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A λ Bence-Jones Protein in Guinea Pigs

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1. The L_2C lymphocytic leukaemia in strain-2 guinea pigs is accompanied by a protein in the urine resembling a homogeneous immunoglobulin light chain. 2. The amino acid sequence over the first 20 residues demonstrates a close analogy with a human λ chain of V region subgroup IV. 3. The protein is apparently synthesized by the leukaemic cells and thus represents a monoclonal light chain, i.e. a Bence-Jones protein.

The L_2C leukaemia of strain-2 guinea pigs is a transplantable tumour maintained by repeated passaging *in vivo* (Nadel *et al.*, 1974). The course of the leukaemia is rapid; after injection of 5×10^6 leukaemic cells into the peritoneal cavity, death follows at about the 12th day, with heavy infiltration of lymphoid organs and a blood white-cell count of $\geqslant300000/\mu l$. We have been particularly interested in the nature of the leukaemic cell, previously shown to be a B lymphocyte (Shevach *et al.*, 1970), and have characterized the surface immunoglobulin as IgM† (Stevenson *et al.*, 1975).

In the present communication we describe the appearance of a λ Bence-Jones protein in the urine of guinea pigs in the terminal phase of the leukaemia. To the best of our knowledge this represents the first available myeloma-type protein in this species.

Urine was collected from guinea pigs after the blood white-cell count exceeded $20000/\mu l$. The animals (600–800g body wt.) were maintained in metabolic cages, and leukaemic and control urines were collected into toluene. Urinary output was usually 15–30ml/24h. The urine was centrifuged at 4°C at 12500*g* for 30min and the supernatant dialysed into 0.02M-Tris–HCl–0.1M-NaCl–0.001M-EDTA, pH8.0, containing 0.02% sodium azide, for 72h. It was then concentrated 100-fold by using an Amicon ultrafiltration apparatus with a PM-10 membrane. The protein concentration, as determined by the method of Lowry *et al.* (1951) with guinea-pig IgG as a standard, was approx. 40mg/ml for concentrated normal urine and 60mg/ml for concentrated leukaemic urine.

The concentrated urines were subjected to cellulose acetate electrophoresis for 20min on a Beckman Microzone apparatus, in sodium barbital buffer, pH8.6, and were stained with Ponceau S. It was found that the leukaemic urine contained an electrophoretically homogeneous protein with a mobility in the slow α region. No corresponding band was seen in normal urine. In order to isolate this material, 2.5ml of concentrated urine was dialysed into 0.01M-Tris–HCl–1.0M-NaCl, pH8.0, and placed on a column (3cm×87cm) of Sephadex G-100 equilibrated with the same buffer. The separation obtained as monitored by E_{280} is shown in Fig. 1. The middle peak, which was characteristic of leukaemic urine and which was responsible for the electrophoretic band, was harvested and concentrated.

Immunodiffusion on Ouchterlony plates demonstrated that the partially purified urinary protein reacted with sheep antiserum raised against pooled guinea-pig light chains. Immunoelectrophoresis resolved this reactivity into a double arc at the anodic end of the distribution range of pooled guinea-pig light chains, suggesting two antigenically identical molecular species with a small difference in net charge.

The protein was then examined by electrophoresis on 5% polyacrylamide gel with a vertical disc-gel-electrophoresis apparatus (Shandon) as described by Smith (1968). The gel buffer was 0.005M-Tris–HCl–0.04M-glycine, pH8.3. After a pre-run of 10min, the samples were loaded and electrophoresed for 30min at 2mA/gel. Protein bands, as detected by the Coomassie Blue Stain, are shown in Plate 1(*a*); evidently there are two principal components, with most of the protein in the leading band.

Further purification was carried out by ion-exchange chromatography. Samples of protein (2.5ml at 5mg/ml in 0.0175M-sodium phosphate buffer, pH6.3) were placed on a column (2.5cm×18cm) of DEAE-cellulose equilibrated with the same buffer. After loading, a column-volume of buffer was run through before starting the linear gradient from 0 to 0.25M-NaCl in the dilute phosphate buffer. Two main peaks were obtained. On analysing these, a partial separation of the electrophoretic components was demonstrated (see Plate 1*b* and 1*c*); peak 1 from

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† Abbreviations: IgM, immunoglobulin M; IgG, immunoglobulin G.

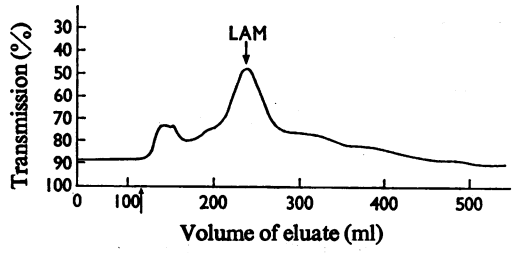


Fig. 1. *Sephadex G-100 chromatography of the protein from the urine of leukaemic guinea pigs*

Protein (2.5 ml, containing 150 mg of protein) was applied to an upward-flowing column (3.0 cm × 87 cm) of Sephadex G-100. Equilibration and elution were with 0.01 M-Tris-HCl–1.0 M-NaCl, pH 8.0. Leukaemia-associated material (LAM) was found in the peak indicated.

the DEAE-cellulose contained all the leading electrophoretic component, but with some trailing component still present, whereas peak 2 contained only the trailing electrophoretic component.

Analyses of the *N*-terminal sequences of peaks 1 and 2 on 2.5 mg samples were carried out in a Beckman 890C automatic sequencer by using the dimethylallylamine fast peptide programme (Beckman Instruments). The phenylthiohydantoin derivatives released on sequencer analysis were identified by g.l.c. (Hewlett-Packard 5830A automatic gas chromatograph) and by t.l.c. on polyamide plates (Summers *et al.*, 1972). The major sequence identified from two runs of peak 1 had a striking homology with the λ IV subgroup of human λ-type light chains:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Human IV Bau	Tyr	Gly	Leu	Thr	Gln	Pro	Pro	Ser	Leu	Ser	Val	Ser	Pro	Gly	Gln	Thr	Ala
Leukaemic urinary protein	Tyr	Val	Leu	Thr	Gln	Pro	(?)	Ser	Met	Ser	Val	Thr	Leu	Gly	Glx	Pro	Val

An analysis of peak 2 permitted a confident identification of sequence to residue 10, to which point there was complete identity with peak 1. We conclude tentatively that the guinea-pig light chain has two electrophoretic components, with the structural basis of this heterogeneity not yet apparent. It is noteworthy that human myeloma light chains have often been described with multiple components demonstrable on gel electrophoresis (Cohen & Porter, 1964; Fahey, 1964).

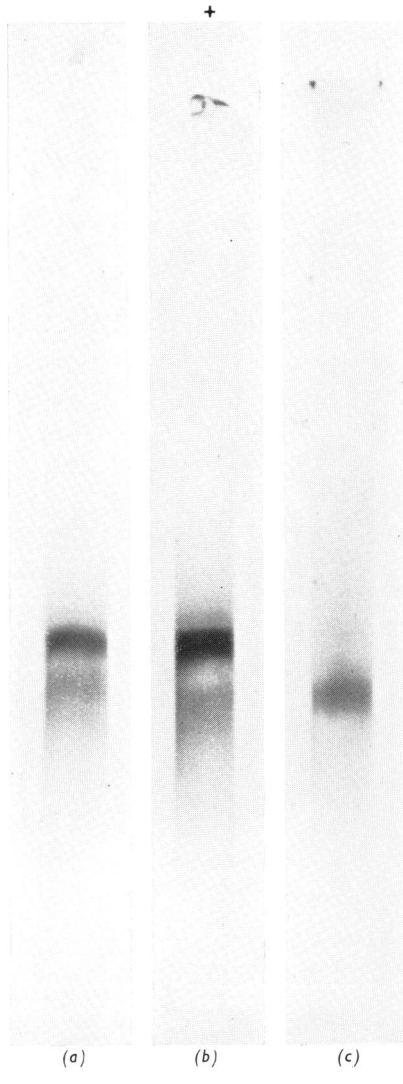
Sequencer analysis coupled with HI back-hydrolysis and amino acid analysis is now being undertaken to quantify these major sequences and confirm the g.l.c. and t.l.c. results.

Further investigations of peak 1 gave results consistent with an immunoglobulin light chain. A molecular weight within the range 21 000–23 000 was estimated from the electrophoretic mobility in polyacrylamide gel in the presence of sodium dodecyl sulphate and β-mercaptoethanol (Weber & Osborn, 1969); five standard globular proteins, including human immunoglobulin light chains, were used. Carbohydrate analysis by the method of Montgomery (1961) with a mannose standard gave a value of 1.8%, compared with 0.8% in guinea-pig pooled light chains. No staining of bands on the polyacrylamide gels could be obtained with the periodate–Schiff reagent (Smith, 1968).

It appears therefore that in the terminal phase of the L_2C leukaemia the host guinea pigs excrete in the urine a protein identifiable by physicochemical and structural features as a Bence-Jones protein (Gally & Edelman, 1962) of the λ type. The likely source of the protein is the neoplastic cells, and indeed we have evidence that the λ chain is the light chain incorporated into the surface IgM of the L_2C cells. Apparently the λ chain is secreted by the tumour cells in excess of the μ chain.

The cause of electrophoretic heterogeneity is not apparent at this stage. Among the identified causes of heterogeneity in myeloma proteins are charge differences acquired after secretion (Awdeh *et al.*, 1970) and monomer–dimer mixtures (Bernier & Putnam, 1963; Deutsch, 1965).

Human Bence-Jones proteins show unusual although well-known behaviour on being heated in solution, namely precipitation at a relatively low temperature (e.g. 55°C) and appreciable dissolution of the precipitate on further heating to near 100°C (Putnam *et al.*, 1959; Stevenson, 1960). The guinea-pig protein exhibited the first of these properties but not the second. A solution of peak-1 protein from the DEAE-cellulose column, at 1.3 mg/ml in 0.2 M-sodium acetate buffer, pH 5.2, was placed in a water bath at 55°C; after 30 min the solution was opalescent and by 60 min a light flocculent precipitate had formed. This precipitate was collected by centrifugation, suspended in the acetate buffer at 1 mg/ml, and placed in a boiling-water bath for 5 min. The amount dissolving under these conditions was <0.04 mg/ml, which is less than one-tenth the minimal solubilization found for six human Bence-Jones proteins (Stevenson, 1960).



EXPLANATION OF PLATE 1

Disc electrophoresis on polyacrylamide gel of protein from the urine of leukaemic guinea pigs before and after separation on DEAE-cellulose

Samples (0.1 ml, containing 150 μg of protein) with 1 drop of glycerol and Bromophenol Blue, were applied to columns of 5% polyacrylamide gel in 0.005 M-Tris–HCl–0.04 M-glycine, pH 8.3. Electrophoresis was for 30 min at 2 mA/gel. Protein was detected by staining with Coomassie Blue. (*a*) Sephadex G-100 peak; (*b*) DEAE-cellulose, peak 1; (*c*) DEAE-cellulose, peak 2.

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