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Immunoglobulin M Biosynthesis

PRODUCTION OF INTERMEDIATES AND EXCESS OF LIGHT-CHAIN IN MOUSE MYELOMA MOPC 104E

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Immunoglobulin M (IgM) biosynthesis was studied with mouse plasma-cell tumour MOPC 104E as a model system. Cell suspensions prepared from solid tumours were incubated *in vitro* with [^{3}H]leucine; the radioactivity incorporated into intracellular and secreted proteins was analysed by sucrose-density-gradient centrifugation and polyacrylamide-gel electrophoresis. The tumour secretes IgM and light chains. 'Pulse–chase' experiments indicated average secretion times of 1.5h for light chain and 2.5h for IgM. The order of disulphide-bond assembly within the cell was shown to be heavy chain+light chain → heavy chain–light chain intermediate → IgMs. The 7S subunit (IgMs) was polymerized into IgM just before or at the time of secretion. Measurements of heavy-chain/light-chain radioactivity ratios in intracellular HL and IgMs and secreted IgM demonstrated the existence of a light-chain pool participating in IgM biosynthesis. The size of the light-chain pool, together with analysis of clones isolated *in vivo*, suggested that the tumour contains cells in which light-chain synthesis is in excess of heavy-chain production.

By using the transplantable mouse plasma-cell tumour MOPC 104E (McIntire, Asofsky, Potter & Kuff, 1965), it has been shown that the IgM* molecule is formed by polymerization of 7S subunits (IgMs) just before or at the time of secretion (Parkhouse & Askonas, 1969). The IgMs accumulates within the cell in the absence of detectable amounts of the fully assembled polymer.

In contrast with the behaviour of subunits artificially prepared by reduction, (Parkhouse, Askonas & Dourmashkin, 1970), isolated intracellular IgMs does not spontaneously polymerize; thiol groups responsible for covalent bonds between the subunits appear to be blocked within the cell (Askonas & Parkhouse, 1971).

In the present paper evidence is presented to show that intracellular IgMs is formed via a disulphide bonded heavy chain–light chain intermediate (HL) and an extremely large light-chain pool. Since the tumour line used in these studies secretes free light chains in addition to IgM, it was possible to measure the rate of secretion of both molecules simultaneously. The size of the light-chain pool, together with analysis of clones isolated *in vivo* (Till & McCulloch, 1961), suggests that there are many cells in which light-chain synthesis is in excess of heavy-chain production.

* Abbreviation: IgM, immunoglobulin M (macroglobulin).

MATERIALS AND METHODS

The MOPC 104E plasma-cell tumour was maintained as described in the preceding paper (Askonas & Parkhouse, 1971).

Preparation of IgM. The IgM was prepared from the serum of tumour-bearing mice as described by Parkhouse & Askonas (1969).

Preparation of μ chains. Purified IgM (3mg/ml) in 0.3M-tris–HCl, pH8.0, containing 1mM-EDTA, was treated with 0.02M-dithioerythritol for 1h at room temperature. Alkylation was accomplished by the addition of iodoacetamide to 0.05M, and heavy and light chains were separated by gel filtration (through Sephadex G-100 equilibrated with 1M-propionic acid).

Preparation of λ chains from urine. A variant of the MOPC 104E tumour which had almost entirely ceased production of IgM (Parkhouse & Askonas, 1969), was kept in Balb/c mice. Light chain was isolated from the urine by gel filtration (on Sephadex G-100 equilibrated with 0.05M-NH_4HCO_3, pH8.0) of the precipitate formed at 30–55%-satd. $(NH_4)_2SO_4$.

Preparation and standardization of antisera. Rabbit antibody against purified proteins was raised as described by Parkhouse & Askonas (1969). Two antisera were used in the present studies. One, raised against purified IgM (anti-IgM), reacted with IgM and free λ chains. The other,

raised against purified μ chains (anti-μ), reacted with IgM but not free light chains.

Antisera were titrated either by the conventional precipitin technique (Kabat & Mayer, 1961), or by measuring inhibition of precipitation of biosynthetically prepared ^{3}H-labelled antigen by increasing quantities of unlabelled antigen. In the latter test, a series of tubes received a constant amount of [^{3}H]leucine-labelled antigen [prepared as described by Askonas & Parkhouse (1971)] and increasing amounts of non-radioactive antigen. A constant volume of antiserum was added and the tubes were incubated and processed as for the standard precipitin test. The sensitivity of the assay was similar to that of the conventional precipitin test. Radioactivity was measured in the immune precipitates. The antiserum titre was given by the highest concentration of antigen at which all the added radioactive material was precipitated.

Labelling in vitro *and analysis of the intracellular and extracellular protein produced by plasma-cell tumour MOPC 104E.* Cell suspensions were incubated with radioactive leucine, harvested and the cells lysed with 1% (w/v) Nonidet P.40 (Shell Chemicals Ltd., London W.C.2, U.K.) containing 0.2M-iodoacetamide. The extracellular fluid and cell lysate were analysed by specific immunological co-precipitation, sucrose-density-gradient centrifugation and sodium dodecyl sulphate–4.25% (w/v) polyacrylamide-gel electrophoresis. Antibody–antigen precipitates formed by goat anti-(rabbit IgG) and rabbit IgG were controls for the specific precipitations. The methods and conditions have been described in detail (Parkhouse & Askonas, 1969).

For polyacrylamide-gel analysis, specific immune precipitates were solubilized at 0.5–2.0 mg of protein/ml in 0.1M-iodoacetamide in 2% (w/v) sodium dodecyl sulphate by heating for 1 min at 100°C (Maizel, White & Scharff, 1968). In the absence of iodoacetamide, boiling in sodium dodecyl sulphate caused appreciable breakdown of subunits (IgMs, HL subunit) into heavy and light chains. Radioactive co-precipitates were reduced by dissolving precipitates in 2% (w/v) sodium dodecyl sulphate containing 0.025M-dithioerythritol and 0.3M-tris–HCl, pH 8.0. Dissolution was effected by heating at 100°C for 1 min and the solution was maintained at 37°C for 60 min. Alkylation was accomplished by the addition of recrystallized iodoacetamide (100% excess over added thiol).

Design and interpretation of the 'pulse–chase' experiment. The basic aim of the experiment was to study the flow of immunoglobulin determinants from the cell interior to the external medium. For a single secretory product, a 'pulse–chase' experiment followed by quantitative assay (e.g. specific immunological co-precipitation) of the secreted material and its intracellular precursor is a simple and convenient approach. However, the MOPC 104E tumour secretes IgM and light chain (Parkhouse & Askonas, 1969) and, as demonstrated in the text, contains intracellular IgMs, HL subunit and light chain. It was therefore necessary to assay for each individual component and this was done by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis of solubilized immune precipitates.

In a typical experiment, a suspension of the myeloma cells at $1–2 \times 10^7$ cells/ml was prepared, and 1.0 ml cultures were incubated with radioactive leucine. The experiment consisted of two series of incubations conducted over a 3 h period, separate cultures being used for each time-point. In one series ('pulse–chase') the cultures were 'pulsed' with high-specific-radioactivity [^{3}H]leucine (L-[4,5-^{3}H]-leucine, 19.7 Ci/mmol; 50 μCi), and then 'chased' with 0.75 mg of unlabelled L-leucine. In the other series ('continuous label'), cells were incubated for the period indicated with low-specific-radioactivity [^{3}H]leucine (L-[4,5-^{3}H]leucine, 1 Ci/mmol; 50 μCi), to demonstrate continued secretion and a constant rate of total protein synthesis over the entire incubation period. Cultures were harvested and the extracellular fluids and cell lysates were assayed by trichloroacetic acid precipitation and specific co-precipitation with anti-IgM serum. Total immunoglobulin precipitated by anti-IgM was obtained by direct counting of the radioactivity in the precipitates. The relative concentration of each biosynthetic component present in the precipitates was given by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis of solubilized immune precipitates. In this way, the rate of secretion of both IgM and light chain was measured simultaneously.

In the continuous-label experiment, the time at which the total amount of secreted protein equals the total amount of its intracellular precursor is twice the time for synthesis and secretion of an average molecule. In the 'pulse–chase' experiment, on the other hand, this time measures the period to secrete one-half of the total immunoglobulin content of a cell, and is therefore a direct measurement of the average synthesis and secretion period.

Cloning in vivo. This was done essentially as described by Till & McCulloch (1961). Myeloma-cell suspensions over the range $10^4–10^7$ cells/ml were prepared and 0.1 ml portions of each cell suspension were injected intravenously into groups of female Balb/c mice. After 2–10 weeks, spleens were removed and examined for foci of myeloma cells. With cell suspensions prepared from the solid tumour no foci were ever detected, even at the higher cell concentrations. When the ascitic form of the tumour was used, however, foci were located in the spleen 2–4 weeks after the injection of 10^4 cells. Foci were excised from spleens bearing not more than three well-separated colonies, and transplanted subcutaneously into mice. The subsequent solid tumours were teased into cell suspensions, incubated with radioactive leucine, and the culture supernatants were analysed by polyacrylamide-gel electrophoresis for IgM and light chains.

RESULTS

Identification of intracellular HL intermediate. Cell suspensions were pulsed with radioactive leucine and the resultant cell lysates were analysed by sucrose-density-gradient centrifugation (Fig. 1), and by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis of specific immune coprecipitates (Fig. 2*a*). Both methods of analysis revealed three peaks of radioactivity corresponding to IgMs, HL subunit and light chain.

The peak with lowest density on the sucrose gradient was not precipitated by specific anti-μ

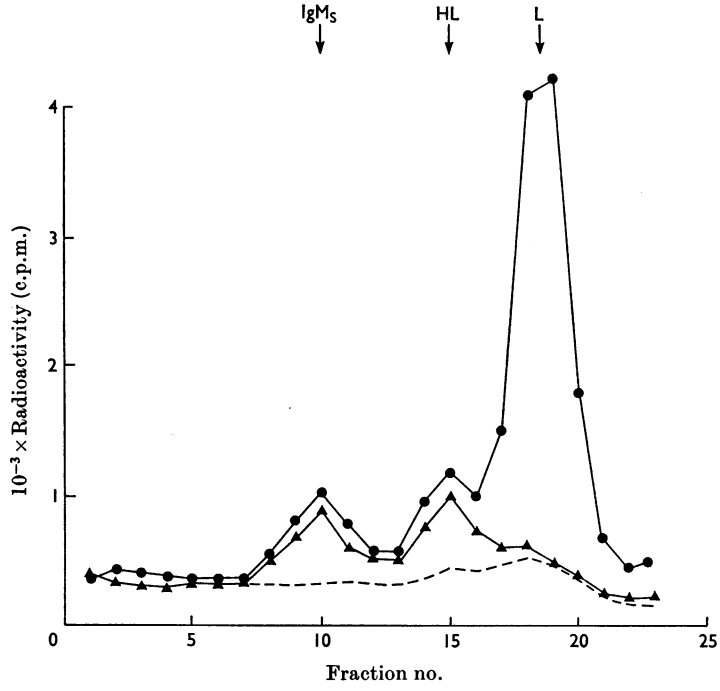

Fig. 1. Sucrose-density-gradient centrifugation of IgMs, HL subunit and light chain in cell lysates of MOPC 104E tumour. The cell suspension was labelled for 10min *in vitro* with [4,5-^{3}H]leucine (40μCi/ml at 19.7Ci/mmol) and intracellular proteins were fractionated on a gradient [5–30% (w/v) sucrose in 130mM-NaCl–4mM-KCl–10mM-sodium phosphate, pH7.4] at 41000rev./min for 22h in a Spinco SW 41 rotor. Fractions were approx. 0.5ml and numbered from the bottom to the top of the gradient. ●, Radioactivity precipitated with specific anti-(mouse IgM); ▲, radioactivity precipitated with specific anti-(mouse μ chain); ----, radioactivity precipitated with anti-(rabbit IgG), i.e. control precipitates.

serum (Fig. 1) and was localized in the position of secreted light chains on sodium dodecyl sulphate–polyacrylamide-gel electrophoresis, and therefore represents light chain. The fastest moving peak ran in the 7S region of the gradient, was coincident with intracellular IgMs on sodium dodecyl sulphate–polyacrylamide-gel electrophoresis (Fig. 2*b*), was precipitated by specific anti-μ serum and clearly represents the intracellular IgMs demonstrated by Parkhouse & Askonas (1969).

The intermediate-sucrose-density fraction was also coprecipitated with specific anti-μ serum. On sodium dodecyl sulphate–polyacrylamide-gel electrophoresis (Fig. 2*c*) the radioactive protein was localized in the same position as the peak with intermediate mobility observed in immune precipitates of total cell lysates (Fig. 2*a*). On reduction the material was converted into two components with the electrophoretic mobilities of heavy (μ) chains and light chains. It is therefore reasonable to assume that the intermediate peak of radioactivity observed on sucrose-density-gradient centrifugation or sodium dodecyl sulphate–polyacrylamide-gel electrophoresis represents intracellular HL subunit; it is not secreted by the cells.

The heavy-chain/light-chain radioactivity ratio was 6:1 for the HL intermediate after the 10min pulse experiment. Similar treatment of the IgMs peak from the same sucrose density gradient resulted in a heavy-chain/light-chain ratio of 7:1. This observation relates to the size of the light-chain pool and is discussed below.

Neither intracellular IgMs nor HL subunit are degradation products of IgM or IgMs. Purified secreted IgM, intracellular IgMs and intracellular HL subunit were coprecipitated with specific antisera and solubilized in sodium dodecyl sulphate (as described in the Materials and Methods section) without evidence of degradation.

Synthesis and secretion of IgM and free light chains. The results of 'pulse–chase' and continuous-label experiments have been analysed to give separate results for light-chain secretion (Fig. 3) and IgM secretion (Fig. 4). Experimental details and interpretation of the 'pulse–chase' experiment are given in the Materials and Methods section.

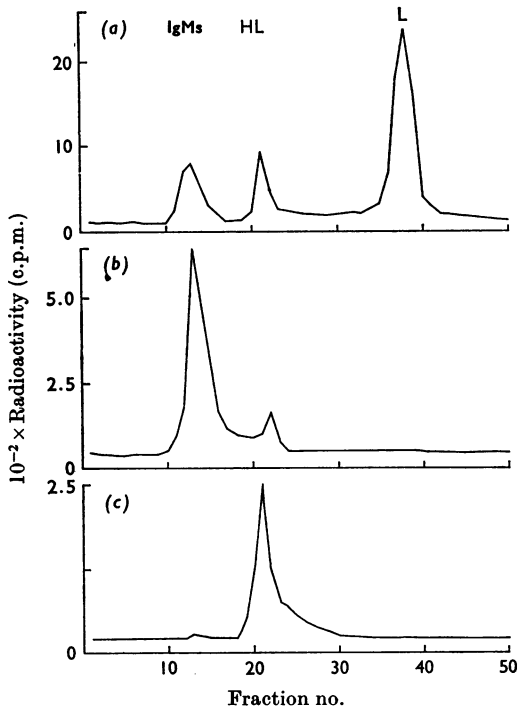

Fig. 2. Polyacrylamide-gel electrophoresis of IgMs, HL subunit and light chain in cell lysates of MOPC 104E tumour. The IgM determinants were precipitated with specific anti-(mouse IgM) and analysed by electrophoresis in 4.25% (w/v) polyacrylamide gels containing sodium dodecyl sulphate as described in the Materials and Methods section. (*a*) MOPC 104E cell lysate after labelling for 30min with [4,5-^{3}H]leucine (40μ Ci/ml at 19.7Ci/mmol); (*b*) the fastest moving peak (IgMs) from the sucrose-gradient separation shown in Fig. 1; (*c*) the intermediate peak (HL subunit) from the sucrose-gradient separation shown in Fig. 1.

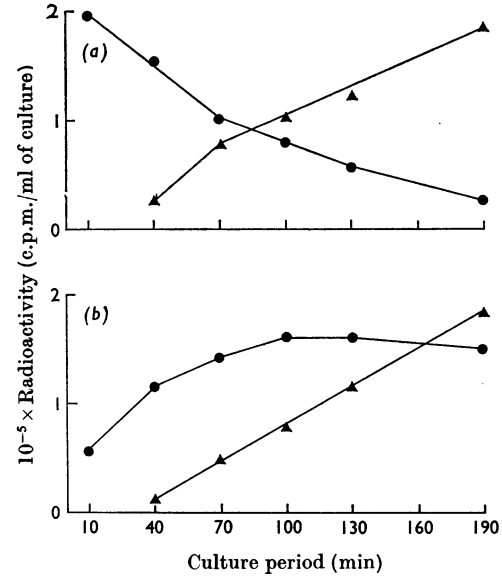

Fig. 3. Secretion of light chains by MOPC 104E tumour. The cells were labelled with [4,5-^{3}H]leucine, and light chain was assayed by electrophoresis of precipitates prepared with specific anti-(mouse IgM) in 4.25% (w/v) polyacrylamide gels containing sodium dodecyl sulphate as described in the Materials and Methods section. The radioactivity is expressed as c.p.m./ml of culture. (*a*) Cells were incubated with radioactive leucine (50μCi/ml at 19.7Ci/mmol) for 10min and then 'chased' with 0.75mg of unlabelled leucine/ml of culture volume ('pulse–chase' experiment). (*b*) Cells were incubated continuously with radioactive leucine (50μCi/ml at 1Ci/mmol) until harvested. ●, Intracellular light chain; ▲, secreted light chain.

Incubation with continuous labelling indicates linear secretion of light chains (Fig. 3*b*) and IgM (Fig. 4*b*) over the period studied, and inferences about the rate of secretion of these molecules from the 'pulse–chase' experiments are therefore justified. Synthesis of IgM and light chains is approx. 20% of total protein synthesis.

For the light chains and IgM both the 'pulse–chase' (Figs. 3*a* and 4*a*) and continuous-label experiments (Figs. 3*b* and 4*b*) show a lag period of 30min before radioactive protein appears in the medium. This lag period before secretion of light chains, IgG or IgM has been well documented (Askonas & White, 1956; Helmreich, Kern & Eisen, 1961; Knopf, Choi & Lennox, 1969; Parkhouse & Askonas, 1969; Melchers, 1970).

Examination of the light-chain results shows equality between amounts of intracellular and extracellular light chain after continuous labelling for 3h (Fig. 3*b*). In consequence, 1.5h represents the average time for synthesis and secretion of a light-chain molecule. An identical conclusion was drawn from the 'pulse–chase' experiment (Fig. 3*a*), where the amounts of intracellular and extracellular light chain were equal after 1.5h. Similar analysis of IgM-secretion results indicates that the average time for synthesis and secretion of an IgM molecule is 2.5h, considerably longer than that for the light chain. Here, the time is given by the point at which amounts of extracellular IgM equals those of intracellular IgMs plus HL intermediate. There was good agreement between the amount of secreted product and decrease of intracellular material. The kinetics are consistent with a precursor–product relationship between intracellular and extracellular light chains, and between intracellular IgMs and extracellular IgM. In addition, after

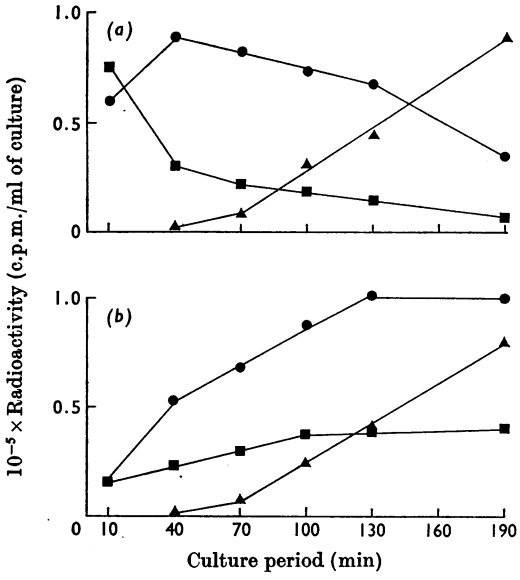

Fig. 4. Secretion of IgM by MOPC 104E tumour. The cells were labelled with [4,5-^{3}H]leucine and HL subunit, IgMs and IgM were assayed by electrophoresis of precipitates prepared with specific anti-(mouse IgM) in 4.25% (w/v) polyacrylamide gels containing sodium dodecyl sulphate as described in the Materials and Methods section. The radioactivity is expressed as c.p.m./ml of culture. The results given in Figs. 3(*a*) and 4(*a*) were derived from the same series of cultures. A separate series of cultures were used for the results shown in Figs. 3(*b*) and 4(*b*). (*a*) Cells were incubated with radioactive leucine (50μCi/ml at 19.7Ci/mmol) for 10min and then 'chased' with 0.75mg of unlabelled leucine/ml of culture volume ('pulse–chase' experiment). (*b*) Cells were incubated continuously with radioactive leucine (50μ-Ci/ml at 1Ci/mmol) until harvested. ■, Intracellular HL; ●, intracellular IgMs; ▲, secreted IgM.

10min with high-specific-radioactivity leucine, the amounts of intracellular IgMs and HL intermediate are approximately equal (Fig. 4*a*). The 'chase' with unlabelled leucine results in a rapid decrease in the relative quantity of the HL intermediate with a compensatory increase in IgMs. A precursor–product relationship between the HL subunit and IgMs is therefore indicated.

Light-chain pool. During the characterization of IgMs and the HL subunit, it was noted that the heavy-chain/light-chain ratio of intracellular IgMs and HL subunit isolated after a 10min pulse was unusually high. The observation was repeated on three separate occasions for a 10min pulse period, giving ratios ranging from 6 to 8, and the obvious interpretation of these results is the existence of a large light-chain pool. Further, when cells were pulsed with radioactive leucine for 3.5min and the intracellular IgMs plus HL subunit was isolated by precipitation with anti-μ serum, reduction revealed a heavy-chain/light-chain ratio of 40. In this and other experiments there was no peak of radioactivity on sodium dodecyl sulphate–polyacrylamide-gel electrophoresis corresponding to the carbohydrate-free heavy-chain intermediate described for the MOPC 104E mouse myeloma by Schubert (1970). Comparison of (1) the natural history of the myeloma and (2) the specificity of the antisera employed in the two laboratories may help to explain this discrepancy.

As an alternative approach for demonstration of the light-chain pool, cell suspensions were incubated with high-specific-radioactivity leucine for 10min and 'chased' with unlabelled leucine as described in the Materials and Methods section. Culture supernatants were harvested at 0–1, 1–2, 2–3, 3–4 and 4–5h after the 'chase', cells in the latter four suspensions being centrifuged down and resuspended in fresh mediumat 1, 2, 3 and 4h respectively. Highly purified IgM, free of light chain, was prepared from each culture supernatant by sucrose-density-gradient centrifugation and then precipitated with anti-μ serum, reduced and alkylated and subjected to electrophoresis on sodium dodecyl sulphate–polyacrylamide gels as described in the Materials and Methods section. The heavy-chain/light-chain radioactivity ratio of each preparation

Table 1. *Heavy-chain/light-chain radioactivity ratio in secreted MOPC 104E IgM*

Cells were incubated for 10min with [4,5-^{3}H]leucine (50μCi/ml at 19.7Ci/mmol), and then 'chased' with 0.75 mg of unlabelled leucine. Culture supernatants were harvested at 0–1, 1–2, 2–3, 3–4 and 4–5h after the 'chase', cells in the latter four suspensions being centrifuged down and resuspended in fresh medium at 1, 2, 3 and 4h respectively. IgM was isolated from the culture supernatants by sucrose-density-gradient centrifugation [5–30% (w/v) sucrose in 130mM-NaCl–4mM-KCl–10mM-sodium phosphate, pH7.4; 41000rev./min ($r_{av.}$ 200000*g*) for 18h in a Spinco SW 41 rotor]. The material was concentrated by precipitation with specific anti-(mouse IgM), reduced and alkylated, and subjected to electrophoresis in 4.25% polyacrylamide gels containing sodium dodecyl sulphate, to measure the heavy-chain/light-chain ratio. Conditions for Expt. 1 and Expt. 2 were identical, but the experiments were done on different days with different cell suspensions.

IgM secreted [time after 'chase' (h)]	Ratio	
	Expt. 1	Expt. 2
0–1	8.8	8.2
1–2	4.0	3.6
2–3	2.9	2.9
3–4	2.5	2.6
4–5	2.5	2.3

was obtained from the electrophoretograms, and results are given in Table 1. The ratio in the earliest secreted IgM (0–1h 'chase' period) is 8.2–8.8 and with continued secretion the ratio progressively diminishes, to 2.3–2.5 for material collected during the 4–5h period.

Relevant to these findings was the fact that out of 27 clones isolated *in vivo*, none deviated from the starting tumour material with respect to the IgM/light chain ratio secreted.

DISCUSSION

It is now apparent that there is no universal order of disulphide-bond formation between the component polypeptide chains of immunoglobulin molecules. In the mouse, immunoglobulin is formed via an HL intermediate in IgG1 (Schubert, 1968; Namba & Hanoaka, 1969), but through an H-H dimer in IgG2a (Askonas & Williamson, 1968; Sutherland, Zimmerman & Kern, 1970; R. M. E. Parkhouse, unpublished work), IgA (Abel & Grey, 1968; Bevan, 1971) and IgG2b (Laskov & Scharff, 1970).

In this paper, evidence is presented to show that the synthesis of murine IgM in the mouse myeloma MOPC 104E proceeds via an HL intermediate. This conclusion was reached by demonstrating the presence of intracellular HL subunits and showing their conversion into intracellular IgMs in the 'pulse–chase' experiment. In confirmation of an earlier suggestion (Parkhouse & Askonas, 1969) a precursor–product relationship was shown to exist between intracellular IgMs and secreted IgM. There was no evidence for appreciable breakdown of either intracellular IgMs, HL subunits or light chain. An identical assembly pathway has been established for IgM secreted by normal mouse lymphoid tissue (R. M. E. Parkhouse, unpublished work). The assembly pathway therefore mirrors the resistance of inter-chain disulphide bonds to reduction (Askonas & Parkhouse, 1971).

The presence of intracellular HL subunits after long labelling periods is perhaps best explained by the lack of non-covalent interaction between HL subunits to form a molecule sedimenting at 7S. When cell lysates were analysed by sucrose-density-gradient centrifugation (Fig. 1), it was apparent that IgMs and HL subunits sedimented as two independent molecular species, the former at about 7S, and the latter significantly slower. Since, therefore, there is no pronounced tendency for non-covalent interaction between HL subunits it is no surprise to find that the equilibrium for the reaction $2HL \rightarrow IgMs$ is not completely in favour of IgMs. Their dimerization would presumably depend on suitable reducing conditions within the cell. Similarly, the interaction between IgMs molecules would be expected to influence the conversion of IgMs into IgM. Here, however, there is an additional controlling factor; the cysteine residues responsible for inter-subunit linkage are apparently blocked (Askonas & Parkhouse, 1971).

For IgG, translation and assembly into the disulphide-linked four-chain molecule occurs in less than 10min (Shapiro, Scharff, Maizel & Uhr, 1966; Askonas & Williamson, 1967*a*,*b*; Fleischman, 1967; Lennox, Knopf, Munro & Parkhouse, 1967). Similarly, completely disulphide-bonded IgMs is found after pulse labelling for 3.5min, and therefore its assembly presumably occurs primarily in the rough endoplasmic reticulum. However, some intracellular HL subunits are found after prolonged periods of labelling (hours), and therefore a small proportion of IgMs assembly within the smooth membranes of the cell cannot be excluded. Since polymerization of IgMs into the pentamer takes place shortly before, or simultaneously with, secretion of the molecules (Parkhouse & Askonas, 1969) it is possible that the polymerization process occurs at the level of the plasma membrane.

The myeloma tumour used for these experiments secretes both IgM and light chains, and it was therefore possible to measure the secretion times of both molecules simultaneously within the same series of cell suspensions. The average time for secretion of a light chain, 1.5h, was found to be in excellent agreement with a previous result for light chain (Knopf *et al.* 1969) whereas the value for IgM, 2.5h, was appreciably longer and close to a value given for IgG (Melchers, 1970). An explanation for this difference is not immediately clear.

In normal lymphoid tissue there is a balanced synthesis of heavy and light chains so that the only secretory product of the cell is fully assembled IgG (Askonas & Williamson, 1967*b*). With the myelomas, however, there is often a departure from balanced synthesis, as evidenced by the secretion of free light chain in addition to assembled immunoglobulin (Shapiro *et al.* 1966; Schubert & Cohn, 1968; Laskov & Scharff, 1970; and the present paper). In such cases is there unbalanced synthesis of heavy and light chains within one cell or, alternatively, are there two cell populations, one normally balanced and secreting fully assembled immunoglobulin, and the other secreting exclusively light chain? For tumour MOPC 104E the latter alternative has been suggested (Schubert & Horibata, 1968), the gradual loss of IgM-secreting potential that occurs on repeated transplantation being attributed to overgrowth of the tumour by light-chain-secreting variants. Results presented in the present paper, on the other hand, argue that the MOPC 104E tumour contains cells in which light chains are synthesized in excess and subsequently

secreted. Although none of the clones isolated *in vivo* were karyotyped, the fact that 27 isolated clones exhibited the same secretory pattern as the original starting cell suspension suggests that most cells are secreting both IgM and light chain. A similar conclusion was reached for MPC 11, an IgG1-secreting myeloma in which secretion of excess of light chain was also noted in isolated clones (Laskov & Scharff, 1970). Further, the heavy-chain/light-chain radioactivity ratios observed for secreted IgM in the 'pulse–chase' experiment are indicative of a very large pool of light chains, and this is taken as evidence for imbalanced synthesis ot light and heavy chains within one cell. In an IgG-synthesizing myeloma where balanced synthesis occurs, the heavy-chain/light-chain radioactivity ratio is approximately the same as the heavy-chain/light-chain weight ratio after 10min labelling (Williamson & Askonas, 1968; R. M. E. Parkhouse, unpublished work). The same is true for IgM synthesis in normal mouse lymphoid tissue (R. M. E. Parkhouse, unpublished work). By comparison, then, the situation in tumour MOPC 104E represents a deviation from the normal situation, in terms of production of excess of light chains, resulting in a large light-chain pool. To explain the gradual conversion of the IgM-secreting tumour into one secreting exclusively light chains it is only necessary to postulate a progressive suppression of heavy chain synthesis.

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