matters arising

GFA protein in C-6 glioma *in vitro*

BISSELL *et al.* reported a 40-fold increase of glial fibrillary acidic (GFA) protein by radioimmunoassay in buffer extracts of C-6 glioma cells maintained in organ culture, compared with the same cells in monolayer and suspension culture[1]. Quantitative data using buffer extracts should be interpreted with caution, since they may indicate differences in solubility rather than in total amounts. GFA protein is only partially soluble in water[2,3], and the factors regulating the ratio between water-soluble and insoluble fractions are still poorly understood. Solubility in buffer is greatly increased by *in situ* proteolysis[4], and may be seen to vary considerably in different regions of the brain, even when a relatively insensitive assay such as double immunodiffusion is used.

Table 1 Solubility of GFA protein in different regions of the brain indicated by the immunodiffusion titre with GFA protein antiserum

	Water-soluble fraction	Urea-soluble fraction (first extract)
Cerebral cortex	1/4	1/4
Cerebral white matter	<1*	1/32
Brain stem	1/8	1/16

Bovine brains were frozen at the slaughterhouse and GFA protein was extracted in 0.05 M sodium phosphate buffer, *p*H 8.0, with and without 6 M urea (w/v, 1:4).
* Precipitin lines were not observed against undiluted GFA protein antiserum unless the well was filled 2–3 times with the white matter extract.

As Table 1 shows, the white matter contains larger amounts than the cerebral cortex, which agrees with its relatively larger content of astroglia. However, the reverse is true for the water-soluble fraction, that is, only small amounts are extractable in buffer from the white matter compared with the cortex.

An additional point is that the radioimmunoassay for GFA protein[5] is difficult to reproduce, since no information is given on the preparation of the standard. Our experience with microcomplement fixation indicates that GFA protein not only precipitates from the standard solution kept at 4 °C or frozen, but also that in these conditions the immunological activity per unit protein steadily decreases in the high-speed supernatant (100,000*g* for 1 h).

A. BIGNAMI

Department of Pathology, Stanford University School of Medicine and Veterans' Administration Hospital, Palo Alto, California 94304

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BISSELL ET AL. REPLY—Bignami[1] raises the question whether our reported data on the quantitative increase of GFA protein in C-6 glioma cells *in vitro*[2] might not indicate differences in solubility rather than in total amounts. We welcome this opportunity to stress that the GFA protein estimations in our study were determined only on the phosphate buffer-soluble fraction. We have of course long been aware[3] of the existence of water-soluble and insoluble GFA protein fractions, and we agree that the chemical relationship between the two and the factors influencing their relative concentrations are still poorly understood. To assign, therefore, any special significance to either is premature. On the other hand, while we cannot exclude the hypothesis that variations in solubility could have played some role in determining minor deviations in the quantitative data obtained in our study, the overall reproducibility of the assays and the fact that they so unequivocally confirm previous observations on the same system using an immunofluorescence technique[4] make it unlikely that solubility differences would account in any significant measure for the striking increases observed. The relevance of Bignami's table in relation to our report is questionable because the heterogeneity of astroglial cell populations that prevail in the different regions of the brain does not apply to the homogeneous cloned cell system used in our study.

A description of the properties of the two-site IRMA assay for the GFA protein and of the stability and immunological identity of the purified and impure antigen, followed by a discussion on the soluble compared with insoluble GFA protein fractions, is to appear soon[5]. Contrary to Bignami's experience, our purified GFA protein standard (prepared from frozen human multiple sclerosis plaques) did not change in two-site IRMA immunoactivity for at least six months. In our report all samples were measured using the same standard.

Stanford University School of Medicine

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Tropomyosin and actin

BECAUSE of changes introduced before publication, Parry's letter[1] was not quite that to which my reply[2] was addressed. I wish to point out that his suggestion that tropomyosin would present pseudo-equivalent aspects to all seven actins is simply a restatement of my original hypothesis[3]. This hypothesis is strengthened by the observation of several charged groups in the sequence[4] repeating at exactly 77 residues. The computed coordinates of these show that some could lie at the correct positions to interact with one strand of actin.

W. LONGLEY

Duke University Medical Center, Durham, North Carolina 27710

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PHA and lymphocyte membrane fluidity

BARNETT *et al.*[1] reported that mitogenic lectins induce changes in the fluidity of lymphocyte membranes, which can be detected by means of the spin label electron spin resonance (ESR) technique. This finding, if substantiated, would be