DISCUSSION

Dr. J. D. JUDAH: After listening to Dr. Whittaker and Dr. de Robertis describing their excellent work, three main questions occurred to me, which perhaps they would be good enough to answer and which one hopes will start a good discussion.

The first point concerns methods. We have seen how successfully the technique of the sucrose gradient has been applied to the isolation of a number of components of the central nervous system and few would deny that the electron micrographs, particularly those of the synaptic junctions, indicate that the structures are remarkably well preserved as to morphology. However, it should be pointed out that the use of such high concentrations of surcose gives rise to misgivings. We heard Dr Abood say that high levels of sucrose inhibit oxidative phosphoration in brain mitochondrial preparations. Not only this, but they also inhibit pyruvate oxidation in liver mitochondria, and furthermore, they clearly bring about serious structural alterations in these particles. For example, if liver mitochondria are suspended in 0·25 M sucrose for more than 3 hr, they will not swell when exposed to Ca ions, thyroxine, inorganic orthophosphate and other well known swelling agents[1]. If the sucrose concentration is higher, these effects are more marked and occur earlier. It is also known that quite small concentrations of sucrose will inhibit the reverse phenomenon of mitochondrial contraction[2]. It is perfectly clear that the isolations which have been described would not have been possible without the use of techniques available, but one wonders whether these same methods will not stultify the ultimate purpose of all such experiments, namely the resolution of the metabolic functions of the structures which are brought to hand. It is of course relatively easy to make these comments, which I am sure must be clear to Drs. Whittaker and de Robertis, and not so easy to suggest methods of evading the difficulty. I hope I will be excused on the grounds that these points may not be so familiar to the audience at large.

The second question is primarily addressed to Dr. Whittaker: I note that the presynaptic vesicles which are beautifully shown in the electron micrographs have not been isolated as such, or at least, we have not been told about a successful isolation. May I ask whether it is the view of Dr. Whittaker that these vesicles have an independent existence? It might be possible for them to be like the holes in Swiss cheese, and come out in the photographs looking like vesicles because of the condensation of some osmophilic substance about their borders.

From the description of their properties, they might also be exceedingly thin walled vesicles, and in this connection, I am reminded of the lysosomes which Dr. de Duve will talk about later. It might help in attempts at their isolation to use agents which protect thin membranes. For example, we find that the adrenolytic agent, dibenzylene[3], is a good stabilizer of liver lysosomes *in vitro,* at concentrations near 10^{-6} M. Furthermore, we have also found that this drug will protect certain tissue culture systems against damage which is now thought to be mediated by the rupture of lysosomes, I refer to experiments with cartilage cultures exposed to vitamin A. The antihistamine drugs also exhibit similar properties[4], and I suggest that agents such as these may well be tried in an effort to preserve the delicate structures which we are considering.

The third point concerns a slide of Dr. de Robertis. I notice that one of his particulate fractions which is free of mitochondria by the electron micrography, exhibits a very considerable succinic dehydrogenase activity, amounting to some 20% I believe of the total. What is the explanation of this? The enzyme is not one which easily leaves the mitochondria, and it is certainly found only in these particles. The finding of the enzyme in a fraction free of identifiable mitochondria suggests that comminution of these has taken place, so that the fragments contaminate another fraction. It is possible that some other explanation exists, and perhaps Dr. de Robertis can tell us what it is.

Finally, and this is not a question but merely a comment, I believe that the aim of all of us is to determine not only the structure but the functional significance of the various subcellular structures. Certain things come immediately to mind in this context. Heald[5] has found for example that cortex slices electrically stimulated *in vitro* exhibit a marked increase in the turnover of a phosphoprotein fraction which on differential centrifugation of sucrose homogenates of the slices comes down with particles which are non-mitochondrial, but which are evidently closely similar in physical properties. We ourselves[6] find that liver slices suspended in sodium-free Ringer show a marked decline in the turnover of cytoplasmic phosphoproteins; this may be reversed by addition of Na in small amounts, and the effect is prevented by strophanthin and by certain antihistamine drugs which inhibit ion movements. It would be of great interest to do experiments of similar type and apply the methods of the two participants especially to a study of the nerve endings. I do not suggest that the work be restricted to phosphoproteins, of course, but also to other components of the acid-insoluble residue, notably the phosphatidic acids.[7]. It would also be worth knowing what happens to the ATP which Dr. Whittaker has shown to be present in some quantity in the presynaptic junctions. I wonder if eith-

er Dr. de Robertis or Dr. Whittaker have undertaken such work or whether they have any comments on the possibilities?

REFERENCES

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Dr. V. P. WHITTAKER: Regarding the remarks by Dr. Abood on the low capacity of mitochondria separated in sucrose density gradients for oxidative phosphorylation and those by Dr. Judah on the failure of mitochondria kept in isotonic sucrose to swell in response to Ca^{++}, it must be recognized that sucrose has a deleterious effect on sub-cellular particles of all kinds. Dr. Gray and I have been examining the morpholog yof particles prepared in isotonic saline and find that the appearance of nuclei, nerve ending particles and mitochondria prepared in this way is much closer to that in whole tissue sections. Thus sucrose induces definite morphological changes. The justification for using sucrose is twofold. (1) Electrolytes cause maceration or clumping of subcellular particles, thus interfering with the differential centrifugal separation of the various particle types. (2) Isotonic density gradients (e.g. those made with ficoll) cannot be substituted for sucrose since the succes of the separation is partly due to a differential loss of water which occurs in the density gradient itself.

The nerve ending particles presumably contain cytoplasm; therefore one would predict a bimodal distribution of soluble cytoplasmic components, such as the enzymes of glycolysis. This has in fact been observed by Mr. M. K. Johnson of Carshalton who finds 1–28% of glycolytic enzymes in the nerve-ending fraction, the remainder in the high-speed supernatant. Experiments are under way to find out if the conditions for releasing this fraction of glycolytic enzymes are similar to those required for acetylcholine release.

Dr. de ROBERTIS: The concept of mitochondria is both a morphological and a biochemical one. Morphologically we can see that although mitochondria tend to swell they do not break or become destroyed. For the assay of mitochondria succinic dehydrogenase is the enzyme of choice because it is bound to the membranes.