The Vectorial Release of Nascent Immunoglobulin Peptides

By MICHAEL J. BEVAN

National Institute for Medical Research, London N.W.7, U.K.

(Received 2 September 1970)

A microsomal preparation from a mouse plasmacytoma, MOPC 47A, that secretes immunoglobulin A was used to study the release of nascent immunoglobulin peptides *in vitro*. Nascent chains were released with puromycin and characterized with specific antiserum against the immunoglobulin product of the tumour. When the tissue had been prelabelled with [^3H]leucine the experiments were complicated by the large background of completed radioactive polypeptides in the microsomal preparation. Up to one-third of the released radioactivity in the microsomal preparation could be recognized as immunoglobulin. With [^3H]puromycin as the radioactive label, however, the results are much easier to interpret, although the proportion of released radioactivity that can be identified as immunoglobulin is lower (up to one-tenth). Both types of experiment demonstrate that all of the recognizable nascent immunoglobulin chains remain in association with the microsomal vesicles after release from the ribosomes.

A secretory tissue such as rat liver probably contains two functionally distinct ribosome populations (Campbell, 1970). A protein such as serum albumin, that is destined to be secreted from the cell, is synthesized preferentially by ribosomes attached to the endoplasmic reticulum whereas ferritin, a protein that is retained inside the cell, is synthesized mainly on free ribosomes unattached to membranes (Takagi & Ogata, 1968; Redman, 1969; Hicks, Drysdale & Munro, 1969; Ganoza & Williams, 1969). The nascent, secretory protein on the membrane-bound ribosomes, when released *in vitro*, either spontaneously (Redman, Siekevitz & Palade, 1966) or on the addition of puromycin (Redman & Sabatini, 1966; Redman, 1967), has been shown to remain associated with the microsomal fraction, probably within the microsomal vesicle, which is the equivalent of the cisternal space *in vivo*. The bound ribosomes of rat liver may also synthesize intracellular enzymes (Pitot *et al.* 1969; Ragnotti, Lawford & Campbell, 1969) and release them to the outside of the microsomal vesicles (Pitot *et al.* 1969). The membrane-bound ribosomes of rat brain, a non-secretory tissue, also appear to release the growing peptide chains to the outside of the vesicles (Andrews & Tata, 1968).

Immunoglobulin is virtually the sole secretory product of mature plasma cells and most plasma-cell tumours. Methods have been devised in this and other laboratories for determining quantitatively the amount of immunoglobulin present in cell lysates; by using a mouse plasma-cell tumour as a model system I have attempted to measure the fraction of nascent peptides released into the micro-

somal vesicles by puromycin treatment that can be identified as immunoglobulin. The difficulties inherent in characterizing puromycin-released peptides pulse-labelled with [^3H]leucine were overcome by using [^3H]puromycin as radioactive label, in which case all of the incorporated radioactivity is in the form of puromycin-peptides.

The results are in accord with the previous studies on liver and pancreas, demonstrating that the immunoglobulin chains remain associated with the microsomal vesicles after release from the ribosomes. However, a large proportion of the trichloroacetic acid-insoluble puromycin-peptide chains that are released to the microsomal vesicles are not identifiable as immunoglobulin. This is in part due to limitations of the method of characterizing nascent chains, but may also be taken as evidence that other, non-immunoglobulin, polypeptides are synthesized by membrane-bound ribosomes and remain with the rough endoplasmic reticulum, even though they are not subsequently secreted.

MATERIALS AND METHODS

Chemicals. Most chemicals were obtained from British Drug Houses Ltd., Poole, Dorset, U.K., and were of AnalaR grade wherever possible. The nucleoside triphosphates were supplied by P-L Biochemicals Inc., Milwaukee, Wis., U.S.A., and creatine phosphate and GSH by Sigma Chemical Co., St Louis, Mo., U.S.A. Creatine kinase was a product of Worthington Biochemical Corp., Freehold, N.J., U.S.A., and had a specific activity of 50 units/mg. Dithiothreitol was purchased from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K. The non-ionic detergent Nonidet P.40 (Borun, Scharff &

Robbins, 1967) was a generous gift from Shell Chemicals Ltd., London W.C.2, U.K. PBS solution was 0.14M-NaCl in 20 mM-sodium phosphate buffer, pH 7.4.

Radiochemicals. L-[4,5-³H]Leucine (specific radioactivity 17 Ci/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. [O-Me-³H]-Puromycin dihydrochloride (specific radioactivity 1 Ci/mmol) was purchased from New England Nuclear Corp., Boston, Mass., U.S.A.

Animals. The plasma-cell tumour MOPC 47A was kindly sent to us by Dr M. Potter and is maintained in Balb/C mice by subcutaneous transfer of 1 mm³ pieces of tissue. The tumour secretes an immunoglobulin half-molecule of the immunoglobulin A class (Potter & Kuff, 1964). This tumour was used in all the experiments reported here.

The MOPC 47B tumour, which secretes only a light chain of the κ -type that is identical with the light chain in the 47A myeloma protein (Potter & Kuff, 1964), was used to prepare pure light chain from the urine of mice bearing this tumour.

Preparation of 47A myeloma protein. This was prepared by the method described by Askonas & Williamson (1968).

Preparation of 47B light-chain myeloma protein. 47B light chain was prepared from the urine of mice bearing this tumour by precipitation with (NH₄)₂SO₄ at 100% saturation. The precipitate was suspended in water (about 10 mg of protein/ml) and dialysed against 10 mM-sodium phosphate buffer, pH 8.0. It was then eluted from a DEAE-cellulose (Whatman DE 11) column with 10 mM-sodium phosphate buffer, pH 8.0.

Preparation of antisera. Antisera against the purified 47A and 47B myeloma proteins were prepared as described by Parkhouse & Askonas (1969), but in this case the rabbits were 'boosted' with three courses of intravenous injections with alum-precipitated protein over a period of 12 months. The titre of the anti-47A serum was 9.0 mg of antibody/ml of serum and that of the anti-(light chain) serum was 4.4 mg of antibody/ml of serum.

Labelling with [³H]leucine. The 13–15-day-old MOPC 47A tumours were excised from mice killed by cervical dislocation and then cut into 1–2 mm³ pieces in cold Eagle's medium minus leucine. The pieces were placed in Falcon Petri dishes in the same medium and incubated at 37°C in an atmosphere of 5% CO₂ in air for 10 min before the addition of [³H]leucine to a final concentration of 100 μ Ci/ml. The incubation was continued for 4 min at 37°C; then the radioactive medium was pipetted off and the tissue centrifuged through 50 ml of ice-cold Eagle's medium.

Preparation of microsomal fraction. About 1 g of tissue, either after incubation with [³H]leucine or without in the [³H]puromycin experiments, was homogenized in 6 ml of medium in an ice-water bath with a Potter homogenizer (all-round clearance 0.16 mm). The homogenizing medium contained (final concentrations) 0.44 M-sucrose, 0.1 mM-MgCl₂ and mM-KCl in 1 mM-tris-HCl buffer, pH 7.4. The homogenate was centrifuged at 6500 rev./min (5000 *g*_{av}) for 10 min in the Sorval SS 34 rotor at 4°C. The supernatant was collected and the Mg²⁺ concentration raised to 1 mM by addition of a concentrated solution of MgCl₂. This was then layered over 2.5 ml of 0.55 M-sucrose in the same ionic medium and centrifuged at 27000 rev./min (50000 *g*_{av}) for 45 min in a Spinco no. 40 rotor at 4°C.

Incubation of the microsomal preparation with puromycin. The pellet from the above centrifugation was rinsed and resuspended in 0.44 M-sucrose in medium containing (final concentrations) 5 mM-MgCl₂ and 50 mM-KCl in 50 mM-tris-HCl buffer, pH 7.4. The suspension was incubated with shaking at 37°C for 15 min with ATP (0.7 mM), GTP (0.17 mM), GSH (13 mM), creatine phosphate (7 mM) and creatine kinase (1.1 units/ml). This preincubation resulted in a more homogeneous suspension and allowed some disputed vesicles to 'heal' before addition of puromycin.

With the [³H]leucine-labelled microsomal preparation duplicate samples of the microsomal suspension, each containing about 0.33 mg of RNA, were further incubated at 37°C for 10 min either with or without puromycin at a final concentration of 0.2 mM.

When [³H]puromycin was to be used two samples of an unlabelled microsomal suspension after preincubation were each incubated for 15 min at 37°C with 2 μ Ci of [³H]puromycin at a final concentration of 4 μ M. After the puromycin incubation the suspensions were centrifuged at 105000 *g*_{av} for 60 min in cellulose nitrate tubes (2 ml) in a Spinco no. 40 rotor at 4°C. The resultant supernatant representing the incubation medium was collected and the pellet rinsed and resuspended in PBS solution containing 1% (w/v) of Nonidet P.40 and 5 mM-MgCl₂. The tubes were left for 15 min on ice and then centrifuged once more at 105000 *g*_{av} for 60 min. This supernatant, representing the proteins from within the microsomal vesicles and the solubilized membranes, was collected and the pellet of ribosomes was rinsed with PBS solution before resuspension in 0.3 M-NaOH and precipitation with 10% (w/v) trichloroacetic acid.

Analysis of the radioactive proteins. Samples of each supernatant representing the incubation medium or the microsomal vesicles were taken to determine the radioactivity in total acid-insoluble protein and in material specifically reacting with antisera prepared against the immunoglobulin product of the tumour.

Total protein radioactivity was determined by precipitation with 10% (w/v) trichloroacetic acid in the presence of carrier bovine serum albumin. These precipitates and the ribosomal pellets were washed three times by dissolution in 0.3 M-NaOH and reprecipitation with 10% (w/v) trichloroacetic acid. Heating at 90°C for 20 min in 5% (w/v) trichloroacetic acid did not decrease the radioactivity in these precipitates.

Larger samples of the supernatants each representing about one-sixth of the volume were used to determine the radioactivity in immunoglobulin. An indirect precipitation procedure was used to facilitate the precipitation of short peptides. The samples were diluted at least three-fold with PBS solution, and two antisera from hyper-immunized rabbits were added, one prepared against purified urinary 47A protein and one against purified urinary 47B light chain. Sufficient antisera were used to precipitate all of the immunoglobulin present, as tested by the recovery of added radioactive immunoglobulin. When attempts were made to inhibit the precipitation of light chain not associated with heavy chain, a known excess of purified urinary light chain was also added. The mixture was incubated at 37°C for 20 min. The rabbit antibody was precipitated with an excess of horse anti-(rabbit immunoglobulin G) serum. Control precipitates

were made by using normal rabbit serum in place of the hyperimmune rabbit sera. The precipitates were left overnight at 4°C and then washed three times with cold PBS solution. The radioactivity in the control precipitates was subtracted from that in the specific antiserum precipitates to determine the amount specifically identifiable as immunoglobulin.

Determination of radioactivity. Each washed protein precipitate was dissolved in 0.3 ml of formic acid and its radioactivity counted at 10% efficiency in Kinard's (1957) scintillation fluid in a Packard Tri-Carb scintillation counter.

Polyacrylamide-gel electrophoresis. The disc-gel electrophoretic system described by Summers, Maizel & Darnell (1965) was used. Immune precipitates were washed in PBS solution and then dissolved in 2% (w/v) sodium dodecyl sulphate containing 0.1 M-iodoacetamide at 100°C for 1 min before electrophoresis (Laskov & Scharff, 1970).

Ethylene diacrylate was used as the cross-linking reagent. After electrophoresis the gels were sliced and dissolved, and their radioactivities counted in Kinard's scintillation fluid (Choules & Zimm, 1965).

Measurement of RNA. RNA in the microsomal fraction was determined by the method of Fleck & Munro (1962).

RESULTS

MOPC 47A tumour. After labelling of cell cultures for up to 3 h with [³H]leucine the extracellular secreted material is over 90% precipitable with antisera prepared against purified urinary MOPC 47A immunoglobulin. Polyacrylamide-gel electrophoresis of the secreted radioactive material in the presence of sodium dodecyl sulphate and urea demonstrates a single radioactivity peak in the position of an immunoglobulin half-molecule (H-L). Thus, unlike most other mouse immunoglobulin A myeloma proteins (Abel & Grey, 1968), this particular one does have covalently linked heavy chains and light chains (Seki, Appella & Itano, 1968).

A pool of free light chain has been shown to exist within plasma cells by a number of workers (Askonas & Williamson, 1966a; Shapiro, Scharff, Maizel & Uhr, 1966). In the 5563 myeloma the light-chain pool acts as an intermediate in the assembly of the

final 7S molecule (Williamson & Askonas, 1968). The pool of completed free light chain in the MOPC 47A tumour was determined after pulse-labelling of a cell suspension with [³H]leucine for 4 and 10 min. The results shown in Table 1 demonstrate that a small pool of free light chain does exist in these cells. Thus at 4 min the light-chain pool accounts for 23% of the total intracellular immunoglobulin and at 10 min this has fallen to 9%. These values show that the light-chain pool in MOPC 47A tumour cells is much smaller than in 5563 ascites cells, where at 4.5 min the light-chain pool is 42% of the intracellular immunoglobulin and at 10 min has fallen to 29% (Askonas & Williamson, 1966a).

The specificity of the precipitation of intracellular immunoglobulin from cellular and microsomal lysates was checked by electrophoresis of immune precipitates dissolved in 2% (w/v) sodium dodecyl sulphate (Laskov & Scharff, 1970) (Fig. 1). All of the peaks, including the shoulder of the H-L peak, are completely reduced to heavy chains and light chains with 50 mM-dithiothreitol.

Puromycin experiments with [³H]leucine-labelled microsomal preparation. Pieces of myeloma tissue were used in the experiments involving the preparation of the microsomal fraction because of the difficulty of homogenizing a cell suspension without damaging the microsomal vesicles. After pulse-labelling of the tissue in culture for 4 min with [³H]leucine, the percentage of the trichloroacetic acid-insoluble radioactivity present in the microsomal fraction that was released from the ribosomes by puromycin varied between 11 and 23% in separate experiments. In Table 2 the results of a typical experiment are presented, showing the distribution of radioactivity in the fractions from microsomal preparations incubated with and without puromycin. The radioactivity found in the incubation medium of the microsomal preparation not treated with puromycin represents soluble protein carried down with the microsomal fraction from the homogenate and also material released from the vesicles during resuspension and incubation. Microsomal preparations that were kept on ice

Table 1. *Relative amounts of radioactive immunoglobulin (H-L) and free light chain in MOPC 47A cells after various times of pulse-labelling with [³H]leucine*

Cytoplasm was prepared as described by Parkhouse & Askonas (1969) from a suspension of 47A tumour cells labelled with [³H]leucine. Samples of the cell lysate were precipitated either with trichloroacetic acid or with rabbit anti-47A serum in the presence and in the absence of excess of purified light chain.

Time of pulse-labelling with [³ H]leucine (min)	...	...	4	10
Total acid-insoluble radioactivity (%)	...	...	100	100
Specifically precipitated immunoglobulin in the absence of excess of light chain (%)	...	...	18.1	18.8
Specifically precipitated immunoglobulin in the presence of excess of light chain (%)	...	...	13.9	17.2

Table 2. *Effect of puromycin on the distribution of radioactivity in the microsomal fraction after pulse-labelling of slices of tissue for 4 min with [^3H]leucine*

Microsomal fractions were prepared from slices of tumour tissue pulse-labelled *in vitro* with [^3H]leucine for 4 min; samples, each containing about 0.33 mg of RNA, were incubated at 37°C with an energy source in the presence and in the absence of puromycin. After incubation the microsomal suspensions were subfractionated into incubation medium, microsomal vesicles and ribosomal pellet.

	Microsomal fraction incubated without puromycin		Microsomal fraction incubated with puromycin	
	Radioactivity (c.p.m.)	% of total radioactivity	Radioactivity (c.p.m.)	% of total radioactivity
Incubation medium:				
Trichloroacetic acid-insoluble	1532	11.6	2438	18.1
Specific antisera precipitation	193	1.46	230	1.71
Control antisera precipitation	105	0.79	141	1.05
Microsomal vesicles:				
Trichloroacetic acid-insoluble	5198	39.3	6606	49.0
Specific antisera precipitation	1895	14.34	2151	15.96
Control antisera precipitation	188	1.42	184	1.37
Ribosomal pellet:				
Trichloroacetic acid-insoluble	6482	49.1	4434	32.9
Total trichloroacetic acid-insoluble	13212	100	13478	100

and not incubated at 37°C contained less radioactivity in this supernatant. Over 95% of the immunoglobulin, however, remains associated with the microsomal vesicles.

Fig. 1. Polyacrylamide-gel-electrophoresis pattern of material precipitated from a radioactive microsomal lysate by anti-immunoglobulin sera. Slices of myeloma tissue were pulse-labelled with [^3H]leucine for 4 min. The microsomal fraction was lysed with 1% Nonidet P.40 and precipitated with rabbit antiserum to the immunoglobulin secreted by this tumour (see the Materials and Methods section). The precipitate was washed with PBS solution and dissolved in 2% (w/v) sodium dodecyl sulphate (Laskov & Scharff, 1970) before electrophoresis on a 7.5% polyacrylamide gel containing sodium dodecyl sulphate and urea. Partially reduced secreted 47A immunoglobulin was subjected to electrophoresis in a parallel gel to serve as a marker for the immunoglobulin half-molecule (H-L) and for the heavy chains (H) and light chains (L).

The effect of adding puromycin in this experiment was to release 16.2% of the total microsomal radioactivity from the ribosomes. The distribution of this released material between the incubation medium and the microsomal vesicles is shown more clearly in Table 3, which presents the range of variation in three separate experiments. The increase in radioactivity specifically identifiable as immunoglobulin occurred almost wholly, and in one experiment completely, in the lysate of the microsomal vesicles. Very little or no increase was found in the material specifically precipitable as immunoglobulin in the incubation medium. One-third or more of the released material insoluble in

Table 3. *Distribution of [^3H]leucine-labelled nascent chains released from ribosomes by puromycin*

The results are expressed as the percentages of acid-insoluble radioactivity released from the ribosomes by puromycin treatment, and are given as the ranges of variation observed in three separate experiments. The radioactivity in the control precipitates was deducted to give the results for specifically precipitated immunoglobulin.

	Distribution (%)	
	Trichloroacetic acid-insoluble	Specifically precipitated immunoglobulin
Released into the incubation medium	32-44	0.0- 1.5
Released into the microsomal vesicles	56-68	10.3-17.4

Table 4. *Distribution of [³H]puromycin radioactivity incorporated in vitro by microsomal fraction*

Unlabelled microsomal fraction was incubated at 37°C for 15 min with an energy source (see the Materials and Methods section) and 2 μ Ci of [³H]puromycin. The microsomal fraction was then subfractionated. Duplicate samples were used, each containing about 0.33 mg of RNA, and duplicate determinations of radioactivity were made on each subfraction.

	Radioactivity (c.p.m.)	% of total radioactivity
Incubation medium:		
Trichloroacetic acid-insoluble	4103	18.0
Specific antibody precipitation	505	2.22
Control antibody precipitation	515	2.26
Microsomal vesicles:		
Trichloroacetic acid-insoluble	14827	65.1
Specific antibody precipitation	1997	8.78
Control antibody precipitation	607	2.67
Ribosomal pellet:		
Trichloroacetic acid-insoluble	3845	16.9
Total trichloroacetic acid-insoluble	22775	100

Table 5. *Distribution of [³H]puromycin-labelled peptides released from the ribosomes*

The values in parentheses represent the ranges of variation observed in five experiments similar to that described in Table 4. The radioactivity in the control antibody precipitates was deducted to give the results for specifically precipitated immunoglobulin.

	Distribution (%)	
	Trichloroacetic acid-insoluble	Specifically precipitated immunoglobulin
Released into the incubation medium	23 (18-29)	0.07 (0.0-0.25)
Released into the microsomal vesicles	77 (71-82)	4.57 (2.4-7.0)

10% trichloroacetic acid, however, was found in the incubation medium of the microsomal preparation. This difference in distribution between total radioactivity released and that which is precipitated by antisera can be accounted for by the presence in the microsomal fraction of free ribosomes that are predominantly synthesizing proteins other than immunoglobulin (Sherr & Uhr, 1970).

Experiments with [³H]puromycin. Whereas after pulse-labelling with [³H]leucine only about 16% (Table 2) of the microsomal radioactivity is released from the ribosomes by puromycin, when an unlabelled microsomal preparation is incubated with [³H]puromycin all of the incorporated radioactivity is in the form of puromycin-peptides, and about 80% of this is released from the ribosomes (Table 4). The 17% of radioactivity remaining in the ribosomal pellet in this experiment could be nascent protein incorporated into the ribosomes, or more probably small peptides that stick to the ribosomes. About

23% of the released radioactivity (Table 5) is found in the incubation medium and the remainder is released from the microsomal fraction only after treatment with detergent. But again all or almost all of the puromycin-peptides that could be identified as immunoglobulin remained with the microsomal fraction after release from the ribosomes.

I have attempted to demonstrate the presence of free light-chain nascent peptides in the microsomal lysate after labelling with [³H]puromycin. A known excess of purified urinary 47B light chain was added to the mixture of microsomal lysate and specific rabbit antisera to inhibit precipitation of any light chains not associated with heavy chains. However, no decrease in the percentage of precipitable peptides was demonstrable. The same result was obtained when the lysate had been passed through a small Sephadex G-25 column in PBS solution to remove most of the detergent and possibly some small inhibitory peptides.

DISCUSSION

The results demonstrate that all of the puromycin-released nascent chains recognizable as immunoglobulin precursors remain associated with the microsomal vesicles after detachment from the ribosomes. A localization of the immunoglobulin in plasma cells to the cisternae of the rough endoplasmic reticulum or to the microsomal fraction has been shown previously both by electron microscopy (Rifkind, Osseman, Hsu & Morgan, 1962; de Petris & Karlsbad, 1965) and by biochemical methods (Swenson & Kern, 1967; Askonas & Williamson, 1967). In the experiments reported here in Tables 1 and 2 it is shown that the myeloma protein accounts for 18 and 33% respectively of the radioactive protein in a total cytoplasmic lysate

and a microsomal lysate after pulse-labelling with [^3H]leucine. The present findings show that immunoglobulins are present discretely in the cisternae as a result of predetermined vectorial release of the heavy chains and light chains.

The use of [^3H]puromycin as opposed to pre-labelling peptides with [^3H]leucine yields much clearer results in this system; pulse-labelling of cell cultures for 4 min with [^3H]leucine results in a large background of completed radioactive polypeptide chains in the microsomal vesicles. Shortening the labelling period with [^3H]leucine increased the fraction of radioactivity released by puromycin, but decreased the actual amount of radioactivity released since uniform labelling of the nascent chains had not been reached before 4 min. With [^3H]leucine-labelled polypeptide chains, the amount of radioactivity released from ribosomes that can be precipitated with trichloroacetic acid or antiserum will depend on the number of radioactive amino acids in the precipitate. However, when the released chains are labelled only with [^3H]puromycin the radioactivity is proportional to the number of puromycin-peptide chains irrespective of their length. Thus, from Table 3, about one-third to one-sixth of the radioactive amino acids released from ribosomes into the microsomal vesicles by puromycin, which are in acid-insoluble form, can be identified as immunoglobulin. The experiments with [^3H]puromycin (Table 5) show that only about one-tenth to one-thirtieth of the puromycin-peptide chains can be identified as immunoglobulin. This difference is in the direction one would expect if the antiserum can only recognize and precipitate immunoglobulin chains above a certain length.

A more strict comparison between the results obtained with the two radioactive compounds is not justifiable. First, different concentrations of puromycin and different incubation times were used in the two sets of experiments. The small amount of radioactivity released by puromycin after pre-labelling the tissue with a radioactive amino acid ruled out the possibility of a double-labelling experiment. Secondly, the release of [^3H]leucine-labelled peptides might be exaggerated by the presence of completed radioactive immunoglobulin chains specifically bound to nascent chains; attachment of either light chains or heavy chains with nascent heavy chains might occur (Shapiro *et al.* 1966; Schubert, 1968; Askonas, Williamson & Awdeh, 1969). Thirdly, the results obtained with [^3H]puromycin do not reflect possible differences in translation rates between mRNA molecules; any such differences would influence the relative [^3H]leucine labelling of each type of polypeptide chain.

The fraction of the acid-insoluble radioactivity released into the microsomal vesicles not identifi-

able as immunoglobulin could comprise both small, unrecognizable, immunoglobulin chains and nascent chains of other polypeptides. Although the procedure for detecting nascent immunoglobulin peptides was designed to maximize specific precipitation, there will inevitably be a proportion of such chains that will be missed. Shorter peptides probably lack sufficient conformation to exhibit antigenicity; it is also possible that the *N*-terminal regions of the immunoglobulin are not immunogenic when antisera are raised in rabbits against whole immunoglobulin molecules. Even allowing for these factors, the vectorial release probably includes nascent chains of non-secretory proteins. Non-immunoglobulin polypeptides that are not destined to be secreted may be incorporated into the microsomal membrane (Ragnotti *et al.* 1969), or may be lysosomal or mitochondrial enzymes (Campbell, 1970).

Failure to specifically demonstrate that light-chain puromycin-peptides are present in the anti-immunoglobulin precipitates is not conclusive. The experiment may be technically difficult, since the light chain will probably have fewer antigenic determinants than the heavy chain; it is therefore possible that the antiserum (though with high Ab titres) can recognize only those light-chain polypeptides that are almost completed. Even if this is not the case one would still expect a larger fraction of light-chain peptides as compared with heavy-chain nascent peptides to be below the minimum size that is recognizable by antiserum. The actual amount of radioactivity detectable in light-chain puromycin-peptides is therefore likely to be fairly low. The molecular weight of the light chain is about 22000 and that of the 47A heavy chain has been estimated to be 40000 (Seki *et al.* 1968). Thus, if there are equal numbers of mRNA molecules for heavy chain and light chain with ribosomes equally spaced out on them, there will be approximately twice as many nascent heavy chains as light chains. The biosynthesis of the complete heavy chains and light chains on separate size classes of polyribosomes has been demonstrated by a number of workers (Askonas & Williamson, 1966b; Williamson & Askonas, 1967; Shapiro *et al.* 1966; Becker & Rich, 1966). Since it is the *N*-terminal region of the heavy chain that interacts with light chain, the nascent heavy-chain peptides may be released in sufficient numbers to interact with the pool of completed light chain and with any light-chain peptides released. I have shown that the free light-chain pool in this tumour is small in comparison with the one in the 5563 ascites tumour. Moav & Harris (1970) have reported their failure to demonstrate light-chain peptides in the material released from ribosomes by the action of puromycin on intact rabbit lymph-node cells.

It is a pleasure to acknowledge the assistance I have received during this work from Dr Brigitte Askonas and Dr Alan Williamson. For the mouse plasma-cell tumour lines I am indebted to Dr M. Potter.

REFERENCES

- Abel, C. A. & Grey, H. M. (1968). *Biochemistry, Easton*, **7**, 2682.
- Andrews, T. M. & Tata, J. R. (1968). *Biochem. biophys. Res. Commun.* **33**, 515.
- Askonas, B. A. & Williamson, A. R. (1966a). *Nature, Lond.*, **211**, 369.
- Askonas, B. A. & Williamson, A. R. (1966b). *Proc. R. Soc. B*, **166**, 232.
- Askonas, B. A. & Williamson, A. R. (1967). *Cold Spring Harb. Symp. quant. Biol.* **32**, 223.
- Askonas, B. A. & Williamson, A. R. (1968). *Biochem. J.* **109**, 637.
- Askonas, B. A., Williamson, A. R. & Awdeh, Z. (1969). *FEBS Symp.* no. 15, p. 105.
- Becker, M. J. & Rich, A. (1966). *Nature, Lond.*, **212**, 142.
- Borun, T. W., Scharff, M. D. & Robbins, E. (1967). *Biochim. biophys. Acta*, **149**, 302.
- Campbell, P. N. (1970). *FEBS Lett.* **7**, 1.
- Choules, G. L. & Zimm, B. H. (1965). *Analyt. Biochem.* **13**, 366.
- de Petris, S. & Karlsbad, G. (1965). *J. Cell Biol.* **26**, 759.
- Fleck, A. & Munro, H. W. (1962). *Biochim. biophys. Acta*, **55**, 571.
- Ganoza, M. C. & Williams, C. A. (1969). *Proc. natn. Acad. Sci. U.S.A.* **63**, 1370.
- Hicks, S. J., Drysdale, J. W. & Munro, H. N. (1969). *Science, N.Y.*, **164**, 584.
- Kinard, F. E. (1957). *Rev. scient. Instrum.* **28**, 293.
- Laskov, R. & Scharff, M. D. (1970). *J. exp. Med.* **131**, 515.
- Moav, B. & Harris, T. N. (1970). *J. Immun.* **104**, 965.
- Parkhouse, R. M. E. & Askonas, B. A. (1969). *Biochem. J.* **115**, 163.
- Pitot, H. C., Sladek, N., Ragland, W., Murray, R. K., Moyer, G., Soling, H. D. & Jost, J.-P. (1969). In *Microsomes and Drug Oxidation*, p. 59. Ed. by Gillette, J. R., Conney, J. H., Cosmides, G. J., Estabrook, R. W., Foats, J. R. & Mannering, G. J. New York and London: Academic Press.
- Potter, M. & Kuff, E. L. (1964). *J. molec. Biol.* **9**, 537.
- Ragnotti, G., Lawford, G. R. & Campbell, P. N. (1969). *Biochem. J.* **112**, 139.
- Redman, C. M. (1967). *J. biol. Chem.* **242**, 761.
- Redman, C. M. (1969). *J. biol. Chem.* **244**, 4308.
- Redman, C. M. & Sabatini, D. D. (1966). *Proc. natn. Acad. Sci. U.S.A.* **56**, 608.
- Redman, C. M., Siekevitz, P. & Palade, G. E. (1966). *J. biol. Chem.* **241**, 1150.
- Rifkind, R. A., Osserman, E. F., Hsu, K. H. & Morgan, C. (1962). *J. exp. Med.* **116**, 423.
- Schubert, D. (1968). *Proc. natn. Acad. Sci. U.S.A.* **60**, 683.
- Seki, T., Appella, E. & Itano, H. A. (1968). *Proc. natn. Acad. Sci. U.S.A.* **61**, 1071.
- Shapiro, A. L., Scharff, M. D., Maizel, J. V. & Uhr, J. W. (1966). *Proc. natn. Acad. Sci. U.S.A.* **56**, 216.
- Sherr, C. J. & Uhr, J. W. (1970). *Proc. natn. Acad. Sci. U.S.A.* **66**, 1183.
- Summers, D. F., Maizel, J. V. & Darnell, J. E. (1965). *Proc. natn. Acad. Sci. U.S.A.* **54**, 505.
- Swenson, R. M. & Kern, M. (1967). *Proc. natn. Acad. Sci. U.S.A.* **57**, 417.
- Takagi, M. & Ogata, K. (1968). *Biochem. biophys. Res. Commun.* **33**, 55.
- Williamson, A. R. & Askonas, B. A. (1967). *J. molec. Biol.* **23**, 201.
- Williamson, A. R. & Askonas, B. A. (1968). *Archs Biochem. Biophys.* **125**, 401.