The Catabolism of Plasma Glycoproteins in Normal and Injured Rats

By G. B. ROBINSON

Department of Biochemistry, University of Oxford

(Received 27 January 1969)

The catabolism of ^{14}C -labelled plasma glycoprotein in rats was studied after injecting homologous plasma protein labelled in the *N*-acetylglucosamine and sialic acid moieties. In normal animals the catabolism was approximately described by a four-compartment model. The fractional rate of catabolism of the plasma-protein amino sugar was found to be 0.0305 hr.^{-1} , corresponding to the degradation of $2.75\text{ }\mu\text{moles/hr.}$ The ^{14}C label was eliminated from the animals largely as carbon dioxide with a small proportion appearing in the urine. Freely circulating amino sugars or glycopeptides did not appear in the plasma as a result of the catabolic processes, and there was no evidence that the protein-bound amino sugars were reutilized in biosynthetic processes. A study of the distribution of ^{14}C label in the carcasses of animals 24 hr. after injection provided evidence that the gastrointestinal tract accounted for 25–38% of the total catabolic pool; the lungs, kidneys, spleen and liver also appeared to contribute to catabolism. Studies were conducted with rats that had been treated with turpentine to induce an inflammatory reaction; the results could not be analysed kinetically, since the metabolism of plasma proteins in these animals did not appear to be in a steady state. The injected plasma protein disappeared from the intravascular pool more quickly than in normal animals, but there were no significant differences in the rates of excretion of the ^{14}C label.

Previously I reported observations on the metabolic fate of labelled glucosamine fed to or injected into rats (Robinson, 1968). Included in this work was a brief study of the fate of ^{14}C label injected as homologous plasma glycoprotein labelled in the *N*-acetylglucosamine and sialic acid residues, when it was found that approx. 23% of the ^{14}C label was eliminated as carbon dioxide 24 hr. after injection. This finding was relevant to a consideration of the catabolism of plasma glycoproteins, but the limited evidence did not permit any detailed conclusions. The present study is an extension of these earlier findings and describes some aspects of the catabolism of this group of glycoproteins.

Since the metabolism of plasma glycoproteins is influenced by inflammatory processes (Winzler, 1965), their catabolism was also investigated in animals with turpentine-induced granulomas.

MATERIALS AND METHODS

$\text{D-[1-}^{14}\text{C]Glucosamine}$ hydrochloride (specific radioactivity 3.1 mc/m-mole) was purchased from The Radiochemical Centre (Amersham, Bucks.).

Rat plasma glycoprotein labelled in the *N*-acetylglucosamine (67% of the total label) and sialic acid (33% of the

total label) residues was prepared as described previously (Robinson, 1968). The specific radioactivity of the protein was approx. 6.6 nc/mg. , varying slightly with different preparations.

Labelled glycopeptide fraction was prepared from the ^{14}C -labelled plasma glycoprotein by incubation with Pronase [Sigma (London) Chemical Co. Ltd., London S.W.6] in 50 mm-sodium borate buffer, pH 9.0, containing 20 mm- CaCl_2 (enzyme/protein ratio 1:12, w/w). The incubation was maintained at 37° for 60 hr. with further additions of Pronase after 24 hr. and 48 hr., each quantity added being the same as that added initially. The digest was applied to a column (1 cm. $\times$ 12 cm.) of Sephadex G-10 gel and eluted with 25 mm- NaCl . All the radioactivity, together with 9.8% of the total amino acids, emerged at the void volume. The radioactive glycopeptide fractions were pooled, freeze-dried and dissolved in sufficient water to give a solution that was 0.15 M with respect to NaCl . The solution was stored at -20° .

Male Wistar rats (240–260 g.) were used and the techniques employed were as described previously (Robinson, 1968). Labelled protein was injected intravenously in doses of 0.15–0.2 μC (20–30 mg.). Turpentine granulomas were induced by sterile injection of a turpentine–corn oil mixture (1:1, v/v) into the hindlimb muscles (0.2 ml. for each limb) and subcutaneously in the suprascapular region (0.4 ml.). Injections were performed under light ether anaesthesia. The animals are referred to in the text as granuloma rats.

Chemical methods, collection of ^{14}C -labelled material and counting techniques were as described previously (Robinson, 1968). The total plasma radioactivity was calculated from measurements of the radioactivity of small portions of plasma corrected for the total plasma volume determined by isotope dilution as described below. Since at all experimental times the total plasma radioactivity was completely precipitable by trichloroacetic acid it was taken to be the total plasma protein radioactivity. The distribution of ^{14}C label in various tissues was determined by removing the tissues from rats exsanguinated by cardiac puncture and macerating them in 0.5M-NaOH (1ml./g. wet wt.). Portions of the homogenates were heated at 70° to dissolve protein and the radioactivity of the solutions was determined. Liver and kidney were perfused to remove residual blood before maceration; bone was macerated and heated in 2.0M-NaOH containing 1.0M-EDTA. In some instances the radioactivity of the tissue was measured by a wet-ashing procedure (Jeffay & Alvarez, 1961) after the tissue had been macerated in water.

The intravascular plasma-glycoprotein space was measured by isotope dilution of intravenously administered labelled glycoprotein at intervals of 5–20 min. after injection. The plasma volume was found to be 10.7 ± 0.4 ml. Measurements taken 20 min. after injection were consistent with those made after 5 min. The plasma volume of granuloma rats (calculated from measurements made after 5 min.) was not found to be significantly greater than that of normal animals.

RESULTS

The fate of ^{14}C label on injection of labelled plasma glycoprotein was assessed by measuring the total radioactivity excreted in the urine and as carbon dioxide. The plasma protein radioactivity was also determined and the total intravascular radioactivity was calculated. The results (Fig. 1) obtained with normal animals confirmed previous results (Robinson, 1968) showing that the ^{14}C label was eliminated largely as carbon dioxide, although a small proportion appeared in the urine. During the early part of the experiment the ^{14}C label was removed from the intravascular compartment but did not appear in the urine or as carbon dioxide. This 'retained' radioactivity is represented by the broken line in Fig. 1. Similar experiments were performed with granuloma rats and the results are illustrated in Fig. 2. The ^{14}C label was rapidly removed from the intravascular space, but was excreted at approximately the same rate as that shown by the normal animals.

Since it was possible that glycopeptide fragments might arise from the degradation of plasma glycoprotein *in vivo*, a glycopeptide fraction prepared from homologous plasma protein was injected intravenously and the metabolic fate of the ^{14}C label was determined after 24 hr. (Table 1). The radioactivity was excreted largely in the urine.

From the results shown in Fig. 1 it was apparent that a proportion of the ^{14}C label, the 'retained'

Fig. 1. Metabolic fate of injected glycoprotein. Normal rats were injected with homologous ^{14}C -labelled plasma glycoprotein (containing radioactive *N*-acetylglucosamine and sialic acid); urine and expired CO_2 were collected for various times. Plasma was prepared from blood obtained by cardiac puncture at the termination of the experiment. The plasma volume was taken as 10.7 ml. for the calculation of total plasma radioactivity. Each result is the mean of at least four separate experiments and the standard deviation is indicated in the figure. ---, Radioactivity retained by the animals; ●, plasma-protein radioactivity; ○, radioactivity in expired CO_2 ; Δ, urine radioactivity.

Fig. 2. Metabolic fate of injected glycoprotein in granuloma animals. The experimental details are as for Fig. 1 except that the animals were treated with injections of turpentine as described in the text. ---, Radioactivity retained by the animals; ●, plasma radioactivity; ○, radioactivity in expired CO_2 ; Δ, urine radioactivity.

radioactivity, was present either in the extra-vascular space or in tissues where catabolism was occurring. Attempts were made to locate the radioactivity in the tissues of four rats 24 hr. after injection of the labelled plasma glycoprotein by counting portions of whole tissues. All the tissues examined, reproductive organs, thymus, skeletal muscle, heart, brain, skin, bone (femur), spleen,

Table 1. *Metabolic fate of ^{14}C -labelled glycopeptide fraction and ^{14}C -labelled glycoprotein injected into rats*

Homologous ^{14}C -labelled plasma glycoprotein was treated with Pronase as described in the text. The resulting labelled glycopeptide fraction or the original labelled glycoprotein (both containing radioactive *N*-acetylglucosamine and sialic acid) were injected intravenously. Expired CO_2 and urine were collected for 24 hr. after injection. Plasma was prepared from blood obtained by cardiac puncture at the termination of the collecting period. The total plasma volume was 10.7 ml. The results are given individually or as means $\pm$ S.D.

Labelled material	No. of animals	% of dose recovered		
		Expired CO_2	Urine	Plasma
^{14}C -labelled glycopeptide fraction (0.2 μC , 3 mg.)	2	11.5, 12.5	55.5, 53.0	3.6, 3.6
^{14}C -labelled glycoprotein (0.2 μC , 30 mg.)	6	21.0 $\pm$ 4.8	2.3 $\pm$ 0.8	33.6 $\pm$ 5.7

kidney, lung, liver and gastrointestinal tract, were radioactive. For most tissues the amounts of radioactivity were low and the specific radioactivities on a wet-weight basis were consistent with the dispersal of the 'retained' ^{14}C label throughout the intravascular and extravascular pools of all tissues, after allowing for the fact that exsanguination of the animals removed only 50% of the blood. The specific radioactivities per g. wet wt. of kidney, liver, gastrointestinal tract, lung and spleen were significantly higher than those found for any of the other tissues and could not be accounted for by assuming that the 'retained' radioactivity and residual plasma radioactivity were evenly distributed throughout the carcasses. The liver, the gastrointestinal tract and the gastrointestinal contents contained $2.0 \pm 0.5\%$, $4.5 \pm 0.3\%$ and $2.3 \pm 0.6\%$ respectively of the total dose of ^{14}C label. It was established that in the gastrointestinal-tract tissue and contents between 20% and 30% of the activity was in trichloroacetic acid-soluble form.

The only analysis made of the radioactive substances excreted in the urine was to establish that between 60 and 70% of the urine radioactivity was not diffusible on dialysis.

CALCULATIONS

Kinetic models have been extensively used to analyse the metabolism of plasma albumin (Reeve & Roberts, 1959), and it was decided to compare the results obtained here with the model system represented in Scheme 1. In this model the intravascular plasma-protein pool (A) is assumed to be in equilibrium with an extravascular pool (B). The catabolite pool (C) is derived directly from the intravascular pool and the products of catabolism are irreversibly eliminated as a pool of excretion products (D).

The radioactivity (α) in the plasma-protein pool (A) at any time t after injection is expressed by the following relationship:

$$\alpha = Z_1 e^{-a_1 t} + Z_2 e^{-a_2 t} \quad (1)$$

Scheme 1. Hypothetical scheme for the catabolism of plasma glycoproteins (Reeve & Roberts, 1959).

Graphic analysis of the logarithmic decay of plasma radioactivity (Fig. 3) showed two exponential functions and provided the following values:

$$\begin{aligned} Z_1 &= 0.501, \\ Z_2 &= 0.467, \\ a_1 &= 0.0172 \text{ hr.}^{-1}, \\ a_2 &= 0.105 \text{ hr.}^{-1}. \end{aligned}$$

These results permitted the calculation of values for k_2 , k_{-2} and k_3 , which are respectively 0.027 hr.^{-1} , 0.065 hr.^{-1} and 0.0305 hr.^{-1} . The radioactivity (β) present in the extravascular pool (B) at any time t is expressed by the following relationship:

$$\beta = Q(e^{-a_1 t} - e^{-a_2 t}) \quad (2)$$

where:

$$Q = k_2/(a_2 - a_1) \quad (3)$$

The value for Q was calculated as 0.308 and it was possible to calculate the radioactivity in the extravascular pool at various times (Fig. 4) by using eqn. (2). When these values are subtracted from the 'retained' radioactivity, i.e. the radioactivity

Fig. 3. Semi-logarithmic plot of the plasma radioactivity from normal animals injected with ^{14}C -labelled glycoprotein. ●, Plasma radioactivity; ○, second extrapolation derived from 'stripping' the first extrapolation of the plasma radioactivity; ----, line used for determining the apparent half-life of the plasma radioactivity.

Fig. 4. Calculated behaviour of the extravascular-pool, breakdown-pool and excreted radioactivities in rats injected with homologous ^{14}C -labelled plasma glycoprotein. The basis for the calculations is described in the text. ●, Observed plasma radioactivity; ○, observed excreted radioactivity; △, calculated extravascular-pool radioactivity; ▲, calculated breakdown-pool radioactivity; ----, calculated excreted radioactivity.

within the animal but which is outside the intravascular pool, the resulting 'residual' radioactivity should represent the breakdown pool C (Fig. 4). Although k_4 cannot be measured directly it can be calculated from eqn. (4), since at the time when the value for the radioactivity (γ) of pool C was at a maximum:

$$(d\gamma)/(dt) = k_3\alpha - k_4\gamma = 0 \quad (4)$$

Taking 18 hr. as the time at which γ was maximal, $k_4 = 0.069 \text{ hr.}^{-1}$. The calculation of this value made it possible to further calculate the cumulative amount of ^{14}C label (δ) that should have been

excreted at any time, so providing theoretical values that may be compared with the experimental results. The excreted radioactivity (δ) was calculated from eqn. (5), and is represented in Fig. 4 as a broken line:

$$\delta = k_4 \int_{t_2}^{t_1} \gamma \cdot dt \quad (5)$$

Calculations based on the model could not be used for the results obtained with granuloma animals. The logarithmic decay of the plasma radioactivity followed a curve that was taken to indicate that the metabolism of the glycoproteins was not in a steady state. This contention was supported by observations showing that the concentrations of plasma glycoproteins in these animals decreased during the course of the experiments.

DISCUSSION

No detailed kinetic analyses of the catabolism of plasma-protein-bound amino sugars have been made, although apparent half-lives have been assessed for bound glucosamine and sialic acid. The measurement of half-life cannot be strictly applied to the decay of plasma-protein radioactivity, since this concept is only valid when a function is described by one exponential term, whereas the catabolic behaviour of plasma proteins is described by two exponential terms. However, to compare the results reported here with other studies an apparent half-life for total radioactivity was calculated for the results obtained during the first 24 hr. of the experiments; this was found to be 17.5 hr. Macbeth, Bekesi, Sugden & Bice (1965), using rats, reported half-lives for plasma-protein-bound glucosamine and sialic acid of 19.4 hr. and 19.0 hr. respectively; Spiro (1959) reported the half-life of plasma-protein-bound glucosamine as 14.0 hr.

A further comparison can be made between the results reported here and those published by Robinson, Molnar & Winzler (1964) and by Molnar, Robinson & Winzler (1965). These studies concerned the kinetic analysis of the incorporation of ^{14}C -labelled glucosamine into plasma proteins in rats. Since glycoprotein metabolism is assumed to be in a steady state (Scheme 1), the rate of synthesis of plasma-protein-bound amino sugar should equal the rate of degradation. The rate of synthesis can be calculated from the papers cited as follows: the animals used here had liver weights of approx. 8.5 g., and thus the microsomal pool of bound glucosamine and sialic acid was $5.7 \mu\text{moles}$ (from Table II in Molnar *et al.* 1965). Only one-half of this pool was metabolically active and the rate constant for

plasma-protein synthesis was 0.02 min.^{-1} (Robinson *et al.* 1964). Therefore the rate of synthesis of glycoprotein amino sugar is calculated as $3.4 \mu\text{moles/hr.}$ The rate of degradation is calculated from the plasma-protein glucosamine and sialic acid pools of the rats, which were found here to be $64.2 \mu\text{moles}$ and $25.6 \mu\text{moles}$ respectively, giving a total of $89.8 \mu\text{moles}$ (G. B. Robinson, unpublished work). Since the rate constant for breakdown (k_3) was 0.0305 hr.^{-1} , the rate of catabolism is calculated to be $2.75 \mu\text{moles/hr.}$, agreeing reasonably well with the calculated synthetic rate.

The validity of the proposed model for catabolism can be assessed by comparing the actual and the calculated excretion of ^{14}C label (Fig. 4). The calculated results fall slightly below the actual results, demonstrating some inconsistency between the observations and the model. It is known that denatured protein is catabolized more rapidly than native protein (Katz, Rosenfeld & Sellers, 1961). Therefore, if a small proportion of the injected glycoprotein were partially denatured, more catabolism would occur at short time-intervals, increasing the rate of excretion of products at early times, obscuring the sigmoid pattern of the excretion curve. The model also assumes a common catabolic pool, which may well be an oversimplification of the situation since the distribution of ^{14}C label in the carcasses 24 hr. after injection suggests that more than one tissue may participate in catabolism. The error resulting from the use of a simplified model for kinetic analysis will depend on the number, size and rate constants of each individual catabolite pool, and these cannot be assessed in this study.

There is controversial evidence on the identity of the tissue or tissues that participate in the catabolism of plasma protein. This problem has been reviewed by Schultze & Heremans (1966). It has been recognized that the gastrointestinal tract is a site of catabolism, but the extent to which it contributes to the total catabolism is not clearly established. The present finding, that the gastrointestinal-tract tissue contained 4.5% of the total administered radioactivity after 24 hr. and that some of this radioactivity was soluble in trichloroacetic acid, supports the view that this tissue is involved in plasma-protein catabolism. Since the residual radioactivity, i.e. the catabolic pool, was 18% of the dose at this time, the results suggest that some 25% of the total plasma-glycoprotein catabolism occurs in this tissue. If the radioactivity of the gut contents (2.3%) is also taken into consideration, the gastrointestinal tract can be calculated to account for 38% of total catabolism. However, a proportion of the radioactivity of the contents may not be reabsorbed (Robinson, 1968) and therefore should be treated as an excretion product rather than as part of the catabolic pool.

It is possible that some of the radioactivity in the gastrointestinal-tract tissue is due to the presence of residual blood, but to explain the high radioactivity in the gastrointestinal-tract tissue it would be necessary to assume that one-third of the residual blood in the carcass was trapped in the tissue, which seems to be very unlikely. Other studies have indicated that the gastrointestinal tract is responsible for 15–25% of albumin catabolism (Tarver, Armstrong, Debro & Margen, 1961), 38% of immunoglobulin G catabolism (Anderson, Glenert & Wallevik, 1963) and 11–22% of caeruloplasmin catabolism (Waldmann, Morell, Wochner, Strober & Sternleib, 1967). The radioactivity present in the liver, 2.0% of the total injected, indicates that 11% of the catabolic pool may reside in this organ. Aronson & de Duve (1968) have shown that rat liver lysosomal enzymes are able to degrade plasma glycoprotein.

In the present study, spleen, kidney and lung all contained significant radioactivity, indicating that these organs may also be involved in catabolism; Katz, Rosenfeld & Sellers (1960) have demonstrated albumin catabolism in the kidney.

There are several ways in which plasma glycoproteins may be degraded by the tissues. The breakdown might be complete, with consequent catabolism of the sugars released from the glycoprotein. The various monosaccharides liberated by hydrolysis could be released into the bloodstream from the tissues and reutilized, or degradation may only be partial with glycopeptides as such being released into the bloodstream. The present experiments indicate that these latter two possibilities are not of major importance. I showed (Robinson, 1968) that both injected glucosamine and *N*-acetylglucosamine are partially excreted in the urine in low-molecular-weight form, and sialic acid is known to be almost quantitatively excreted by this route (Johnson, Kistler & McCluer, 1961). In these experiments only 2–3% of the ^{14}C label is excreted in the urine in low-molecular-weight form after 48 hr. This implies that, at most, only 10% of the metabolism occurs by pathways involving the release of monosaccharides into the bloodstream. Accordingly there can be little reutilization, for plasma-protein synthesis, of amino sugar derived from plasma-protein catabolism in tissues other than liver, which is the principal site of glycoprotein synthesis (Robinson *et al.* 1964). The kinetic analysis further supports the contention that reutilization of the amino sugar does not occur to any large extent, a conclusion reached by Robinson *et al.* (1964). Catabolism of the proteins to glycopeptides does not appear to be a major pathway, since a large proportion of injected glycopeptide was excreted in the urine whereas only 4–5% of the total protein radioactivity appears in the urine in

large-molecular-weight form; Bourrillon & Cornillot (1964) have reported the presence in urine of glycopeptides similar to those obtained from plasma proteins. The results therefore indicate that the plasma-glycoprotein amino sugars are catabolized to carbon dioxide at the site of glycoprotein hydrolysis.

The results obtained with granuloma animals can only be analysed in qualitative terms, since the plasma-glycoprotein metabolism was not in a steady state. The principal difference between these animals and the controls is in the rate of removal of the ^{14}C label from the intravascular space; this is approximately twice as fast as that observed in normal rats. The rapid disappearance would imply a more speedy catabolism of the glycoprotein, but the rate of excretion of the ^{14}C label is not significantly greater than that found in normal animals. This situation may be explained by assuming a marked increase in the extravascular space in these animals, possibly due to altered capillary permeability or oedema in the injured tissues, resulting in a loss of protein into the extravascular compartment. Alternatively the glycoproteins could be hydrolysed to monosaccharides, which, instead of being oxidized, may be utilized for the synthesis of macromolecules within the tissues. The low urine radioactivity precludes the possibility that the monosaccharides recirculate in the blood. A more detailed consideration of these possibilities would be relevant to the problems of altered plasma-glycoprotein metabolism found in inflammatory conditions (Winzler, 1965).

I thank Miss J. Byrne for her skilful assistance.

REFERENCES

- Anderson, S. B., Glenert, J. & Wallevik, K. (1963). *Nature, Lond.*, **199**, 1096.
- Aronson, N. N. & de Duve, C. (1968). *J. biol. Chem.* **243**, 4564.
- Bourrillon, R. & Cornillot, P. (1964). In *The Protides of Biological Fluids*, vol. 12, p. 300. Ed. by Peeters, H. Amsterdam, London and New York: Elsevier Publishing Co.
- 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.
- Katz, J., Rosenfeld, S. & Sellers, A. L. (1960). *Amer. J. Physiol.* **198**, 814.
- Katz, J., Rosenfeld, S. & Sellers, A. L. (1961). *Amer. J. Physiol.* **200**, 1301.
- 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.
- Reeve, E. B. & Roberts, J. E. (1959). *J. gen. Physiol.* **43**, 415.
- Robinson, G. B. (1968). *Biochem. J.* **108**, 275.
- Robinson, G. B., Molnar, J. & Winzler, R. J. (1964). *J. biol. Chem.* **239**, 1134.
- Schultze, H. E. & Heremans, J. F. (1966). *Molecular Biology of Human Proteins*, vol. 1, p. 450. Amsterdam, London and New York: Elsevier Publishing Co.
- Spiro, R. G. (1959). *J. biol. Chem.* **234**, 742.
- Tarver, H., Armstrong, F. B., Debro, J. R. & Margen, S. (1961). *Ann. N.Y. Acad. Sci.* **94**, 23.
- Waldmann, T. A., Morell, A. G., Wochner, R. D., Strober, W. & Sternleib, I. (1967). *J. clin. Invest.* **46**, 10.
- Winzler, R. J. (1965). In *The Amino Sugars*, vol. 2A, p. 338. Ed. by Balazs, E. A. & Jeanloz, R. W. New York: Academic Press Inc.