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PRACTICAL APPLICATIONS OF NEURONAL TISSUE CULTURE IN IN VITRO TOXICOLOGY

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PRACTICAL APPLICATIONS OF NEURONAL TISSUE CULTURE IN *IN VITRO* TOXICOLOGY

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SUMMARY

1. Primary chick embryo forebrain neurones are relatively easy to culture and are quite resilient to treatment manipulation. These characteristics have allowed application in a surprisingly diverse number of areas.

2. These cultures have been used to investigate the anticholinesterase potencies of many organophosphate (OP) nerve agents and insecticides.

3. These cultures have been used to quantitate levels of OP in 'spiked' unknowns and in OP-contaminated soil samples.

4. These cells have also been used to test a variety of compounds using the MTT and neutral red cytotoxicity assays.

Key words: acetylcholinesterase, cell culture, cytotoxicity, neurones, neurotoxicity, organophosphate.

It is a fact that it is not feasible to perform extensive animal testing on the thousands of chemicals which are present in society and the environment. Nevertheless, it is essential that these chemicals be screened in some manner in order to assess their potential toxicity and, in the case of commercial product development, their efficacy with respect to a wide variety of endpoints. Tissue culture models are favoured alternatives to animal testing and, in the author's laboratory, primary cultures of chick embryo forebrain neurones have been found to be particularly useful. These cultures are economical, relatively easy to initiate and maintain technically, and are quite resilient to treatment manipulation. These features have allowed applications using these cultures in a surprisingly diverse number of areas.

The efficacy of this system with respect to cytotoxicity testing has been investigated through participation in the Multicentre Evaluation of *In Vitro* Cytotoxicity (MEIC) exercise (Weiss & Sawyer 1993). In this study 50 test chemicals were assayed using the neutral red (NR) and MTT assays. The NR assay was consistently more sensitive to chemical toxicity. However, both assays were equally predictive when compared with *in vivo* toxicity data obtained from the Registry of Toxic Effects of Chemical Substances. High correlations were obtained when comparing the *in vitro* data with intraperitoneal (i.p.) rodent toxicity data, but these correlations decreased significantly when oral toxicity data were used. The predictive value of the *in vitro* data for oral human toxicity was generally poor, but comparable with its value in predicting oral rodent toxicity. In a limited study with 10 of the MEIC test chemicals, the cytotoxicity of some compounds was dependent on the degree of differentiation of the neurone cultures, suggesting that this culture system may not only be sensitive to the basal cytotoxicity (chemical toxicity to cellular functions and structures that are basic to all types of cells) of chemicals, but also to toxic effects specific to the specialized differentiated functions of the central nervous system. Further analysis of this system is being carried out by the MEIC study committee and involves comprehensive statistical analysis of the data compared against other *in vitro* data, rodent data and human data that incorporates the toxicokinetics of each test compound.

Two classes of compounds whose toxicity is dependent upon biochemistry unique to excitatory tissue are the carbamate and organophosphate (OP) anticholinesterases. These compounds exert their primary toxic effects through inhibition of the enzyme acetylcholinesterase (AChE), causing massive overstimulation of the cholinergic nervous system. We have studied the effects of a variety of anticholinesterases using the chick embryo neurone system. These cultures have been found to have significant levels of AChE and increases in this enzyme with time in culture were found to closely follow its development *in ovo* and *in vivo* (Sawyer & Weiss 1993). Furthermore, a close relationship was found between the anticholinesterase activities of a series of OP nerve agents *in vitro* and their toxicities *in vivo* (Sawyer *et al.* 1992c), thus allowing their use in estimating the potential *in vivo* toxicity of test articles including pure standards, complex mixtures or OP adsorbed onto complex matrices (Sawyer *et al.* 1992a).

The OP nerve agent work has been extended to include the OP insecticides paraoxon and parathion (Sawyer *et al.* 1992b). These studies found that an exogenous source of metabolic activation (in the form of hepatic S9 fractions) was required in order for the anticholinesterase activities of these two compounds to be predictive of their relative *in vivo* toxicities. Additional studies have investigated the anticholinesterase activities of 24 OP and six carbamate insecticides (unpubl. data). Although the median inhibitory concentrations of these compounds ranged over several orders of magnitude, once again these values did not correlate with their literature reported *in vivo* rodent toxicities. Pre-incubation of an arbitrary selection of 18 of these insecticides with rat hepatic S9 fractions altered their anticholinesterase activities so that they became predictive of their relative *in vivo* toxicities.

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In summary, primary chick embryo neurone cultures offer a valuable tool with which to study nervous system toxicants. Not only can these cultures be used in routine cytotoxicity studies, but they can also be used to investigate compounds whose mechanism(s) of action may be unique to excitatory tissue, such as the anticholinesterases. The very high sensitivity of these cultures towards the anticholinesterase effects of OP nerve agents allow their use in a semi-quantitative sense when assaying for OP adsorbed onto, or dissolved into complex matrices. Furthermore, since a high correlation has been demonstrated between the potencies of nerve agents as inhibitors of AChE *in vitro*, and their toxicities *in vivo*, these cultures can also provide an estimation of the potential toxicity of test samples *in vivo*. The OP and carbamate insecticides, whose ultimate toxicities often depend on prior metabolism, can also be assayed in this culture system by employing a pre-incubation step with hepatic S9 fractions.

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