# IMMUNOGLOBULIN SYNTHESIS AND SECRETION

## I. Biosynthetic Studies of the Addition of the Carbohydrate Moieties

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ABSTRACT

This study was designed to determine the time in the intracellular life of immunoglobulin when the carbohydrate moieties are added. Plasma cells from a mouse myeloma tumor were exposed to glucosamine-$^{3}$H (a "bridge" sugar), galactose-$^{3}$H, or leucine-$^{3}$H. With each of the above isotopes, the percentage of total radioactive immunoglobulin that has been secreted after different periods of labeling and the extent to which puromycin prevented incorporation into immunoglobulin were determined. The results indicate that both galactose and glucosamine (in its *N*-acetyl form) become covalently incorporated into immunoglobulin G late in its intracellular life and suggest that glucosamine is also added onto nascent polypeptide chains (i.e., on polyribosomes).

### INTRODUCTION

Immunoglobulin G (IgG) is a heteropolymeric glycoprotein consisting of two heavy (H) and two light (L) polypeptide chains joined by disulfide bonds and one or more carbohydrate moieties (1). For mouse IgG, the sugar residue which links the carbohydrate to an aspartyl residue of the H chain is D-glucosamine, presumably in its *N*-acetyl form (2–4).

Previous studies with a mouse plasma cell tumor $MPC_{11}$ (5) indicated that incorporation of mannose and galactose into IgG continued after protein synthesis was completely inhibited by the addition of puromycin, suggesting that these sugars can be incorporated on completed polypeptide chains. Glucosamine-$^{14}$C, the "bridge" sugar, however, was incorporated into polyribosomes of such cells and radioactivity was liberated by the in vitro addition of puromycin, suggesting that some of the glucosamine residues were incorporated into nascent polypeptide chains on polyribosomes.

The purpose of the present studies was to investigate further the time in the intracellular life of IgG when glucosamine and galactose are incorporated into the carbohydrate portion of the molecule. The approaches used were to measure: (*a*) the extent to which puromycin can prevent incorporation of galactose or glucosamine into intracellular and secreted IgG; (*b*) the percentage of total radioactive IgG secreted after different periods of time of labeling with leucine, galactose, or glucosamine.

The rationale is as follows: If a given sugar is incorporated on nascent polypeptide chains only, puromycin should completely prevent such incorporation. If, on the other hand, the sugar is added on completed chains and "late" in the intracellular life of the molecule, then puromycin should reduce incorporation only partially for a given short period of incubation since chains completed prior to the addition of puromycin will continue to have the sugar attached to them.

The percentage of total labeled IgG secreted after various intervals should also give information on the time of incorporation of the sugars. Thus, if incorporation of a sugar occurs on nascent chains, the proportion secreted should be similar to that of leucine-labeled IgG whereas if the sugar is added "late," a higher percentage of sugar labeled IgG should be secreted.

The results suggest that galactose is added onto completed polypeptide chains late in the intracellular life of the molecule, whereas glucosamine is attached early, presumably on nascent polypeptide chains on polyribosomes and also late, on completed chains.

## MATERIALS AND METHODS

### *Cell Suspension*

$LPC_1$, kindly provided by Dr. John Fahey, NIH, is a transplantable plasma cell tumor producing gamma $G_2B$ which is carried in Balb/c mice. 2–3 wk old subcutaneous tumors were removed, and cells were teased from them by means of scalpels with No. 15 blades. The cells were then filtered through a stainless steel screen, washed three times with Eagle's medium without glucose, and suspended in this medium at a concentration of $2 \times 10^7$ cells per ml. For the purpose of labeling with leucine, the medium was free of leucine. The cells were preincubated at 37°C for 30 min prior to labeling.

### *Labeling*

Cells were incubated with either L-leucine-4,5-$^3$H (45 Ci/mmole), D-galactose-1-$^3$H (6.9 Ci/mmole), or D-glucosamine-6-$^3$H (1.1 Ci/mmole) (New England Nuclear Corp., Boston, Mass.). For labeling with leucine, 100 $\mu$Ci/ml was used; for the two sugars, 250 $\mu$Ci/ml. In some cases, puromycin was added to a final concentration of $6 \times 10^{-4}$ M 5 min before labeling.

### *Preparation of Cell Lysate and Secretion for Electrophoresis*

At intervals after start of incubation with the radioactive material, incorporation was stopped by chilling the cells to 4°C. They were then pelleted, and the supernatant containing the IgG secreted by the cells (secretion) was saved. The cells were then washed once in Eagle's medium and treated with 0.5% Nonidet P-40 (NP-40) (Shell Chemical Co., New York) for 10 min at 0°C in order to obtain cell cytoplasm free of membranes. The nuclei were then pelleted by centrifugation at 1000 rpm for 5 min. The supernatant was dialyzed overnight against 0.01 M phosphate-buffered saline pH 7.2. This cell lysate was treated with 0.1 ml of rabbit antiserum specific to mouse immunoglobulin, and the mixture was incubated at 37°C for 30 min followed by an additional 4 hr at 0°C. The resulting precipitate was washed two times in cold phosphate-buffered saline and the precipitate obtained, after centrifugation was dissolved in 0.25 N acetic acid (HAC) and then treated with 1% sodium dodecyl sulfate (SDS) and 0.5 M urea. The solubilized precipitate was dialyzed overnight against 0.5 M urea and 0.1% SDS and was then electrophoresed on acrylamide gel (5% in 0.1% SDS) at 15 ma/gel for 150 min (6). Gels were divided according to the method of Maizel (7), and the gel aliquots were counted in a Beckman scintillation counter in cocktail "D" solution (8). Cell secretions were usually treated exactly as the cell lysates, except in some early experiments in which precipitation with antibody was omitted. When secretions were precipitated, carrier IgG (40 $\mu$g/ml) was added.

### *Chromatographic Analysis of Cell Lysates and Secretions*

Removal of free radioactive precursors from either secretions or lysates was accomplished by "sieving" through Sephadex G-25 (Pharmacia Fine Chemicals, Uppsala, Sweden). Sephadex G-50 or G-75 was employed for the separation of H and L chains of reduced and alkylated IgG. All Sephadex columns were prepared according to the technical manual published by Pharmacia (9). Reduction and alkylation was performed according to the method of Porter (10) using 0.28 M 2-mercaptoethanol (ME), 0.05 M Tris buffer pH 8.6, and 0.56 M iodoacetamide. Specific precipitates that were to be reduced and alkylated were first dissolved in 0.5 M Tris HCl buffer that was 8 M in urea and 1% in SDS. Dissolution was complete after incubation for 10 min at 37°C.

Hexoses or their *N*-acetyl derivatives from experimentally obtained IgG fractions (lysates or secretions) were separated from an acid hydrolysate of the glycoprotein (6 hr, 100°C 4 N HCl) by the use of Dowex-50 (H+ form) according to the method of Boas et al. (11).

Further identification of these sugars was done by paper chromatography of the fractions from the Dowex procedure on Whatman 3 MM paper, with the pyridine:$H_2O$; *n*-butanol (BuOH) solvent as irrigant (descending, 18 hr. Glucosamine *N*-acetyl glucosamine, and galactose (Mann Research Laboratories, Inc., New York) were used as standards in order to determine the respective $R_f$ values. Visualization was done by spraying with Partridge's reagent (12), and radioactivity was measured by liquid-scintillation counting of suitable segments of the paper in cocktail D (Beckman Instruments, Fullerton, Calif.) with a

TABLE I

*Effect of Puromycin on Incorporation of Leucine and Sugars into Immunoglobulin (IgG)*

| Duration of incubation | Isotope-$^3$H | Intracellular IgG | | | Secreted IgG | | |
|---|---|---|---|---|---|---|---|
| | | Control | Puromycin* | Decrease | Control | Puromycin* | Decrease |
| *min* | | *cpm* | *cpm* | *%* | *cpm* | *cpm* | *%* |
| 30 | Leucine | 259,896 | 0 | 100 | 9,174 | 0 | 100 |
| | Galactose | 3,170 | 1,407 | 55 | 3,104 | 1,536 | 50 |
| | Glucosamine | 906 | 241 | 73 | 418 | 174 | 58 |
| 60 | Leucine | 576,747 | 0 | 100 | 99,134 | 0 | 100 |
| | Galactose | 4,139 | 1,494 | 63 | 10,600 | 4,448 | 58 |
| | Glucosamine | 2,702 | 296 | 89 | 1,866 | 870 | 53 |

* $6 \times 10^{-4}$ M, 5 min before labeling.

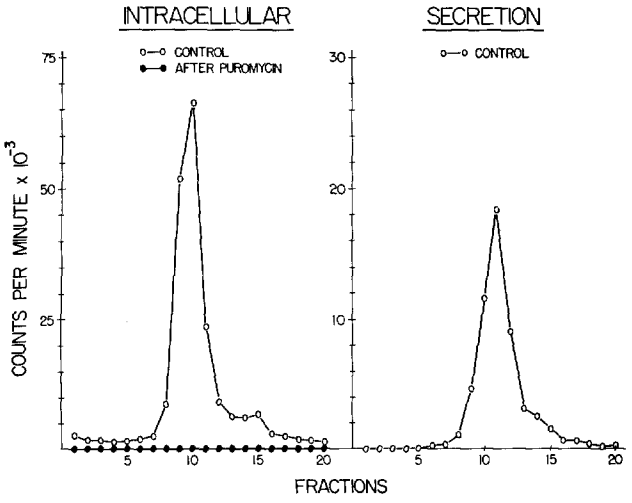


FIGURE 1 Electrophoretic analyses of leucine-labeled intracellular and secreted IgG of $LPC_1$ cells. Cells were exposed to leucine-$^3$H for 60 min. One aliquot received $6 \times 10^{-4}$ M puromycin 5 min prior to labeling. Cell lysates and secretions were precipitated with rabbit anti-mouse IgG, and the dissolved precipitates were electrophoresed in 5% acrylamide gel in 0.1% SDS (sodium dodecyl sulfate) (see Materials and Methods).

Beckman LS-250 scintillation counter. Counting was done to the 95% confidence level.

## RESULTS

### *Electrophoretic Analysis of Cell Lysates and Secretions*

In a representative experiment, $LPC_1$ cell suspensions, some of which were pretreated with puromycin ($6 \times 10^{-4}$ M) 5 min before labeling, were exposed to leucine-$^3$H, galactose-$^3$H, or glucosamine-$^3$H. Aliquots were removed at time 0, 30, and 60 min after labeling, and radioactive IgG was measured in cell lysates and secretions (see Materials and Methods).

The results of this experiment are summarized in Table I, and the analyses of the electrophoresis of the 60-min samples of the cell lysates and secretions are shown in Figs. 1–3. The amounts of radioactivity represented by the IgG peaks in the electrophoretic patterns were used for the calculations in the Table.

As can be seen in Fig. 1 and Table I, puromycin prevented incorporation of leucine-$^3$H into IgG. In contrast, incorporation of glucosamine and galactose into IgG was *not* completely abolished (Figs. 2, 3). Indeed, both these sugars continue to be incorporated into IgG 30–60 min after protein synthesis has stopped. Thus, some of the residues of each of these two sugars are incorporated into completed chains.

It is difficult to predict, however, the degree to

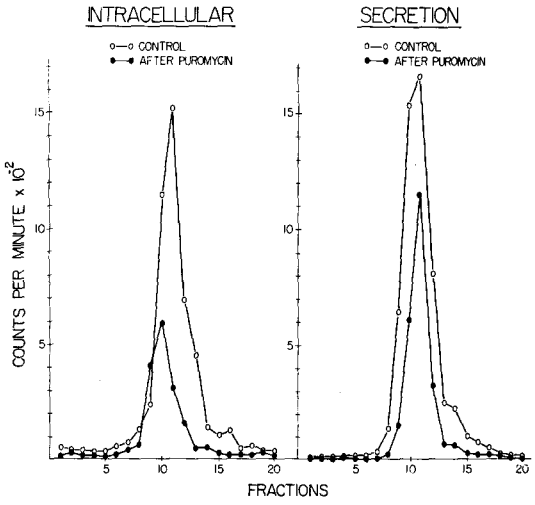


FIGURE 2 Electrophoretic analyses of galactose-$^3$H-labeled intracellular and secreted IgG of $LPC_1$ cells (see legend to Fig. 1).

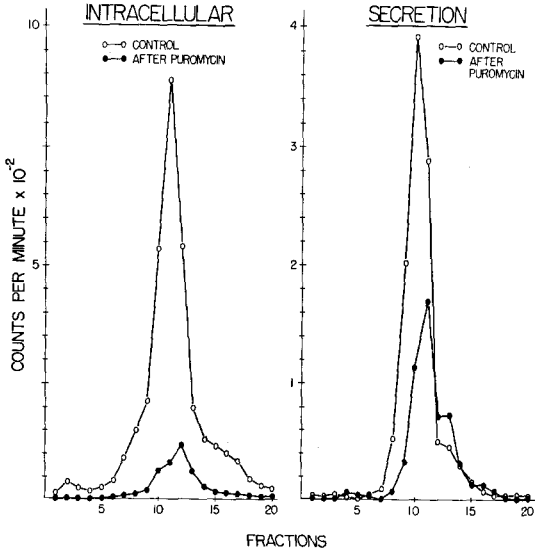


FIGURE 3 Electrophoretic analyses of glucosamine-$^3$H-labeled intracellular and secreted IgG of $LPC_1$ cells (see legend to Fig. 1).

which puromycin would depress incorporation of sugars into completed IgG chains over a given period of time for two reasons: (*a*) The supply of IgG molecules to which sugar is to be added decreases with time. This explains why puromycin had less effect on incorporation of sugars into IgG that was secreted than that which remained intracellular. On the average, IgG molecules that are found in the secretion are synthesized earlier than those not yet secreted; hence this former population is less dependent on new protein synthesis. (*b*) Puromycin has toxic effects on the cells as evidenced by alteration in ultrastructure seen after 1 hr of incubation.[1] Despite these problems, the experiment shows that the effect of puromycin was more pronounced on the incorporation of glucosamine into intracellular IgG than that on galactose incorporation at both time points studied. The difference is particularly apparent after 60 min of incubation when 89% of glucosamine incorporation into IgG has been prevented by puromycin compared to 63% for galactose.

Table II summarizes the percentages of total radioactive IgG secreted by control cells (i.e., not treated with puromycin) from the above experiment. As can be seen in Table II, IgG labeled by either galactose or glucosamine appears more rapidly in secretions than that labeled by leucine. Thus, after 30 min of labeling, virtually no IgG labeled by leucine has been secreted (4%); in contrast, 32% and 50% of IgG labeled by glucosamine and galactose, respectively, have been secreted. A similar pattern is present after 60 min of labeling.

TABLE II

*Percentage of Total Radioactive IgG Secreted after Labeling with Leucine and Sugars*

| Isotope-$^3$H | % Total radioactive IgG secreted | |
|---|---|---|
| | 30 min | 60 min |
| Leucine | 4 | 15 |
| Galactose | 50 | 72 |
| Glucosamine | 32 | 40 |

The above experiment was repeated twice with essentially similar results.

These experiments indicate, therefore, that a portion of the galactose and glucosamine residues are added some time after the IgG chains are synthesized, i.e., nearer to the time of their secretion. The striking finding that, in a continuous labeling experiment, 72% of galactose-labeled

[1] D. Zagury, I. Schenkein, and J. W. Uhr. Unpublished observations.

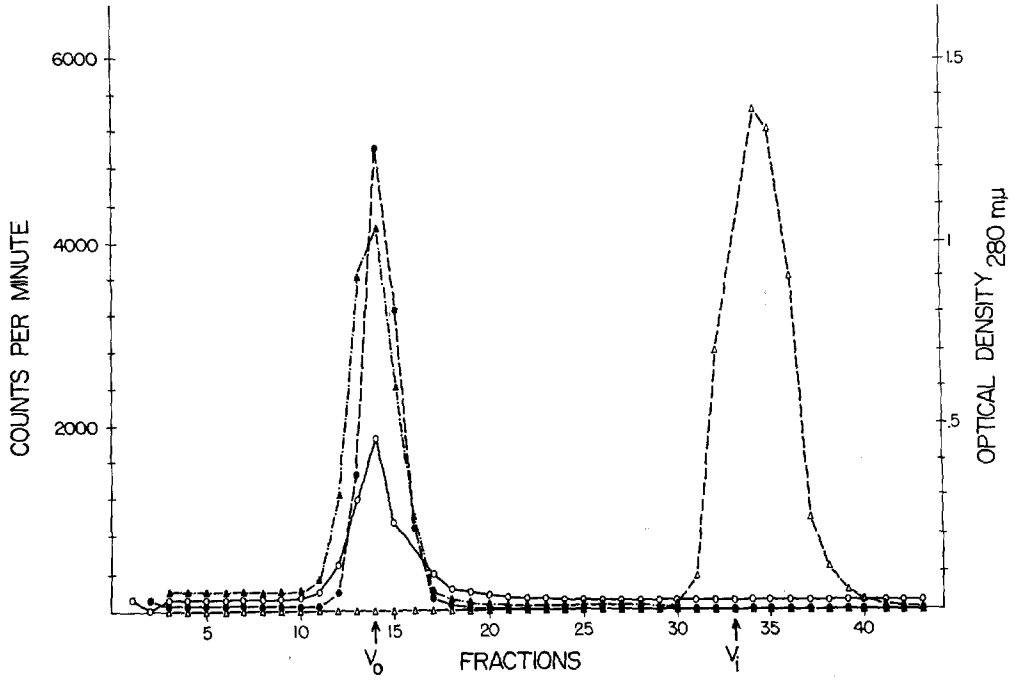


FIGURE 4 Molecular sieving on Sephadex G-25 of galactose-$^3$H and glucosamine-$^3$H–labeled IgG. The secretion from cells labeled for 60 min with galactose-$^3$H and a lysate from cells labeled for 60 min with glucosamine-$^3$H after pretreatment with puromycin were each precipitated with antibody to mouse IgG before filtration. The figure combines the results of the two individual sieving procedures. A mixture of purified mouse IgG and free galactose-$^3$H was used to establish void volume ($V_o$) and internal volume ($V_i$) of the columns. ●-----● Secretion, galactose-$^3$H. ○——○ Intracellular, glucosamine-$^3$H, after puromycin. ▲——▲ OD of mouse IG to establish $V_o$. △-----△ Free galactose-$^3$H to establish $V_i$.

IgG has been secreted at a time when only 15% of leucine-labeled IgG has been secreted, suggests that galactose is added on completed chains and close to the time of their secretion. As with the previous experiment, there is a marked difference between glucosamine and galactose, i.e., a smaller proportion of glucosamine-labeled IgG is secreted compared to galactose-labeled IgG at both time periods studied. This observation, which is more fully discussed later, and the results of the puromycin studies are consistent with the idea that glucosamine is attached to both nascent and completed chains.

### *Chromatographic Analysis of Cell Lysates and Secretions*

The validity of the measurements in the preceding labeling experiments depends upon the demonstration that the radioactivity associated with IgG is incorporated into its structure.

For this purpose, both lysates and secretions from cells incubated for 60 min with either glucosamine-$^3$H or galactose-$^3$H with and without puromycin (i.e., eight samples representing all possible combinations) were examined by the techniques to be described in this section. It was found that lysates and secretions gave identical results in terms of chromatography and that pretreatment with puromycin did not affect such results. The first procedure was to subject the above materials to molecular sieving on Sephadex G-25 with $H_2O$ used as eluant (or 1 N HAC for the specific precipitates). Preliminary experiments had shown that this maneuver completely removed free radioactive glucosamine-$^3$H from purified cold IgG with which it had been incubated for 3–6 hr at 37°C. Recovery of either material in these control experiments was virtually complete. Dialysis of the fractions prior to the molecular sieving did not essentially alter the results of the procedure. Between 2–5 mg of "carrier" mouse IgG was added to experimental fractions in order to detect emer-

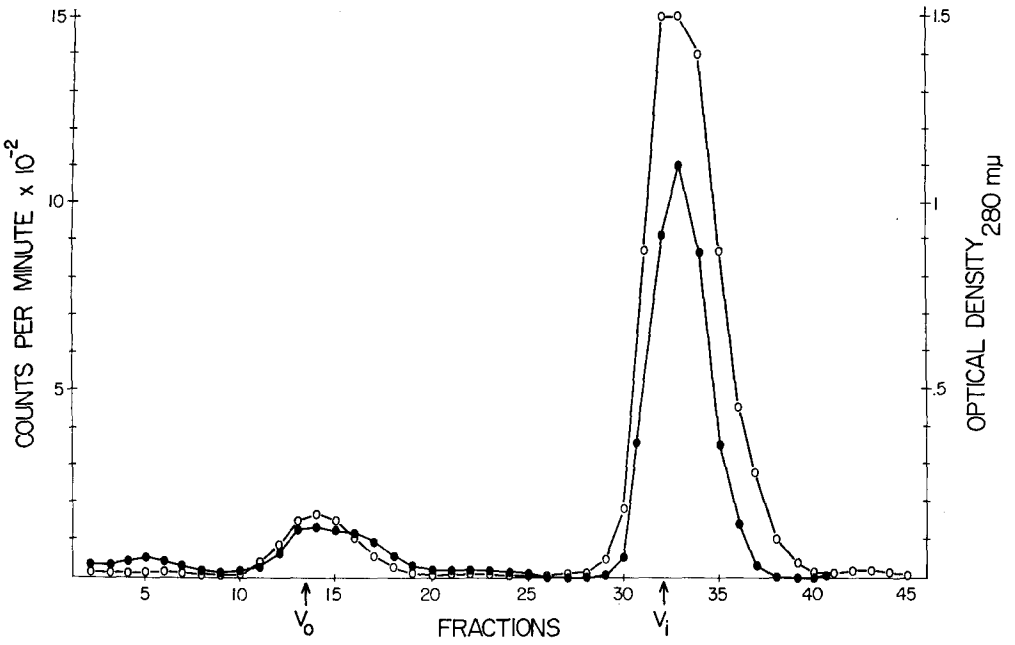


FIGURE 5 Molecular sieving on Sephadex G-25 of glucosamine-$^3$H–labeled intracellular material not precipitable by anti-mouse IgG. The cells were exposed for 60 min to glucosamine-$^3$H after pretreatment with puromycin. Intracellular material was precipitated with anti-mouse IgG and the supernatant was used. $V_o$ and $V_i$ of column established independently (see Fig. 4). O——O glucosamine-$^3$H, after puromycin. ●----● $OD_{280\ m\mu}$.

gence of materials from columns by scanning of the OD at 280 mμ.

Representative results of scans of radioactivity and optical density are illustrated in Fig. 4 which shows a galactose-labeled secretion and a glucosamine-labeled lysate after pretreatment of cells with puromycin. The $V_0$ (void volume) in all cases coincided with the actual emergence of material absorbing at 280 mμ and radioactivity.

These findings are a strong indication that the radioactivity is covalently bound to the macromolecules in these preparations. All the lysates had been specifically precipitated with goat-anti-mouse gamma globulin prior to chromatography and, as shown previously, gave single radioactivity peaks that corresponded to the known position of IgG on acrylamide gel electrophoresis. Some of the secretions had not been previously precipitated with specific antiserum. In these cases, materials eluting in the region of the $V_0$ were combined, lyophilized, and then precipitated with anti-mouse IgG. Solubilization of these in 1 N HAC and filtration on G-25 equilibrated with 1 N HAC indicated that no significant changes occurred in the total radioactivity, strengthening the conclusion that radioactivity found in the $V_0$ of the first molecular sieving procedure was covalently bound to IgG.

In order to determine the extent to which non-IgG proteins become labeled with radioactive glucosamine and galactose, the *supernatants* resulting from specific precipitation with anti-gamma globulin of the appropriate lysates were also examined after Sephadex G-25 filtration. No carrier IgG was added.

The results with lysates from cells labeled with glucosamine are shown in Fig. 5. No significant UV-absorbing material or radioactivity was present in the higher molecular weight fraction ($V_0$, Fig. 5). Similar results were obtained with lysates from cells labeled with galactose. These findings indicate that at least 95% of the cytoplasmic macromolecules which become labeled with either glucosamine or galactose for the period of time studied is IgG.

The $V_i$ (internal volume) of these fractions gave, in all cases, a significant quantity of material absorbing at 280 mμ. Spectrophotometric analy-

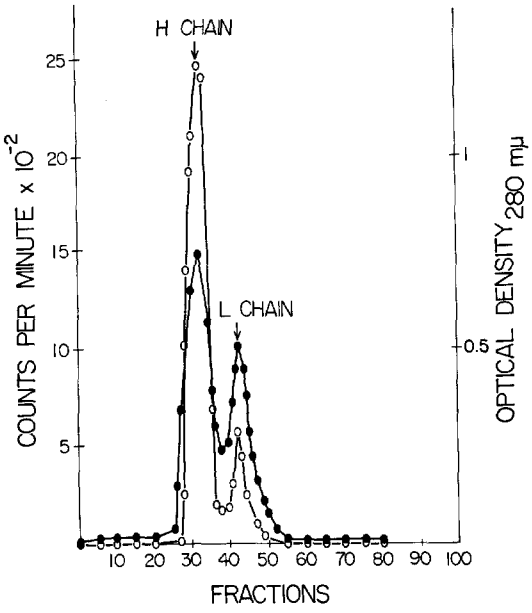


FIGURE 6 Chromatography on Sephadex G-75 of reduced and alkylated intracellular IgG from glucosamine-$^3$H-labeled cells after pretreatment with puromycin. The material was taken up in 1 N acetic acid; the column was equilibrated with this solvent prior to addition of sample. Flow rate was 15–20 ml/hr. ○——○ glucosamine-$^3$H, intracellular after puromycin. ●——● $OD_{280m\mu}$.

sis of this material indicated that it was not protein.

The third aspect examined was whether or not the radioactivity found associated with IgG was identifiable as being in the sugar which served as "precursor" in the incubations. The method followed was to perform a reduction and alkylation of the fraction under study (ME + iodoacetamide) and separate the resulting alkylated H and L chains on either Sephadex G-50 or G-75 columns.

Figs. 6–8 show the results with lysate obtained from cells pretreated with puromycin and labeled with glucosamine. In Fig. 6, it can be seen that adequate separation was obtained of H and L chains whose positions in the chromatogram are in accord with their molecular weights. Radioactivity was mostly associated with H chain, though small amounts of radioactivity were present in the chromatographic area corresponding to L chain. The incorporation of carbohydrate into both L and H chains of a myeloma protein was previously reported (5).

The material comprising the H chains was hydrolyzed in 4 N HCl and exchanged on Dowex 50. Previous studies had shown that it was possible to separate *N*-acetyl glucosamine from glucosamine by this method since the former was not retained by Dowex 50 columns, whereas the latter was and required 4 N HCl for elution. Recovery of radioactivity present in H chain by the Dowex procedure was greater than 90%.

The results (Fig. 7) indicate two peaks, one of which was identified as glucosamine since it could be eluted in the same position as glucosamine-$^{14}$C.

The materials in the regions corresponding to these peaks were concentrated by flash evaporation and then subjected to descending paper chromatography utilizing BuOH – pyridine and $H_2O$ (6:4:3 v/v) as the resolving solvent system. Glucosamine-$^{14}$C was again admixed with the

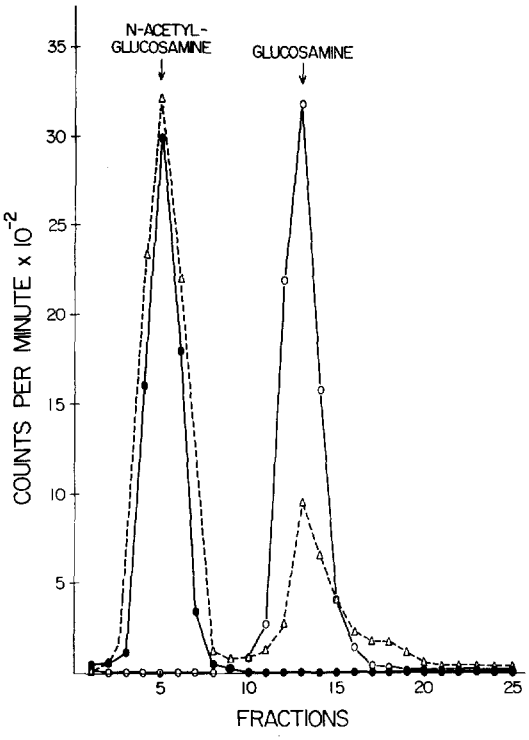


FIGURE 7 Dowex 50 (H+ form) ion exchange chromatography of acid hydrolysate (dried in vacuo) of H chains (obtained from reduced and alkylated IgG, shown in Fig. 6). *N*-acetyl glucosamine was not held by resin; glucosamine eluted with 4 N HCl. ●——● *N*-acetyl-glucosamine-$^{14}$C. ○——○ glucosamine-$^{14}$C. △-----△ glucosamine-$^3$H, intracellular, after puromycin.

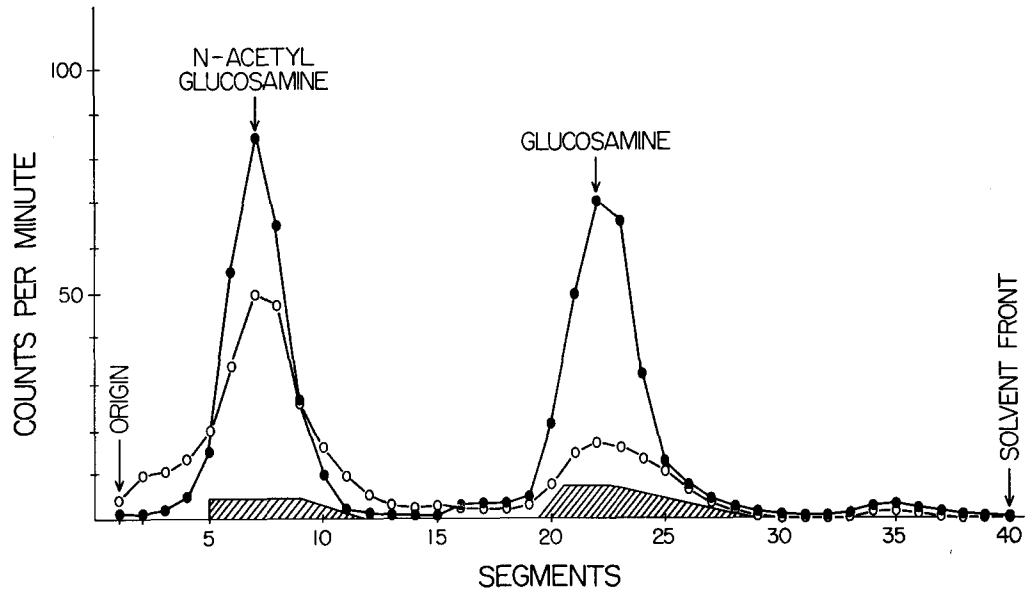


FIGURE 8 Composite plot of radioactivity present in consecutive 1 cm segments of developed paper chromatogram. A mixture of *N*-acetyl glucosamine-$^{14}$C and glucosamine-$^{14}$C was chromatographed independently as well as co-chromatographed with the sugars obtained from the Dowex 50 (H+) ion-exchange procedure (see Fig. 7). Color obtained by the reagent described by S. M. Partridge (11). ●——● *N*-acetyl-glucosamine and glucosamine, mixture. ▨ Spots of two sugars on paper chromatogram. O—O Glucosamine-$^{3}$H, intracellular, after puromycin (fractions from Dowex 50).

presumed labeled glucosamine-$^{3}$H. The run was completed in 16 hr. Previous runs had shown good separation between *N*-acetyl glucosamine and glucosamine.

Fig. 8 shows the results of this experiment. The $R_f$ values of the radioactivity of the experimental fractions coincide precisely with those of standard *N*-acetyl glucosamine and glucosamine, respectively. Furthermore, co-chromatography of these two sugars with the experimental fractions showed no separation. Hence, glucosamine-$^{3}$H added as precursor is incorporated into IgG primarily as *N*-acetyl glucosamine. The small amount recovered as glucosamine most likely represents deacetylation occurring in vitro. Similar results were obtained using lysates or secretions from cells labeled with glucosamine and not pretreated with puromycin.

The results of Dowex ion exchange chromatography of a lysate obtained from cells labeled with galactose-$^{3}$H are shown in Fig. 9. There was co-chromatography with standard galactose-$^{14}$C. The paper chromatogram of this eluate is shown in Fig. 10. Coincidence of standard sugar and sugar from cell lysates was obtained both in terms of radioactivity and by visualization of the spot.

## DISCUSSION

The data presented provide additional biochemical evidence concerning the time in the intracellular life of an immunoglobulin G (IgG) molecule when glucosamine and galactose are incorporated. The data consist of the percentage of total radioactive IgG that has been secreted as a function of time of labeling with leucine, galactose, or glucosamine, and the proportion of such synthesis and secretion which is prevented by prior treatment with puromycin.

The meaningfulness of these measurements depends upon (*a*) the precision with which intracellular or secreted IgG can be measured, and (*b*) the labeled sugar being covalently bound to IgG and recognizable as such. These questions were each investigated, and the evidence indicates that these criteria are met. Thus, a single peak of radioactivity corresponding to IgG was obtained on acrylamide gel electrophoresis from either intracellular lysates or secretion after precipitating these with rabbit antibody specific to mouse IgG. Chromatographic studies of these precipitates established that both sugars used become covalently attached to IgG (primarily to the H chains)

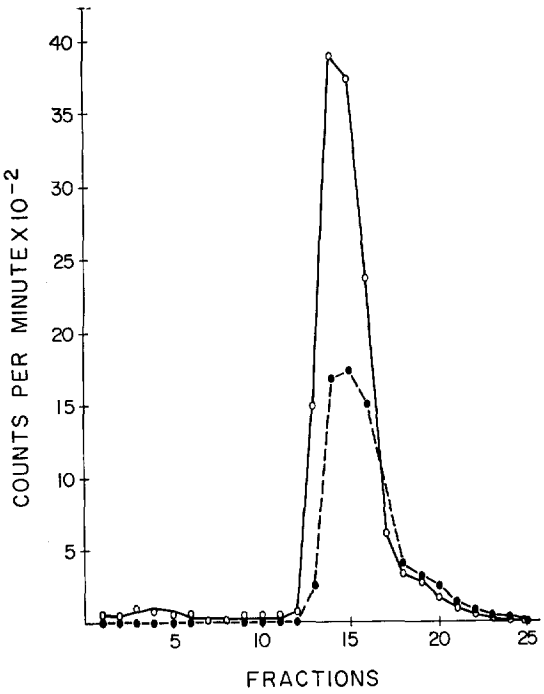


FIGURE 9 Dowex 50 (H+ form) ion exchange chromatography of acid hydrolysate of H chains from reduced and alkylated IgG obtained from cells labeled with galactose-$^{3}$H. Galactose eluted with 4 N HCl. O——O Galactose-$^{14}$C (standard). ●——● galactose-$^{3}$H, intracellular.

and that all the attached label can be accounted for in the respective sugars used. In the case of glucosamine, incorporation occurs primarily if not exclusively as the *N*-acetylated form.

It is also of interest that virtually all radioactive galactose or glucosamine incorporated into macromolecules in the cytoplasm during a period of 1 hr is into IgG.

It was, therefore, possible to interpret unambiguously the observations that (*a*) a concentration of puromycin which completely prevented IgG synthesis did not completely abolish incorporation of glucosamine or galactose into either secreted or intracellular IgG, and (*b*) that a higher percentage of total radioactive IgG was secreted after labeling with glucosamine or galactose compared to that secreted after leucine labeling. These findings each indicate that a substantial portion of the residues of each of these two sugars is added on completed polypeptide chains and late in the intracellular life of the molecule (i.e., nearer to the time of secretion). In the case of galactose, the findings suggest that all the galactose is added late because of the very high proportion of galactose-labeled IgG secreted compared to that of leucine, e.g., 72% of galactose-labeled IgG was secreted after 1 hr of labeling compared to 15% of leucine-labeled IgG. This conclusion is similar to that reached by Melchers and Knopf (13) with regard to fucose incorporation into mouse IgG and to that of Kern and Swenson (14) with regard to galactose and glucosamine incorporation into rabbit IgG.

In addition, however, there was a difference between the results of labeling with galactose and glucosamine in each of the above experiments. Compared with results with galactose, glucosamine incorporation into IgG was suppressed to a greater extent by pretreatment with puromycin (e.g., 89% compared to 63% with galactose after 60 min of labeling). Further, the percentage of total glucosamine-labeled IgG secreted was smaller than that observed with galactose under similar conditions (e.g., 40% compared to 72%, respectively, after 60 min of labeling). Stated in another way, the results with glucosamine were intermediate to those of leucine and galactose in these experiments.

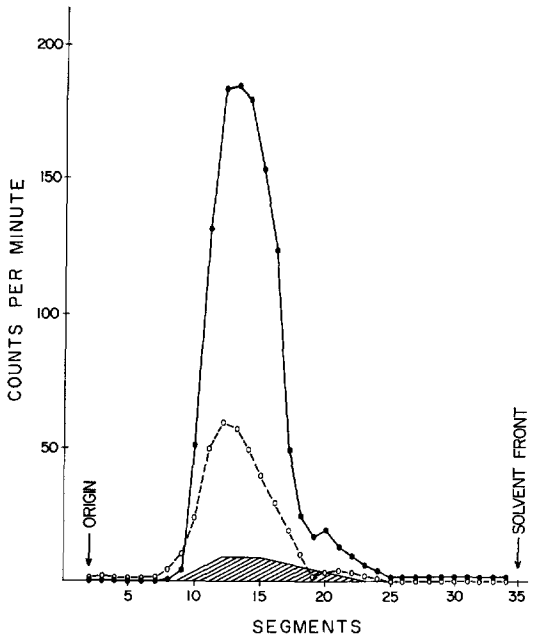


FIGURE 10 Paper chromatogram of a mixture of standard galactose-$^{14}$C and the sugar obtained from the Dowex 50 (H+) procedure (see Fig. 9). ●——● Standard galactose-$^{14}$C. ▨ Spot of galactose on paper chromatogram. O——O Galactose-$^{3}$H, intracellular.

These differences however cannot be uniquely interpreted since several additional assumptions have to be made: (*a*) Puromycin does not significantly alter the rate or availability of the enzymes concerned with the transport, metabolism, and addition to IgG of galactose and glucosamine. This assumption is probably warranted because of the short period of observation involved. (*b*) Penetration of galactose and glucosamine into the cell and their incorporation into IgG is rapid and similar for both sugars. This assumption has been partially tested. Thus, electron microscope radioautography (15) revealed that incorporation of both sugars into macromolecules takes place as early as 5 min after their addition to the medium. Also, labeled IgG was detectable intracellularly and in secretions by acrylamide gel electrophoresis at 15 min. (*c*) Changes in the specific activity (cpm/mg sugar) of the immediate precursor (UDP-*N*-acetyl glucosamine and UDP-galactose) with time occurs at approximately similar rates for both sugars. At present, there is no direct information regarding this point. However, it should be noted that the same relative differences between glucosamine and galactose labeling in the above experiments were observed at both 30 and 60 min.

We, therefore, interpret the above evidence as indicating that galactose is incorporated into IgG primarily on completed chains and late in the intracellular life of the molecule, whereas glucosamine is added onto both nascent and completed chains. The data, of course, do not provide direct information about the intracellular sites at which incorporation occurs. The following paper, however, establishes the sites of addition of these two sugars as well as the critical steps in the process of intracellular transport.

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