Requirements for Neurone Culture

from our Neurochemistry Correspondent

ONE of the mysteries occasionally experienced by the experimentalist attempting to study biological systems *in vitro* is the apparent necessity, after all else has resulted in failure, for the addition of an obscure "factor X" in order to get the system to work. Nerve-growth factor (NGF), a protein isolated from such unlikely sources as the venom from certain snakes and the salivary gland of the mouse, has for several years seemed an irrational requirement of this sort for the successful culture of mammalian neural tissue. Thus, growth and differentiation of neurones in cultures of chick embryonic sympathetic ganglia are vastly increased by NGF, but, in spite of extensive investigation in recent years, very little is known about the mechanisms by which these effects are achieved. A recent report by P. Burnham, C. Raiborn and S. Varon (*Proc. US Nat. Acad. Sci.*, **69**, 3556; 1972), showing that, in cultures of neurones derived from certain dissociated ganglia, NGF may be effectively replaced by non-neuronal cells from the same ganglia, promises to throw light not only on the way in which NGF acts, but also on the part played by glial cells in the normal development and maintenance of neurones.

In the experiments of Burnham *et al.* dissociated chick embryo sympathetic ganglia and dorsal root ganglia from newborn mice or rats were used. Cell suspensions were initially prepared from the isolated ganglia, and "non-neuronal" cultures were developed by growing aliquots of dorsal root ganglia cell suspensions without NGF. In the case of preparations from chick and rat, the absence of NGF alone was sufficient to prevent the growth of neurones, and in mouse preparations this was achieved by removing neurones before attachment. In order to examine the effect of additional non-neuronal cells on the performance of dissociated neurones in culture, separate cultures were prepared using dorsal root ganglia cell suspensions (with or without NGF), either in normal conditions or over previously-attached non-neuronal cells. Three criteria were used to describe the responses of the neurones during growth of the cultures: attachment (total number of neurones attached to the culture floor), fibre production, and longer-term survival (number of neurones living for more than one day in culture).

Results showed that in terms of the first two of these criteria, cultures grown over non-neuronal cells, but without NGF, showed the same degree of stimulation as was caused by NGF, but did not survive as long as those supported by NGF. This latter failure, however, could be overcome by increasing the number of non-neuronal cells present. When NGF and supplementation of non-neuronal cells were used together, the effect produced was greater than when either treatment alone was applied, but a maximal enhancement of neuronal performance could be obtained with a large excess of non-neuronal cells.

As shown by the authors, the similarities between the effects of NGF and non-neuronal supplementation do not necessarily imply that both are achieved in the same way. But the results of the study do allow suggestions to be made about the possible ways in which NGF may operate. Thus, it may act as a supplement to NGF-like supporting substances produced by non-neuronal cells, or, alternatively, it may act on the non-neuronal cells themselves to increase their capacity to stimulate neuronal growth by some totally different means. The evidence for a dependence of neurones on other types of cells in close proximity to them is of great interest. The authors have not yet established what cell types within the non-neuronal population are responsible for the supportive action, but it is possible that information derived from their system may lead to further knowledge about the interactions between neurones and glia under normal conditions *in vivo*.

COMPLEX CARBOHYDRATES

New Society

THE Society for Complex Carbohydrates has been organized by scientists interested in fostering research and communication in the areas of glycoproteins, glycolipids and glycosaminoglycans (mucopolysaccharides). Biological, pathological, chemical and physical aspects of complex carbohydrates are included within the scope of the society's interests.

Correspondence relating to membership and meetings should be directed to the secretary, Professor John D. Gregory, Rockefeller University, York Avenue at 66th Street, New York City 10021.

PHOTOSYSTEMS

In Series or in Parallel?

from our Photosynthesis Correspondent

RECENT work on photosynthesis carried out partly in Professor Arnon's laboratory at the University of California, Berkeley, seems to cast considerable doubts on controversial ideas which originated from this laboratory. Although most workers in photosynthesis accept the model that two light reactions, system one (S1) and system two (S2), act in series to drive electrons from water to $NADP^+$, Arnon and his colleagues have had other ideas: they have argued ever since the mid-nineteen-sixties that the two photosystems S1 and S2 act in parallel rather than in series when bringing about carbon fixation (*Nature*, **207**, 1367; 1965). They maintained that S2 alone is capable of reducing $NADP^+$ and that the function of S1 is to produce adenosine triphosphate (ATP).

In a more recent report from Arnon's laboratory (*Proc. US Nat. Acad. Sci.*, **67**, 1404; 1970) the parallel model was extended to include two photoreactions in S2, called S2a and S2b. These two photoreactions were thought to act in series being joined by a chain of electron carriers including the copper containing protein, plastocyanin. Evidence for the parallel scheme came from spectroscopic studies of electron carriers in spinach chloroplasts and on results derived from studies with subchloroplast fragments enriched in S2. This scheme, however, was unable to explain the antagonistic effect of S2 and S1 light on the oxidation and reduction levels of Cyt f, P700 and plastoquinone.

In a recent report Malkin and Bearden (*Biochim. Biophys. Acta*, **292**, 169; 1973) present new results which are not consistent with the parallel scheme and strongly suggest that plastocyanin acts as an electron carrier between S2 and S1. These workers describe how electron paramagnetic resonance (EPR) can be used to monitor oxidized bound plastocyanin within spinach chloroplasts. Until this report, studies of the role of plastocyanin in photosynthetic electron transport had been confined primarily to effects of externally-added plastocyanin on reactions in plastocyanin-depleted chloroplast fragments. Using the EPR signal, Malkin and Bearden were able to study light-induced changes of this copper-containing protein at physiological temperatures, and concluded that it participates in non-cyclic electron flow between H_2O and $NADP^+$. As predicted by both the parallel and series schemes S2 light-reduced plastocyanin but, in contradiction to the parallel model, S1 light-oxidized this electron carrier. Moreover, in the presence of 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU) S2 light photo-oxidized plastocyanin.