Biochem. J. (1970) **119**, 765-772
Printed in Great Britain

Biosynthesis of the Carbohydrate Portion of Immunoglobulins

KINETICS OF SYNTHESIS AND SECRETION OF [^{3}H]LEUCINE-, [^{3}H]GALACTOSE- AND [^{3}H]MANNOSE-LABELLED MYELOMA PROTEIN BY TWO PLASMA-CELL TUMOURS

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(*Received* 20 *April* 1970)

The kinetics of incorporation of leucine, galactose and mannose into intracellular and secreted myeloma protein, MOPC 21 IgG_1 and MOPC 46 κ-type light chain, by cell suspensions of two myeloma plasma-cell tumours, MOPC 21 and MOPC 46, were similar. Radioactive galactose was incorporated to over 90% into galactose residues of intracellular and secreted protein, mannose to over 90% into glucosamine and mannose residues of intracellular protein and to over 90% into glucosamine, mannose and fucose residues of secreted protein, but not into galactose residues. The results show that specific residues in the carbohydrate portion of myeloma proteins can be labelled by specific radioactive monosaccharides, and suggest that fucose residues are added, while myeloma protein is in its final stage of secretion from the plasma cell. The kinetics of incorporation indicate at least three sequential precursor–product relationships between different intracellular forms and the secreted form of myeloma protein.

Immunoglobulins are glycoproteins that are synthesized and secreted by plasma cells. IgG* has been found to contain at least two, probably identical, carbohydrate groups with a molecular weight of 2500–3000 attached to each of the two heavy chains at identical points within their *C*-terminal halves (Fleischmann, Pain & Porter, 1962; Edelman *et al.* 1969). In some cases, additional points of attachment for carbohydrate have been found (Utsumi & Karush, 1965; Smyth & Utsumi, 1967).

It has been suggested, more generally for all glycoproteins (Eylar, 1966), that the biosynthetic steps by which carbohydrate is attached to the protein might be part of the processes by which plasma cells transport and secrete immunoglobulins (Melchers & Knopf, 1967; Swenson & Kern, 1967). After its synthesis on polyribosomes (Scharff, Shapiro & Ginsberg, 1967; Askonas & Williamson, 1967) a lag of approx. 30min was observed before the appearance of immunoglobulin outside the plasma cells (Helmreich, Kern & Eisen, 1961). During this lag period the immunoglobulin to be secreted has been shown to migrate from the membranous structures of the rough endoplasmic reticulum into those of the smooth endoplasmic reticulum and from there out of the plasma cells (Rifkind, Osserman, Hsu & Morgan, 1962; de Petris, Karlsbad & Pernis, 1963; Jamieson & Palade, 1966, 1967*a*,*b*; Knopf, Choi & Lennox, 1969). There is evidence that during this migration attachment of carbohydrate to immunoglobulin polypeptide chains proceeds in several steps at different sites within the plasma cell. Glucosamine may already be added to growing or unreleased polypeptide chains on polyribosomes (Melchers & Knopf, 1967; Shenkein & Uhr, 1969). Most of the glucosamine and mannose residues are attached to completely synthesized immunoglobulin chains isolated from rough membranes, whereas galactose residues are found only in protein from smooth membranes (Melchers, 1969*b*) and fucose residues only in secreted protein (Melchers & Knopf, 1967).

The suggested stepwise addition of carbohydrate residues to immunoglobulin chains implies several precursor–product relationships between different forms of intracellular and secreted protein. The two plasma-cell tumours transplantable in Balb/c-mice, namely MOPC 21, producing and secreting IgG myeloma protein (Potter, 1967), and MOPC 46, producing and secreting a κ-type light chain with carbohydrate attached to it (Melchers, Lennox & Facon, 1966; Melchers, 1969*a*), have been taken as model substances to study the kinetics of incorporation of radioactive leucine, mannose and galactose into the carbohydrate portion of immunoglobulins.

* Abbreviation: IgG, immunoglobulin G (World Health Organization, 1964).

MATERIALS AND METHODS

The plasma-cell tumours MOPC 21 and MOPC 46, transplantable in Balb/c-mice, were given to Dr E. S. Lennox by Dr M. Potter, National Institutes of Health, Bethesda, Md., U.S.A. MOPC 21 TC 10/18 is a derivative of MOPC 21 that grows in tissue culture. I am grateful to Dr Kengo Horibata, The Salk Institute, for allowing me to use this tissue-culture line of MOPC 21. MOPC 46 was used in transplantation numbers 43–48. Tumours were used 2–3 weeks after transplantation.

Cell suspensions were prepared by teasing apart the excised tissue from small tumours with two pairs of forceps in cold (4°C) modified Eagle's medium (Vogt & Dulbecco, 1963) containing 10% (w/v) of foetal calf serum (Grand Island Biological Co., Grand Island, N.Y., U.S.A.). The cells were filtered free from connective tissue through a 200-mesh stainless-steel screen and washed twice with the same cold medium by centrifugation for 15min at 120*g* and 4°C. Viability of cells was determined by the uptake of fluorescein dibutyrate (Nutritional Biochemical Corp., Cleveland, Ohio, U.S.A.; Rotman & Papermaster, 1966). Only cell suspensions with viability counts greater than 80% were used in incubations with radioactive material.

For the incorporation of radioactive leucine, mannose and galactose conditions similar to those described by Knopf *et al.* (1969) were used. The procedure for the incorporation of L-[4,5-$^3H_2$]leucine (19.7Ci/mmol) (batch 21; The Radiochemical Centre, Amersham, Bucks., U.K.) was as follows. Cell suspensions of 5×10^6 cells/ml in modified Eagle's medium containing 40μM-leucine, 25μCi of [4,5-$^3H_2$]leucine/ml and 3% of foetal calf serum were incubated at 37°C in plastic Petri dishes in an incubator containing $CO_2+O_2+N_2$ (10:7:83). The procedure for the incorporation of D-[1-^{3}H]mannose (562.5mCi/mmol) (batch 5; The Radiochemical Centre), D-[U-^{14}C]-mannose (91mCi/mmol) (lot no. 6701; Schwarz Bio-Research Inc., Orangeburg, N.Y., U.S.A.), D-[1-^{3}H]-galactose (2.9Ci/mmol) (batch 8; The Radiochemical Centre) and D-[U-^{14}C]galactose (34.7mCi/mmol) (New England Nuclear Corp., Frankfurt am Main, Germany) was as follows. Cell suspensions of 1×10^7 cells/ml (for mannose incorporations) or 5×10^6 cells/ml (for galactose incorporations) in modified Eagle's medium with 1μM-glucose and containing 3% of foetal calf serum as well as the radioactive monosaccharide ([1-^{3}H]mannose and [1-^{3}H]galactose: 50μCi/ml; [U-^{14}C]mannose and [U-^{14}C]-galactose: 5μCi/ml) were incubated as described for leucine.

Determination of total trichloroacetic acid-precipitable radioactivity incorporated by plasma cells after various incubation times, separation of plasma cells from their supernatant medium, lysis of the plasma cell pellets with Nonidet P-40 (Shell Chemical Co., London S.E.1, U.K.) non-ionic detergent and precipitation by specific antiserum of radioactivity incorporated into myeloma protein was carried out essentially as described by Knopf *et al.* (1969). The serologically precipitated [^{3}H]leucine-labelled material was examined by polyacrylamide-gel electrophoresis (Maizel, 1966; Choules & Zimm, 1965) and shown to consist of heavy chains and light chains from MOPC 21 cells and of light chains only from MOPC 46 cells.

Radioactive material incorporated into myeloma protein was identified as follows. Radioactive myeloma protein was purified from dialysed extracellular supernatant medium and from dialysed intracellular lysates by using the indirect immune-precipitation method. Radioactive mouse myeloma protein was complexed with an excess of antiserum prepared in rabbits against the corresponding myeloma protein purified from the serum [MOPC 21; the method was analogous to the one described by Knopf, Parkhouse & Lennox (1967) for the Adj-PC-5 IgG_{2a}-protein] or from the urine [MOPC 46; Melchers *et al.* (1966)] of tumour-bearing mice. These mouse myeloma proteins–rabbit anti-(mouse myeloma) antibody complexes were then precipitated with an equivalent amount of pig (or goat) anti (rabbit IgG) serum. This immune precipitate was washed once, then hydrolysed with 0.175M-H_2SO_4, and neutral hexoses and hexosamines were isolated from the hydrolysate by the method of Kim, Shome, Liao & Pierce (1967). These conditions of hydrolysis are somewhat milder than those that are usually needed (Spiro, 1966) to obtain a quantitative release of all the monosaccharides from glycoproteins containing the general type of carbohydrate moiety that is present in MOPC 46 κ-type light chain. However, it has been demonstrated (Melchers *et al.* 1966) that they result in the release of amounts of sugars comparable with those found when this Bence–Jones protein is hydrolysed under more vigorous conditions (0.5M-H_2SO_4 at 100°C for 8h). Quantitative determination of radioactivity in the different neutral hexoses and in hexosamines was done by column chromatography [neutral hexoses: Dowex 2 (X8) (Walborg, Christensson & Gardell, 1965); hexosamines: Amberlite CG-120 (Jollès, Samour & Lederer, 1963)]. The radioactivities of fractions from the columns as well as all other radioactive samples were counted in a liquid-scintillation counter (Beckman LS 250) in plastic counting vials (Fa. Hormuth, Heidelberg, Germany) in Bray's (1960) solution B-3 containing 3.5% (w/v) of Aerosil 200 (Degussa, Frankfurt am Main, Germany). Fractions from the Amberlite CG-120 column in 0.33M-HCl were neutralized with NaOH before measurement of their radioactivity.

RESULTS

Cell suspensions of the two plasma-cell tumours MOPC 21 and MOPC 46 were incubated with radioactive leucine or sugars for various times up to 10h under conditions described in the Materials and Methods section. The kinetics of synthesis and secretion of the protein and carbohydrate moieties of the two myeloma proteins were studied and compared by following the incorporation of radioactive leucine, mannose and galactose into intracellular and extracellular material precipitable by trichloroacetic acid as well as that precipitable by specific antiserum. In experiments with radioactive galactose and mannose the radioactivity incorporated into myeloma protein was analysed as described in the Materials and Methods section.

Incorporation of [^{3}H]*leucine.* The kinetics of incorporation of [^{3}H]leucine into total MOPC 21 and MOPC 46 intracellular and extracellular acid-precipitable material as well as into intracellular

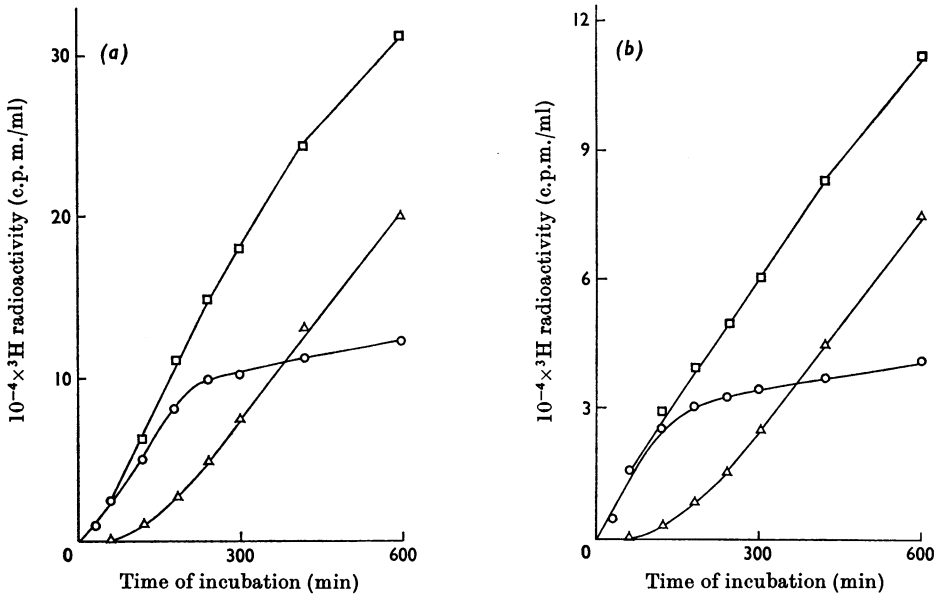

Fig. 1. Time-course of incorporation of L-[4,5-$^3H_2$]leucine into total (□), intracellular (○) and secreted (△) myeloma protein obtained by precipitation with specific antiserum. (*a*) MOPC 21 IgG_1; (*b*) MOPC 46 light chain. For details see the text.

and extracellular myeloma protein (MOPC 21 IgG_1 and MOPC 46 κ-type light chain) were similar to those reported by Knopf *et al.* (1969) for MOPC 46 plasma cells. Incorporation into total acid-precipitable material was linear for the first 4–5h in both tumours, then slowed down to about 25% of the initial rate. Both myeloma proteins were synthesized at a constant rate for 7h, but thereafter the rate of synthesis decreased (Figs. 1*a* and 1*b*). In both tumours the amount of intracellular [^{3}H]-leucine-labelled myeloma protein increased from within the first 5 min for about 4h (see also Fig. 2, which shows the incorporation into MOPC 21 myeloma protein at a different time-scale). In the next 6h only a small increase in intracellular myeloma protein was observed, the rate of increase being 5–10% of the initial rate in the first hour. This suggests that 90–95%, but possibly not all, of the synthesized myeloma protein or (with MOPC 21 IgG_1) of possible free subunits precipitated by the anti-IgG_1 antiserum is also secreted from the cells. It is assumed that there is no turnover of the intracellular myeloma protein.

There was a lag of 20–30min with both types of plasma cells before [^{3}H]leucine-labelled myeloma protein could be detected in the extracellular medium (Fig. 2 for MOPC 21 myeloma protein). A constant rate of secretion of [^{3}H]leucine-labelled myeloma protein was reached after 4h (Fig. 1).

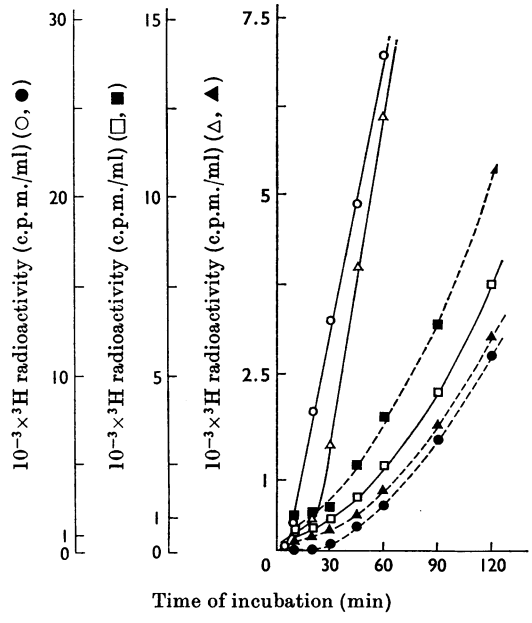

Fig. 2. Time-course of incorporation of L-[4,5-$^3H_2$]leucine (○ and ●), D-[1-^{3}H]galactose (□ and ■) and D-[1-^{3}H]-mannose (△ and ▲) into intracellular (——) and secreted (– – – –) MOPC 21 IgG_1 myeloma protein within the first 120min of incubation. For details see the text.

Between 1 and 10h after initiation of labelling with leucine 80–90% of the labelled material in the extracellular medium of both MOPC 21 and MOPC 46 plasma-cell suspension was myeloma protein. The steady-state rate of secretion of [^{3}H]leucine-labelled myeloma protein from MOPC 21 and MOPC 46 cells amounted to 20% of the initial rate of total incorporation of [^{3}H]leucine into these cell suspensions (Table 1). In the first 3h of [^{3}H]leucine incorporation 22–23% of the total acid-precipitable radioactivity inside MOPC 21 cells (23% inside MOPC 46 cells) was myeloma protein. The initial rate of incorporation of [^{3}H]leucine into intracellular myeloma protein thus appeared to be higher than the final steady-state rate of secretion of [^{3}H]leucine-labelled myeloma protein. This again suggests that about 90%, but possibly not all, of the [^{3}H]leucine-labelled myeloma protein or free subunits of it synthesized inside the plasma cell are also secreted.

Incorporation of [^{3}H]*galactose.* The rate of incorporation of [^{3}H]galactose into total acid-precipitable material as well as the rate of secretion of myeloma protein from MOPC 21 and from MOPC 46 plasma cells (Figs. 3*a* and 3*b*) increased for the first 3–4h, then became constant for the next 6–7h. The final rate of secretion of [^{3}H]galactose-labelled MOPC 21 myeloma protein was about 30% (MOPC 46, 50%) of the rate of total incorporation of [^{3}H]galactose into acid-precipitable material (Table 1). About 80% of the total acid-precipitable secreted galactose-labelled material was myeloma protein for both types of plasma cells. In the first 5h of [^{3}H]galactose incorporation about 30% of the total acid-precipitable radioactivity inside MOPC 21 cells (about 50% inside MOPC 46 cells) was myeloma protein. This indicates that all galactose-labelled myeloma protein was secreted from the plasma cells. The initial rate of incorporation of [^{3}H]galactose into intracellular myeloma protein was therefore equal to the final steady-state rate of secretion of [^{3}H]galactose-labelled myeloma protein.

Intracellular as well as secreted [^{3}H]- and [^{14}C]-galactose-labelled myeloma protein was analysed for radioactivity in its protein and carbohydrate moieties. [1-^{3}H]Galactose as well as [U-^{14}C]galactose was found to be incorporated to over 90% into galactose positions within the carbohydrate moiety of myeloma protein.

Incorporation of [^{3}H]- *and* [^{14}C]-*mannose.* The kinetics of incorporation of [1-^{3}H]- and [U-^{14}C]-mannose into MOPC 21 and MOPC 46 cell suspensions and into myeloma protein (Fig. 4, shown only for MOPC 21 protein, since MOPC 46 protein showed practically identical kinetics) were similar to each other. The incorporation rate into total acid-precipitable material was linear for about 5h, then changed to continue at about 35–50% of the initial rate. In both tumours intracellular [^{3}H]- and [^{14}C]-mannose-labelled myeloma protein

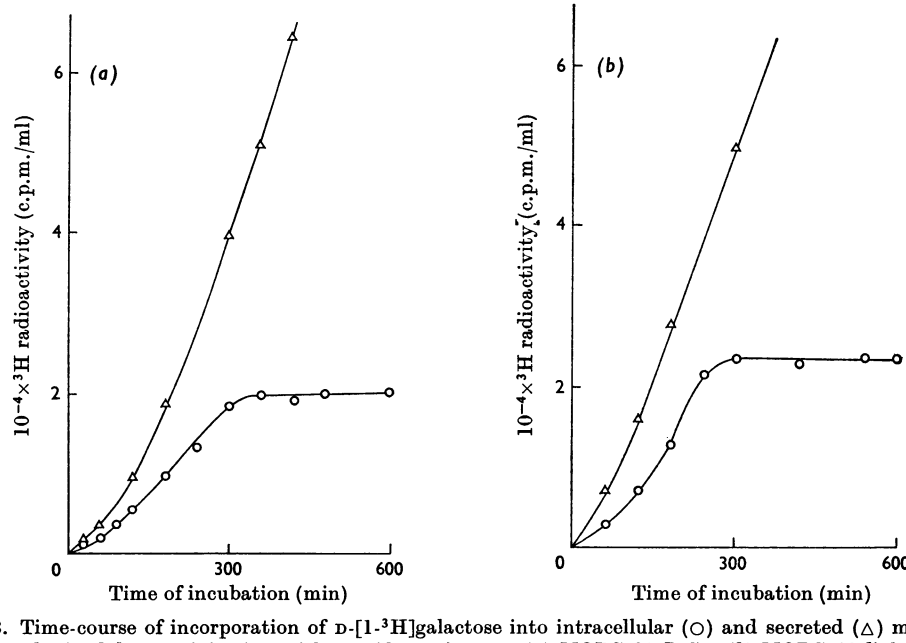

Fig. 3. Time-course of incorporation of D-[1-^{3}H]galactose into intracellular (○) and secreted (△) myeloma protein obtained by precipitation with specific antiserum. (*a*) MOPC 21 IgG_1; (*b*) MOPC 46 light chain. For details see the text.

Table 1. *Radioactivity incorporated into MOPC 21 IgG_1 and MOPC 46 light chain within 5h at steady state of secretion*

Cell concentrations (per ml of medium) are given in the Materials and Methods section.

	10^{-4} × Radioactivity incorporated (c.p.m./5h)			
	MOPC 21 plasma cells		MOPC 46 plasma cells	
Label	Total	Secreted	Total	Secreted
[^{3}H]Leucine	62.5	12.5 (20%)	25.0	5.0 (20%)
[^{3}H]Mannose	7.5	1.8 (24%)	7.5	1.9 (25%)
[^{14}C]Mannose	15.0	2.5 (17.5%)	15.0	3.0 (20%)
[^{3}H]Galactose	16.0	5.0 (32%)	16.0	8.0 (50%)

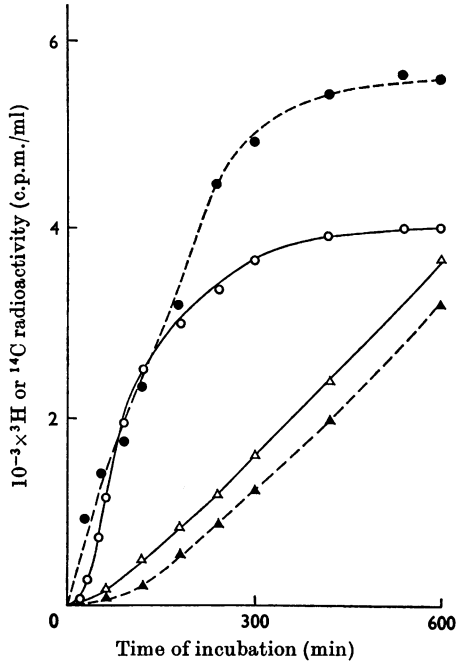

Fig. 4. Time-course of incorporation of D-[1-^{3}H]mannose (——) and D-[U-^{14}C]mannose (- - - -) into intracellular (○ and ●) and secreted (△ and ▲) MOPC 21 IgG_1 myeloma protein obtained by precipitation with specific antiserum. For details see the text.

increased for 4–6h and levelled off within the next 4h. In contrast with [^{3}H]leucine-labelled intracellular myeloma protein there was a lag of over 20min during which only little [^{3}H]mannose was incorporated into intracellular myeloma protein (shown in more detail in Fig. 2). After 20min intracellular [^{3}H]mannose-labelled myeloma protein started to follow kinetics similar to those of intracellular [^{3}H]leucine-labelled protein. In the first 30min only very little radioactivity was found in secreted myeloma protein. The steady state of secretion was reached in approximately the same time (3–4h) as for [^{3}H]leucine-labelled myeloma protein. The final rate of secretion of [^{3}H]mannose-labelled MOPC 21 and MOPC 46 myeloma protein was about 25% of the rate of incorporation of total acid-precipitable [^{3}H]mannose (Table 1). About 90% of the total acid-precipitable [^{3}H]mannose-labelled secreted material was myeloma protein. In the first 5h of [^{3}H]mannose incorporation 25–30% of the total acid-precipitable radioactivity inside MOPC 21 and MOPC 46 cells was in myeloma protein. The initial rate of incorporation of [^{3}H]mannose into myeloma protein therefore appeared to be higher than the final steady-state rate of secretion of [^{3}H]mannose-labelled myeloma protein. This indicates that not all of the [^{3}H]mannose-labelled myeloma protein was secreted from the cells, especially if one considers that (as shown below) only extracellular, but not intracellular, [^{3}H]mannose-labelled myeloma protein contains radioactive fucose.

Intracellular and secreted myeloma protein that had been labelled with [1-^{3}H]mannose as well as [U-^{14}C]mannose was analysed for its radioactivity (see the Materials and Methods section). The results obtained with [^{3}H]mannose-labelled MOPC 21 myeloma proteins are shown in Fig. 5. In intracellular protein only mannose and glucosamine residues were labelled to any great extent. Mannose residues in the carbohydrate moiety reached a constant specific radioactivity after 4–5h, glucosamine residues after 2–3h. Less than 3% of the label incorporated into intracellular as well as secreted myeloma protein was found in galactose. [There is a constant increase of a minor amount of radioactive fucose in intracellular protein from about 0.03% of the total radioactivity in carbohydrate positions at 2h to about 3% at 10h (Fig. 5).] I was therefore able to label specifically either galactose or mannose plus glucosamine positions of intracellular myeloma protein by incorporating either [1-^{3}H]galactose or [1-^{3}H]mannose into plasma-cell suspensions. Only secreted, but not intracellular

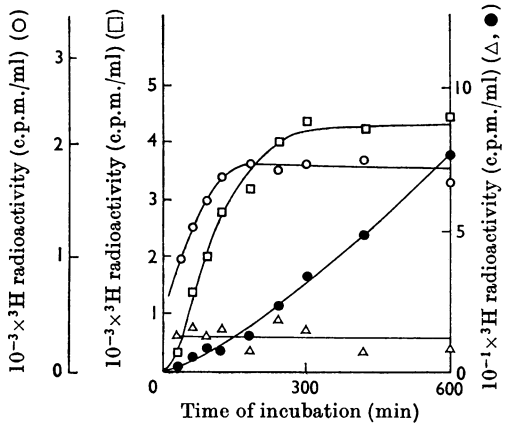

Fig. 5. Distribution of radioactivity in carbohydrate residues (○, glucosamine; □, mannose; △, galactose; ●, fucose) of intracellular MOPC 21 IgG_1 myeloma protein labelled with D-[1-^{3}H]mannose for different periods of time. It should be noted that the scale for radioactivity in glucosamine and mannose is different from that for radioactivity in galactose and fucose. For details see the text.

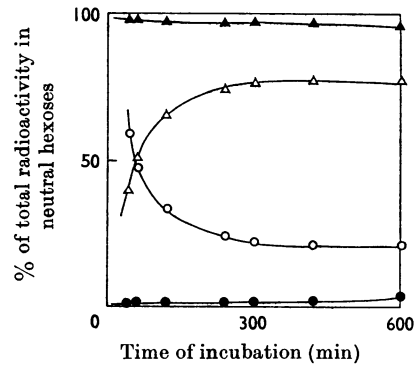

Fig. 6. Distribution of radioactivity (%) in mannose (△ and ▲) and fucose (○ and ●) residues of intracellular (○ and △) and secreted (● and ▲) MOPC 21 IgG_1 myeloma protein labelled with D-[1-^{3}H]mannose for different periods of time. The total radioactivity recovered in neutral hexoses (mannose+fucose+galactose) is taken as 100%. For details see the text.

myeloma protein contained an appreciable amount of radioactivity in fucose. The initial ratio of radioactivity in fucose residues to that in mannose residues in secreted protein changed from 60:40 at 45min to a constant value of 22:78 at 4–5h and later (Fig. 6). Possible radioactivity in *N*-glycollylneuraminic acid residues (Melchers *et al.* 1966) has not been determined in these analyses. These results support previous findings (Melchers & Knopf, 1967) that most of the fucose is found attached to the carbohydrate moiety of secreted but not of intracellular myeloma protein. They suggest that addition of some of the residues of the carbohydrate group is stepwise; mannose (and glucosamine) residues seem to be added earlier than fucose residues.

Although [1-^{3}H]mannose was incorporated to over 90% into known carbohydrate residues of myeloma protein, this was not the case for [U-^{14}C]-mannose. The ratio of the radioactivity in mannose residues to that in glucosamine residues (to fucose residues in secreted protein) was the same, however; 20–25% of the total radioactivity was found in material that was adsorbed on Dowex 1 (X8) and Dowex 50 (X8) together with glucosamine. On Amberlite CG-120 columns this 20–25% of the total radioactivity was eluted in several peaks before and after the glucosamine peak (see the Materials and Methods section). Although not yet identified, these peaks probably represent amino acids or peptides.

Very similar results have been obtained with the [^{3}H]- and [^{14}C]-mannose-labelled MOPC 46 myeloma protein, suggesting that the carbohydrate moieties of MOPC 21 and MOPC 46 myeloma proteins have very similar compositions of hexose and hexosamine residues.

Results of previous analyses of MOPC 46 light chain, labelled under different conditions of incubation with D-[U-^{14}C]mannose (Melchers, 1969*b*), are discrepant from the results of the analysis reported in this paper, since a considerable amount of radioactivity was found incorporated into galactose residues. The reasons for this difference are not clear at present. In earlier analyses of D-[U-^{14}C]-galactose-labelled MOPC 46 light chain radioactivity was found in galactose residues as well as in an unidentified compound. The increase in the sulphuric acid concentration from 0.125M to 0.175M during hydrolysis of the glycoprotein (see the Materials and Methods section) led to all the radioactivity of this compound being associated with a substance that migrated at a position coinciding with that of galactose on Dowex 2 (X8) column chromatography.

DISCUSSION

The kinetics of synthesis and secretion of [^{3}H]-leucine-labelled MOPC 46 light chain reported in this paper are similar to the results of Knopf *et al.* (1969). They are strikingly similar to those of [^{3}H]leucine-labelled IgG. This similarity is an indication that MOPC 46 plasma cells, producing and secreting a light chain with carbohydrate covalently attached to the variable portion of its polypeptide chain (Melchers, 1969*a*), can be used as a model system to study the biosynthesis of the carbohydrate portion of immunoglobulins (Melchers & Knopf, 1967).

One can calculate the secretory activity of the two types of myeloma plasma cells as the number of molecules of myeloma protein secreted per unit time by one cell. For this calculation it is assumed that all cells possess the same secretory activity and that the specific radioactivity of the [^{3}H]leucine residues in the myeloma protein secreted at steady state equals the specific radioactivity of [^{3}H]leucine in the incubation medium (25 μCi of [^{3}H]leucine/ml with 19.7 Ci/mmol diluted in medium containing 40 μmol of unlabelled leucine = 0.62 Ci/mmol). Fifteen leucine residues have been found per molecule of MOPC 46 light chain (Melchers *et al.* 1966), and with its molecular weight of approx. 150000 MOPC 21 IgG_1 is assumed to contain approximately 100 leucine residues per molecule. From the amount of radioactivity secreted at steady state by the input number of plasma cells one can calculate the secretory activity of one MOPC 21 plasma cell to be about 7×10^3 molecules of IgG_1/s, and of one MOPC 46 plasma cell to be 1.9×10^4 molecules of light chain/s. In my experiment MOPC 46 plasma cells therefore secreted, on average, three times as many light-chain molecules as MOPC 21 plasma cells did IgG_1 molecules. These numbers, although only approximate in view of the assumptions made, are in reasonable proximity to numbers given by Fahey & Finegold (1967) for human lymphoid-cell lines.

The half-time to reach a steady state of secretion of [^{3}H]leucine-labelled myeloma protein is about 2h for both types of tumour plasma cells. Therefore one MOPC 21 plasma cell contains about 10^8 molecules of IgG_1, one MOPC 46 plasma cell about 2.75×10^8 molecules of light chain.

The immunoglobulin- and haemoglobin-synthesizing cell systems have been considered in parallel (cf. Symposium on Differentiation and Growth of Hemoglobin- and Immunoglobulin-Synthesizing Cells, Oak Ridge, Tenn., U.S.A. 1966). The mean rate of synthesis of haemoglobin has been calculated to be 800 molecules/s per cell. An average erythrocyte contains 29 pg or 3×10^8 molecules (cf. Itano, 1966; in this Symposium). These numbers are strikingly similar to the ones calculated above for plasma cells.

Incorporation of radioactive galactose, mannose, or both, into glycoproteins including immunoglobulins has been studied in several laboratories and similar results have been obtained in analysing the radioactivity incorporated (McGuire, Jourdian, Carlson & Roseman, 1965; Swenson & Kern, 1967; Schenkein & Uhr, 1969; Herscovics, 1969).

From my kinetic data (Figs. 2*a* and 2*b*) it seems possible that a minor fraction (approx. 5–10%) of the myeloma protein synthesized inside plasma cells is not secreted. Similar observations have been made by Knopf *et al.* (1969) in [^{3}H]leucine pulse–chase experiments with MOPC 46 plasma cells. They found that the final amount of intracellular [^{3}H]-leucine-labelled light chain was not zero even after 4h of chase time. The residual light chains represented about 10% of the initial amount of [^{3}H]-leucine-labelled light chain. In my experiments the amount of [^{3}H]galactose-labelled intracellular myeloma protein did not increase in 5h after reaching a constant value (Figs. 3*a* and 3*b*), in contrast with [^{3}H]leucine- and [^{3}H]mannose-labelled intracellular myeloma protein. If a fraction of intracellular myeloma protein exists that is not secreted from the two plasma cell types, this fraction might incorporate radioactive mannose but not radioactive galactose at the same rate as protein destined to be secreted. It has been suggested that the attachment of carbohydrate to immunoglobulin may be prerequisite for its secretion from plasma cells (Eylar, 1966; Melchers & Knopf, 1967; Swenson & Kern, 1968). It is therefore possible that a fraction of intracellularly synthesized myeloma protein is not secreted from plasma cells because certain parts of the carbohydrate portion are not attached to the protein.

An alternative explanation for the inability of the cell suspensions to secrete all their immunoglobulin may be that some cells have been damaged during the preparation of the cell suspensions and thus have lost the capacity to secrete.

Comparison of the kinetics of incorporation of [^{3}H]leucine, [^{3}H]galactose and [^{3}H]mannose suggests several precursor–product relationships between different forms of intracellular myeloma protein and between intracellular and secreted myeloma protein (see Fig. 2 for MOPC 21 protein). There are two groups of similar kinetic patterns, namely the kinetics of synthesis of [^{3}H]leucine- and [^{3}H]mannose-labelled intracellular myeloma protein forming group 1, and the kinetics of secretion of [^{3}H]galactose-, [^{3}H]mannose- and [^{3}H]leucine-labelled protein as well as the kinetics of synthesis of [^{3}H]galactose-labelled intracellular protein forming group 2. The two groups of kinetics suggest that a precursor–product relationship exists between all forms of myeloma protein behaving as group 1 and all forms behaving as group 2. The rate-limiting step in the kinetics of secretion of myeloma protein from plasma cells has to be sought in the conversion of myeloma protein behaving as group 1 into that behaving as group 2.

With group 1 the kinetics of synthesis of [^{3}H]-mannose-labelled myeloma protein show a lag period of approx. 20min. This indicates either that there is a pool of glucosamine, mannose, or both, in the cells, which is equilibrated with radioactive mannose in the observed lag time, or, since several residues of mannose and glucosamine exist in one carbohydrate group of the two myeloma proteins (Melchers & Knopf, 1967), a pool of unlabelled

myeloma protein might exist in the cells to which some, but possibly not all, of the mannose and glucosamine residues have already been attached. The time required by the plasma cells to label every mannose and glucosamine position in the carbohydrate portion of the myeloma protein with the same specific radioactivity would thus be measured as a lag in the incorporation of [^{3}H]mannose into intracellular myeloma protein.

Within group 2 [^{3}H]galactose-labelled protein is synthesized and secreted from the beginning, whereas [^{3}H]leucine-labelled protein is secreted with a well-documented lag (Helmreich *et al.* 1961; Knopf *et al.* 1969) of 20–30 min, suggesting that only after this lag-time is myeloma protein capable of accepting galactose.

The intracellular product, [^{3}H]galactose-labelled protein, appears to be synthesized from the intracellular precursor, [^{3}H]mannose-labelled protein. The conversion into the galactose-containing forms seems to be the rate-limiting step in the secretion of myeloma protein from plasma cells. This supports the kinetic results of Knopf *et al.* (1969), showing that the rate-limiting step of secretion is the transfer of myeloma protein from rough to smooth membranes within plasma cells, and analyses (Melchers, 1969*b*) showing that during this transfer galactose is added to the carbohydrate moiety of myeloma protein.

Preliminary experiments in this laboratory indicate that similar kinetics of synthesis and secretion with similar incorporation of radioactive mannose and galactose into the carbohydrate moiety of immunoglobulin can also be obtained with lymphoid cells from lymph nodes and spleens of hyperimmunized mice. Therefore I consider that myeloma plasma cells are suitable model cells for the study of the kinetics of radioactive leucine, mannose and galactose into immunoglobulin.

Part of this work was done at the Salk Institute for Biological Studies, La Jolla, Calif. 92112, U.S.A., and was supported by a U.S. Public Health Service Grant AI-06544 to Dr E. S. Lennox. I thank Dr Edwin S. Lennox and Dr Paul M. Knopf, The Salk Institute, for continuous suggestions, criticism and discussions of this work and Dr Thomas A. Trautner, Dr Christof Spatz and Dr Walter Messer for critical evaluation of the manuscript. The technical help of Miss D. Jablonski is gratefully acknowledged.

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