**Discussion**

**Dr Brachfeld:** May I ask a question, please? Do you have any control biopsies from patients who were not myopathic?

**Dr Peters:** Yes; the controls were surgical biopsies from patients with Fallot's tetralogy or patients who were having valve replacement.

**Dr Schwartz:** Dr Olsen's first paper gave us some information about his much more extensive studies on localization of the specific changes of the cells in hypertrophic cardiomyopathy; I was wondering whether you could comment on to what extent these hypertrophied and specially recognizable fibres are reachable by our present methods of biopsy, and to what extent might sampling be a problem?

**Dr Olsen:** It depends on the stage the disease had reached. One can have a superficial layer of relatively normal muscle which may not be penetrated by the bioptrme. If the disease has progressed to the stage where abnormal fibres extend to the subendocardial region—and this must have been the stage reached by the four patients in whom a positive result was obtained—a confirmatory result can be expected. The abnormal, recognizable myocardial fibres often extend over a large area and in these cases there should be no problem with sampling. With regard to the recognition of these fibres, we have applied the HHI (Histological HOCM Index; Van Noorden, Olsen and Pearse, 1971) and the highest results obtained were in the region of 65%.

**Dr Richardson:** Can I ask Dr Olsen if he feels we should do biopsies, if we suspect HOCM, always from the left ventricle.

**Dr Olsen:** Yes, but the distribution pattern does depend on the stage the disease has reached. I have seen hearts of patients where the abnormal fibres extend throughout the interventricular septum, but in the earlier stages of the disease, the left ventricular biopsy would certainly be better. The abnormal fibres often extend in one continuous sheet from the apex to the region of the aortic valve. This was established from previous analysis of specimens obtained at open heart operation or at post-mortem.

**Professor Muir:** I would like to ask two questions. I think you are unwise to refer to calcium stimulated ATPase activity as a marker for the myofibril, and I was, therefore, wondering if you studied the 'myofibrillar' fraction with a mitochondrial ATPase inhibitor present and in the presence of high ionic strength. This would separate the myofibrillar ATPase from the mitochondrial and microsomal ATPase activity. My second question is how are you going to fractionate cardiac myofibrils in such small quantities on sucrose gradients? We have had enough difficulty getting pure preparations of cardiac subcellular fractions using whole dog hearts. and you are now proposing to use many orders of magnitude less. I would be very interested to know how you propose to do it.

**Dr Peters:** Calcium activated ATPase—we measured this using 2 mmol ATP in the presence of 10 mmol of calcium. The literature is very complicated and very confusing. We have tried the effect of measuring calcium-activated ATPase in the presence of magnesium using an oxide as an inhibitor and get the same sort of figures. We would like to reserve our judgment on the specificity until we have done the fractionation.

**Chairman:** Does Dr Nayler want to comment?

**Dr Nayler:** Yes; this calcium-activated ATPase. Are you sure that you have got the optimal conditions for the enzymes in both situations? Because one really cannot say that the activity is diminished unless one is sure one is assaying in the optimum conditions for both enzymes. Secondly, I think one is on even dicier ground using Na- and Ca-activated ATPase with the plasma enzymes.

**Dr Schwartz:** May I make another comment? As a North American I am pleased to have shared in these very fascinating experiments. I am also pleased that I was not a patient from whom little snips of tissue were taken. I cannot agree or understand how one can extrapolate from 1 mm³ of tissue to anything. This is still a remarkable piece of work, and I will be fascinated to look at the details.

**Dr Peters:** But I think we have come against the problem of congestive cardiomyopathy; we can either try to apply new techniques to it, or we can just give up.

**Dr Richardson:** I wonder if I might just ask Dr Peters how he ensures that the samples consist entirely of myocardium?

**Dr Peters:** We have taken multiple biopsies and pooled them; one problem has been the presence of blood clot, but we are able to distinguish myocardial tissue by the use of the dissecting microscope.

**Dr Richardson:** I would have thought this poses quite a problem for you, because the biopsy samples are often associated with fibrin and blood clot. It must be difficult to be certain that what you are dealing with is cardiomyopathic tissue.

**Chairman:** Does anyone want to comment on Dr Brachfeld's beautiful pictures?

**Dr Rona:** I am glad that in the preceding discussion some question was raised about the selection for metabolic studies. I felt that this is also very true for the selection of the ultrastructural studies. Dr Brachfeld's permeability studies are very important. However, I would be careful in assessing the microscopic presentation, because the pictures for this particular study were not as high in quality as they have to be. Studying the contractile component in these slides shows that these are fixed by immersion and not by perfusion, which means that the cardiac muscle cells were fixed in a state of contraction instead of relaxation. Immersion fixation produces a tremendous number of artefacts, particularly if you want to evaluate this kind of subtle change;
I think the pictures shown by Dr Brachfeld, all the electronmicrographs as well as the injection studies, all carry a danger of artefacts. The conclusions, although they might be right, are not fully supported by the morphological presentation.

**Dr Brachfeld:** I suspect that all the electronmicrographs that were shown this morning were made by immersion and not by perfusion.

**Dr Rona:** Yes.

**Dr Brachfeld:** So I do not see what validity that has in terms of comparing my sections with anybody else's. Control material was presented for comparison. I think that even a very poor histologist would recognize that there were very significant differences between mitochondria in the control and in the experimental specimens, although they may not have achieved the technical perfection possible in Dr Rona's laboratory. There is very little possibility that there could have been confusion between the abnormal and the normal sections. I have been disturbed in the past by the lack of correlation between histological appearance, clinical presentation, and biochemical findings in specific cardiac disease. This is not to say that the findings of individual disciplines represented by specialized workers are not optimal, but that there is little co-ordination between such findings. In this study we evaluated haemodynamic, histological and metabolic performance of the heart. We also tried to correlate findings with electronmicrographic and vascular injection studies, so that we could even evaluate the vascular pattern. These studies were all performed in one laboratory by myself and my assistants.

**Dr Rona:** I think that Dr Brachfeld made a very correct statement, and the data he presented are pertinent. Dr Oakley stressed that we want to find some way of ascertaining the pathogenesis of cardiomyopathy, particularly that of the congestive form. The kind of work that Dr Brachfeld presented is designed to answer this kind of question.

**Dr Schwartz:** I do agree with the concept that Dr Brachfeld is trying to present, but truly it is rather difficult and requires a lot of expertise.

May I make a general comment and congratulate Dr Peters for a very heroic effort. I know why he intended to indicate that biopsies should be done—on the contrary I do not think we should sit by and just use the same old techniques. What I was trying to say is that I cannot quite conceive now how one can extrapolate with all the various errors. I do not deny that you can obtain the data. May I just echo Dr Richardson's comment, or question really, and ask it again—how certain are you that in this sampling technique you are, in fact, getting common myocardial working tissue? I believe that is what you meant. How certain are you that you are getting the proper tissue?

**Dr Peters:** As I say, a lot of the control work was done on tissue from open heart surgery, and there is absolutely no doubt that we are getting the right material. If certain enzymes give the same sort of level in heart muscle obtained by open heart surgery, and in the cardiomyopathic heart, muscle obtained by biopsy, and if, in the same tissue, say, the calcium ATPase is low, then the result is significant.

**Dr Schwartz:** In Chicago, Rabinowitz had clearly shown that normal heart contains maybe 20 or 25% of non-working myocardial tissue, i.e. connective tissue, while in surgery it seems to me one does not know what one gets.

**Dr Peters:** The other problem, for example, is how do you express your results. We have expressed our results as mu/mg of protein—should we express it, for example, as mu/mg of DNA? If there is a question of hyperplasia or hypertrophy, perhaps we should express the result as mu of enzyme/mg of creatin? There are a lot of technical and almost philosophical problems in expressing the results of this sort of experiment.

**Dr Schwartz:** There is one other problem, and that is the problem that I think Dr Burch raised; at what stage is it appropriate to take samples? I still do not have an answer. It is not my area but I will just raise it here as a point for discussion—why not stick to animals?

**Dr Burch:** I do not want to push my point too strongly, but I would like to ask Dr Olsen this question. If the clinicians do not agree with the histologists, what happens then; what becomes of the diagnosis?

**Dr Olsen:** That I cannot answer completely. I make my assessment without any knowledge of clinical history. Subsequently, the results of the pathology are discussed with the clinician and the results are grouped as either confirmed—in which case the diagnosis stays the same—or excluding one or more possible diagnoses— in which case one diagnosis may become definite. Follow-up in some patients has suggested that the result of the biopsies, in the 60% of the patients where the pathological diagnosis has been of use, has made a difference to the clinical management. This may have resulted in further investigations, such as open heart surgery. In these few cases the diagnosis has also changed.

**Professor Goodwin:** I think we are getting confused between making the diagnosis by biopsy in individual patients, which at the moment is not really feasible, and doing cardiac biopsies to advance our knowledge. There are much better ways than biopsy of making a diagnosis, certainly of hypertrophic cardiomyopathy.

**Dr Olsen:** I would absolutely agree, but diagnostic feasibility has so far not been worked out properly. In the majority of cases there are other ways of making a diagnosis, but in a few patients there still exists serious clinical doubt, where direct examination of endomyocardial tissue may be of help. This also applies to hypertrophic cardiomyopathy where, although rarely, such examination is relevant. Of course, we are undertaking many other investigations on this biopsy material, for example cytochemistry, in the hope that some abnormal factor or factors may help to elucidate the pathogenesis of cardiomyopathy.

**Dr Oakley:** I think this is the crux of the problem. We do not understand so we should try to discover. That is why we are here.

**Chairman:** Is there such a thing as hypertrophic obstructive cardiomyopathy, which can be diagnosed by biopsy alone, and nothing else?

**Professor Goodwin:** Well, I think you prejudge the issue by putting the label on it in the first place, for,
thereby, you have presupposed that there are other means of making a diagnosis. If you had put the question 'Are there people with a disorder of heart muscle in whom it is impossible to arrive at a diagnosis by clinical haemodynamic and other means?', the answer of course is 'Yes'. There are such people, but I have yet to find that a biopsy will give a definitive answer in the majority if serious clinical doubt had existed.

DR SOMERVILLE: Let us say that before the biopsy you did not know what it was, apart from heart muscle disease.

PROFESSOR GOODWIN: Yes, I see what you mean.

CHAIRMAN: And then Dr Olsen comes along with the biopsy and he says it is HOCM!

PROFESSOR GOODWIN: Occasionally, yes.

CHAIRMAN: At this point one can say that in certain instances biopsy may be important, and we must try to identify its areas of usefulness.