available data for λ -chains do not offer as yet such clear-cut classification. There is some suggestion that well-defined fundamental sequences may occur in λ -chains also, but this is very tentative. Thus if one considers the four proteins Sh, Bo, Ha and X there are several positions where the residue shared by proteins X and Sh is different from that shared by proteins Bo and Ha. In Table 23 are tabulated positions that contain two varieties of residues, two proteins having residue A and the other two having residue B. There are seven such positions, and in six of them the same pairs (X-Sh and Bo-Ha) are involved, but in one (position 88) the pairs are different. The addition after residue 27 is also common to proteins Bo and Ha, though the residues added are different.

Cohn (1968) has in fact suggested three fundamental sequences for λ -chains. In our opinion the data are still insufficient to propose a meaningful and statistically significant group of linked residues in λ -chains, for any fundamental sequence. Until this is possible it cannot be established whether λ -chains are more or less variable than κ -chains.

Note added in proof. Since this paper was submitted the sequences of two λ Bence-Jones proteins have been published (Ponstingl, Hess & Hilschmann, 1968; Langer, Steinmetz-Kayne & Hilschmann, 1968). The size difference discussed in this paper is also observed in the two proteins studied by the above authors.

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APPENDIX

A Simple Procedure for the Fractionation of the Tryptic Peptides of the C-Terminal Half of Immunoglobulin λ -Chains

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Immunoglobulin light chains consist essentially of an 'invariable' and a 'variable' section, each being one-half of the chain. This conclusion has been substantiated in studies of both κ -chains (Hilschmann & Craig, 1965; Titani, Whitley, Avogardo & Putnam, 1965; Milstein, 1966a) and λ -chains (Milstein, Clegg & Jarvis, 1967; Wikler, Titani, Shinoda & Putnam, 1967). However,

variants of the 'invariable' part of the molecule occur in both types of chain. Thus in κ -chains a substitution in position 191 (Hilschmann & Craig, 1965) was found to be correlated with the Inv antigenic marker (Milstein, 1966a,b; Baglioni, Alescio Zonta, Cioli & Carbonara, 1966). In λ -chains a substitution frequently occurs at the position equivalent to position 190 of κ -chains

(Appella & Ein, 1967; Milstein, 1967). In addition isolated variants in the *C*-terminal half of λ -chains have been described (Milstein, 1967; Ponstingl, Hess, Langer, Steinmetz-Kayne & Hilschmann, 1967). It is therefore desirable to obtain precise information on the frequency of variants in the *C*-terminal half. The method described here for the detection of variants of the *C*-terminal half of λ -chains is very simple and is suitable for the screening of large numbers of samples. A small amount of material only is required to provide the amino acid composition of the tryptic peptides. The amino acid sequences of the variants can also be determined after fractionation of the tryptic peptides by the same method. It must be emphasized that since the *N*-terminal half is variable it is likely that in some proteins a variable peptide will not be separated from an 'invariable' peptide. In such isolated cases an additional fractionation may be required. Similarly, variants of the *C*-terminal half may give tryptic peptides that do not occupy the same position as the corresponding 'invariable' peptide. In these cases further location and characterization of the variants may require more extensive studies.

The method is based on the use of ^{14}C -labelled proteins obtained by reduction in 6*M*-guanidine and carboxymethylation with iodo[^{14}C]acetate. After tryptic digestion the peptides are fractionated by a three-dimensional 'fingerprint' procedure. The radioactive peptides are eluted after radioautography and the non-radioactive peptides are stained with dilute ninhydrin solution and eluted. 'Fingerprint' procedures described previously failed to detect all the tryptic peptides of the *C*-terminal half (Baglioni & Cioli, 1966; Putnam & Easley, 1965).

The light chain (10mg.) is dissolved in 1ml. of 0.5*M* - tris - 6*M* - guanidine - 1*mM* - EDTA, pH 8.6 (Milstein, 1966b), and 50 μl . of a 10mg./ml. solution of dithiothreitol is added after the tube has been flushed with nitrogen for a few minutes. The reduction is allowed to proceed for 1 hr. at 37°; 100 μl . of 0.1*M*-iodo[^{14}C]acetate (0.5mc/m-mole) is then added, and the mixture is allowed to react for 1½ hr. at 37°. The reaction is stopped by the addition of a small amount of a thiol (e.g. 1 μl . of mercapto-ethanol) and dialysed exhaustively against 1% (w/v) ammonium hydrogen carbonate. Then 100 μg . of trypsin is added, and the material is freeze-dried after digestion for 3-4 hr. at 37°. If the ammonium hydrogen carbonate is not completely removed, the digest is freeze-dried again after addition of water. The digest is applied in a band 10cm. wide in the middle of a piece of Whatman no. 3MM paper with markers at the side. The paper is subjected to electrophoresis at pH 6.5 at 60 v/cm. for about 1 hr., or until a marker spot of

First dimension, pH 6.5

Acidic peptides: Second dimension, pH 3.5

Neutral + 'core' peptides: Second dimension, BAWP; third dimension, pH 3.5

Basic peptides: Second dimension, BAWP

Fig. 1. Position of the tryptic peptides of the *C*-terminal half of Bence-Jones protein X in a three-dimensional 'map'. Peptides are numbered starting at the *C*-terminus. The sequence of each peptide and the overlaps are discussed in the main paper (Milstein, Clegg & Jarvis, 1968). The peptides of the *N*-terminal half are not included in the Figure.

red Pentel-pen ink reaches the buffer compartment. It is then radioautographed; a marker strip at each side is stained with ninhydrin to determine the position of the neutral band, and the unstained strip (about 8cm. wide) is divided into three sections corresponding to the acidic, neutral and basic regions. Each portion is sewn on to a new sheet of paper; the acidic and basic sections are then run at right angles to the original run, and the neutral band in the same direction. The acidic

peptides are subjected to electrophoresis at pH 3.5 at 60 v/cm. for 2 hr., and the basic peptides to chromatography in BAWP* overnight. The neutral peptides (which may include the insoluble radioactive 'core') are also subjected to chromatography in BAWP, care being taken that a marker of ϵ -DNP-lysine does not run off the paper. A marker strip is then stained, and the unstained part of the paper containing the neutral peptides is sewn on to a new sheet of paper and subjected to electrophoresis at pH 3.5 at 60 v/cm. for 1½ hr. The papers are radioautographed, the radioactive peptides are eluted with water, and the rest of the paper is stained with 0.03% ninhydrin to locate the spots to be eluted with 6N-HCl (except peptide T8; see below) for analysis.

The positions of the different peptides from the C-terminal half of protein X (but not of those from the N-terminal half) are shown in Fig. 1. Peptide T8 is insoluble on electrophoresis at pH 6.5 and its position may not coincide with the neutral band. This is easily established by radioautography after the first dimension. In such cases it may be recovered as the insoluble 'core' from the acidic section after electrophoresis at pH 3.5 (second

dimension) and purified by chromatography in BAWP. The procedure for the elution of peptide T8 from the paper was described in the main paper (Milstein *et al.* 1968).

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* Abbreviation: BAWP, butan-1-ol-acetic acid-water-pyridine (15:3:12:10, by vol.)