of the star is less than 0.7 $M_{\odot}$, and have constructed a corresponding neutron star model of the system. But this upper limit is curiously relevant to Vauclair's discussion of white dwarf models for Cen X-3. On the face of things, it seems at present that the white dwarf models win out on the grounds of simplicity, although without Vauclair's work proponents of the models might have found van den Heuvel and Heise's upper limit uncomfortably restricting.

Although this speculation is interesting because it shows how easily slightly different white dwarf models can be fitted to the observations of Cen X-3, and even to data put forward in support of neutron star models, there must remain some doubt about the value of the exact figures derived in any such calculation. There seems good evidence to support the suggestion that Cen X-3 is an eclipsing binary system in which the white dwarf accretes matter which is lost from its companion, overflowing that star's Roche lobe. In that case, although the energy mechanism for X-ray production is conveniently provided, any detailed calculations based on radial pulsations of non-rotating white dwarf models must be taken as of qualitative value only.

ACETYLCHOLINE

Receptors Resolved

from our Molecular Biology Correspondent

THE acetylcholine receptor has been remorselessly stalked in all quarters of the globe, and in spite, perhaps, of the odd fruitless swish of the butterfly net, several specimens seem to have been captured alive. A modest amount of information has now been compiled about their physical attributes.

For the most part the source of the material has been the electric organ of the electric eel, *Electrophorus*, or of the ray, *Torpedo*. The techniques so far used have involved some form of affinity labelling in the intact cells followed by solubilization with detergent, and fractionation. Amongst recent reports, Meunier *et al.* (*Biochemistry*, **11**, 1200; 1972) used a snake venom α-toxin, into which a tritium label had been introduced, as a marker for the *Electrophorus* receptor, to which it binds with extremely high affinity. On addition of a non-ionic detergent the complex is solubilized in the form of an aggregate of high molecular weight. Such behaviour, in fact, seems to be universal for membrane-embedded receptors, such as those for a variety of hormones. What is more remarkable is that in the presence of the anionic detergent, sodium dodecyl sulphate (SDS), which is a more or less universal denaturing and disaggregating agent, the toxin seems to remain bound to the receptor, which, as will be seen, is evidently in a monomeric state in these conditions. Electrophoretic mobility in polyacrylamide gel in the presence of this detergent leads to an estimate for the molecular weight of about 55,000, whereas that of the toxin alone is much lower.

Reiter *et al.* (*Proc. US Nat. Acad. Sci.*, **69**, 1168; 1972), on the other hand, obtained a more direct estimate of the molecular weight of the same species based on an affinity-labelling procedure, developed earlier in the same laboratory. The receptor contains a disulphide bond, which is readily reduced, and one of the resulting thiol groups is then highly susceptible to reaction with an acetylcholine analogue, containing the thiol-specific N-benzylmaleimide group. When this reagent is labelled with tritium, a peak of radioactivity emerges from a broad background in SDS-polyacrylamide gels. If the thiol group is protected, by exposure either to the affinity reagent, dithiobischoline, which evidently enters the binding site and annihilates the free thiol, or to cobrotoxin, or if an excess of the inhibitor, hexamethonium, is present, the peak is suppressed. Further, because the affinity reagent binds rapidly and specifically, the presence of a moderate concentration of ^{14}C-labelled N-ethylmaleimide does not significantly interfere. This reagent, on the other hand, labels free thiol groups throughout the membrane. Again, against the broad background of label, in an SDS-polyacrylamide gel, a peak of high tritium : ^{14}C ratio stands out, which is lost on treatment with thiobischoline. Evidently then the peak of radioactivity in the polyacrylamide gels represents the veritable receptor, covalently labelled.

The molecular weight estimated from the electrophoretic mobility is some 42,000, corresponding, it may be supposed, to the single polypeptide chain. Reiter *et al.* have, moreover, taken a more critical view of the method than others before them: by showing that the molecular weight, relative to a calibration based on standard proteins, does not change with polyacrylamide concentration, they have eliminated, as far as is possible, any likelihood that their protein contains any large quantity of polysaccharide (or lipid), for in such a case less detergent would be bound, and the molecular weight estimate would be in error to an extent that depends on the gel concentration. Bearing in mind the uncertainty in the estimate of the molecular weight of Meunier *et al.*, whose preparative procedure makes it imperative to maintain the receptor in its active state, and therefore precludes the addition of reducing agent to the electrophoresis samples (for otherwise the labelled ligand would dissociate), the agreement (though possibly fortuitous, as the protein is evidently not unfolded) is remarkably good: if one toxin molecule, of molecular weight 7,000, is bound to each receptor chain, the molecular weight of the latter comes out as 48,000.

Eldefrawi and Eldefrawi (*ibid.*, 1776) solubilized the receptor of *Torpedo* with non-ionic detergent, and examined the extract by isolectric focusing. This is, of course, less informative, for it does not lead to an estimate of molecular weight, and in any case the material is presumably in an aggregated state. Among the multiple protein bands that appear, the receptor activity is confined to a fraction with an isoelectric point of *p*H 4.5. It seems, however, that in this system phospholipids remain associated with the receptor, for treatment with phospholipase causes loss of activity. The receptor is rather unstable at this *p*H, and its activity is also diminished by reduction.

Mammalian DNA Repair Endonuclease?

THE so-called excision patch repair mechanism by which microorganisms maintain the integrity of their DNA has been well characterized; it involves three basic steps—the removal of a segment of DNA by an endonuclease, synthesis of a replacement segment by DNA polymerase and sealing of the nicks by ligase. In eukaryotes there is evidence, albeit less complete and compelling, of a similar repair mechanism, and in next Wednesday's *Nature New Biology* (October 11) Brent describes the identification of a putative DNA repair endonuclease in extracts of HeLa cells.

To assay HeLa cell extracts for an endonuclease which preferentially attacks DNA damaged by ultraviolet irradiation, Brent isolated superhelical double stranded circular DNA from the bacteriophage PM2 and irradiated it with a dose of ultraviolet light estimated to cause radiation damage without actually breaking phosphodiester bonds. He then measured the ability of his HeLa cell extracts to convert irradiated and unirradiated PM2 DNA from the superhelical form to the open circular form.

He found that HeLa extracts would extensively nick irradiated DNA, but that they had little effect on unirradiated DNA and had no exonuclease activity. The endonuclease activity in these extracts is heat labile and is independent of divalent cations, which is also a property of at least one bacterial repair endonuclease. Using normal and xeroderma pigmentosum skin fibroblasts, Brent is now investigating the role of this activity in DNA repair in human cells.