I want to tell you a little bit about the x-ray diffraction of muscle structure.

I'm afraid despite your Chairman's remarks, I'm not an expert in this particular field. I have been doing x-ray diffraction work on protein structure in general; fibrous protein with a little bit of work in muscle.

The electron micrographs that you saw in the previous paper were indicating structures of the order of several thousand angstroms in size. The sarcomere length for example, the distance between one Z line and another is a distance of about a couple thousand angstroms. X-ray diffraction will look at structures which are very much smaller than this. The order of a couple of angstroms is the order of one to two angstroms. If we know the number of phenomena interferences which gives rise to the interference pattern, it would give us indication of structures which are the order of several angstroms up to several hundreds of angstroms.

We are looking at a different order of structure for one thing, and therefore of necessity, are concentrating on a different kind of problem. The problem which has been of primary concern is, I think, the establishment of the nature of the polypeptide chain structures that exist in the filaments of muscle.

Let me first tell you a little bit about the x-ray diffraction techniques. I don't know what extent you are familiar with it. It basically is, of course, a fracture of interference phenomena. When anyone has a molecule of scattered centers and the intensity upon this is a monochromatic beam of x-rays, for instance, there is diffracted or scattered a very disordered array of beams. If one were to take a simple organic substance or something of this type, the kind of pattern that would be observed is the following, if this very simple experiment is done.

The experimental aspect of the technique is almost trivial. Let this be a muscle fiber, for instance, perpendicular to a beam, very fine beam of x-rays, monochromatic perhaps by filtering and behind this you put a piece of photographic film. You would see the type of pattern that is shown on slide one.

Now, the sample is somewhere out here. This just happens to be a beamstock to catch the very strong, direct beam. So, it doesn't completely fog the photographic film. What you see is an array of spots which represent the diffracted beams of x-rays. The problem is, from such data can one get information on the structure of the original substance? The information that is available is of two kinds. First, the geometry of this diffraction pattern; namely, the position of the various spots in relation of course, to the known specimen of film substance. The second, the known wavelength of
x-rays. The second bit of information that is available is the relative intensity of these spots, which some, you will notice are stronger than others.

In studying protein structure, the next slide shows a picture at the opposite end of the spectrum, so to speak. This is a diffraction from an alpha, the protein that occurs in horn, hair, nails; materials of this type, as we'll see, are the basis for the structure of one of the filaments in structure; namely, the myosin filament. This was the diffraction pattern obtained. This is very poor, as one sees only several diffuse regions of scattering here and there.

Let me briefly indicate two important directions on this film because they indicate something about the structure. The vertical line we call the meridian in the pattern. Any sequence of spots along this direction are indicative of the periodicity of the longitudinal direction of the fiber. On the horizontal direction, namely the equator of the pattern, spots will represent periodicities. Due to lateral ordering, and the originality of the material, somewhat intermediate between these two patterns is the pattern shown in the next slide.

This is another fibrous protein, feather keratin, the protein in the quill and rachis of feathers. It is a very ordered structure. It happens we are working on the structure of this particular protein. It happens to be of interest for various reasons. I think you'll see we have many more spots; much more detail. From this one we will try to determine or induce the structure. I don't have time to go into the methods by which this is done. They are rather complicated. They are certainly not trivial.

In the case of proteins, which are very complicated materials, one has to resort to the following type of technique. This is true also for muscle. That is, to get some idea on the basis of many types of data. To get some idea what the structure might be, we are talking about structure of individual proteins. They are three dimensional. One has to get some idea about this structure and then make a test of diffraction pattern that would be predicted by it. You have to use the inverse procedure which is a very unique one.

You can always calculate a diffraction pattern. You hypothesize a structure; you can then test out its pattern. There are various ways of doing this pattern. It is a kind of optical procedure. This happens to be a diffraction pattern of collagen on the left and this happens to be the optical diffraction pattern given by a mold whose structure is believed to correspond to the polypeptide chain structure in collagen. A small chain will make a large chain in the pattern. One can by this method get some confidence in the correctness or otherwise of the structure that you hypothesize.

Let me turn very briefly to some of the results, just to remind you what the situation is with our present knowledge of protein structure.

The next slide shows results of studies on small molecules. This is a polypeptide chain. It has the sequence of C, R, and H, and we know the dimension in such a chain fairly well from very careful studies of small
organic molecules. This would be a substantially stretched out form of the polypeptide chain, from which work that has been done by polypeptide in this group. One has also the extended structure taken up by such a chain. One of these is shown on the next slide.

This is similar to the one I have just shown, except it is impossible to fully stretch out such a chain. The chain intends to take a bent or pleated form in order to produce, or in order to enable proper or satisfactory hydrogen bonding to occur. A weak bond is shown between the oxygen on the CO group and the hydrogen on the NH group.

One type of structure which can be formed is an extended structure of this sort. Another structure shows, and for which there has been a great deal of evidence accumulated, that there is a kind of folded or helical structure. This is only helix. I prepared two of them. This is the only one for which there is good experimental evidence. The chain folds in such a way upon itself that hydrogen bonds are made within. That is between one portion of the chain and another portion of the chain, rather than within and between the chains as in the case of the extended chain structure; and furthermore, one knows as is shown in the next slide, there is a tendency for such helices to super coil. Each one represents an alpha-helix and the axis instead of being straight, may itself take a very slow coiling with the result that one can form such aggregates of the super coils.

Now, what has this to do with the muscle and what sorts of things can one do with the x-ray diffraction of the muscle? Let me first show you a few of the x-ray diffraction patterns.

The next one shows one for a muluxon muscle. These are patterns described by Dr. Worthington. Now, you've got to turn your head sideways by ninety degrees or you have to keep in mind that the slide is oriented at ninety degrees. The axis of the muscle is horizontal rather than vertical. So, the periodicities that we have seen are what were along the fiber. It is a very rich pattern relatively speaking. The pattern has the following characteristics: First of all, it shows the kind of scattering -- (these broad areas and these have strong reflections here), -- which are associated with the alpha-helix. This coiled structure, which I just showed you on the previous slide, has a very fine series of spacing going down at very low angles, which means very large distances because the angle of scattering is extensively reciprocal to the periodicities you are experimenting. This pattern is of the whole muscle and shows a super position of the myosin compound and the actin compound. One, of course, can compare this in greater detail. One can find out the periodicities corresponding to these various lines here.

The next pattern shows a very strong myosin pattern. In other words, we have a super position of these two structures and we have to somehow disentangle the results. The information we get from them are just brief summaries. We find out something about the axial period, that is the period in which there is an identical repetition of some sort of scattering material in the thin filament, the actin filament, 410 angstroms; and in the thin filaments for vertebral muscle it is 435 angstroms going up to about 475. The problems we are mainly interested in tackling is the molecular structure of this compound.
We know the thick filaments are composed of alpha-helixes. They are probably present in the super coiled form. The details of this structure and how they're organized in this filament are not known; the same for the actin filament, the thin one. At the present time there is no knowledge on this whatsoever. What this means is the following: We have investigated and we do know, for instance, in various fibrous proteins, proteins like collagen, what the polypeptide structure is. This must represent something quite different from myosin and actin. This diffraction pattern in no way corresponds to this. From information of this type, we do get some knowledge of periodicities and can form some density about the molecular structure.

One thing we did find out though, if you stretch a muscle or allow it to contract, there is no change in the x-ray pattern; no change whatsoever. This is presumptive evidence for the validity for this interdigitating mold you saw on the previous slides. There is, of course, a great deal of electron microscopy on this subject. It is not completely acceptable by everyone in the field. Certainly the evidence does in a sense help to verify the ideas brought out by the electron microscope. In fact, it can do a little bit more.

The next slide shows the very close region in the equator. This represents the stock right in here and one sees these two spots which are representative of very large lateral spacings. The order now of a couple hundred angstroms representing the packing of the thick filament. These are patterns which are taken of the sarcomere length in microns on the right. These two patterns should be inverted with respect to the distance shown. What one observes is a change in the relative intensity of these spots shown in the next slide.

One can't make the measurement terribly accurate. The importance of this is the following: The importance is that we can tell that in one case where the muscle is, the sarcomere length is shown. We can tell that in between the set of thick filaments are a set of thin filaments. The fracture pattern and relative intensities are not affected by the removal of the thin filaments within the array of thick filaments. The intensity changes, the periodicities are not affected. The distribution of scattering matters are quite different and this has its main effect upon the relative intensities.

Let me show you one thing. I have been trying to do a little work on the actin and its elements. Bear and his collaborators were able to produce the one thing that one can get out of an x-ray pattern besides the periodicities. One can get the symmetry of the structure and the relationship of the scattering units. You may not have any idea what the scattering units are. You may not have any idea whether to look at the units in this fashion or in fashion to each other, but you can at least say something about the rough symmetry, the units of scattering and how they are positioned along the fiber.

Now, this only represents a sort of what Bear calls a cell. That is, if you wish you can take this and wrap it around itself in a T; X-ray diffraction pattern won't tell you this. It will simply tell that the symmetry is such that if it be helicel there are fifteen units in seven turns of a unit before you repeat it by identical translation.
The last slide is purely speculative. It is on the structure of the actin and what it is that may give rise to this periodicity. Don't take it for any more than speculation. The feeling is, it may be based on an extended chain structure represented by these lines which form as it were, a kind of hollow cylinder. By means of hydrogen bonding with each other and that certain residues, particularly protein, which may be very important to the determination of the structure, repeat periodically and in fact much of the data which I won't go into, consistent and giving exactly this symmetry that I indicated.

In other words, if you took the center of a plane of proteine and went around, you would go around seven times; seven times of such a helix and there would be fifteen of such units before you obtain the translation.

I hope I have given a little indication of the kind of things that can be done and are being done. The technique is certainly not one that visualizing as it were, either the atom or protein change. There is a lot of thickening that goes on in between.

The important thing of what we are trying to arrive at is the special, the three dimensional structure of the atoms in the molecule which comprise these larger units; mainly, the filaments you see in the micrograms.

(Applause)

MR. BRISKEY: Thank you very much, Dr. Krimm. We will move on to our next speaker, C. E. Bodwell from Michigan State University. C. E. was a graduate of Oklahoma State University, 1956; was a recipient of a Fulbright Scholarship and worked at the University of Cambridge in England and obtained his M.S. Degree there in 1959. I'm sure many of you are familiar with his fine publications. At the present time he is completing his Ph.D. at Michigan State under Al Pearson. We are very pleased to introduce C. E. to this conference. C. E.