or N-acetylglucosamine, (2) the metabolism

of intestinally absorbed D-glucosamine and N-acetyl-D-glucosamine is by different paths.

CHAPTER V

SUMMARY

1. The absorption and metabolism of D-glucosamine and N-acetyl-glucosamine in the intact rat has been investigated.
2. Both D-glucosamine and N-acetyl-D-glucosamine are absorbed from the intestine at the same rate; however, the rate is much slower than the absorption rate for D-glucose.
3. A portion of the D-glucosamine in the intestinal lumen is apparently degraded by the intestinal flora in rats not treated with neomycin.
4. N-acetyl-D-glucosamine is not degraded by the intestinal flora in similar animals.
5. Absorbed D-glucosamine and N-acetyl-D-glucosamine are efficiently incorporated into hexosamine-containing tissues of the body.
6. A portion of the absorbed N-acetyl-D-glucosamine is deacetylated after leaving the lumen of the intestine.
7. A small amount of absorbed N-acetyl-D-glucosamine and D-glucosamine is oxidized to carbon dioxide. More N-acetyl-D-glucosamine is oxidized to carbon dioxide than is D-glucosamine.
8. In addition to incorporation into tissue hexosamines or oxidation to carbon dioxide, absorbed D-glucosamine and N-acetyl-D-glucosamine are metabolized to amino acids. There is apparently little

conversion to hexoses.

9. A portion of absorbed D-glucosamine and N-acetyl-D-glucosamine is excreted unchanged in the urine.

10. Insulin apparently facilitates the entry of D-glucosamine and N-acetyl-D-glucosamine into the cells; however, it does not increase their incorporation into hexosamines of the tissues.

11. Phlorizidin inhibits the absorption of N-acetyl-D-glucosamine from the intestine and stimulates the absorption of D-glucosamine.

12. The effects of phlorizidin on the incorporation of D-glucosamine and N-acetyl-D-glucosamine into tissues are variable.

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APPENDIX

TABLE 1

RADIOACTIVITY[a] ASSOCIATED WITH CHROMATOGRAPHICALLY DETECTED SUGARS[b] IN INTESTINAL CONTENTS THREE HOURS AFTER FEEDING

	Sugar Fed	Sugar Identity and Radioactivity (c/m)		
A.	Untreated Animals	Glucose	Glucosamine	N-Acetylglucosamine
	D-Glucosamine-1-C^{14}	(-) 10	(+) 240	(-) 2
	N-Acetyl-D-Glucosamine-1-C^{14}	(+) 6	(-) 1	(+) 329
B.	Neomycin Sulfate Treated Animals			
	D-Glucosamine-1-C^{14}	(-) 35	(+) 689	(-) 13
	N-Acetyl-D-Glucosamine-1-C^{14}	(+) 10	(-) 5	(+) 235

[a]Radioactivity detected by cutting out aminobiphenyl positive areas of the chromatogram and counting the area in the liquid scintillation counter.

[b]A (+) indicates that the sugar was detected with the aminobiphenyl reagent.

A (-) indicates that the sugar was not detected with the aminobiphenyl reagent; however, the area was counted.

TABLE 2

ABSORPTION OF D-GLUCOSAMINE, N-ACETYL-D-GLUCOSAMINE

AND D-GLUCOSE DURING A THREE HOUR PERIOD

Sugar Fed and Label	No. of Animals	Animal Weights gms.	Treatment	Millimoles Sugar Absorbed	Absorption Coefficient
Acetylglucosamine-1-C^{14}	9	116 (110-125)	None	0.267 ± 0.079 (0.152 - 0.397)	0.077 ± 0.023 (0.043 - 0.120)
Acetylglucosamine-1-C^{14}	9	137 (123-149)	Neomycin	0.276 ± 0.059 (0.204 - 0.365)	0.068 ± 0.016 (0.048 - 0.087)
Acetyl-1-C^{14}-Glucosamine	8	121 (102-136)	None	0.256 ± 0.072 (0.188 - 0.305)	0.070 ± 0.018 (0.046 - 0.103)
Glucosamine-1-C^{14}	9	111 (86-122)	None	0.307 ± 0.062 (0.227 - 0.388)	0.104 ± 0.017 (0.080 - 0.135)
Glucosamine-1-C^{14}	9	150 (136-163)	Neomycin	0.276 ± 0.027 (0.210 - 0.298)	0.062 ± 0.007 (0.050 - 0.073)
Glucose-1-C^{14}	9	96 (91-100)	None	0.996 ± 0.009 (0.985 - 1.009)	---

[a]Millimoles sugar absorbed per 100 gm. body weight per hour.

Values given are the means and their standard deviations. Values in parentheses are the ranges.

TABLE 3

ABSORPTION OF D-GLUCOSE DURING A ONE HOUR PERIOD

Label	No. of Animals	Animal Weights gms.	Treatment	Millimoles Sugar[a] Absorbed
Glucose-1-C^{14}	3	111 (100-118)	None	0.919 (0.903-0.935)
Glucose-1-C^{14}	3	113 (96-126)	Insulin	0.966 (0.952-0.975)
Glucose-1-C^{14}	3	110 (107-112)	Phlorizidin	0.949 (0.937-0.972)

[a]Values given are means; the range is in parentheses.

TABLE 4

RADIOACTIVITY[a] IN SERUM FOLLOWING ORAL ADMINISTRATION OF LABELED SUGARS

Sugar Absorbed and Label	No. of Animals	Absorption[c] Coefficient	Specific Activity of Sugar (c/m per millimole)	Percentage Absorbed Radioactivity in Serum	Percentage Nondialyzable Radioactivity in Serum
N-Acetyl-D-Glucosamine-1-C^{14} [b]	9	0.068 ± 0.016 (0.048 - 0.087)	4.3×10^6	1.4 ± 0.1 (1.2 - 1.7)	19.7 ± 3.5 (14.8 - 24.9)
N-Acetyl-1-C^{14}-Glucosamine	8	0.070 ± 0.018 (0.046 - 0.103)	12.1×10^6	1.2 ± 0.2 (1.0 - 1.4)	26.1 ± 7.0 (19.2 - 41.2)
D-Glucosamine-1-C^{14} [b]	9	0.062 ± 0.007 (0.050 - 0.073)	17.8×10^6	2.0 ± 0.2 (1.7 - 2.2)	33.2 ± 8.5 (22.8 - 50.0)
D-Glucose-1-C^{14}	9	0.996 ± 0.009[d] (0.985 - 1.009)	9.5×10^6	3.0 ± 0.1 (2.8 - 3.0)	28.8 (26.9 - 30.1)

[a]Values given are means and their standard deviations. Values in parentheses are the ranges.

[b]Animals treated with neomycin.

[c]Millimoles sugar absorbed per 100 gm. body weight per hour.

[d]Millimoles sugar absorbed.

TABLE 5

RADIOACTIVITY[a] IN LIVER FOLLOWING ORAL ADMINISTRATION OF LABELED SUGARS

Sugar Absorbed and Label	No. of Animals	Absorption[c] Coefficient	Specific Activity of Sugar (c/m per millimole)	Percentage Absorbed Radioactivity in Liver	Percentage Nondialyzable Radioactivity in Liver
N-Acetyl-D-Glucosamine-1-C^{14} [b]	9	0.068 ± 0.016 (0.048 - 0.087)	4.3×10^6	2.2 ± 0.3 (1.6 - 2.5)	37.3 ± 3.4 (31.7 - 41.8)
N-Acetyl-1-C^{14}-D-Glucosamine	8	0.070 ± 0.018 (0.046 - 0.103)	12.1×10^6	1.7 ± 0.1 (1.5 - 1.8)	33.8 ± 3.6 (25.3 - 36.0)
D-Glucosamine-1-C^{14}[b]	9	0.062 ± 0.007 (0.050 - 0.073)	17.8×10^6	7.1 ± 0.8 (6.0 - 8.6)	16.2 ± 2.0 (14.3 - 20.3)
D-Glucose-1-C^{14}	9	0.996 ± 0.009[d] (0.985 - 1.009)	9.5×10^6	19.7 ± 5.3 (9.4 - 25.8)	45.0 ± 7.2 (32.8 - 58.5)

[a]Values given are means and their standard deviations. Values in parentheses are ranges.

[b]Animals treated with neomycin.

[c]Millimoles sugar absorbed per 100 gm. body weight per hour.

[d]Millimoles sugar absorbed.

TABLE 6

RADIOACTIVITY[a] IN SMALL INTESTINAL TISSUE FOLLOWING ORAL ADMINISTRATION OF LABELED SUGARS

Sugar Absorbed and Label	No. of Animals	Absorption[c] Coefficient	Specific Activity of Sugar (c/m per millimole)	Percentage Absorbed Radioactivity in Small Intestine	Percentage Nondialyzable Radioactivity in Small Intestine
N-Acetyl-D-Glucosamine-1-C^{14} [b]	9	0.068 ± 0.016 (0.048 - 0.087)	4.3×10^6	8.7 ± 2.6 (4.3 - 13.6)	28.6 ± 7.8 (18.8 - 46.9)
N-Acetyl-1-C^{14}-D-Glucosamine	8	0.070 ± 0.018 (0.046 - 0.103)	12.1×10^6	9.9 ± 3.8 (5.8 - 16.4)	45.3 ± 12.3 (23.4 - 60.7)
D-Glucosamine-1-C^{14}[b]	9	0.062 ± 0.007 (0.050 - 0.073)	17.8×10^6	12.4 ± 3.0 (9.3 - 17.7)	23.2 ± 5.9 (12.4 - 30.7)
D-Glucose-1-C^{14}	9	0.996 ± 0.009[d] (0.985 - 1.009)	9.5×10^6	6.8 ± 2.7 (3.3 - 10.4)	99.8 ± 14.5 (77.7 - 119.4)

[a]Values given are means and their standard deviations. Values in parentheses are the ranges.

[b]Animals treated with neomycin.

[c]Millimoles sugar absorbed per 100 gm. body weight per hour.

[d]Millimoles sugar absorbed.

TABLE 7

PERCENTAGE OF RADIOACTIVITY EXPIRED AS CARBON DIOXIDE FROM NEOMYCIN-TREATED RATS AND UNTREATED RATS FOLLOWING ORAL ADMINISTRATION OF LABELED SUGARS

Sugar Fed and Label	No. of Animals	Absorption Period(Hours)	Neomycin Treated	Absorption[a] Coefficient	Specific Activity of Sugar (c/m per millimole)	Percentage Absorbed Activity Expired as CO_2
N-Acetyl-D-Glucosamine-1-C^{14}	9	3	No	0.077±0.023 (0.043-0.120)	4.2×10^6	10.1(10.0-10.3)
N-Acetyl-D-Glucosamine-1-C^{14}	9	3	Yes	0.068±0.016 (0.048-0.087)	4.3×10^6	9.0(6.9-11.2)
N-Acetyl-1-C^{14}-D-Glucosamine	8	3	No	0.070±0.018 (0.046-0.103)	12.1×10^6	29.9(28.2-31.6)
D-Glucosamine-1-C^{14}	9	3	No	0.104±0.017 (0.080-0.135)	7.8×10^6	17.9(14.8-19.6)
D-Glucosamine-1-C^{14}	9	3	Yes	0.062±0.007 (0.050-0.073)	17.8×10^6	4.5(4.1-5.0)
D-Glucose-1-C^{14}	9	3	No	0.996±0.009[c] (0.985-1.009)	10.9×10^6	31.4(30.4-32.5)
D-Glucose-1-C^{14}	3	3	No	---	20.2×10^8	42.6[b]
D-Glucose-1-C^{14}	3	1	Yes	0.919[c] (0.903-0.935)	10.6×10^6	6.4

[a]Millimoles sugar absorbed per 100 gm. body weight per hour.
Values given are means and their standard deviations. Values in parentheses are ranges.
[b]Rats injected intraperitoneally with 2 microcuries D-glucose-1-C^{14} in saline.
[c]Millimoles sugar absorbed.

TABLE 8

RADIOACTIVITY[a] IN SERUM FOLLOWING ORAL ADMINISTRATION OF LABELED SUGARS

TO RATS TREATED WITH INSULIN OR PHLORIZIDIN

(All Animals Treated with Neomycin)

Sugar Absorbed and Label	No. of Animals	Absorption[b] Coefficient	Specific Activity of Sugar (c/m per millimole)	Percentage Absorbed Radioactivity in Serum	Percentage Nondialyzable Radioactivity in Serum
A. No Treatment					
N-Acetyl-D-Glucosamine-1-C^{14}	9	0.068±0.016 (0.048-0.087)	4.3×10^6	1.4±0.1 (1.2-1.7)	19.7±3.5 (14.8-24.9)
D-Glucosamine-1-C^{14}	9	0.062±0.007 (0.050-0.073)	17.8×10^6	2.0±0.2 (1.7-2.2)	33.2±8.5 (22.8-50.0)
B. Insulin Treated					
N-Acetyl-D-Glucosamine-1-C^{14}	9	0.062±0.024 (0.030-0.099)	4.3×10^6	1.4±0.4 (0.9-2.2)	21.8±5.9 (13.4-30.0)
D-Glucosamine-1-C^{14}	9	0.056±0.011 (0.041-0.070)	13.4×10^6	2.2±0.4 (1.7-2.9)	39.4±13.7 (24.3-59.6)
C. Phlorizidin-Treated					
N-Acetyl-D-Glucosamine-1-C^{14}	9	0.026±0.015 (0.013-0.053)	4.2×10^6	1.7±0.5 (1.1-2.7)	45.9±12.1 (33.3-72.1)
D-Glucosamine-1-C^{14}	9	0.080±0.016 (0.071-0.105)	14.6×10^6	1.0±0.2 (0.8-1.2)	55.1±15.4 (22.9-80.9)

[a]Values given are means and their standard deviations. Values in parentheses are ranges.

[b]Millimoles sugar absorbed per 100 gm. body weight per hour.

TABLE 9

RADIOACTIVITY[a] IN LIVER FOLLOWING ORAL ADMINISTRATION OF LABELED SUGARS TO RATS TREATED WITH INSULIN OR PHLORIZIDIN

(All Animals Treated with Neomycin)

Sugar Absorbed and Label	No. of Animals	Absorption[b] Coefficient	Specific Activity of Sugar (c/m per millimole)	Percentage Absorbed Radioactivity in Liver	Percentage Nondialyzable Radioactivity in Liver
A. No Treatment					
N-Acetyl-D-Glucosamine-1-C^{14}	9	0.068±0.016 (0.048-0.087)	4.3×10^6	2.2±0.3 (1.6-2.5)	37.3±3.4 (31.7-41.8)
D-Glucosamine-1-C^{14}	9	0.062±0.007 (0.050-0.073)	17.8×10^6	7.1±0.8 (6.0-8.6)	16.2±2.0 (14.3-20.3)
B. Insulin-Treated					
N-Acetyl-D-Glucosamine-1-C^{14}	9	0.062±0.024 (0.030-0.099)	4.3×10^6	2.4±0.7 (1.4-3.5)	40.0±8.8 (24.1-51.4)
D-Glucosamine-1-C^{14}	9	0.056±0.011 (0.041-0.070)	13.4×10^6	6.8±1.5 (4.5-10.2)	20.5±5.3 (13.9-27.6)
C. Phlorizidin-Treated					
N-Acetyl-D-Glucosamine-1-C^{14}	9	0.026±0.015 (0.013-0.053)	4.2×10^6	2.1±1.1 (0.1-3.9)	38.7±11.4 (25.8-57.2)
D-Glucosamine-1-C^{14}	9	0.080±0.016 (0.071-0.105)	14.6×10^6	5.0±0.6 (4.2-6.3)	15.5±2.6 (11.0-19.1)

[a]Values given are means and their standard deviations. Values in parentheses are ranges.

[b]Millimoles sugar absorbed per 100 gm. body weight per hour.

TABLE 10

RADIOACTIVITY[a] IN SMALL INTESTINE TISSUE FOLLOWING ORAL ADMINISTRATION OF LABELED SUGARS TO RATS TREATED WITH INSULIN OR PHLORIZIDIN

(All Animals Treated with Neomycin)

Sugar Absorbed and Label	No. of Animals	Absorption[b] Coefficient	Specific Activity of Sugar (c/m per millimole)	Percentage Absorbed Radioactivity in Small Intestine	Percentage Nondialyzable Radioactivity in Small Intestine
A. No Treatment					
N-Acetyl-D-Glucosamine-1-C^{14}	9	0.068±0.016 (0.048-0.087)	4.3×10^6	8.7±2.6 (4.3-13.6)	28.6±7.8 (18.8-46.9)
D-Glucosamine-1-C^{14}	9	0.062±0.007 (0.050-0.073)	17.8×10^6	12.4±3.0 (9.3-17.7)	23.2±5.9 (12.8-30.7)
B. Insulin-Treated					
N-Acetyl-D-Glucosamine-1-C^{14}	9	0.062±0.024 (0.030-0.099)	4.3×10^6	12.4±4.5 (7.5-18.6)	30.2±9.5 (21.8-48.4)
D-Glucosamine-1-C^{14}	9	0.056±0.011 (0.041-0.070)	13.4×10^6	8.7±2.5 (5.5-13.2)	26.0±8.5 (20.4-42.3)
C. Phlorizidin-Treated					
N-Acetyl-D-Glucosamine-1-C^{14}	9	0.026±0.015 (0.013-0.053)	4.2×10^6	25.9±10.1 (9.6-32.4)	19.1±5.3 (12.7-29.5)
D-Glucosamine-1-C^{14}	9	0.080±0.016 (0.071-0.105)	14.6×10^6	10.2±3.2 (6.4-15.2)	24.4±9.9 (12.7-46.4)

[a]Values given are means and their standard deviations. Values in parentheses are ranges.

[b]Millimoles sugar absorbed per 100 gm. body weight per hour.

TABLE 11

EFFECT OF INSULIN AND PHLORIZIDIN ON THE ABSORPTION OF D-GLUCOSE, D-GLUCOSAMINE AND N-ACETYL-D-GLUCOSAMINE AND THEIR CONVERSION TO CARBON DIOXIDE IN NEOMYCIN-TREATED RATS

Sugar Fed and Label	No. of Animals	Period of Absorption (Hours)	Absorption[a] Coefficient	Specific Activity of Sugar (c/m per millimole)	Percentage Absorbed Activity Expired as CO_2
A. No Treatment					
N-Acetyl-D-Glucosamine-1-C^{14}	9	3	0.068±0.016 (0.048-0.087)	4.3×10^6	9.0 (6.9-11.2)
D-Glucosamine-1-C^{14}	9	3	0.062±0.007 (0.050-0.073)	17.8×10^6	4.5 (4.1-5.0)
D-Glucose-1-C^{14}	3	1	0.831 (0.778-0.935)	10.6×10^6	6.4
B. Insulin-Treated					
N-Acetyl-D-Glucosamine-1-C^{14}	9	3	0.062±0.024 (0.030-0.099)	4.3×10^6	11.8 (9.3-14.9)
D-Glucosamine-1-C^{14}	9	3	0.056±0.011 (0.041-0.070)	13.4×10^6	8.2 (7.2-9.3)
D-Glucose-1-C^{14}	9	1	0.868	10.6×10^6	9.9
C. Phlorizidin-Treated					
N-Acetyl-D-Glucosamine-1-C^{14}	9	3	0.026±0.015 (0.013-0.053)	4.2×10^6	11.5 (9.1-14.6)
D-Glucosamine-1-C^{14}	9	3	0.080±0.016 (0.071-0.105)	14.6×10^6	3.1 (3.0-3.1)
D-Glucose-1-C^{14}	3	1	0.863 (0.847-0.876)	11.6×10^6	5.0

[a]Values given are means and their standard deviations. Values in parentheses are ranges. Millimoles sugar absorbed per 100 gm. body weight per hour.

TABLE 12

RADIOACTIVITY[a] ASSOCIATED WITH CHROMATOGRAPHICALLY DETECTED SUGARS[b] IN URINE FROM RATS FED D-GLUCOSE-1-C^{14} AND TREATED WITH NEOMYCIN, INSULIN OR PHLORIZIDIN

Absorption Period (Hours)	Treatment			Sugar Identity and Radioactivity (c/m)					
				Uronic Acid	Glucosamine	Galactose	Glucose	Mannose	Acetyl-glucosamine
1	Neomycin	---	---	(-)4	(-)0	(-)0	(-)4	(-)1	(-)0
1	Neomycin	Insulin	---	(-)1	(-)0	(-)3	(-)6	(-)3	(-)6
1	Neomycin	---	Phlorizidin	(-)1	(-)2	(+)13	(+)395	(+)65	(-)4
3	---	---	---	(-)0	(-)0	(-)0	(-)0	(-)0	(-)0

[a]Radioactivity detected by cutting out aminobiphenyl positive areas of the chromatograms and counting the area in the liquid scintillation counter.

[b]A (+) indicates that the sugar was detected with the aminobiphenyl reagent.
A (-) indicates that the sugar was not detected with the aminobiphenyl reagent; however, the area was counted.

TABLE 13

RADIOACTIVITY[a] ASSOCIATED WITH CHROMATOGRAPHICALLY DETECTED SUGARS[b] IN URINE FROM RATS FED N-ACETYL-D-GLUCOSAMINE-1-C^{14} and N-ACETYL-1-C^{14}-D-GLUCOSAMINE AND TREATED WITH NEOMYCIN, INSULIN OR PHLORIZIDIN

Sugar and Label Fed	Treatment			Sugar Identity and Radioactivity (c/m)					
				Uronic Acid	Glucosamine	Galactose	Glucose	Mannose	Acetyl-glucosamine
A. N-Acetyl-D-glucosamine-1-C^{14}	---	---	---	(+)11	(-)0	(-)3	(+)0	(-)2	(+)60
	Neomycin	---	---	(+)4	(-)0	(-)4	(+)3	(-)1	(+)86
	Neomycin	Insulin	---	(+)7	(-)7	(-)7	(+)5	(-)4	(+)135
	Neomycin	---	Phlorizidin	(+)0	(+)9	(+)1	(+)7	(+)2	(+)35
B. N-Acetyl-1-C^{14}-D-Glucosamine	---	---	---	(+)1	(-)0	(-)3	(+)2	(-)4	(+194)

[a]Radioactivity detected by cutting out aminobiphenyl positive areas of the chromatogram and counting the areas in the liquid scintillation counter.

[b]A (+) indicates that the sugar was detected with the aminobiphenyl reagent.

A (-) indicates that the sugar was not detected with the aminobiphenyl reagent; however, the area was counted.

TABLE 14

RADIOACTIVITY[a] ASSOCIATED WITH CHROMATOGRAPHICALLY DETECTED SUGARS[b] IN URINE FROM RATS FED D-GLUCOSAMINE-1-C^{14} AND TREATED WITH NEOMYCIN, INSULIN OR PHLORIZIDIN

Treatment			Sugar Identity and Radioactivity (c/m)					
			Uronic Acid	Glucosamine	Galactose	Glucose	Mannose	Acetyl-glucosamine
---	---	---	(-)0	(+)50	(-)1	(-)0	(-)0	(-)0
Neomycin	---	---	(+)34	(+)591	(-)16	(+)6	(-)4	(-)8
Neomycin	Insulin	---	(-)2	(+)227	(-)5	(-)2	(-)2	(-)6
Neomycin	Insulin	Phlorizidin	(+)7	(+)259	(+)5	(+)2	(+)1	(-)3

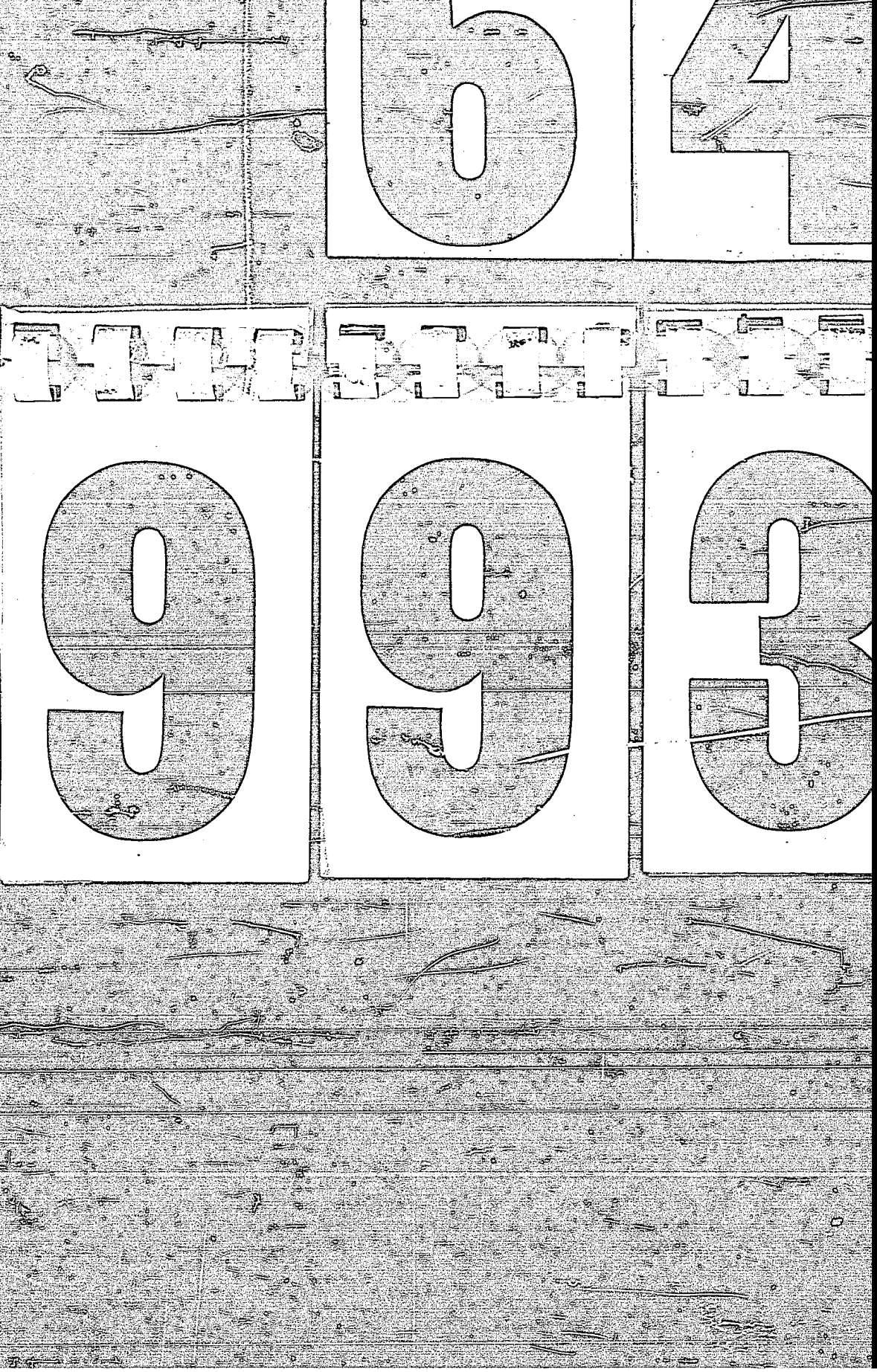
[a]Radioactivity detected by cutting out aminobiphenyl positive areas of the chromatogram and counting the areas in the liquid scintillation counter.

[b]A (+) indicates that the sugar was detected with the aminobiphenyl reagent.
A (-) indicates that the sugar was not detected with the aminobiphenyl reagent; however, the area was counted.

TABLE 15

RADIOACTIVITY OF TISSUE HYDROLYSATE FRACTIONS

Tissue Hydrolyzed and Source of Label	Animal Treated with Neomycin	Total Radioactivity of Column Eluate	Percentage Total Radioactivity in Column Fractions		
			Water Wash	Hexosamines	Amino Acids
A. D-Glucosamine-1-C^{14}					
Serum	No	479	2.5	58.5	38.6
Serum	Yes	222	1.4	78.8	19.8
Liver	No	1042	14.5	24.4	61.1
Liver	Yes	194	7.7	68.0	24.2
Small Intestine	No	1057	2.8	65.7	31.4
Small Intestine	Yes	513	2.0	86.0	12.1
B. N-Acetyl-D-Glucosamine-1-C^{14}					
Serum	No	51	7.8	45.1	47.1
Serum	Yes	47	2.1	36.2	61.7
Liver	No	234	15.0	30.3	54.7
Liver	Yes	163	7.4	35.0	57.7
Small Intestine	No	218	1.8	65.1	33.0
Small Intestine	Yes	175	3.4	68.6	28.0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99
3

