Biosynthesis of Immunoglobulin A (IgA)

SECRETION AND ADDITION OF CARBOHYDRATE TO MONOMER AND POLYMER FORMS OF A MOUSE MYELOMA PROTEIN

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Cell suspensions of mouse plasma-cell tumour MOPC 315 secreting predominantly IgA (immunoglobulin A) monomer and dimer were incubated with radioactive leucine, mannose, galactose and fucose for various periods of time. The amounts of secreted and intracellular immunoglobulins were measured by co-precipitation with specific antibody, and the molecular species present were assessed by electrophoresis in polyacrylamide gels. Analysis of the secreted myeloma protein demonstrated that monomer and dimer IgA molecules are identical with respect to carbohydrate composition and rate of secretion. Within the cell, the myeloma protein is almost entirely accounted for by monomer units which either leave the cell as such or are polymerized with the addition of J chain close to the time of secretion. The results support the concept of a stepwise addition of carbohydrate residues to IgA immunoglobulin during the process of secretion. Similar patterns of carbohydrate assembly were found for the monomer or dimer molecules. Mannose residues are added at an early stage, whereas fucose is added close to the time of secretion. Galactose is also added early, but some may also be incorporated at a later stage. Control of IgA polymerization is considered unlikely to reflect regulation at the level of carbohydrate addition, and it is suggested that the critical controlling factor is the J chain.

Immunoglobulins, like other secreted glycoproteins, are synthesized on membrane-bound polyribosomes. They are vectorially released into the cisternae of the endoplasmic reticulum and pass to the outside via the Golgi apparatus. During passage through the membranous elements of the cells there is a stepwise addition of carbohydrate at different subcellular sites, and a general rule is that mannose and hexosamine are added early, whereas fucose and galactose are added late. Two recent reviews, one confined to immunoglobulins (Bevan *et al.*, 1972) and the other covering glycoproteins from a variety of sources (Hughes, 1973), describe these processes in detail.

A unique feature of IgA‡ among immunoglobulins is its molecular-weight heterogeneity. In normal human serum, IgA is present mainly as a 7S monomer, but 10.5S, 13S and 16S oligomers also occur (Vaerman *et al.*, 1965a). The same observation is true with myeloma IgA both in human (Fahey, 1963; Levin *et al.*, 1964) and mice (Fahey, 1961), although higher-molecular-weight forms can predominate (Fahey, 1961; Levin *et al.*, 1964; Cummings & Franklin,

1965; Vaerman *et al.*, 1965b). The degree of polymerization observed is characteristic and stable for a given myeloma protein (Fahey, 1963; Abel & Grey, 1968), raising the question of what controls this variable degree of polymerization. It is particularly interesting that myeloma cells that secrete polymeric IgA certainly contain all the biochemical machinery for polymerization and yet still secrete a proportion of monomer form.

The molecular heterogeneity of secreted IgA contrasts with the uniform pentameric product formed by IgM-secreting cells, in spite of the fact that polymerization in both cases occurs from monomeric (H_2L_2) subunits just before, or at the time of, secretion (Parkhouse & Askonas, 1969; Parkhouse, 1971a, b; Bevan, 1971; Bargellesi *et al.*, 1972). At this time, J chain (Halpern & Koshland, 1970; Mestecky *et al.*, 1971) is incorporated into IgM (Parkhouse, 1972) and polymeric, but not monomeric, IgA (Halpern & Coffman, 1972; Parkhouse, 1972).

In order to explain control of polymerization we have chosen to investigate the role played by carbohydrate addition, J chain and an enzyme catalysing disulphide interchange. Here we report the results of an analysis of secreted products formed by mouse myeloma cells incubated with radioactive leucine, mannose, galactose and fucose. We conclude that the rate of secretion of IgA is independent of the fraction

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‡ Abbreviations: IgA, immunoglobulin A; IgM, immunoglobulin M; IgG, immunoglobulin G.

polymerized and that the various molecular species contain identical amounts of carbohydrate. Addition of carbohydrate is therefore unlikely to control polymerization of IgA.

Materials and Methods

The MOPC 315 plasma-cell tumour that secretes IgA was kindly provided by Dr. M. Potter (National Institutes of Health, Bethesda, Md., U.S.A.) and maintained in Balb/C mice by subcutaneous transfer of 1 mm³ pieces from tumour grown 3 weeks after transplantation.

Preparation of serum IgA (MOPC 315)

Because the myeloma protein has antibody activity towards 2,4-dinitrophenol, it was purified directly from whole serum by affinity chromatography on a column of ϵ -N-2,4-dinitrophenyl-lysine-Sepharose (Goetzl & Metzger, 1970). The only modification was that the serum was not reduced and alkylated, so that the final product consisted predominantly of a mixture of monomer and dimer IgA.

Preparation and standardization of antisera to the MOPC 315 IgA

Rabbits were immunized with the purified IgA at 0.5–1.0 mg/ml in complete Freund's adjuvant. Portions (0.5 ml) were injected into the left and right thighs on days 1 and 7 respectively. A further 1.0 ml was injected subcutaneously in several sites after 5 weeks. The rabbits were bled 1–2 weeks after the final injection and sera were standardized by precipitation with various amounts of IgA according to the standard precipitin test (Kabat & Mayer, 1961).

Labelling in vitro of intracellular and extracellular protein by plasma-cell tumour MOPC 315

Cell suspensions containing 1×10^7 cells were prepared as previously described (Parkhouse & Askonas, 1969) and incubated with L-[4,5-³H]leucine (1 Ci/mmol, 40 μ Ci/ml), D-[1-³H]mannose (3.8 Ci/mmol, 50 μ Ci/ml), D-[1-³H]galactose (5.2 Ci/mmol, 50 μ Ci/ml) or L-[1-³H]fucose (0.92 mCi/mmol, 50 μ Ci/ml) under conditions previously defined (Parkhouse & Melchers, 1971). Determination of total trichloroacetic acid-precipitable radioactivity incorporated by plasma cells, separation of plasma cells from the medium and lysis of cell pellets with 1% (w/v) Nonidet P.40 (Shell Chemical Co., London S.E.1, U.K.; a non-ionic detergent) containing 0.2 M-iodoacetamide were performed as described by Parkhouse & Askonas (1969).

The amount of intracellular and secreted myeloma protein in cell lysates and culture fluids was determined by direct precipitation with rabbit anti-(MOPC 315 myeloma protein) and added homologous carrier protein; antibody-antigen precipitates formed by goat anti-(rabbit IgG) and rabbit IgG in similar proportions served as non-specific controls (Parkhouse & Askonas, 1969).

To determine the molecular sizes of radioactive secreted and intracellular myeloma protein, the specific antibody-antigen precipitates were solubilized in 0.1 M-iodoacetamide in 2% (w/v) sodium dodecyl sulphate by heating for 2 min at 100°C (Maizel *et al.*, 1968). The samples were then analysed by electrophoresis in sodium dodecyl sulphate-polyacrylamide gels.

The presence or absence of J chain in radioactive secreted and intracellular myeloma protein was determined by electrophoresis of reduced and alkylated specific antibody-antigen precipitates in alkaline urea-polyacrylamide gels. Under the conditions used the J chain runs considerably faster than other proteins present (Halpern & Koshland, 1970). For reduction, antibody-antigen precipitates were dissolved in 9 M-urea–10 mM-dithiothreitol–0.1 M-Tris–HCl, pH 8.0, and incubated at 56°C for 90 min. Samples were alkylated by addition of an equal volume of 9 M-urea–50 mM-iodoacetamide.

Polyacrylamide-gel electrophoresis

Electrophoresis was carried out with 8 cm gel columns in glass tubes (0.6 cm internal diam.) with ethylene diacrylate as the cross-linking agent (Choules & Zimm, 1965). The gels were cut into 1 mm slices, then dissolved and counted for radioactivity in Kinard's scintillation fluid (Choules & Zimm, 1965). Fractions are numbered from the negative to the positive electrode.

Electrophoresis in the presence of 0.1% (w/v) sodium dodecyl sulphate (Summers *et al.*, 1965) was carried out in 4.25% (w/v) polyacrylamide gels, and alkaline urea-polyacrylamide gels at 6% (w/v) acrylamide were prepared as described by Reisfeld & Small (1966).

Analysis of the radioactivity incorporated into the carbohydrate portions of MOPC 315

Radioactive intracellular and secreted proteins were precipitated with rabbit anti-(MOPC 315 myeloma proteins) and added homologous carrier protein. Precipitates were washed three times with ice-cold phosphate-buffered saline (130 mM-NaCl–4 mM-KCl–10 mM-sodium phosphate, pH 7.4).

For hydrolysis, precipitates were suspended at about 0.1 mg of protein/ml in HCl prepared from con-

stant-boiling HCl distilled from glass. The amount of protein in the antibody-antigen precipitates was estimated from the amount of carrier myeloma protein added and assuming a combining ratio of three for the antibody. Tubes were flushed with N₂ and hydrolysed at 100°C. For analysis of hexoses, samples were hydrolysed for 3 h in 2M-HCl, and for hexosamine analysis the conditions were 4M-HCl for 4 h. The hydrolysates were dried down over P₂O₅ and NaOH at 2–4°C, redissolved in deionized water and similarly dried down a further two times. Samples were taken up in deionized water containing a standard sugar mixture (glucosamine, galactosamine, mannose, galactose and fucose) and chromatographed on Whatman no. 1 paper with butan-1-ol-pyridine-water (6:4:3, by vol.) (Gottschalk, 1955) as solvent. The paper was dried and sugars were revealed with alkaline AgNO₃ by the dipping technique (Trevelyan *et al.*, 1950). The entire paper was cut into 10–20 mm pieces in the direction of chromatography, and the paper segments were counted for radioactivity in a scintillation fluid composed of PPO (2,5-phenyloxazole) (10g) and POPOP [1,4-bis-(5-phenyloxazol-2-yl)benzene] (0.25g) dissolved in toluene (2.5litres). Recovery of radioactivity (Table 1) in identified hexoses and hexosamines from the specifically precipitated radioactivity was 69 % for mannose, 80 % for galactose and 86 % for fucose.

Results

Kinetics of incorporation of radioactive leucine, mannose, galactose and fucose into intracellular and secreted MOPC 315 myeloma proteins

The incorporation of leucine into intracellular and secreted IgA (Fig. 1a) follows the now well-established kinetics for immunoglobulin synthesis and secretion (Askonas & White, 1956; Helmreich *et al.*, 1961; Parkhouse & Askonas, 1969; Melchers, 1970; Choi *et al.*, 1971a), there being an immediate incorporation into intracellular immunoglobulin and a lag period of 30 min before the appearance of secreted protein. After 4 h the rate of secretion of labelled MOPC 315 myeloma protein is constant and balances the rate of synthesis, so that the amount of radioactive intracellular immunoglobulin (30 % of the total radioactivity precipitated by trichloroacetic acid from the cell lysate) remains constant. The time to secrete an amount of immunoglobulin equivalent to the contents of one cell is given by the intersection between curves for intracellular and extracellular myeloma protein.

The observed time for MOPC 315, 3½ h, was similar to that observed for an IgG₁ myeloma protein and for a carbohydrate-containing light chain (Melchers, 1970; Choi *et al.*, 1971a), but slower than that found for an IgM myeloma protein (Parkhouse, 1971a) and a free light chain lacking carbohydrate (Melchers,

Fig. 1. Incorporation of radioactive leucine, mannose, galactose and fucose in MOPC 315 myeloma cell suspensions

Incorporation of [³H]leucine (a), [³H]mannose (b), [³H]galactose (c) and [³H]fucose (d) into intracellular (○) and secreted (●) MOPC 315 myeloma protein. Preparation of cell suspensions and measurement of radioactivity in myeloma protein by specific precipitation with anti-(MOPC 315 myeloma protein) are described in the Materials and Methods section.

1971a). This variability in secretion times may simply be a feature of the neoplastic nature of myeloma cells, as previous experiments on secretion of antibody by rabbit lymph nodes indicated a more time-ordered, rapid secretion of immunoglobulin (Helmreich *et al.*, 1961).

Incorporation of radioactive mannose into intracellular and secreted MOPC 315 myeloma protein had not reached the steady state during the time-course of the experiment (Fig. 1b). There was a linear incorporation into intracellular immunoglobulin from zero time, but amounts of secreted immunoglobulin were relatively low. At 4 h most of the radioactivity in intracellular (74 % of the total radioactivity) and secreted (62 % of the total radioactivity) myeloma protein was found in mannose (Table 1), although galactose and fucose, but not hexosamine, also contained radioactivity. At this time 32 % of the trichloroacetic acid-precipitable radioactivity in the cell lysate was precipitated by specific antisera to the myeloma protein. There was insufficient radioactivity at earlier time-points for carbohydrate analysis. However, by analogy with other studies on the incorporation of mannose into myeloma proteins (Melchers, 1970; Parkhouse & Melchers, 1971), it is likely that at earlier time-points a relatively greater amount of radioactivity would be incorporated as fucose in the secreted protein.

Table 1. *Analysis of radioactivity incorporated into the carbohydrate of MOPC 315 myeloma protein*

MOPC 315 cell suspensions were incubated with radioactive hexoses and the intracellular and secreted immunoglobulins were isolated by precipitation from 4h cultures with specific antisera. The precipitates were hydrolysed and analysed by paper chromatography. Details are given in the Materials and Methods section. The sensitivity of detection was such that a value of 0 represents <2 % of the total radioactivity recovered in the major radioactive component.

Immunoglobulin sample	Radioactive precursor	Percentage of total recovered radioactivity			
		Mannose	Galactose	Fucose	Hexosamine
Secreted	Mannose	74	23	3	0
Intracellular	Mannose	62	34	4	0
Secreted	Galactose	5	95	0	0
Secreted	Fucose	0	0	100	0

The kinetics of mannose incorporation are similar to those obtained with an IgG₁- and IgM-producing mouse myeloma tumour (Melchers, 1970; Parkhouse & Melchers, 1971), and they suggest that mannose is added to the polypeptide chain at an early stage in biosynthesis.

Radioactive galactose was incorporated into MOPC 315 myeloma protein almost entirely without conversion into other sugars (Table 1), and at 4h 34 % of the trichloroacetic acid-precipitable radioactivity present in the cell lysate was myeloma protein. The kinetics of incorporation (Fig. 1c) were similar to those found for leucine (Fig. 1a), there being an immediate incorporation into intracellular immunoglobulin and a lag of 30 min before the appearance of radioactive secreted protein. Thus some galactose, at least, must be added to the molecule early in biosynthesis. This result contrasts with findings obtained with mouse myeloma cells that secrete IgG₁, light chain and IgM (Melchers, 1970; Parkhouse & Melchers, 1971), where there was an immediate appearance of galactose-labelled immunoglobulin into the medium and considerably smaller amounts of radioactive intracellular myeloma protein. In the latter cases, therefore, galactose addition can be considered to be a late biosynthesis event.

Radioactive fucose was incorporated entirely as such (Table 1) and at 4h 29 % of the trichloroacetic acid-precipitable radioactivity present in the cell lysate was myeloma protein. The kinetics of incorporation (Fig. 1d) were similar to those previously observed for myelomas secreting IgG₁, light chain and IgM (Melchers, 1970, 1971b; Parkhouse & Melchers, 1971). There was an immediate appearance of radioactive secreted protein, and the amount of intracellular myeloma protein was low relative to the amount of secreted protein, particularly at longer times of incubation. Thus fucose is added to the IgA molecule late in biosynthesis, a not surprising finding in view of its terminal position in carbohydrate pro-

thetic groups of immunoglobulins (Clamp *et al.*, 1968; Dawson & Clamp, 1968; Spragg & Clamp, 1969). A similar conclusion was drawn from short-term experiments with a mouse myeloma tumour secreting predominantly monomeric IgA (Cowan & Robinson, 1972).

Analysis of intracellular and secreted radioactive immunoglobulin

Secreted and intracellular immunoglobulin from 4h cultures incubated with radioactive leucine, mannose, galactose and fucose were isolated by precipitation with specific antibody and electrophoresed in polyacrylamide gels containing sodium dodecyl sulphate and alkaline urea to measure the relative concentrations of the different molecular species and J chain. For the purpose of clarification, it should be stressed that there are no disulphide bonds between heavy chains and light chains in mouse IgA (Abel & Grey, 1968). Further, the light chains in freshly secreted IgA consist of a mixture of monomer and dimer, the monomer continuing to dimerize after secretion (Bevan, 1971). Thus under the dissociating conditions of the sodium dodecyl sulphate-polyacrylamide-gel electrophoresis, IgA monomers are resolved into α_2 , L₂ and L subunits, and dimers dissociate to α_4 , L₂ and L subunits. Thus electrophoresis of IgA in sodium dodecyl sulphate-polyacrylamide gels allows the computation of heavy-chain/light-chain ratios and monomer (α_2)/dimer (α_4) ratios.

After labelling with radioactive leucine for 4h the major products of the cell are monomer and dimer (Fig. 2a), whereas the intracellular IgA is largely monomer (Fig. 2b). This observation demonstrates that polymerization of the monomer occurs just before, or at the time that, the molecule is secreted, and confirms previous reports (Bevan, 1971; Parkhouse, 1971b; Bargellesi *et al.*, 1972).

By using this methodology it was possible to mea-

Fig. 2. Intracellular and secreted myeloma protein of MOPC 315 plasma-cell tumour

Electrophoresis in sodium dodecyl sulphate–polyacrylamide gels of (b) intracellular and (a) secreted MOPC 315 myeloma protein obtained after labelling with radioactive leucine. Secreted and intracellular immunoglobulins were precipitated with anti-(MOPC 315 myeloma protein) from cultures incubated for 4h and electrophoresed. Details are given in the Materials and Methods section. The position of the various chains and their aggregates are indicated.

sure the ratio of radioactivity in monomer (α_2) to dimer (α_4) in intracellular and secreted subunits from the radioactivity profiles of the fractionated gels (Table 2). The essential point to be made about the secreted immunoglobulin is that the monomer: dimer ratio is essentially the same for all labelled precursors. We would therefore conclude that the carbohydrate moieties of the monomer and dimer have a similar if not identical carbohydrate composition. Analysis of the intracellular immunoglobulin (Table 2) supports the conclusions drawn from the kinetic data (Fig. 1). It is clear from the ratios of monomer to dimer that there is very little leucine-labelled intracellular dimer, but

the small amount that is present is evidently rich in fucose. In contrast, the intracellular monomer is relatively low in fucose content. Molecules destined to be secreted as monomers must therefore acquire fucose just before release into the exterior milieu. Thus the addition of fucose, like the polymerization process, is a late event in biosynthesis. On the other hand, most of the intracellular mannose-labelled immunoglobulin is monomer (Table 2), as would be predicted if mannose is added to the immunoglobulin molecule early in biosynthesis. It is possible that a significant proportion of the radioactivity found in the secreted dimer cultures incubated with radioactive mannose is actually fucose and galactose, since mannose is converted into these sugars (Table 1). Unfortunately, there was not enough radioactive material to test this. Analysis of the galactose-labelled intracellular material shows a situation intermediate between those found for mannose and fucose (Table 2). This would support the notion of an early addition of galactose as suggested by the kinetic evidence, while also indicating some incorporation late in the intracellular life of immunoglobulin.

Most of the radioactivity in carbohydrate was located in the heavy chains (Table 3), and the fraction associated with the light-chain peak was largely accounted for by radioactivity found in the J chain peak on alkaline-urea–polyacrylamide gels (Table 3). Although the agreement (between radioactivity in light chain and J chain) is not very good, reflecting inaccuracy inherent in measuring relatively small amounts of radioactivity, the conclusion that the carbohydrate label found in the light-chain peak resides in the J chain is strengthened by the following evidence. (1) Under the conditions of electrophoresis in sodium dodecyl sulphate–polyacrylamide gels, monomeric light chain and J chain travel at the same position (R. M. E. Parkhouse, unpublished work). (2) In the light chain secreted after 4h incubation with [^3H]leucine 19% of the light-chain radioactivity was found in the light-chain dimer (Fig. 2a), whereas there was no radioactivity incorporated into the light-chain dimer when the cells were incubated with [^3H]mannose, [^3H]galactose or [^3H]fucose. (3) There is no hexosamine in the light chain of MOPC 315 myeloma protein (Underdown *et al.*, 1971). (4) Human J chain contains 7.6% total carbohydrate (Niedermeier *et al.*, 1972). A final assessment of whether MOPC 315 light chain does, or does not, contain carbohydrate must, however, await chemical analysis.

As would be predicted on the basis of previous studies (Halpern & Coffman, 1972; Parkhouse, 1972), alkaline-urea–polyacrylamide gel electrophoresis did not reveal J chain in reduced and alkylated intracellular immunoglobulin precipitated with specific anti-(MOPC 315 myeloma protein). There is therefore an absence of non-covalent interactions

Table 2. *Monomer to dimer radioactivity ratio in secreted and intracellular MOPC 315 myeloma protein, obtained after labelling with radioactive leucine, mannose, galactose or fucose*

Secreted and intracellular immunoglobulins were precipitated with anti-(MOPC 315 myeloma protein) from cultures incubated for 4h and electrophoresed in sodium dodecyl sulphate–polyacrylamide gels as described in the Materials and Methods section. Gels were fractionated and counted for radioactivity. The ratio of monomer to dimer was obtained by summing the radioactivity under the α_2 and α_4 peaks.

Radioactive precursor . . .	Monomer/dimer radioactivity ratio			
	Leucine	Mannose	Galactose	Fucose
Secreted IgA	0.61	0.63	0.60	0.63
Intracellular IgA	52.5	10.8	6.0	1.9

Table 3. *Distribution of radioactivity in total heavy chains, total light chains and J chain in secreted MOPC 315 myeloma protein after labelling with radioactive leucine, mannose, galactose and fucose*

Secreted immunoglobulins were precipitated with anti-(MOPC 315 myeloma protein) from cultures incubated for 4h, and electrophoresed in sodium dodecyl sulphate–polyacrylamide gels to compute the relative radioactivity in heavy and light chains. Parallel co-precipitates were reduced and alkylated and electrophoresed in alkaline-urea–polyacrylamide gels to measure radioactivity in J chains as a percentage of the total recovered radioactivity. Details are given in the Materials and Methods section.

Radioactive precursor . . .	Per cent of total recovered radioactivity			
	Leucine	Mannose	Galactose	Fucose
Heavy chains	74.9	88.0	94.0	97.7
Light chains	25.1	12.0	6.0	2.3
J chain	3.3	9.0	9.8	5.3

between intracellular J chain and the intracellular monomer IgA.

A final point deduced from gel analysis of secreted leucine-labelled MOPC 315 myeloma protein relates to the ratio of polymeric forms secreted at different times of incubation. Over the period studied, 0.5–4h, there was no change in the monomer/dimer ratio, showing that there is no progressive polymerization or degradation in the culture fluid, and that the rate of secretion of IgA is independent of the fraction polymerized. Similar conclusions have been reached previously (Bargellesi *et al.*, 1972).

Discussion

The molecular-weight heterogeneity of secreted IgA poses a fascinating regulatory problem. As with IgM, the final assembly into polymeric forms occurs close to the time of secretion and, as demonstrated above, there is a stepwise addition of carbohydrate to the molecule, which is terminated by the addition of fucose. A definite role for the carbohydrate still remains controversial. If it is required to indicate that the protein is to be secreted, then it is

surprising that there are examples of secreted proteins that do not contain carbohydrate (Winterburn & Phelps, 1972). On the other hand, since the polymerization of intracellular 7S subunits and addition of fucose occur simultaneously, close to the time of secretion, there is the possibility that these two processes might be correlated. We therefore considered the possibility that secreted monomer and polymers were different with respect to carbohydrate. Thus secreted IgA monomers and polymers might differ with respect to their carbohydrate moieties, there being, for example, more (or less) fucose in the monomer than in the dimer. That this is not the case was shown by the results of incorporation of radioactive mannose, galactose, fucose and leucine into secreted monomer and dimer IgA (Table 2). It is clear that the radioactivity ratios of monomer to dimer were identical, irrespective of the radio-isotopic precursor used to label the immunoglobulin. Without excluding subtle difference in structural aspects of the carbohydrate moieties, nonetheless we can certainly conclude that there are no obvious compositional differences in carbohydrate between IgA monomers and dimers secreted by a myeloma cell suspension. In order to argue that the carbohydrate on the monomer and

dimer are absolutely identical, it is necessary to rely on reasoned argument as follows. The myeloma tumour is a clone of cells and therefore the amino acid sequence of heavy and light chains is the same in monomer and dimer. The predominant intracellular form of the myeloma protein is the monomer, and it is likely that a homogeneous molecule will receive the same sequences of monosaccharide units during its passage through the membranous elements of the cell. There are, however, examples (e.g. blood-group substances) where different sugar sequences can result from competition for substrate by a mixture of glycosyltransferases (Hughes, 1973). It is clear that just before secretion monomer units receive fucose and leave the cell as such or are polymerized with the addition of J chain. Further the rate of secretion of IgA is independent of the fraction polymerized.

On the basis of our experimental findings and considerations given, we would therefore argue that the control of IgA polymerization cannot reflect regulation at the level of carbohydrate addition. The obvious difference between monomer and dimer IgA is the presence of J chain in the dimer and not in the monomer (Parkhouse, 1972); thus the role of J chain is likely to be the critical factor in controlling polymerization, and evidence supporting this contention is given in the accompanying paper (Della Corte & Parkhouse, 1973).

As discussed in the text, analysis of the intracellular and secreted molecular forms of IgA together with the kinetic data supports the contention that there is a stepwise addition of carbohydrate residues to immunoglobulins during the process of secretion. In common with other mouse myeloma proteins (Melchers, 1970; Schenkein & Uhr, 1970; Uhr & Schenkein, 1970; Choi *et al.*, 1971b; Parkhouse & Melchers, 1971) mannose is added early in biosynthesis, whereas fucose is added late, about the time of secretion. Analysis of galactose incorporation into MOPC 315 IgA protein shows that a substantial proportion of galactose must be added early, although some may be incorporated later. This is not the case in previously studied immunoglobulins, the galactose being added late in biosynthesis. This result may simply reflect structural features of the MOPC 315 myeloma carbohydrate, i.e. the location of certain galactose residues close to the core of the carbohydrate prosthetic groups. Alternatively, the sites of carbohydrate addition may vary between different myeloma tumours.

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