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DR. GOLL: Thank you, Harry, for the very clear picture you have given us concerning some of the rather difficult techniques which are available to study fibrous proteins. Earlier this morning you heard a discussion of the structure of muscle. I think you will find that many of the structural characteristics or structural features found in a muscle cell are those which we would ordinarily expect in a normal cell. A muscle cell has a cell membrane, cytoplasm, sarcoplasmic reticulum or endoplasmic reticulum, mitochondria, nuclei -- many of the same structural features which you would expect in an ordinary cell. The presence of the myofibril makes muscle and muscle cells a highly specialized cell, and Bob Cassens outlined for us some of the unique features of muscle cells. Dr. Szent-Gyorgyi then went into the characteristics of the proteins, especially the proteins which make up the myofibrils. We learned some of the unique structural characteristics proceeding to the molecular level in our discussion, particularly in the case of myosin, about which there appears to be more information than about the other two major proteins found in the myofibril. Then our last speaker this morning, Dr. Snyder, outlined for us in general terms the methods which are available to separate and study the fibrous proteins which make up the bulk of muscle.

I think we're just about right on time, so we should have approximately fifteen to twenty minutes left for discussion. Are there any questions from the floor? I might add that you please state your name and your institution before asking a question for the benefit of our recorder.

DR. PAUL: I am Pauline Paul from the University of California. I am very much interested in the problem of protein separations and in looking through the literature you find the sort of thing that Dr. Szent-Gyorgyi mentioned concerning a ten per cent stroma content of rabbit muscle. Then you look at the kind of thing that has been done with the beef round muscles where you find somewhere in the neighborhood of maybe two to three per cent connective tissue. I am curious about what else comes into this stroma to bring it up. Does anyone have any information on this? We're trying to match up some of these basic data and get some idea of how much we should find in these different factions, you see, before we start.

DR. GOLL: This is an interesting topic. Perhaps Dr. Szent-Gyorgyi would care to comment on it. I understand the nature of the proteins in the Z-band is not altogether known and this may contribute in part to the disparity.

DR. SZENT-GYORGYI: I am afraid it will depend on what type of muscle you are using. You certainly will have protein in the Z-band. People suspect that part of it may be due to tropomyosin, but you may have various amounts of connective tissue. I don't think one can give a straightforward answer about what you will find or what you should expect.

DR. PAUL: Well, the thing I'm puzzled about is that the rabbit source has about ten per cent stroma protein on this kind of a basis. The beef round muscles which would be expected to have a lot more connective tissue seem to have a lot less. Of course they are using different methods of separation.

DR. SZENT-GYORGYI: It would depend entirely on what method of extraction you use. You may be employing methods for extracting actin or actomyosin or tropomyosin. But if you use 20% urea the per cent of soluble protein will certainly be much larger than if you use simply high ionic strength KCI. If you use potassium iodide of some sort you can make an extraction of 95% to 96%. So it entirely depends on how you define your extraction, how you identify your proteins and then you have to find out where these proteins come from.

DR. PAUL: Has there been any work to try and identify what they call these stroma proteins?

DR. SZENT-GYORGYI: Not at all. Part of the stroma protein fraction may be tropomyosin, which was called a stroma protein about fifteen years ago. Now it is called a fibrillar protein or a protein of the myofibril. It is not associated with the sarcolemma at least, and now we start to suspect it may be actually part of the Z-band but it is a long process, you see. I think you call it a stroma protein if you don't know anything about it.

(Laughter)

DR. TRAUTMAN: I am from Oscar Mayer & Company. I would like to direct a question to Dr. Szent-Gyorgyi. At rigor mortis how constant do you consider the stoichiometric combination of the molecular ratios of actin and myosin, and can this be influenced by the physiological condition of the muscle tissue at that time?

DR. SZENT-GYORGYI: Well, the amount of actin and myosin in the muscle is not a question. I think the question is what are the number of cross linkages present during rigor mortis, and I have to speculate here. It has been concluded by others, that the development of rigidity, or rigor, coincides with the disappearance of ATP. It develops only when the ATP level decreases. I cannot answer your question directly, however, I would like to know the answer.

DR. TAKI: Taki, from the University of Florida. I am a little bit confused about the method of extraction for the

different muscle proteins. Different people seem to use different procedures for extraction of the different protein components, such as myofibrillar proteins. What is the difference in the extraction, whether we use, say pH 6, or an ionic strength of 0.1 or 0.5? Are we extracting the same protein or not? Also, concerning sarcoplasmic protein extraction, should we use water or buffer for the extraction of these proteins? What is our reference? What is the standard procedure, or isn't there any?

DR. SNYDER: I think you hit on it. The trouble is we don't have any reference, and there is no standard procedure. You have to consider the fact that there are many, many proteins involved. I don't think it is surprising that each of these proteins would require just a little bit of change in ionic structure or pH or temperature or some of the other conditions to get them out exclusively in relation to all of the other proteins that are in muscle. I don't think this is anything to worry about. It is to be expected. But of course the problem is to find the right condition for the protein you want, and this is strictly quesswork as far as I know.

DR. TAKI: Do we get all the protein out of the muscle, all of the myofibrillar protein out, for instance, using a certain volume at a certain strength or not? Using the micro-kjildahl determination we say that 16% of muscle is protein. Is this determination true? Do we get all this protein out of the muscle?

DR. SNYDER: Maybe Dr. Goll could answer that.

DR. GOLL: I didn't hear the question.

DR. SNYDER: I just don't know. I can't say whether you can get all of the myofibrillar proteins out of muscles. It depends on how you grind the thing up to start with, and different workers are using different methods, yet they all arrive at almost the same answer.

DR. GOLL: They have used numerous different solutions. I believe that complete extraction of myofibrillar protein has been demonstrated by using strong KI extracting solutions. This is your best bet, if you want quantitative extraction of the myofibrillar proteins.

DR. TAKI: How is one sure that he gets all of the protein out of the muscle?

DR. SNYDER: I don't think you would be sure, not at the state of the knowledge we have right now.

DR. SZENT-GYORGYI: You measure it. That is how you are sure.

DR. GOLL: You measure what is left?

DR. SZENT-GYORGYI: No, you measure the ATPase activity of the entire muscle and then the ATPase activity of the extracted protein. If these are not the same, then you have not extracted all of the myofibrillar protein for almost all of the ATPase activity is in the myofibrillar protein fraction. If you have not extracted all of the protein, then you check your extract procedure. You know that extract procedure does not destroy ATPase activity. By this procedure you can measure, somewhat, how complete your extraction has been. With other proteins, it's a bit tougher, but it can be done. If you do this measurement, how much there was and how much is remaining, then you know. But for extracting the other myofibrillar proteins you need different procedures.

DR. TAKI: Well, in your classification of muscle proteins, there is a general statement that you can extract all of these proteins by using a buffer of high ionic strength, about 0.5 or so. If one used this strength, should one expect to get all these proteins?

DR. SZENT-GYORGYI: If you cut the muscle up before extraction. If you don't cut it up, you don't expect anything, or very little--say ten per cent of what is there. By varying the degree to which the muscle is minced before extraction, you may change the amount of protein extracted considerably. But whether completely extracted or not, that depends upon the muscles used, how you do your experiments, how long you extract and so on. But you can determine what is present in this type of muscle and what is left after extraction. If you don't determine this, nobody guarantees you that you get 100 per cent extraction either.

DR. GOLL: Just a couple more questions.

DR. WANDERSTOCK: Dr. Szent-Gyorgyi, I wonder if you would elaborate on the most current evidence describing the mechanics of rigor.

DR. SZENT-GYORGYI: As far as rigor mortis is concerned, I think the best study is still Schiller's, where they followed the appearance of rigor and measured muscle metabolism during the development of rigor or rigidity of the muscle. They found that it was correlated only with the disappearance of ATP, and I think I am correct in saying that the simplest picture is that rigor is due to formation of actomyosin, because of the fact that ATP disappears. ATP present in the resting state of muscle is necessary to keep the links between actin and myosin separated, and you have got to have enough ATP, of course, to make it contract. Muscle cannot be stretched in rigor. The muscle will rather break than stretch. What happens later is difficult to say. It is probably due to some proteolytic changes.

DR. MULLINS: Mullins, from Louisiana. I would like to direct a question to Dr. Snyder. Is it possible to isolate reticulin from collagen?

DR. SNYDER: I am going to pass on that one. The expert is up on the podium as far as collagen is concerned. In my own estimation, reticulin hasn't been well characterized. I don't have information on it myself.

DR. GOLL: I would say it would be extremely difficult. Perhaps you could make different use of the thermostability of protein.

I think at this point that I will turn the program back to Dr. Henrickson.

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DR. HENRICKSON: It appears that we have only spectators in the other room. The reciprocators are all in this room. I don't know where Chairman Briskey is. Are there any announcements, Quinn?

DR. KOLB: We are very pleased to have the Usinger Sausage people serve as hosts to the charter members of the American Meat Science Association this noon at 12:00 o'clock over at Lowell Hall.

DR. HENRICKSON: We are adjourned until 10:15.

(Recess)

DR. HENRICKSON: Gentlemen, Prof. Quinn Kolb has an announcement before we proceed with the program. Quinn?

PROF. KOLB: Thank you, Bob. We have one thing that I hope we can get across to you, if we can talk to the coffee drinkers and others too. When we assigned you parking places we hoped you would continue to use those parking places that were assigned to you. If your car is in another spot you may well be in a place where the owner of that parking lot may have you removed at your expense. So this creates a little problem that we think we can alleviate by working with you, and so we won't pick out individuals but if your car is not in the place originally assigned to you, would you try by noon or shortly after to get it back in that original spot. We have a couple of fraternities who feel that using their spot perhaps just isn't exactly Hoyle. So if you folks who perhaps have changed your parking place could get back in your original spot, it would help the problems of our parking committee a great deal and then we perhaps wouldn't have the problem of trying to locate your car in one of the police lots here in the city. So we want you to enjoy your stay here. The other announcement we want to make again, in case you missed it, was that all of you people and your guests that you may have here are invited to the noon luncheon and I pointed this out to you before that the Usinger people are pleased to serve as hosts to the charter members and their guests of the American Meat Science Association. So we will look for you at 12:00 o'clock over at Lowell Hall and we wish you would get this message to all of your guests and friends here. The wives are not invited to the luncheon this noon.

One other announcement, tonight we are serving well over 300 at the banquet at the Memorial Union and we would urge you to go over as soon as you can after the session is finished this afternoon. We are going to try and serve at 6:00 o'clock, on time, and our folks at the Union are quite punctual. We will need your cooperation to get in the building up the stairs and into the main hall at 6:00 o'clock. Very fine. Enjoy yourself this afternoon and this evening.

DR. HENRICKSON: Thank you, Quinn. Proceeding with our program, you note that the other selected topic was physiological phases of the basic research on muscle tissue. To head this section is Robert Sayre of the Western Utilization Research and Development Division of ARS, USDA at Albany, California.

DR. SAYRE: Thank you. I would like to take this opportunity to express my appreciation to the other members of my committee for their excellent help and advice during the development of this program. Dr. Fred Deatherage, Dr. Harold Hedrick, Dr. Paul Lewis and Dr. Jim Stouffer.

We are quite fortunate to have with us Dr. John Whitaker from the University of California. He is presently on a one year sabbatical leave and is working in the Department of Chemistry at Northwestern University. Dr. Whitaker received his Ph.D. degree under Dr. Deatherage at Ohio State and after a tour in the army has been at the University of California at Davis. His Ph.D. work was in the line of protein hydrolisis on ion exchange resins, and work since then has been with proteolytic enzymes. At this time it gives me great pleasure to present Dr. John Whitaker.

DR. WHITAKER: Thank you, Dr. Sayre.

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