Distribution of Isotopic Label after the Oral Administration of Free and Bound ^{14}C -Labelled Glucosamine in Rats

By G. B. ROBINSON

Department of Biochemistry, University of Oxford

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The metabolic fate of [$1\text{-}^{14}\text{C}$]glucosamine, of *N*-acetyl[$1\text{-}^{14}\text{C}$]glucosamine and of glycoproteins labelled with [$1\text{-}^{14}\text{C}$]glucosamine was studied in rats for a period of 24 hr. after these materials were given orally or injected. When [$1\text{-}^{14}\text{C}$]glucosamine was injected 26.3% of the label was excreted in the urine, 19.7% was expired as carbon dioxide and 12.7% was incorporated into plasma proteins. When the same compound was given orally, 49.2% of the label was expired as carbon dioxide, with little appearing in the urine or in the plasma. When *N*-acetyl[$1\text{-}^{14}\text{C}$]glucosamine was injected, 51.3% of the label was excreted in the urine with 12.3% appearing in carbon dioxide, but there was little incorporation into plasma protein. When this compound was given orally, 46.5% of the label was expired as carbon dioxide, 7.4% was recovered in the urine and 1.7% was incorporated into plasma protein. After the injection of ^{14}C -labelled glycoprotein 21.0% of the label was expired as carbon dioxide, whereas when it was given orally 49.8% of the label was recovered in carbon dioxide. The differences observed between the metabolic fate of the amino sugars when they were given orally and their fate when injected could not be accounted for by the action of the intestinal microflora or by the rate of administration of the material. It is concluded that amino sugars undergo metabolic alteration or degradation during absorption.

The amino sugar D-glucosamine is widely distributed in Nature, occurring principally as *N*-acetylglucosamine, a component of many mucopolysaccharides and glycoproteins. The intermediary metabolism of glucosamine in many organisms has been studied and interest has recently focused on the mechanisms of biosynthesis of macromolecules containing glucosamine (see Jeanloz & Balazs, 1966). Little attention has been given to the assimilation and consequent metabolism of glucosamine when it is ingested by mammals, though the sugar occurs commonly in the diet. Capps, Shetlar & Bradford (1966) have shown that both glucosamine and *N*-acetylglucosamine can be absorbed by rats, but their studies gave little indication of the subsequent metabolic fate of the compounds. The fate of injected glucosamine has been investigated by Shetlar, Bradford, Hern, Endecott & Schilling (1962), by Shetlar, Capps & Hern (1964) and by Kohn, Winzler & Hoffman (1962), who also studied the fate of injected *N*-acetylglucosamine.

In the present work, the metabolic fate of orally administered [$1\text{-}^{14}\text{C}$]glucosamine, *N*-acetyl[$1\text{-}^{14}\text{C}$]glucosamine and [$1\text{-}^{14}\text{C}$]glucosamine-labelled glycoproteins was studied after they had been fed to rats in order to establish the extent to which dietary

glucosamine could be absorbed and utilized. For comparison the fate of amino sugar intravenously injected as these various forms was investigated. A preliminary communication of this work has been published (Robinson, 1967).

MATERIALS AND METHODS

D-[$1\text{-}^{14}\text{C}$]Glucosamine hydrochloride (specific activity 3.1 mc/m-mole) was obtained from The Radiochemical Centre (Amersham, Bucks.). *N*-Acetyl-D-[$1\text{-}^{14}\text{C}$]glucosamine (specific activity 3.1 mc/m-mole) was prepared by the method described by Kuhn & Bister (1958) and the identity and purity of the product were confirmed by paper chromatography as described below. Plasma glycoprotein labelled in the *N*-acetylglucosamine and sialic acid (*N*-acetylneuraminic acid) moieties was obtained from rats by injecting [$1\text{-}^{14}\text{C}$]glucosamine intraperitoneally in five consecutive $2\mu\text{C}$ doses at hourly intervals. Blood was collected by cardiac puncture 2 hr. after the last injection and the plasma was separated by centrifugation. The plasma was dialysed against distilled water (two changes) to remove all radioactive material soluble in 5% (w/v) trichloroacetic acid. The plasma protein was freeze-dried, dissolved in 0.15 M-NaCl (30 mg./ml.), centrifuged to remove denatured material and stored at -20° . Analysis of the glycoprotein by the methods described below showed that 67% of the label was present in *N*-acetylglucosamine and 33% in

sialic acid. The specific activity of the glycoprotein was $6.6\mu\text{C}/\text{mg}$.

Male Wistar rats (240–260 g. body wt.) were used; food and water were given *ad libitum* during the experiments. The animals were caesarian-derived stock from Scientific Products Ltd. (Ash, Canterbury, Kent).

Gastric intubation was employed for the oral administration of the labelled compounds. $[1\text{-}^{14}\text{C}]\text{glucosamine}$ and $N\text{-acetyl}[1\text{-}^{14}\text{C}]\text{glucosamine}$ were given dissolved in 0.15M-NaCl ; ^{14}C -labelled plasma glycoprotein was denatured by addition of 1 vol. of 10% (w/v.) trichloroacetic acid, and the precipitated protein was washed twice with 95% (v/v) ethanol and suspended in water (15mg./ml.). Radioactive sugars and native plasma glycoprotein were injected in solution in 0.15M-NaCl into the femoral vein, under light ether anaesthesia.

After receiving the labelled compounds, animals were kept in metabolic cages to facilitate the collection of urine, faeces and CO_2 . CO_2 -free air was circulated through the cages and expired CO_2 was trapped in ethanolamine. Measurements of the efficiency of CO_2 recovery were made by slowly releasing CO_2 from known amounts of $\text{NaH}^{14}\text{CO}_3$ solution within the metabolic cages and subsequently measuring the radioactivity of the ethanolamine; 98–100% of the label was recovered. Immediately after termination of the collections the animals were anaesthetized with ether and blood was obtained by cardiac puncture. In some experiments the gastrointestinal tracts were removed from the animals and the contents of these organs were recovered by washing out with water. The contents, including the faeces, and the gastrointestinal-tract tissues were homogenized in water separately for the measurement of radioactivity.

The radioactivity of samples was measured by scintillation counting with internal standardization of samples. Whole plasma (0.4ml.) and plasma proteins were dissolved in Hyamine hydroxide (1ml.) before the addition of scintillation liquid (6ml.). The radioactivity of homogenates of gastrointestinal-tract tissue and of its contents was measured by the oxidation method of Jeffay & Alvarez (1961). Samples of ethanolamine-trapped $^{14}\text{CO}_2$ and urine were added directly to scintillation liquid. Portions of paper chromatograms were counted immersed in scintillation fluid.

Sialic acid was released from biosynthetically ^{14}C -labelled glycoprotein by hydrolysis in 5% (w/v) trichloroacetic acid (5ml.) at 80° for 1 hr. after the protein had been previously precipitated in 5% trichloroacetic acid. The insoluble protein was removed from the hydrolysate by centrifugation and the supernatant was extracted with ether ($5 \times 1\text{ vol.}$) to remove the trichloroacetic acid. The extracted solutions were applied to $1\text{cm.} \times 10\text{cm.}$ columns of Dowex 1 (Cl-form; 200–400 mesh) resin, and the columns were washed with water to recover hexoses and hexosamines. The sialic acid was then eluted from the columns with 0.2N-HCl (10ml.) and the eluates were dried *in vacuo* and redissolved in water for counting, paper chromatography and colorimetric determination (Warren, 1959) with $N\text{-acetylneuraminic acid}$ as a standard. For chromatography, the solvent employed was butan-1-ol–propan-1-ol– N-HCl ($1:2:1$, by vol.). The residue from the trichloroacetic acid hydrolysis was further hydrolysed in 3N-HCl for 4 hr. at 100° . These hydrolysates were combined with the above aqueous washings and were dried *in vacuo*, redissolved in water and the hexosamines were isolated on columns

($1\text{cm.} \times 10\text{cm.}$) of Dowex 50 (H^+ form) resin (Boas, 1953). The column eluates were dried *in vacuo* and redissolved for counting and colorimetric determinations (Boas, 1953). The identity of the amino sugar was confirmed by paper chromatography in ethyl acetate–pyridine–water ($10:4:3$, by vol.) and butan-1-ol–acetic acid–water ($4:1:5$, by vol.) on Whatman no. 1 paper. The former solvent separated galactosamine and glucosamine sufficiently well to show that more than 90% of the radioactivity was associated with glucosamine, but glucosamine could not be separated from mannosamine.

In some experiments the presence of micro-organisms in the gastrointestinal tract was examined by suspending the total caecal contents from each animal in 50ml. of 0.15M-NaCl – $0.05\text{M-KH}_2\text{PO}_4$ buffer, adjusted to $\text{pH}7.4$ with 2N-KOH , containing glucose (0.5%). Samples (0.1ml.) of suspension were cultured in brain–heart infusion broth, cooked–meat broth and on blood–agar and nutrient–agar (Oxoid Ltd., London, S.E. 1). The cultures were examined for growth after various times of incubation (maximum time 2 days) at 37° and 22° under aerobic and anaerobic conditions.

RESULTS

A problem encountered in the studies where labelled compounds were given orally was a wide variability in the results obtained in experiments of short duration (up to 6 hr.). This variation was most marked in experiments where glycoprotein was given orally, and was found to be due to variations in the stomach emptying times of individual animals. On dissection, it was found that in one animal no stomach emptying had occurred after 6 hr. whereas in others various degrees of emptying had occurred with a maximum evacuation of 95% of the label. To allow for this variation, experiments were always conducted over a 24 hr. period.

The results of experiments in which ^{14}C -labelled glucosamine was administered orally as $[1\text{-}^{14}\text{C}]\text{-glucosamine hydrochloride}$, as $N\text{-acetyl}[1\text{-}^{14}\text{C}]\text{-glucosamine}$ or as ^{14}C -labelled glycoprotein are given in Table 1, and the results obtained with the injected ^{14}C -labelled compounds are shown in Table 2. When the ^{14}C -labelled compounds were given orally more radioactivity was expired as carbon dioxide compared with the results obtained after injection, and with both $[1\text{-}^{14}\text{C}]\text{glucosamine}$ and $N\text{-acetyl}[1\text{-}^{14}\text{C}]\text{glucosamine}$ less radioactivity was recovered in the urine. Retention of radioactivity in the plasma was most marked after injection of $[1\text{-}^{14}\text{C}]\text{glucosamine}$ and ^{14}C -labelled glycoprotein.

Providing the animals with 0.5% neomycin sulphate in the drinking water for 48 hr. decreased the number of bacteria in the caecum to about 2% of normal, and after 96 hr. treatment no bacteria had survived. When animals treated with neomycin for either 48 or 96 hr. were given $[1\text{-}^{14}\text{C}]\text{glucosamine}$

Table 1. *Metabolic fate of [1-¹⁴C]glucosamine, N-acetyl[1-¹⁴C]glucosamine or ¹⁴C-labelled glycoprotein given orally to rats*

The ¹⁴C-labelled compounds were given by gastric intubation. The ¹⁴C-labelled glycoprotein, which was rat plasma glycoprotein labelled in the *N*-acetylglucosamine and sialic acid moieties, was denatured with trichloroacetic acid, washed with ethanol and given orally as a suspension in water. Expired CO₂ and urine were collected for 24 hr. after administration of the labelled compounds. Blood was obtained by cardiac puncture at the end of this period and plasma was separated by centrifugation. The plasma volume was taken as 10.7 ml. The gastrointestinal tract and its contents were homogenized separately in water and samples of the homogenates were taken for the determination of radioactivity. Results are given as means $\pm$ s.d. for the numbers of experiments indicated.

Labelled compound	No. of animals	% of dose recovered				
		Expired CO ₂	Urine	Plasma	Gastrointestinal tract	Gastrointestinal contents and faeces
[1- ¹⁴ C]Glucosamine (2.3 μ moles, 1 μ c)	6	49.2 $\pm$ 12.0	3.2 $\pm$ 1.6	2.8 $\pm$ 0.8	3.2 $\pm$ 0.9†	9.8 $\pm$ 5.4†
<i>N</i> -Acetyl[1- ¹⁴ C]glucosamine (0.3 μ mole, 1 μ c)	4	46.5 $\pm$ 21.8	7.4 $\pm$ 4.4	1.7 $\pm$ 0.5	—	—
¹⁴ C-labelled glycoprotein* (30 mg., 0.2 μ c)	10	49.1 $\pm$ 8.5	2.3 $\pm$ 1.1	3.5 $\pm$ 1.3	4.1 $\pm$ 2.2†	8.0 $\pm$ 4.7†

* Containing 2.7 μ moles of hexosamine.

† Four experiments only.

Table 2. *Metabolic fate of [1-¹⁴C]glucosamine, N-acetyl[1-¹⁴C]glucosamine or ¹⁴C-labelled glycoprotein injected into rats*

The ¹⁴C-labelled compounds were injected into the femoral vein. The ¹⁴C-labelled glycoprotein administered was rat plasma glycoprotein labelled *in vivo* in the *N*-acetylglucosamine and sialic acid moieties. Expired CO₂ and urine were collected for 24 hr. after injection. Plasma was prepared from blood obtained by cardiac puncture at the termination of the collection period. The total plasma volume was taken as 10.7 ml. Results are given as means $\pm$ s.d. for the numbers of experiments indicated.

Labelled compound	No. of animals	% of dose recovered		
		Expired CO ₂	Urine	Plasma
[1- ¹⁴ C]Glucosamine (2.3 μ moles, 1 μ c)	5	19.7 $\pm$ 2.8	26.3 $\pm$ 2.3	12.7 $\pm$ 2.3
<i>N</i> -Acetyl[1- ¹⁴ C]glucosamine (0.3 μ mole, 1 μ c)	5	12.3 $\pm$ 7.4	51.3 $\pm$ 13.6	0.7 $\pm$ 0.2
¹⁴ C-labelled glycoprotein* (30 mg., 0.2 μ c)	6	21.0 $\pm$ 4.8	2.3 $\pm$ 0.8	33.6 $\pm$ 5.7

* Containing 2.7 μ moles of hexosamine.

or ¹⁴C-labelled glycoprotein orally the metabolic fate of these substances was found to be essentially the same as that observed in normal animals (Table 3).

Injected radioactivity entered the bloodstream immediately whereas that derived from the diet presumably entered considerably more slowly over a period of hours. As this difference could have affected the fate of the label, it was investigated by infusing rats under Nembutal anaesthesia with glucosamine (2.3 μ moles, 1 μ c in 1 ml.) into the peritoneal cavity. Since earlier observations (Molnar, Robinson & Winzler, 1965) showed that glucosamine given by intraperitoneal injection was used as effectively as intravenously injected glucosamine for plasma glycoprotein synthesis, this

mode of administration was adopted on account of its greater ease. The mean result from two experiments terminated after a 6 hr. period of infusion was that 4.8% of the radioactivity of the dose appeared in carbon dioxide, 38.9% was in the urine and 32.0% was retained in the plasma. Thus major proportions of the label appeared in the urine and plasma, in agreement with the results for intravenous injection, and it was concluded that the period over which the ¹⁴C-labelled compounds were administered did not markedly influence their utilization.

The nature of the radioactive material present in the plasma of animals given the ¹⁴C-labelled compounds orally and by injection was investigated as described; in all cases the label was associated

Table 3. *Effect of neomycin treatment on the metabolic fate of [1-¹⁴C]glucosamine and ¹⁴C-labelled glycoprotein given orally to rats*

Animals were given neomycin sulphate, 0.5% in their drinking water, for 48 or 96 hr. before and during the experiment. Other experimental details were as given in Table 1. Results are given as means ($\pm$ s.d. where appropriate) for the numbers of experiments indicated.

Treatment	Labelled compound	No. of animals	% of dose recovered		
			Expired CO ₂	Urine	Plasma
Control	[1- ¹⁴ C]Glucosamine	6	49.2 $\pm$ 12.0	3.2 $\pm$ 1.6	2.8 $\pm$ 0.8
Neomycin, 48 hr. pretreatment	[1- ¹⁴ C]Glucosamine	4	53.0 $\pm$ 9.5	3.4 $\pm$ 1.2	3.6 $\pm$ 0.8
Neomycin, 96 hr. pretreatment	[1- ¹⁴ C]Glucosamine	2	54.7	3.1	2.2
Control	¹⁴ C-labelled glycoprotein	10	49.1 $\pm$ 8.5	2.3 $\pm$ 1.1	3.5 $\pm$ 1.3
Neomycin, 48 hr. pretreatment	¹⁴ C-labelled glycoprotein	2	43.8	1.75	1.5

Table 4. *Effects of increasing the oral dose of [1-¹⁴C]glucosamine on its metabolic fate*

Experimental details were as given in Table 1. Results are given as means ($\pm$ s.d. where appropriate) for the numbers of experiments indicated.

[1- ¹⁴ C]Glucosamine dose (μ moles)	No. of animals	% of dose recovered		
		Expired CO ₂	Urine	Plasma
2.3	6	49.2 $\pm$ 12.0	3.2 $\pm$ 1.6	2.8 $\pm$ 0.8
46.0	2	44.6	2.9	2.1
460	2	43.6	4.3	3.1

with trichloroacetic acid-insoluble material. When [1-¹⁴C]glucosamine was injected approx. 30% of the radioactivity of the plasma was associated with protein-bound sialic acid and 70% was ascribed to protein-bound glucosamine. Similar results were obtained when ¹⁴C-labelled glycoprotein was injected. When [1-¹⁴C]glucosamine was given orally 30% and 60% of the label in the plasma were present in protein-bound sialic acid and glucosamine respectively. The remaining 10% was found in other components of the plasma protein; some 5–6% of the radioactivity appeared to be present in hexoses, since it was not retained on either a Dowex 1 (Cl⁻ form) or a Dowex 50 (H⁺ form) column. When ¹⁴C-labelled glycoprotein was given orally 70% of the radioactivity was present in protein-bound glucosamine, 10% was in protein-bound sialic acid and 20% was associated either with hexoses or with amino acids.

The molecular size of the radioactive components appearing in the urine was investigated by gel filtration of urine samples on Sephadex G-25 gel. When [1-¹⁴C]glucosamine was injected only 2% of the radioactive material in the urine was excluded on gel filtration, and when it was given orally 10% of the radioactive material was excluded by the gel, showing that in both instances the label is in material excreted predominantly in low-molecular-weight form. In contrast, when ¹⁴C-labelled glycoprotein was either given orally or injected, some 50% of the radioactive material in the urine was excluded by the gel.

In several experiments the metabolic fate of orally administered [1-¹⁴C]glucosamine was followed when the dose was increased (Table 4). Increasing the dose from 2.3 μ moles to 460 μ moles had little effect on the metabolic fate, except that perhaps less of the label was expired as carbon dioxide.

DISCUSSION

The present finding, that within 24 hr. after the injection of [1-¹⁴C]glucosamine into rats 19.7% of the label appeared in carbon dioxide with 26.3% in the urine and 12.7% in the plasma protein, is in general agreement with the results reported by Kohn *et al.* (1962) and by Shetlar *et al.* (1962, 1964). The finding that only 12.3% of the label from injected *N*-acetyl[1-¹⁴C]glucosamine appeared in carbon dioxide in 24 hr. is markedly different from the value obtained by Kohn *et al.* (1962). These authors showed that the expired radioactivity was decreased by giving glucose to the starved animals, and thus, in the present studies with fed animals, preferential utilization of glucose could account for the low value obtained. The longer time-interval employed in the present study would account for the finding that more radioactivity was excreted in the urine than was reported by Kohn *et al.* (1962).

Within 4 hr. of [1-¹⁴C]glucosamine injection some 25% of the injected radioactivity is incorporated into plasma protein as glucosamine and as sialic acid (Shetlar, Hern, Bradford & Endecott, 1961; Robinson, Molnar & Winzler, 1964). In the

present work only 12.7% of the injected radioactivity was found in plasma glycoprotein after 24 hr., indicating a rapid catabolism of plasma glycoprotein; the half-life of plasma protein-bound glucosamine in rats has been given as approx. 19 hr. (Macbeth, Bekesi, Sugden & Bice, 1965). The finding that 66% of the radioactivity of the injected ^{14}C -labelled plasma glycoprotein was removed from the plasma in 24 hr. is also in accord with this conclusion. Kohn *et al.* (1962) and Richmond (1963) have reported that *N*-acetylglucosamine is only slowly incorporated into glycoprotein, a result confirmed here.

When $[1\text{-}^{14}\text{C}]$ glucosamine or *N*-acetyl $[1\text{-}^{14}\text{C}]$ glucosamine was given orally to rats, 50% of the label was recovered in carbon dioxide, less than 3% was retained in the plasma and 3% ($[1\text{-}^{14}\text{C}]$ glucosamine) or 7% (*N*-acetyl $[1\text{-}^{14}\text{C}]$ glucosamine) appeared in the urine. These results are markedly different from those obtained when these substances were injected. That there was a difference between the fates of orally administered and injected glucosamine was first indicated by Kawabe (1934), who found that only 15% of an oral dose of glucosamine appeared in the urine of rabbits whereas 25–30% of an injected dose was excreted by this route, and the present experiments confirm such a difference. With $[1\text{-}^{14}\text{C}]$ glucosamine and ^{14}C -labelled glycoprotein the results were unaffected by pretreating animals with neomycin, which destroyed a large proportion of the intestinal microflora, and slow infusion of $[1\text{-}^{14}\text{C}]$ glucosamine as opposed to injection had no influence on the fate of the label. Thus neither the activity of the intestinal microflora nor the rate of release of radioactive material into the bloodstream explains this difference.

Capps *et al.* (1966) gave glucosamine in doses of 1 m-mole orally to starved animals and found after 3 hr. that 30% of the dose had been absorbed and that of this 18% had been oxidized to carbon dioxide. They further reported that oral neomycin treatment decreased the extent to which the glucosamine was converted into carbon dioxide, in contrast with the results obtained in the present study. It is difficult to compare results from studies performed over markedly different time-periods with different doses of glucosamine, but Capps *et al.* (1966) found that the absorption coefficient of glucosamine was decreased in the neomycin-treated animals, and a slowed or delayed absorption in these animals might account for the decreased rate of oxidation observed over a short time-interval.

The differences in the results obtained on oral administration and injection may be explained by assuming that at least a proportion of the ^{14}C -labelled compounds given orally were either metabolized by the gastrointestinal tract to carbon dioxide or were altered by the gastrointestinal tract

to substances other than glucosamine that were subsequently converted into carbon dioxide. If a proportion of the amino sugar were absorbed intact, it would account for the finding that when $[1\text{-}^{14}\text{C}]$ glucosamine is given orally some radioactive glucosamine is incorporated into plasma glycoprotein and some radioactivity is excreted in the urine. On the basis of this assumption, then by analogy with the results obtained on injection, where 12% of the label was found in the plasma after 24 hr., the 3% found in the plasma after oral administration would represent an absorption of some 25% of the administered dose in the form of glucosamine. Similarly, if some *N*-acetyl $[1\text{-}^{14}\text{C}]$ glucosamine were absorbed unaltered, by analogy with the results of injected animals, more radioactivity would be found in the urine than would be retained in the plasma, as was found to be the case.

In discussing these findings it is relevant to consider the dietary intake of glucosamine by the rat in relation to the rate at which glucosamine is utilized. In 24 hr. the animals consumed 20–30 g. of a diet containing 5.4 μmoles of amino sugar/g. (G. B. Robinson, unpublished work). Assuming the amino sugar to be glucosamine this represents a maximum intake of 162 μmoles in 24 hr. with 25%, i.e. 40.5 μmoles , being absorbed intact. The total plasma protein-bound glucosamine pool of the animals is 64.2 μmoles (G. B. Robinson, unpublished work) and, taking the half-life as 19 hr. (Macbeth *et al.* 1965), the turnover time ($t_1 \times 1.44$) is 27.3 hr. Thus the glucosamine requirement for the plasma protein synthesis is 56.4 $\mu\text{moles}/24\text{ hr.}$, which cannot be supplied entirely from the diet. It is not possible, from the data available, to assess the total daily rate of glucosamine utilization in the rat, but, since all tissues contain glucosamine bound in macromolecules, the total amount of glucosamine used must be greater than that required for plasma protein biosynthesis. Consequently there must be an endogenous synthesis of glucosamine unless amino sugar, resulting from the degradation of macromolecules, is reutilized.

The fate of orally administered ^{14}C -labelled glycoprotein is very similar to the fate of orally administered $[1\text{-}^{14}\text{C}]$ glucosamine. This suggests that the glycosidases present in the gastrointestinal tract are capable of degrading glycopeptide-bound carbohydrates. Though the glycoprotein contains labelled sialic acid it is unlikely that this is absorbed intact, since injected sialic acid is almost quantitatively excreted in the urine (Johnson, Kistler & McCluer, 1961), and little radioactivity was recovered in the urine of animals given ^{14}C -labelled glycoprotein orally.

In feeding experiments with $[1\text{-}^{14}\text{C}]$ glucosamine and with ^{14}C -labelled glycoprotein, approx. 10% of the label was recovered in the faeces and intestinal

contents. It is not clear from these experiments if this radioactivity represents material that remains unabsorbed in the intestinal lumen or whether it is due to radioactive components that have been incorporated into glycoproteins in the intestinal tract and subsequently secreted back into the lumen.

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REFERENCES

- Boas, N. F. (1953). *J. biol. Chem.* **204**, 553.
- Capps, J. C., Shetlar, M. R. & Bradford, R. H. (1966). *Biochim. biophys. Acta*, **127**, 194.
- Jeanloz, R. W. & Balazs, E. A. (1966). *The Amino Sugars*, vol. 2, part B. New York: Academic Press Inc.
- Jeffay, H. & Alvarez, J. (1961). *Analyt. Chem.* **33**, 612.
- Johnson, G. A., Kistler, C. R. & McCluer, R. H. (1961). *Proc. Soc. exp. Biol., N.Y.*, **106**, 124.
- Kawabe, K. (1934). *J. Biochem., Tokyo*, **20**, 243.
- Kohn, P., Winzler, R. J. & Hoffman, R. (1962). *J. biol. Chem.* **237**, 304.
- Kuhn, R. & Bister, W. (1958). *Liebigs Ann.* **617**, 92.
- Macbeth, R. A. L., Bekesi, J. G., Sugden, E. & Bice, S. (1965). *J. biol. Chem.* **240**, 3707.
- Molnar, J., Robinson, G. B. & Winzler, R. J. (1965). *J. biol. Chem.* **240**, 1882.
- Richmond, J. E. (1963). *Biochemistry*, **2**, 676.
- Robinson, G. B. (1967). *Biochem. J.* **104**, 61P.
- Robinson, G. B., Molnar, J. & Winzler, R. J. (1964). *J. biol. Chem.* **239**, 1134.
- Shetlar, M. R., Bradford, R., Hern, D. L., Endecott, B. & Schilling, J. A. (1962). *Proc. Soc. exp. Biol., N.Y.*, **109**, 335.
- Shetlar, M. R., Capps, J. C. & Hern, D. L. (1964). *Biochim. biophys. Acta*, **83**, 93.
- Shetlar, M. R., Hern, D. L., Bradford, R. H. & Endecott, B. (1961). *Biochim. biophys. Acta*, **53**, 615.
- Warren, L. (1959). *J. biol. Chem.* **234**, 1971.