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Biosynthesis, Intracellular Transport, and Secretion of Immunoglobulins. Effect of 2-Deoxy-D-glucose in Tumor Plasma Cells Producing and Secreting Immunoglobulin G1†

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ABSTRACT: 2-Deoxy-D-glucose, at concentrations between 0.5 and 3 mg/ml, completely inhibits attachment of radioactive glucosamine, mannose, galactose, and fucose as glucosamine, mannose, galactose, fucose, and *N*-glycolylneuraminic acid residues to intracellular carbohydrate moieties including those of an IgG1 myeloma protein in tumor plasma cells. Subcellular localization of IgG1 in the absence and presence of 2-deoxy-D-glucose shows that 2-deoxy-D-glucose inhibits the migration of newly synthesized IgG1 polypeptide chains from membrane-bound polyribosomes into the cisternae of the rough endoplasmic reticulum. It also inhibits the transfer of IgG1 molecules from rough to smooth membranes. It does not inhibit the transfer of IgG1 molecules from the smooth membranes to the outside of cells. Thus, attachment of galactose, fucose, and *N*-glycolylneuraminic acid to IgG1 molecules located in smooth membranes is not a prerequisite for their secretion from plasma cells. Glycosylation of IgG1 molecules and/or of other intracellular carbohydrate moieties is, however, necessary to draw newly synthesized IgG1 molecules into rough membranes and to transport them from there into smooth membranes.

Immunoglobulins (Ig)[1] are glycoproteins. In an IgG1 produced and secreted by the mouse plasma cell tumor MOPC 21, carbohydrate is attached at one asparagine or aspartic acid in each heavy chain within the F_c portion of the molecule (Melchers, 1971b). Carbohydrate moieties of Ig are probably of branched structure, in which *N*-acetylglucosamines and mannoses form a core, while galactoses occupy semiterminal and fucoses and *N*-glycolylneuraminic acid residues occupy terminal positions (for an example see Kornfeld *et al.*, 1971).

The carbohydrate groups of Ig are assembled in a stepwise manner at different subcellular sites during transport of the protein through plasma cells (Melchers, 1970, 1971a; Sherr and Uhr, 1970; Schenkein and Uhr, 1970; Uhr and Schenkein, 1970; Choi *et al.*, 1971; Parkhouse and Melchers, 1971). After its synthesis on membrane-bound polyribosomes (Scharff *et al.*, 1967; Askonas and Williamson, 1967; Cioli and Lennox, 1970; Sherr and Uhr, 1970), IgG migrates into the cisternae of the rough endoplasmic reticulum, from there into membranous structures of the smooth endoplasmic reticulum, and from there out of the plasma cell (Rifkind *et al.*, 1962; de Petris *et al.*, 1963; Jamieson and Palade, 1967a,b; Choi *et al.*, 1971; Uhr, 1970). Chemical analyses of the carbohydrate composition of IgG1 and incorporation of radioactive monosaccharides into IgG1 found in different subcellular fractions and secreted from plasma cells (Melchers, 1970, 1971a,b) have suggested that at least four sequential precursor–product relationships exist between different intracellular forms and the secreted form of IgG1. Ig associated with polyribosomes contains very little, if any, glucosamine and/or mannose (Melchers and Knopf, 1967; Cioli and Lennox, 1970). IgG1 in the rough endoplasmic reticulum contains most of its glucosamine and mannose residues, but only traces of galactose and no fucose residues, while IgG1 in the smooth endoplasmic reticulum contains glucosamine, mannose, galactose, and *N*-glycolylneuraminic acid residues, but only traces of fucose residues. Only the secreted form of IgG1 has the full complement of all carbohydrate residues.

It has been suggested that the biosynthetic steps by which carbohydrate residues are attached to Ig might be part of the processes by which plasma cells transport and secrete Ig (Eylar, 1965; Melchers and Knopf, 1967; Swenson and Kern, 1967). While the experimental evidence cited above favors such a hypothesis, secretion of Ig light chain subunits without detectable carbohydrate attached to them (Melchers, 1971c; Parkhouse and Melchers, 1971) and secretion of full IgG1

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[1] Abbreviations used are: IgG, immunoglobulin G (World Health Organization, 1964); RM, rough membranes; SM, smooth membranes; CS, cytoplasmic supernatant.

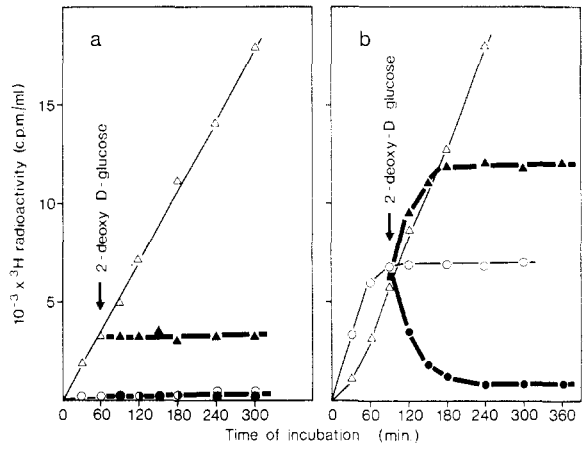

FIGURE 1: Time course of incorporation of (a) L-[1-^{3}H]fucose and (b) D-[1-^{3}H]galactose into intracellular (○) and secreted (△) MOPC 21 IgG1 myeloma protein in the absence (—) and in the presence (—) of 2-deoxy-D-glucose (3 mg/ml). The arrow denotes the time of addition of 2-deoxy-D-glucose to plasma cell suspension. For details see Materials and Methods.

molecules with incomplete carbohydrate moieties (Melchers, 1971a) argue against it.

It has been found that 2-deoxy-D-glucose inhibits synthesis and secretion of some glycoproteins (Farkas *et al.*, 1970; Liras and Gascon, 1971; Ghandi *et al.*, 1972). Although the precise mechanism of this inhibition is not known, it appears that 2-deoxy-D-glucose blocks the biosynthesis of the carbohydrate moieties of glycoproteins. Such an inhibitor appeared useful for testing the role of attachment of carbohydrate residues to Ig in synthesis, transport, and secretion. Synthesis, transport, and secretion of the polypeptide portions and of the carbohydrate portions of Ig are monitored independently by the incorporation of radioactive leucine and of radioactive monosaccharides into IgG1 found in different subcellular fractions and secreted from plasma cells of the mouse plasma cell tumor MOPC 21 in the absence and presence of 2-deoxy-D-glucose.

Materials and Methods

The plasma cell tumor MOPC 21, transplantable in Balb/c mice, was provided by Dr. M. Potter, National Institutes of Health, Bethesda, Md. It was used in transplantation numbers 50–80 at 2–3 weeks after transplantation. Cell suspensions were prepared and incubated with either 10 μCi/ml of L-[4,5-$^3H_2$]leucine (batch 36; 46 Ci/mmol), 100 μCi/ml of D-[1-^{3}H]galactose (batch 12; 18.4 Ci/mmol), 100 μCi/ml of D-[1-^{3}H]mannose (batch 6; 3.8 Ci/mmol), 100 μCi/ml of L-[1-^{3}H]-fucose (batch 4; 1.8 Ci/mmol), or 100 μCi/ml of D-[1-^{3}H]glucosamine-HCl (batch 5; 2.3 Ci/mmol) (all from the Radiochemical Centre, Amersham, Bucks, U. K.), as described before (Melchers, 1970; Melchers, 1971a). Viable cells (5×10^6/ml) were incubated in modified Eagle's medium (Vogt and Dulbecco, 1963) containing 10% fetal calf serum (Ip-tested, batch R8050Q, Grand Island Biological Co., Grand Island, N. Y.). The fetal calf serum had been dialyzed against the balanced salt solution of the Eagle's medium. For labeling with [^{3}H]-leucine, unlabeled leucine was supplied in $^1/_{40}$th of the normal amount in the Eagle's medium. For labeling with [^{3}H]fucose 250 μM unlabeled D-galactose and D-glucosamine were added to the Eagle's medium in which glucose was omitted. For labeling with [^{3}H]galactose, 250 μM unlabeled D-glucosamine and L-fucose were added to the glucose-free Eagle's medium. For labeling with [^{3}H]glucosamine, 250 μM unlabeled D-galactose and L-fucose were added to the glucose-free Eagle's medium, while no unlabeled sugars were added when cells were labeled with [^{3}H]mannose. Cell suspensions were incubated in plastic petri dishes in an incubator containing $CO_2 + O_2 + N_2$ (10:7:83). Viability of the cell suspensions after 5 hr of incubation in all experiments was at least better than 75% of the viability of the cells at the start of incubation. Determination of total trichloroacetic acid precipitable radioactivity incorporated by plasma cells, separation of plasma cells from the medium, lysis of cell pellets with 0.5% (v/w) Nonidet P-40 (Shell Chemical Co., Zürich, Switzerland), and determination of serologically precipitable radioactivity with rabbit antisera specific (recognizing κ-type light chain as well as IgG-F_c determinants) and nonspecific (recognizing *Escherichia coli* β-galactosidase determinants) for the IgG1 myeloma protein secreted by plasma cell tumor MOPC 21 were carried out as described previously (Melchers, 1970). The pig anti-(rabbit Ig) antibody fraction used in the coprecipitations of rabbit antiserum–mouse myeloma protein complexes was kindly prepared by Dr. F. Franek, Czechoslovakian Academy of Sciences, Prague, Czechoslovakia. Values obtained by serological precipitations with IgG specific antisera were corrected by subtracting from them the values obtained by precipitating with nonspecific antiserum. The values obtained with nonspecific antiserum did not exceed 5% of those obtained with specific antiserum. Unless otherwise stated, 2-deoxy-D-glucose (Calbiochem, Lucerne, Switzerland) dissolved in the appropriate medium was added to plasma cell suspensions to a final concentration of 3 mg/ml. Subcellular fractions of plasma cells were prepared and fractionated as described (Melchers, 1971b) using method 2 with a Potter-Elvejhem tissue homogenizer (Kontes Glass Co., Vineland, N. J.). Packed cells (1 ml) in 3 ml of 0.05 M Tris-HCl–0.025 M KCl–0.005 M $MgCl_2$, pH 7.5, containing 0.25 M sucrose were homogenized at 0° by 30 strokes with the large clearance pestle. Quantitative determinations of radioactivity in different neutral hexoses and hexosamines were done by column chromatographies on Dowex 2-X8 (neutral hexoses; Walborg *et al.*, 1965) and on Amberlite CG-120 (hexosamines, Jollés *et al.*, 1963) with hydrolysates of serological precipitates of radioactive myeloma protein as described (Melchers, 1970). Polyacrylamide gel electrophoreses were performed as described previously (Parkhouse and Melchers, 1971). Simultaneous determination of ^{14}C and ^{3}H radioactivities on samples was done as described (Melchers, 1971a).

Results

Kinetics of synthesis and secretion of the protein and the carbohydrate moieties of MOPC 21 IgG1 myeloma protein were followed with the incorporation of radioactive leucine and of radioactive mannose, glucosamine, galactose, and fucose by tumor plasma cell suspensions into intracellular and secreted material, which could be precipitated by IgG1-specific antisera (see Materials and Methods). In the absence of inhibitor, these kinetics were the same as those published and discussed previously (Melchers, 1970, 1971a). Analyses of the radioactivities incorporated into IgG1 from the four radioactive sugar precursors ascertained that radioactive fucose labeled only fucose residues (Melchers, 1971a) and radioactive galactose only galactose residues (Melchers, 1970), while radioactive glucosamine labeled glucosamine

and *N*-glycolylneuraminic acid residues of IgG1 (Knopf, Sasso, Destree and Melchers, manuscript in preparation). Radioactive mannose labeled mannose, glucosamine, and galactose residues of intracellular IgG1 and, in addition, fucose residues of extracellular IgG1 (Melchers, 1970).

Effect of 2-Deoxy-D-glucose on Synthesis and Secretion of Sugar-Labeled MOPC 21 IgG1 Myeloma Protein. 2-Deoxy-D-glucose was added at a final concentration of 3 mg/ml either before or after the addition of radioactive sugars to tumor plasma cell suspensions. Addition of 2-deoxy-D-glucose after the addition of radioactive sugars was timed such that the intracellular pools of IgG1 protein had been equilibrated with radioactive IgG1 molecules and a steady state had been reached in the rate of secretion of radioactive IgG1 molecules.

2-Deoxy-D-glucose inhibited the incorporation of radioactive mannose, glucosamine, galactose, and fucose into acid-precipitable material and into IgG1 myeloma protein in all experiments completely (Figures 1 and 2, a and b). In the following experiments, the effect of 2-deoxy-D-glucose on intracellular transport and secretion of IgG1 molecules was studied with tumor plasma cells in which the pools of intracellular IgG1 molecules had been prelabeled with the four radioactive sugars. Since 2-deoxy-D-glucose inhibited the incorporation of radioactive sugars completely, such experiments could be regarded as pulse-chase experiments, although the radioactive sugars remained in the medium. In fact, transfer of sugar-prelabeled cells into nonradioactive medium at the time of addition of the inhibitor yielded the same kinetics of synthesis and secretion of sugar-labeled MOPC 21 IgG1 myeloma protein as those reported below.

Fucose and Galactose. Secretion of fucose-labeled IgG1 ceased after the addition of 2-deoxy-D-glucose, while intracellular IgG1 remained to be labeled only in trace amounts (Figure 1a). Galactose-labeled IgG1 continued to be secreted for about 1 hr after the addition of 2-deoxy-D-glucose. The intracellular pool of IgG1 molecules, prelabeled with galactose, was depleted at the rate at which galactose-labeled IgG1 was secreted (Figure 1b). Between 10 and 20% of the total intracellular pool of galactose-prelabeled IgG1 remained inside the cells and was not secreted within the next 3 hr. These results suggest that inhibition of the incorporation of galactose into IgG1 by 2-deoxy-D-glucose does not inhibit the secretion of 80–90% of all intracellular IgG1 molecules previously labeled with galactose. Since 2-deoxy-D-glucose also inhibits the addition of fucose to IgG1, these molecules therefore also do not need the addition of fucose residues to be secreted.

Mannose and Glucosamine. Only between 25 and 35% of the intracellular IgG1 prelabeled with radioactive mannose was secreted and found outside the cells within the first hours after addition of 2-deoxy-D-glucose. The remaining 65–75% labeled IgG1 was not secreted from the cells within the next 3 hr (Figure 2a). The nonsecreted form of intracellular IgG1 prelabeled with radioactive mannose contained 20% of its radioactivity in glucosamine residues and 70% of it in mannose residues, with no detectable radioactivity in galactose and fucose residues. Similarly to IgG1 prelabeled with radioactive mannose, only 40% of IgG1 prelabeled with glucosamine could be secreted within the first hour after addition of 2-deoxy-D-glucose (Figure 2b). For the next 3 hr thereafter, 60% remained inside. More than 90% of the total radioactivity in IgG1 remaining inside the cells was found in glucosamine residues, while the IgG1 secreted in the presence of 2-deoxy-D-glucose had its radioactivity distributed between glucosamine (60% of total) and *N*-glycolylneuraminic acid residues (35% of total). It is concluded that in the presence of 2-deoxy-D-glucose, *e.g.*, in the absence of further carbohydrate addition to IgG1, intracellular forms of IgG1 can be secreted, which contain mannose, glucosamine, galactose, and *N*-glycolylneuraminic acid residues. Secretion of intracellular forms of IgG1 containing only mannose and glucosamine, but no galactose, fucose, and/or *N*-glycolylneuraminic acid residues is inhibited.

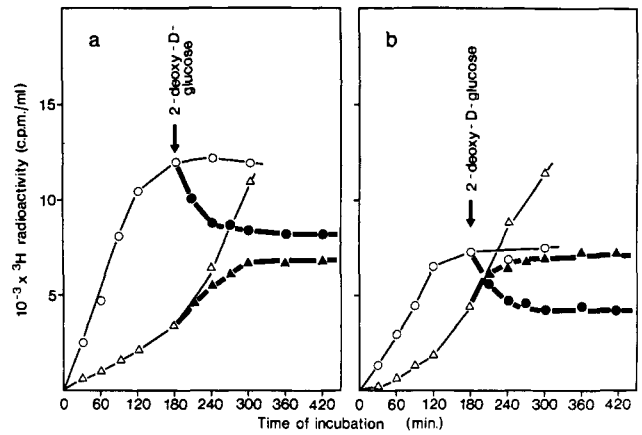

FIGURE 2: Time course of incorporation of (a) D-[1-^{3}H]mannose and (b) D-[1-^{3}H]glucosamine into intracellular (○) and secreted (△) MOPC 21 IgG1 myeloma protein in the absence (—) and in the presence (—) of 2-deoxy-D-glucose (3 mg/ml). The arrow denotes the time of addition of 2-deoxy-D-glucose to plasma cell suspension. For details see Materials and Methods.

Intracellular Localization of IgG1 Molecules Remaining Inside the Plasma Cells in the Presence of 2-Deoxy-D-glucose. From the carbohydrate analyses of mannose- and glucosamine-prelabeled IgG1 remaining inside the cells in the presence of 2-deoxy-D-glucose it appeared possible that 2-deoxy-D-glucose could inhibit the transfer of IgG1 molecules from rough to smooth membranes (Choi *et al.*, 1971; Melchers, 1971b). Therefore, subcellular fractions were prepared and separated on sucrose gradients (Melchers, 1971b) from cells labeled with either radioactive mannose or radioactive galactose to equilibrium (a) before and (b) after a subsequent 4-hr chase in cold medium containing 2-deoxy-D-glucose. Gradient fractions enriched for rough membranes (RM), smooth membranes (SM), and the nonsedimenting cytoplasmic supernatant (CS) were pooled and the amount of labeled IgG1 protein in the detergent-lysed subcellular fractions determined by serological precipitations with specific antiserum (Table I).

From the 94% of the total intracellular galactose-labeled IgG1 distributed between the SM (72% of total) and the CS (22% of total) subcellular fractions, over 90% (86% of total) could be chased out of the cells in the presence of 2-deoxy-D-glucose. This agrees with the results described above (Figure 1b) and shows that 2-deoxy-D-glucose does not inhibit the secretion of galactose-labeled IgG1 from the SM and the CS subcellular fractions.

In the presence of 2-deoxy-D-glucose, 85% (17% of total) of the mannose-labeled IgG1 located in the SM fraction could be secreted. Thus mannose-labeled IgG1 can be secreted from plasma cells once the molecules have reached the SM subcellular fraction in their intracellular migration.

However, only 20% (12% of the total) radioactivity of the mannose-labeled IgG1 found in the RM subcellular fraction (62% of the total) could be secreted from the cells in the pres-

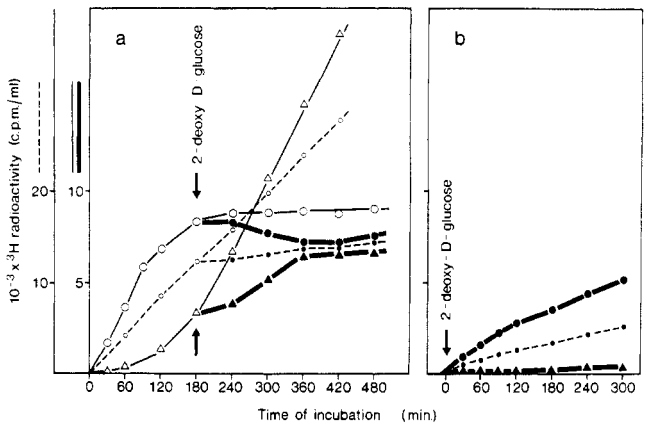

FIGURE 3: Time course of incorporation of L-[4,5-^{3}H]leucine into intracellular (○), secreted (△), and total intracellular plus secreted (- - -) MOPC 21 IgG1 myeloma protein in the absence (—) and in the presence (—) of 2-deoxy-D-glucose (3 mg/ml). Time of addition of 2-deoxy-D-glucose to plasma cell suspensions was (a) 180 min and (b) 0 min after initiation of labeling (see arrows). For details see Materials and Methods.

ence of 2-deoxy-D-glucose. 2-Deoxy-D-glucose freezes around 80% (50% of total) of the mannose-labeled IgG1 in the RM subcellular fraction and inhibits its secretion from the cells. Thus, 2-deoxy-D-glucose blocks the transfer of IgG1 from rough to smooth membranes.

Effect of 2-Deoxy-D-glucose on Synthesis and Secretion of the Leucine-Labeled Protein Portion of MOPC 21 IgG1 Protein. In contrast to the incorporation of radioactive sugars, 2-deoxy-D-glucose did not totally inhibit the synthesis of leucine labeled proteins including IgG1 myeloma protein. The rate of synthesis of IgG1 decreased, however, as can be seen from the incorporation of radioactive leucine into the sum of intracellular and secreted IgG1 in Figure 3a and b. The effect of 2-deoxy-D-glucose could be seen more clearly when it was added to plasma cell suspensions prior to the addition of radioactive leucine (Figure 3b). 2-Deoxy-D-glucose completely inhibited the secretion of newly synthesized IgG1 for a period of at least 5 hr. However, intracellular material precipitable by IgG-specific antisera continued to be synthesized at a declining rate for at least 5 hr. This material contained H γ and L chains, as shown by polyacrylamide gel electrophoresis in sodium dodecyl sulfate–urea after full reduction and alkylation (Figure 4). Compared to H γ chains of MOPC 21 IgG1 secreted from cells in the absence of 2-deoxy-D-glucose the intracellular H γ chains made in the presence of 2-deoxy-D-glucose had a smaller apparent molecular weight in the gels. This could be expected from a H γ chain with less or no carbohydrate (Schubert, 1970).

The effects of 2-deoxy-D-glucose on incorporation of radioactive sugars and on secretion of newly synthesized, leucine-labeled IgG1 were observed down to concentrations as low as 0.5 mg/ml. No differential inhibition of either incorporation of radioactive sugars or secretion of newly synthesized leucine-labeled IgG1 could be observed in the inhibitory concentration range between 0.5 and 3 mg/ml.

Subcellular Localization of Leucine-Labeled MOPC 21 IgG1 Myeloma Protein Synthesized in the Presence of 2-Deoxy-D-glucose. Subcellular fractions were prepared and separated on sucrose gradients from cells labeled either in the absence or in the continuous presence of 2-deoxy-D-glucose and the serologically precipitable leucine-labeled IgG1 protein determined in the different subcellular fractions (Table II). It is

TABLE I: Distribution of MOPC 21 IgG1 Protein Labeled for 4 hr with [^{3}H]Mannose or [^{3}H]Galactose in RM, SM, and CS Subcellular Fractions of MOPC 21 Tumor Plasma Cells[a] before and after Chase in Cold Medium Containing 2-Deoxy-D-glucose.[b]

Subcellular Fraction[c]	% Total Radioactivity in Intracellular IgG1 from Cells Labeled with [^{3}H]Mannose[d]		[^{3}H]Galactose[d]	
	Before	After	Before	After
RM	62	50	2	2
SM	20	3	72	5
CS	4	2	22	3

[a] 5 × 10^6 cells. [b] 3 mg/ml. [c] The subcellular fraction containing nuclei, mitochondria, and any unbroken cells at the bottom of the sucrose gradients has not been analyzed. [d] Values are in per cent of the total radioactivity in all intracellular IgG, analyzed by serological precipitation with specific antiserum in detergent lysates of aliquots of whole labeled cells prior to chase in cold medium containing 2-deoxy-D-glucose and prior to homogenization and fractionation of subcellular fractions; values are before and after chase.

evident that only a minor part of the IgG1 molecules synthesized in the presence of 2-deoxy-D-glucose appear associated with either smooth or rough membrane fractions of tumor plasma cells, but are mainly found in the cytoplasmic supernatant fraction.

Discussion

2-Deoxy-D-glucose inhibits glycosylation of macromolecules in plasma cells. The target for this inhibition is not known, although it has been reported that 2-deoxy-D-glucose competitively inhibits the enzyme glucose–phosphate–isomerase (Mahler and Cordes, 1961). Preliminary experiments in our laboratory show that the action of 2-deoxy-D-glucose on plasma cells is reversible and that glycosylation can also be inhibited in other lymphocytes synthesizing IgM (Parkhouse and Melchers, 1971; Andersson and Melchers, 1973). The general inhibitory action on the synthesis of all carbohydrate moieties makes it impossible to invoke a direct relationship between the glycosylation of IgG and its intracellular transport and secretion from plasma cells (Eylar, 1965; Melchers and Knopf, 1967). We can, however, specify that glycosylation of intracellular structures including IgG1 is *not* necessary to transport IgG1 from the smooth membranes including Golgi apparatus to the outside of plasma cells. From the secretion of IgG1 molecules under conditions of glucose starvation of plasma cells, it was concluded earlier that addition of fucose residues was not necessary to secrete IgG1 molecules (Melchers, 1971a). We can now conclude that attachment of fucose, galactose, and/or *N*-glycolylneuraminic acid residues, which are still missing in the population of IgG molecules in the smooth membrane fractions, is not necessary for secretion of IgG1.

In addition to the pool of molecules in the smooth membranes, two other intracellular pools of IgG1 can be defined in plasma cells. One is the pool of molecules containing only

TABLE II: Distribution of MOPC 21 IgG1 Protein Labeled for 4 hr with [^{3}H]Leucine in the Presence and Absence of 2-Deoxy-D-glucose in RM, SM, and CS Subcellular Fractions of Tumor Plasma Cells.[a]

Subcellular Fraction	% Total Radioactivity in MOPC 21 IgG1 Protein from Cells Labeled with [^{3}H]Leucine with	
	2-Deoxy-D-glucose	
	Absent	Present
RM	55–65	10
SM	25–30	5
CS	10–15	80

[a] Details are described under Materials and Methods and in the footnotes to Table I.

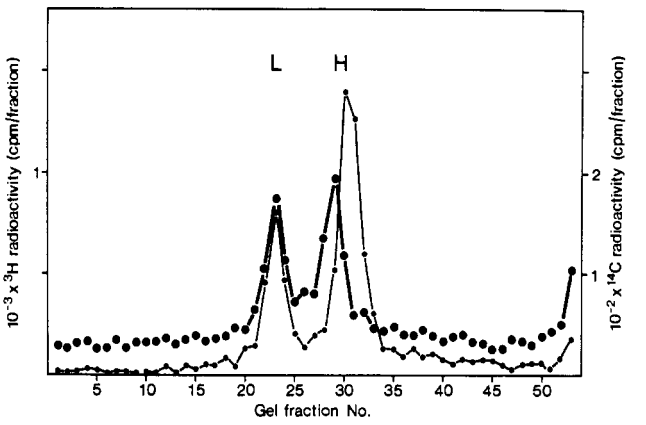

FIGURE 4: Polyacrylamide gel electrophoresis of [^{14}C]leucine-labeled MOPC 21 IgG1 myeloma protein secreted by plasma cells in normal medium (—), admixed with [^{3}H]leucine-labeled MOPC 21 IgG1 myeloma protein, produced by plasma cells in the presence of 2-deoxy-D-glucose (—) (see Figure 3b). The labeled proteins were serologically precipitated with specific antiserum after mixing extracellular fluids containing the ^{14}C-labeled protein with intracellular lysates containing the ^{3}H-labeled protein. Reduction and alkylation to H and L chains, gel electrophoresis, and simultaneous counting of ^{14}C and ^{3}H radioactivities in the gel fractions were done as described previously (Melchers, 1971a). Fractions are numbered from the positive to the negative electrode.

the "core" sugars, mannoses and glucosamines, localized in the rough membranes (Melchers, 1971b). The other is the pool of growing and/or unreleased IgG1 polypeptide chains, containing very little if any sugar residues, associated with membrane-bound polyribosomes (Melchers and Knopf, 1967; Cioli and Lennox, 1970). Migration of IgG1 molecules occurs from the site of synthesis on membrane-bound polyribosomes (pool 1) into the cisternae of the rough endoplasmic reticulum (pool 2), from there into the cisternae of the smooth endoplasmic reticulum (pool 3), and from there out of the cells (Choi *et al.*, 1971; Melchers, 1971b). Our results clearly indicate that 2-deoxy-D-glucose, inhibiting glycosylations, also inhibits the transfer of IgG1 molecules from rough (pool 2) to smooth membranes (pool 3). It is also clear that 2-deoxy-D-glucose inhibits IgG1 molecules to be drawn from the polyribosomes on the one side of the membranous interphase (pool 1) into the cisternae on the other side of the membranous interphase of the rough endoplasmic reticulum (pool 2). (For a discussion of the polarity of membranes of the rough and smooth endoplasmic reticulum, see Rifkind *et al.*, 1962; de Petris *et al.*, 1963; Hirano *et al.*, 1972.)

It is not clear at which intracellular site the IgG1 molecules are located which are synthesized in the presence of 2-deoxy-D-glucose. Staining with fluorescent IgG-specific antisera of live MOPC 21 plasma cells, kept in culture for 5 hr in the presence of 2-deoxy-D-glucose, did not show any change in the number of cells with surface-bound IgG1 and/or an increase of the amount of surface-bound IgG1 on positive cells (Forni, L., and Melchers, F., unpublished observations). These observations may even be taken as indications that secretion and deposition of Ig in the outer membrane are effected by separate routes inside plasma cells.

It might be suspected that inhibition of glycosylation would impair the structural integrity of intracellular membranes. An increased fragility of such membranes may yield a different fractionation of subcellular fractions, with most of the IgG1 molecules being released into the nonsedimenting cytoplasmic supernatant fractions (see distribution in Table II).

Although inhibition of glycosylation by 2-deoxy-D-glucose of IgG1 molecules in pool 1 prevents these IgG1 molecules from being drawn into the cisternae of the rough endoplasmic reticulum (pool 2) and to be transported from the rough to the smooth membranes (pool 3), it remains to be clarified if glycosylation of IgG1 *directly*, or of some other intracellular carbohydrate moieties *indirectly*, is needed for these transport processes. It is, however, clear from our results that glycosylation of *either* IgG1 *and/or* some other component of plasma cells is necessary to select the newly synthesized IgG1 molecules for intracellular transport and secretion.

The results also leave open the problem why free light chains can be secreted from plasma cells without detectable carbohydrate attached to them although they seem to traverse the same subcellular sites on their way out of the cells (Melchers, 1971c). It should finally be emphasized that other serum proteins are secreted from other cells without attachment of carbohydrate and by a subcellular migration quite similar to that of IgG1 (*e.g.*, albumin, Peters *et al.*, 1971).

It can be predicted that 2-deoxy-D-glucose will prove a useful inhibitor in biosyntheses of carbohydrate moieties of cell constituents for investigations of their biological functions.

Acknowledgments

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References

Andersson, J., and Melchers, F. (1973), *Proc. Nat. Acad. Sci. U. S. 70*, 416.

Askonas, B. A., and Williamson, A. R. (1967), *Cold Spring Harbor Symp. Quant. Biol. 32*, 223.

Choi, Y. S., Knopf, P. M., and Lennox, E. S. (1971), *Biochemistry 10*, 668.

Cioli, D., and Lennox, E. S. (1970), *Proc. Int. Congr. Biochem., Interlaken, Switzerland*, 298.

de Petris, S., Karlsbad, G., and Pernis, B. (1963), *J. Exp. Med. 117*, 849.

Eylar, E. H. (1965), *J. Theor. Biol. 10*, 89.

Farkas, V., Svoboda, A., and Bauer, S. (1970), *Biochem. J. 118*, 755.
Gandhi, S. S., Stanley, P., Taylor, J. M., and White, D. O. (1972), *Microbios. 1*, 41.
Hirano, H., Parkhouse, B., Nicholson, G. L., Lennox, E. S., and Singer, S. J. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 2945.
Jamieson, J. D., and Palade, G. E. (1967a), *J. Cell Biol. 34*, 577.
Jamieson, J. D., and Palade, G. E. (1967b), *J. Cell Biol. 34*, 597.
Jollés, P., Samour, D., and Lederer, E. (1963), *Biochim. Biophys. Acta 18*, 342.
Kornfeld, R., Keller, J., Baenziger, J., and Kornfeld, S. (1971), *J. Biol. Chem. 246*, 3259.
Liras, P., and Gascon, S. (1971), *Eur. J. Biochem. 23*, 160.
Mahler, H. R., and Cordes, E. H. (1961), Biological Chemistry, New York, N. Y., Harper and Row, p 422.
Melchers, F. (1970), *Biochem. J. 119*, 765.
Melchers, F. (1971a), *Biochem. J. 125*, 241.
Melchers, F. (1971b), *Biochemistry 10*, 653.
Melchers, F. (1971c), *Eur. J. Immunol. 1*, 330.
Melchers, F., and Knopf, P. M. (1967), *Cold Spring Harbor Symp. Quant. Biol. 32*, 255.
Parkhouse, R. M. E., and Melchers, F. (1971), *Biochem. J. 125*, 235.
Peters, T., Fleischer, B., and Fleischer, S. (1971), *J. Biol. Chem. 246*, 240.
Rifkind, R. A., Osserman, E. F., Hsu, K. C., and Morgan, C. (1962), *J. Exp. Med. 116*, 423.
Scharff, M. D., Shapiro, A. L., and Ginsburg, B. (1967), *Cold Spring Harbor Symp. Quant. Biol. 32*, 235.
Schenkein, J., and Uhr, J. W. (1970), *J. Cell Biol. 46*, 42.
Schubert, D. (1970), *J. Mol. Biol. 51*, 287.
Sherr, C. J., and Uhr, J. W. (1970), *Proc. Nat. Acad. Sci. U. S. 66*, 1183.
Swenson, R. M., and Kern, M. (1967), *J. Biol. Chem. 242*, 3242.
Uhr, J. W. (1970), *Cell. Immunol. 1*, 228.
Uhr, J. W., and Schenkein, J. (1970), *Proc. Nat. Acad. Sci. U. S. 66*, 952.
Vogt, M., and Dulbecco, R. (1963), *Proc. Nat. Acad. Sci. U. S. 49*, 171.
Walborg, E. F., Christensson, L., and Gardell, S. (1965), *Anal. Biochem. 13*, 177.
World Health Organization (1964), *Bull. W. H. O. 30*, 447.

Developmental Changes in Microtubule Protein of Chick Brain†

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ABSTRACT: A time-decay colchicine-binding assay and two quantitative polyacrylamide gel electrophoresis systems have been employed to determine the quantities of microtubule protein in the soluble and particulate fractions of chick brain during development. Results with all three methods were identical. The microtubule protein concentration in the soluble fraction of sonicated brains increased from 20% at 5–7 days of development to a maximum concentration of 42% at 13 days of development, then decreased slowly to 25% in the adult. Specificity experiments demonstrated that the binding of colchicine in the particulate fraction of brain was to microtubule protein. No particulate microtubule protein was detected prior to 13 days of development. The particulate microtubule protein concentration then increased slowly from 0.9% of total brain protein at 13 days of development to approximately 2–3% in the adult. The maximum concentration of microtubule protein, as a percentage of total brain protein, was 23–24%, observed between 9 and 17 days of development. The adult concentration was 11–12%. The colchicine binding affinity of microtubule protein appeared to remain constant between 5 days of development in the embryo and the adult. However, the half-life for loss of colchicine binding activity of the protein decreased with increasing age. These results suggest that a change in microtubule protein may be taking place during development of the chick brain.

Microtubules are widely distributed in all eucaryotic cells and tissues, but considerably higher concentrations of microtubule (neurotubule) proteins appear to exist in cells of the central nervous system than in those of other organ systems or tissues (Borisy and Taylor, 1967; Dutton and Barondes, 1969). Estimates of neurotubule protein concentrations in soluble fractions of mammalian brain have ranged between 10 and 40%, and brain has served as a rich source material in the purification of colchicine-binding microtubule proteins (*cf.* Weisenberg *et al.*, 1968; Falxa and Gill, 1969; Bryan and Wilson, 1971; Eipper, 1972).

As an initial approach to questions of regulation of microtubule assembly and function in the central nervous system, we have utilized a time-decay colchicine binding assay procedure, and two independent quantitative polyacrylamide gel electrophoresis procedures to determine accurately the con-

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