somes, and the antagonism of both by bicuculline. Thus, even in the absence of intact neurons, $^{36}Cl^-$ flux that is mediated by receptors for GABA and barbiturates can be measured in vitro (*6*). Our results (*6*) are also in good agreement with those obtained from primary cultures of intact embryonic chick neurons (*7*). We would caution, however, that although GABA receptor–mediated Cl^- conductance is believed to be confined to neurons (*8*), even the "filtered" synaptoneurosome preparation used in our experiments contains a few oligodendrocytes and erythrocytes (Table 1), both of which possess specific disulfonylstilbene-sensitive mechanisms for anion transport (*9*). Contrary to the statement made by Harris and Allan (*1*, reference 3), the synaptoneurosome preparation should prove suitable for biochemical and pharmacological studies of GABA receptor–effector function.

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1 July 1985; accepted 26 December 1985

Response: Paul *et al.* raise two points. First, they suggest that the γ-aminobutyric acid (GABA)–stimulated chloride flux reported by us (*1*) may be due to intact cells, rather than isolated membrane vesicles. Second, they assert that a slightly different preparation (synaptoneurosomes) is suitable, and in fact superior to our preparation, for the study of GABA-operated chloride channels.

In support of the first point, Paul *et al.* counted the types of particles in preparations made by their method (*2*) and by the method of Daly *et al.* (*3*). The latter preparation was similar to that used in our study. When one calculates the data of Paul *et al.* as percentages, their preparation contained 1.4% erythrocytes, and the Daly preparation contained 2%. For nonneuronal cells the figures are 1.6% and 4.8%, respectively, and for neuronal cells, 0 and 2.4%, respectively. Thus, the measurements of Paul *et al.* indicate that both preparations contained a small percentage of intact cells, but the Daly preparation contained more than the synaptoneurosome preparation. However, the Daly preparation used is not identical to that used in our study (*1*). Paul *et al.* do not present their methodology, but they do mention that they used guinea pig cortex. We used mouse brain in our studies. Daly *et al.* (*3*) used a different type of homogenization and fewer washes than we did (*1*). To determine the importance of these differences, we examined our preparation by light and electron microscopy. We had great difficulty finding any intact cells in our preparation, and all intact cells together constituted less than 1% of the vesicles (*4*). In addition, we fractionated brain membranes by sucrose and Ficoll density gradient centrifugation (*5*) and found the highest activity of GABA-stimulated chloride flux in the synaptosomal fraction (*4*), a membrane population that clearly does not contain intact cells. Thus, we conclude that the GABA-activated chloride flux reported by us is not due to the presence of intact cells in our preparation.

The second issue raised by Paul *et al.* is whether or not the synaptoneurosome preparation is suitable for measurement of GABA-stimulated chloride flux. We stated (*1*, reference 3) that no one had shown GABA-stimulated chloride flux with a cell-free system and pointed out that Paul's group had shown barbiturates "to stimulate the uptake of $^{36}Cl^-$ by isolated membrane vesicles." Paul *et al.* provide evidence in their comment that a GABA agonist, muscimol, can also enhance chloride flux in their preparation. However, these data had not been published when our report appeared. In addition, the published report of Schwartz *et al.* (*2*) indicates that the GABA receptor antagonist bicuculline reduces flux of chloride across synaptoneurosomes in the absence of added GABA. This suggests that the preparation contains sufficient endogenous GABA to activate the receptor and channel without addition of exogenous agonists. If this is true, it will limit the usefulness of their preparation because it will not be possible to determine accurate concentration-response curves for GABA agonists or kinetics of activation and desensitization if there are significant but unknown amounts of GABA present in the assay mixture. We conclude that additional studies are required to establish the suitability of the synaptoneurosome preparation for the study of GABA-stimulated chloride flux.

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2 June 1986; accepted 16 June 1986