2. The main hydrolysis products were 4-*O*-(β -D-glucopyranosyl)-D-ribitol 1-phosphate (III) and its isomeric 2-phosphate (IV). These were separated from each other and their structures determined by periodate oxidation.

3. Minor hydrolysis products included the 1:5- and 2:5-diphosphates (V and VI) of glucosylribitol. The structures assigned to these followed from periodate oxidation. The oxidation product from the 2:5-diphosphate gave a ribitol diphosphate when treated with alkali.

4. The structure previously suggested for this ribitol teichoic acid is confirmed by these studies.

5. Oxidation of the teichoic acid with buffered periodate before and after removal of alanine ester residues has shown that the amino acid is attached to a hydroxyl at either the 2- or 3-position in the ribitol residues.

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Formation of Bence-Jones Protein and Myeloma Protein *in vitro* by the Plasma-Cell Tumour MPC-2

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Malignant diseases of plasma cells are associated with high concentrations of myeloma proteins in the serum or Bence-Jones protein of smaller molecular weight in the urine. Both types of anomalous proteins are often present in cases of multiple myeloma. The origin of these proteins and the relationship of the urinary protein to the larger-molecular-weight myeloma protein has not been certain. Strong evidence has pointed to the malignant plasma cells as the sites of synthesis of serum myeloma protein. Studies both *in vivo* (Nathans, Fahey & Potter, 1958) and *in vitro* (Askonas, 1961) have shown that the mouse-plasma-cell neoplasm 5563 is the site of synthesis of the γ -myeloma protein found in high concentrations in serum of mice bearing the 5563 tumour. Meyer (1957) has reported briefly that bone-marrow

aspirate from a patient with multiple myeloma incorporated radioactive amino acid into the γ -globulin fraction of urine with solubility characteristics of Bence-Jones protein.

Observations on the Bence-Jones proteins of human disease have been reviewed recently by Putnam (1960). In general, urinary Bence-Jones proteins have about one-third to one-quarter the molecular weight of serum myeloma globulins or normal γ -globulins; they are related immunologically to the serum γ -globulins and myeloma proteins and therefore may represent a portion of the larger molecules. This has raised the question whether the Bence-Jones proteins are precursor molecules of the larger-molecular-weight globulins which accumulate in neoplastic cells. Putnam & Miyake (1958) have shown that, after administration of radioactive amino acids to patients with multiple myeloma, the Bence-Jones protein in the urine

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became labelled more rapidly than the serum myeloma protein. The urinary protein was therefore not derived by breakdown of the serum myeloma protein but the incorporation curves were consistent with the view that the two proteins were formed independently or had a precursor-product relationship.

Recently Fahey & Potter (1959) reported that mice carrying plasma-cell tumour line MPC-2 had a β -myeloma protein in the serum (S_{20} , 6.5) and excreted Bence-Jones protein of γ -globulin mobility (S_{20} , 3.4) in the urine. Through the kindness of Dr Potter we obtained the MPC-2 tumour line; this provided us with tissue to study the relationship between the synthesis of the small-molecular-weight Bence-Jones protein and the serum myeloma protein. The Bence-Jones protein and serum myeloma protein were purified by chromatography on anion-exchange diethylaminoethylcellulose. Incorporation of [^{14}C]amino acids into both myeloma protein and Bence-Jones protein by slices of tumour tissue was followed at various time intervals. The malignant plasma cells appeared to form both proteins independently with no evidence for Bence-Jones protein as a precursor. In addition, the pool of Bence-Jones and myeloma protein associated with the tumour tissue was determined by means of isotope dilution methods by using ^{131}I -labelled Bence-Jones and myeloma protein.

EXPERIMENTAL

MPC-2 Tumour. BALB/c mice carrying the MPC-2 tumour were sent to us through the kindness of Dr M. Potter. The tumour was transplanted subcutaneously into the F_1 generation by means of a trocar, by using 3–4 small pieces of tumour, and tumour tissue was utilized for experimentation after about 3–4 weeks when the tumour had grown to a size of 2–4 g.

Purification of myeloma protein and Bence-Jones protein. Myeloma protein was purified from serum in two steps. Serum from mice bearing MPC-2 tumour was concentrated threefold and zone electrophoresis was carried out with polyvinyl chloride (Geon 425, British Geon Ltd., London, W. 1), under the conditions described by Fahey & Horbett (1959). The section of the block containing the β -globulin region was cut out, and the protein eluted. Material from 10–15 ml. of serum was pooled and concentrated to 2–3 ml. by ultrafiltration (Sober, Gutter, Wyckoff & Peterson, 1956). Further purification was carried out on columns of anion-exchange diethylaminoethylcellulose (DEAE-cellulose) (Peterson & Sober, 1956). The electrophoretically prepared β -globulins and myeloma proteins were dialysed against 0.04 M-potassium phosphate buffer, pH 8, and put on a DEAE-cellulose column (5 g.), and the myeloma protein was obtained by elution with a gradient to 0.4 M-potassium phosphate pH 8. The system of gradient elution described by Fahey & Horbett (1959) was employed. A total volume of 150 ml. was used for elution, 95 ml. of initial buffer in a cylindrical mixing chamber and 55 ml. of the final buffer in

a conical reservoir. Fractions of 3 ml. were collected and the elution of the protein was followed by measuring the extinction of the fractions at 280 m μ . Fractions between 52 and 84 % of the effluent volume contained the myeloma protein.

Urine was collected from mice bearing the MPC-2 neoplasm and concentrated by ultrafiltration 10- to 20-fold. The Bence-Jones protein was purified by chromatography on DEAE-cellulose columns with gradient elution as described above. The starting buffer was 0.01 M-potassium phosphate, pH 8, and the limit buffer concentration was 0.30 M-potassium phosphate, pH 8. Bence-Jones proteins were eluted between 0 and 30 % of the effluent volume. The myeloma and Bence-Jones protein fractions, obtained by chromatography, were concentrated by ultrafiltration, dialysed against 0.9 % NaCl-phosphate buffer, pH 7.4, and then characterized by immunoelectrophoresis, starch-gel electrophoresis (Smithies, 1955) and ultracentrifuging. Some of the analytical results are presented in this paper but a detailed characterization, including comparison of fragments obtained by papain digestion, will be reported subsequently (B. A. Askonas & J. L. Fahey, in preparation).

Preparation of antisera. Rabbits were immunized with Bence-Jones protein or myeloma protein purified as described above to obtain specific antisera. The proteins in Freund's adjuvant (Freund & McDermott, 1942) were injected intramuscularly into both thighs at 2 weeks' interval. One month later the rabbits received a course of five intravenous injections of alum-precipitated protein. A total of about 6–8 mg. of protein was given to each rabbit. Serum was collected 1–2 weeks later. The intravenous course could be repeated after 6–8 weeks to obtain more antisera.

Incubation of tumour tissue. Tumour was sliced with scissors into small cubes (about 1–2 mm.) and 300 mg. of tissue (wet weight) was incubated at 37° for various time intervals in 4 ml. of Hanks (1948) balanced salt medium containing the amino acid supplement of Eagle's (1955) medium, 100 units of penicillin and streptomycin/ml. and [^{14}C]amino acids. A hydrolysate of [^{14}C]algal protein (250 μC /mg.) obtained from The Radiochemical Centre, Amersham, Bucks., was used after purification of the amino acids on a Zeo-Karb 225 column as described by Askonas (1961). The incubation mixture was gently rocked and gassed with $\text{O}_2 + \text{CO}_2$ (95:5) to maintain the pH at about 7.4. After incubation, 20 mg. of non-radioactive amino acid was added, the tissue was homogenized in a glass homogenizer with a Teflon pestle, and clear supernatant containing the Bence-Jones and myeloma protein was obtained by centrifuging for 10 min. at 13 000 g in the cold.

Isolation of the Bence-Jones and myeloma proteins from incubation fluid. The above tumour extract and incubation fluid contained small amounts of the two proteins. A large amount of nucleoprotein and tissue protein was removed by adjusting the extract to pH 5.8, centrifuging off the precipitate and re-neutralizing the extract. The myeloma protein and Bence-Jones proteins, which remained in solution, were subsequently separated by chromatography on small columns of DEAE-cellulose (7 cm. $\times$ 0.6 cm.). The supernatant and adsorbent were equilibrated with 0.01 M-potassium phosphate buffer, pH 8, and the protein was applied to the column. Elution was effected as follows:

(A) 0.01M-potassium phosphate, pH 8 (this permits collection of the Bence-Jones protein that is not adsorbed on DEAE-cellulose at pH 8) (fraction A); (B) 0.05M-potassium phosphate, pH 8; (C) 0.4M-potassium phosphate, pH 8 (fraction C). This last effluent contained the myeloma protein. Ten fractions of 2.5 ml. were collected from each step. This method of separation and the recovery of protein were worked out by using Bence-Jones and myeloma proteins labelled with ^{131}I (McFarlane, 1958). Most of the protein (70–80%) was eluted in the three first fractions from the DEAE-cellulose column (Fig. 3, Table 1). Each protein was isolated therefore from the pool of the three main fractions by precipitation with specific antiserum at the equivalence zone.

Precipitation of Bence-Jones protein in eluate A. Although immunization of several rabbits was attempted with purified Bence-Jones protein by repeated courses of injections, the rabbits produced only traces of antibody against the Bence-Jones protein. Bence-Jones protein strongly cross-reacted with antiserum against a γ myeloma protein from a different tumour line [neoplasm 5563 (Potter, Fahey & Pilgrim, 1957)]. Since the amount of Bence-Jones protein in the MPC-2 tumour tissue was very small, it was co-precipitated with 500 μg . of 5563-myeloma protein yielding 3.5 mg. of antibody-antigen precipitate. As is shown in Table 1, 90% of the iodinated Bence-Jones protein was co-precipitated in this way.

Precipitation of myeloma protein from eluate C. A considerable amount of intracellular and extracellular myeloma protein was associated with the tumour slices. Since the antibody/antigen ratio varied in the presence of excess of antibody or antigen, the myeloma protein had to be precipitated at equivalence. In each experiment the amount of myeloma protein present in a sample of the tumour extract was titrated in the presence of traces of ^{131}I -labelled myeloma protein. Samples of eluate C (control incubations) were pipetted into a series of tubes and increasing amounts of antiserum were added. The precipitates were washed and dissolved in 2.5 ml. of 0.1N-NaOH, and the ^{131}I content and protein content determined. In this way the amount of antiserum needed to precipitate the myeloma protein at equivalence point could be determined. By this technique 95% of the ^{131}I -labelled myeloma protein was precipitated at an antibody/antigen ratio of 8:1 (Table 3). In the experiments with the ^{14}C amino acids, portions of eluate C were chosen to yield 3.5–4 mg. of antibody-antigen precipitate.

Determination of radioactivity. The ^{131}I content was determined by counting liquid samples (1–3 ml.) in a scintillation counter with NaI crystal. In a given experiment the volume to be counted and size of tubes were kept constant to provide comparable data.

The ^{14}C -labelled, dried protein powders were counted on 0.3 cm.² disks at infinite thickness on an automatic recording Geiger counter with a thin end window to an accuracy of $\pm 5\%$. Counts were converted into counts/min./cm.².

RESULTS

Purification and characterization of the MPC-2 myeloma and Bence-Jones proteins

The MPC-2 myeloma protein, which migrates in the β -globulin region of serum protein on paper electrophoresis (Fahey & Potter, 1959), was purified from MPC-2 serum by a combination of zone electrophoresis on polyvinyl chloride particle (Geon) blocks and chromatography on columns of DEAE-cellulose. When gradient elution is employed with buffers from 0.04 to 0.40M-potassium phosphate, pH 8, most of the β -globulins are eluted in the first major peak and the myeloma proteins are eluted relatively late in the chromatogram effluent as indicated by the line in Fig. 1. MPC-2 myeloma proteins prepared in this way were compared by starch-gel electrophoresis with normal mouse serum and serum from a mouse with the MPC-2 tumour (Plate 1). Ultracentrifuging of the MPC-2 myeloma protein revealed a major component with a sedimentation constant of 6.4 at a concentration of 0.5 g./100 ml. in 0.15M-NaCl. A small amount of protein, less than 10% of the total, was found in a faster-sedimenting peak (9.5s).

The Bence-Jones protein was purified from concentrated urine by chromatography on columns of DEAE-cellulose with buffers, pH 8, and a gradient from 0.01 to 0.30M-potassium phosphate. The Bence-Jones proteins are eluted very early in the chromatogram effluent (Fig. 2), being slightly or not at all retarded at 0.01M-potassium phosphate

Table 1. *Reaction of antisera with Bence-Jones protein and myeloma protein*

Antiserum	Antigen added			Percentage of ^{131}I -labelled antigen precipitated by antibody (in slight excess)
	Bence-Jones protein	MPC-2 myeloma protein	5563 myeloma protein	
R5 (anti-MPC-2 myeloma protein)	—	+	—	95
R5	+	+	—	38
R5	+	—	—	10
R90 (anti-5563 myeloma protein)	—	—	+	95
R90	+	—	+	85–93
R90	+	—	—	60–70

* Added proteins had been iodinated with ^{131}I by the method of McFarlane (1958).

Fig. 1. Purification of myeloma protein from serum of mice bearing MPC-2 neoplasm. The β fraction of the serum was prepared by zone electrophoresis before chromatography was carried out on DEAE-cellulose columns (Peterson & Sober, 1956) as described in the Experimental section. Gradient elution with 0.04-0.4M-potassium phosphate buffer, pH 8, was used and 3 ml. fractions were collected. The line indicates fractions containing the myeloma protein (see Plate 1).

Fig. 2. Purification of Bence-Jones protein from urine of mice bearing MPC-2 neoplasm. Chromatography was carried out on a column (1.3 cm. $\times$ 10 cm.) of ion-exchange DEAE-cellulose (Peterson & Sober, 1956). Urine was concentrated about tenfold, and dialysed against the starting buffer, 0.01M-potassium phosphate, pH 8. Gradient elution was carried out with 0.3M-potassium phosphate, pH 8, as described in the Experimental section, and 3 ml. fractions were collected. The line on the elution pattern indicates fractions containing the heterogeneous Bence-Jones protein (see Plate 1).

concentration. Three Bence-Jones protein components are discernible by anion-exchange cellulose chromatography or starch-gel electrophoresis (Fig. 2 and Plate 1). These components have been shown to be very closely related (unpublished observations) and in the present study are treated as a single group. Ultracentrifuging of the preparation gave a single symmetrical boundary with an S_{20} of 3.56. Dr Charlwood calculated the molecular weight of the main component to be 45 000 by using the Archibald method. In addition to the ultracentrifugal distinction, the MPC-2 urinary protein differs from the serum β -myeloma protein by migrating in the γ -globulin region on paper electrophoresis and in being eluted at relatively low ionic strength on DEAE-cellulose chromatography. Since the MPC-2 serum myeloma protein requires a considerably higher ionic strength for elution from DEAE-cellulose, this difference has been utilized to separate these two proteins in the biosynthesis studies.

Recovery of Bence-Jones and myeloma proteins after incubation of tumour tissue

The quality of separation and the recovery of individual proteins from the incubation fluid was determined by the use of ^{125}I -labelled samples of previously purified protein. Results of one typical experiment are described in detail.

Chopped tumour tissue (300 mg.) was added to a series of flasks containing incubation medium. Four control flasks were set up without ^{14}C -amino acids; these flasks were used to calculate the pool size of myeloma and Bence-Jones protein. Control flasks 1 and 2 were frozen immediately, and flasks 3 and 4 were incubated for 5 hr. and then frozen. ^{125}I -Labelled myeloma protein (about 0.1 mg.; 5 $\mu\text{Ci}/\text{mg.}$) was added to control flasks 2 and 4 and similar amounts of ^{125}I -labelled Bence-Jones protein were added to control flasks 1 and 3.

Table 2 illustrates the procedures followed to facilitate discussion of the results with the ^{125}I -labelled proteins. Sediments I and II carried down 8-10% of the iodinated proteins and supernatant II, after dialysis, contained 85-90% of the protein. Fig. 3 shows the distribution of radioactivity in the effluent when supernatant II from flasks 1 and 2 was chromatographed by a stepwise elution system on small DEAE-cellulose anion-exchange columns. The upper half of Fig. 3 shows that 90% of the ^{125}I -labelled Bence-Jones protein is eluted with 0.01M-phosphate buffer, pH 8, and that no Bence-Jones protein is in eluate C. In flask 2, to which ^{125}I -labelled myeloma protein had been added, 95% of the ^{125}I -labelled myeloma protein is eluted with 0.4M-phosphate in eluate C; eluate A contains no ^{125}I -labelled myeloma protein. The stepwise elution system thus was shown to have separated the Bence-Jones and myeloma proteins efficiently. In addition, the fractionation was highly reproducible and chromatography of supernatant II of flasks 3 and 4 (incubated for 5 hr.) gave identical patterns.

Starch-gel electrophoresis of MPC-2 myeloma protein. Myeloma protein and Bence-Jones protein were purified by chromatography on DEAE-cellulose (see Figs. 1 and 2). Starch-gel electrophoresis was carried out in 0.03 M 2-amino-2-hydroxymethylpropane-1,3-diol-ethylene-diamine-tetra-acetate borate buffer (Aronsson & Grönwall, 1958).

Table 2. Scheme for isolation of myeloma protein and Bence-Jones protein

Fig. 3. Chromatographic separation of myeloma and Bence-Jones proteins after tissue incubation. Myeloma protein and Bence-Jones protein were separated by chromatography of supernatant II (see Table 1) on the DEAE-cellulose columns by three elution steps with potassium phosphate buffer, pH 8: (A) 0·01 M; (B) 0·05 M; (C) 0·4 M. Fractions (2·5 ml.) were collected. (a) ¹²⁵I-Labelled Bence-Jones protein; (b) ¹²⁵I-labelled myeloma protein. Brackets indicate recovery of the two proteins from the column.

The stepwise elution procedure also concentrated the bulk of the proteins into three effluent fractions: 75% of the Bence-Jones protein in eluate *A*, and 78% of the myeloma protein in eluate *C*. The three fractions in each eluate were pooled and the proteins were isolated from these 7.5 ml. pools to avoid an excessive volume for antibody precipitation. The myeloma protein was then isolated by precipitation with specific antisera, and the Bence-Jones protein obtained by co-precipitation with an antigenically closely related γ myeloma protein as described above. Corrections for recovery were made in estimating the amount of [^{14}C]amino acid incorporated into the two proteins.

Fig. 4. Incorporation of [^{14}C]amino acids into serum myeloma protein and urinary Bence-Jones protein by tumour slices *in vitro*. O, Radioactivity of myeloma protein precipitate; x, radioactivity of Bence-Jones protein precipitate. Minced tumour (300 mg.) was incubated in the presence of 2.5 μC of [^{14}C]algal protein hydrolysate as described in the Experimental section. Equal quantities of antibody-antigen precipitates (3.5 mg.) were isolated after separation of the myeloma and Bence-Jones protein and their specific radioactivities determined.

Incorporation of [^{14}C]amino acids into myeloma protein and Bence-Jones protein

The procedure outlined above was applied to tumour tissue incubated in the presence of [^{14}C]amino acids. Five flasks containing 300 mg. of chopped tumour were incubated for various time intervals (0–5 hr.) in the presence of [^{14}C]amino acids. Subsequently Bence-Jones and myeloma proteins were isolated as described above and the amount of radioactive amino acid incorporated into equivalent amounts of antibody-antigen precipitates was determined. The Bence-Jones and myeloma proteins obtained for ^{14}C measurement included the 0.9% NaCl-soluble proteins within the cells as well as proteins secreted into the incubation medium.

The time curve for incorporation *in vitro* of [^{14}C]amino acid into the two proteins by the tumour tissue is shown in Fig. 4. Both Bence-Jones and myeloma proteins are formed by the tumour tissue. The amount of radioactivity incorporated is linear for both proteins over the period of 5 hr. investigated. The slopes of the time curves are different with no evidence of crossing. This suggests that the two proteins are formed independently at different rates.

Pool size of Bence-Jones and myeloma proteins associated with tumour tissue

A known amount of iodinated myeloma or Bence-Jones protein had been added to the incubation flasks to calculate the pool of protein (which includes intracellular as well as extracellular protein) associated with the tissue by the isotope-dilution method. Table 3 shows the specific radioactivity (due to ^{131}I) of antibody-antigen precipitates when the ^{131}I -labelled proteins are precipitated with antibody in the absence and in the presence of the incubation mixtures after preliminary purification. The added ^{131}I -labelled

Table 3. Amount of Bence-Jones and myeloma protein associated with MPC-2 tumour tissue

^{131}I -Labelled Bence-Jones protein (0.052 mg.) and 0.09 mg. of ^{131}I -labelled myeloma protein (MPC-2) had been added to the incubation fluid containing 300 mg. of tissue. Procedure for isolation of both proteins is described in Table 1 and Fig. 3. MPC-2 myeloma protein was precipitated with specific antiserum. Bence-Jones protein was precipitated with antiserum to 5563 myeloma protein (Table 2).

	Specific radio- activity due to ^{131}I (counts/mg. of protein/100 sec.)	Antibody- antigen ratio	Dilution of ^{131}I -labelled protein
^{131}I -labelled myeloma protein (MPC-2)	2 090 000	—	—
^{131}I -labelled myeloma protein-antibody ppt.	260 000	8	—
^{131}I -labelled myeloma protein-antibody ppt. from eluate <i>C</i> (flask 2)	20 400	—	12.7-fold
^{131}I -labelled Bence-Jones protein	2 200 000	—	—
^{131}I -labelled Bence-Jones protein-antibody ppt.	147 000	15	—
Bence-Jones protein-antibody ppt. from eluate <i>A</i> (flask 1)	44 500	—	3.3-fold

myeloma protein (0.09 mg.) was diluted 12.7-fold by myeloma protein associated with 300 mg. of tissue; hence 1 g. of tumour tissue contains about 3.6 mg. of myeloma protein. Much less Bence-Jones protein, however, is found in the tissue. ^{131}I -Labelled Bence-Jones protein (0.052 mg.) added to 300 mg. of tissue was diluted 3.3-fold; hence 1 g. of tumour tissue contains about 0.40 mg. of Bence-Jones protein, which is about one-ninth of the amount of myeloma protein. For this determination the ^{131}I -labelled Bence-Jones protein was precipitated directly with antiserum R 90 (anti 5563 myeloma protein, cf. Table 1).

Similar values per gram of tissue (0.35 mg. for Bence-Jones and 3.2 mg. for myeloma protein) are obtained when the tissue pools are calculated from the amount of antibody-antigen precipitate obtained (corrected for recovery) by quantitative precipitation of the proteins in eluates *A* and *C*.

DISCUSSION

The results have shown that the urinary Bence-Jones protein (mol.wt. 45 000) and the serum β -myeloma protein (mol.wt. 160 000) originate in the plasma-cell tumour tissue. The progress of incorporation of radioactivity by the tissue into the two proteins is linear over the period of 5 hr. The incorporation curves have different slopes, but no evidence for crossing or a precursor-product relationship was found. The proteins isolated for radioactivity determination included the Bence-Jones and myeloma proteins secreted into the medium as well as the material readily extracted with 0.9% sodium chloride-phosphate from the broken cells. It does not include any of the protein still bound to subcellular particles at the site of synthesis; so that it can only be stated that the readily extractable or extracellular Bence-Jones protein is not a precursor of the myeloma protein, but it could still be an incomplete part of the myeloma protein at the site of synthesis.

The two proteins are formed at different rates. More myeloma protein is synthesized than Bence-Jones protein. This agrees with the findings of Putnam & Miyake (1958) where up to five times as much total radioactivity was incorporated into the myeloma protein as into the Bence-Jones protein by a patient with multiple myeloma. Since the molecular weight of the Bence-Jones protein is 1/3.5 that of serum myeloma protein, approximately two (1.7-2.4) molecules of Bence-Jones protein are formed per molecule of myeloma protein in the tumour tissue. This is an average of two experiments.

Our results are consistent with the findings of Putnam & Hardy (1955) and Putnam & Miyake (1958). The kinetic analysis of their data in patients

with myeloma suggested that Bence-Jones and myeloma protein were formed independently but the possibility that Bence-Jones protein was a precursor could not be excluded. Osserman, Graff, Marshall, Lawlor & Graff (1957) came to the same conclusions with similar experiments. The relatively small amount of Bence-Jones protein observed by us in the tumour tissue pool, and the fact that Bence-Jones protein is not present in serum in detectable amounts, explain the high specific radioactivity of the urinary Bence-Jones protein reported by Putnam & Miyake (1958). The newly formed myeloma protein is diluted by all the myeloma protein in the tissue pool and the serum and would thus be expected to have a much lower specific radioactivity than the Bence-Jones protein, which is obviously cleared very rapidly from the animal by the kidney.

The question as to whether the Bence-Jones and myeloma proteins are formed within the same neoplastic plasma cell or whether different plasma cells of the MPC-2 tumour line secrete the different protein products could not be settled. Originally it had been hoped to study this by staining the tissue with specific fluorescein-labelled antiserum. However, Bence-Jones and MPC-2 myeloma protein cross-react immunologically (see Table 1) and it therefore has not been possible to study this problem by the fluorescent method so far.

The significance of formation of the small-molecular-weight Bence-Jones protein by the neoplastic plasma cells of the MPC-2 line and some patients with multiple myeloma is not clear. The differently sized proteins formed by the plasma cells do share antigenic groups but Bence-Jones protein has also antigenic groups in common with normal mouse γ -globulin, not evident in the MPC-2 myeloma globulin. In addition, myeloma globulin has antigenic features not present in Bence-Jones protein (J. L. Fahey & B. A. Askonas, to be published). Similar observations have been recorded for the corresponding proteins of human multiple myeloma (Korngold & Lipary, 1956; Putnam, 1960). It is not clear whether we are dealing with the formation of incomplete globulins due to an anomaly in protein synthetic ability of the neoplastic cell or whether the difference between normal and neoplastic cells is merely a quantitative one. Similar findings have been reported, for example, in investigations of the thyroid gland. Thyroid neoplasms form a small iodoprotein (S_{20} , 4) beside the thyroglobulin. This was thought to be an 'abnormal' protein but on further investigation it was found that normal thyroid glands also contain a small amount of iodoprotein (S_{20} , 4), and Robbins, Wolff & Rall (1959) suggest that the difference between normal and cancerous thyroid tissues is quantitative rather than qualitative. It is possible that

we have a similar situation with Bence-Jones and myeloma proteins, particularly since Stevenson (1960) has reported that Bence-Jones-like proteins may be present in normal human urine.

SUMMARY

1. The serum myeloma protein and urinary Bence-Jones protein of mice carrying the MPC-2 plasma-cell neoplasm have been purified by chromatography on ion-exchange diethylaminoethyl-cellulose and characterized.

2. The neoplastic plasma cells were found to form both proteins. Biosynthesis was studied by incubating sliced tumour tissue *in vitro* and following the incorporation of [¹⁴C]amino acids into the Bence-Jones protein and myeloma protein isolated by chromatography on diethylaminoethyl-cellulose and by precipitation with rabbit antiserum. The time course of incorporation of radioactive amino acid into the two proteins is consistent with the view that Bence-Jones and myeloma proteins were formed independently by the neoplastic plasma cells and that neither protein was a precursor or breakdown product of the other. Approximately two molecules of Bence-Jones protein were formed for each molecule of myeloma protein.

3. The pool of extracellular plus intracellular protein associated with MPC-2 tumour tissue was determined by isotope-dilution techniques employing ¹³¹I-labelled Bence-Jones and ¹³¹I-labelled myeloma protein. About 3.6 mg. of myeloma protein and 0.4 mg. of Bence-Jones protein were found in 1 g. of tumour tissue (wet weight). This difference is probably caused by the rapid clearance of Bence-Jones protein from the plasma and extracellular fluid by excretion through the urinary tract.

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Induced Formation of Leucine Decarboxylase in *Proteus vulgaris*

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The formation of many microbial enzymes is directly stimulated or 'induced' by the presence of the corresponding substrate or some related compound. Inducers must presumably combine with

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some specific receptor within the cell in order to exert their effects. The earliest and simplest suggestion was that this receptor was the enzyme itself (Yudkin, 1938). If small amounts of an enzyme exist in equilibrium with large amounts of its precursors a substrate or competitive inhibitor