Muscle Activity

A HISTOCHEMIST'S VIEW

BSI interviews Dr. V.R. Edgerton

In many elementary and advanced texts of physiology and anatomy, the section on skeletal musculature is crowned by a series of drawings which show the human skeleton as a complex mechanism replete with hinges, pulleys, beams, trusses, etc. The muscles appear as winches, engines, cables or springs, and the main point seems to be an analysis of the complexity of human movement into a form of energy release which is ultimately shortenings and lengthenings.

This approach has its value, but directs one's attention to movement as a whole phenomenon by presenting muscle itself in a mechanical, oversimplified fashion.

It is clearly asking too much that every individual's knowledge of the human body should include all the latest facts in every area. But it is desirable, even necessary, I believe, that the

overall maps or models by means of which one integrates his store of information at least have room for such facts as he may discover. The chief deficiency of a model of the human body which presents muscle as cables or springs, is the oversimplification of what muscle metabolism is and how it relates to the rest of the body's activity.

Dr. V. Reggie Edgerton is a young scientist engaged in histochemical research into muscle tissue at U.C.L.A. In the following interview he gives us an idea of the complexity of this field.

Laurence E. Davis

Just what is histochemistry? I think most of us have a fair idea of what chemistry is, but how is histochemistry different?

Histochemistry is just a combination of histology and chemistry. Histology is and has been for many years the science that deals with the microscopic anatomy of tissues. We do this simply by removing tissues, treating them so they will not degenerate, and then taking thin sections of these "fixed" tissues. We cut them with a microtome--a knife-- into thin sections, place these sections on a glass slide, and then stain them. The staining differentiates acidic or basic portions of the cell and this helps to identify organelles within the cell. We use light microscopy (rather than electron microscopy) for our work, and are primarily concerned with the makeup of cells rather than the structure of those elements--for example mitochondria--which constitute the cells.

The "chemistry" portion is the use of chemical techniques to study the various parts of the cell. Our work is primarily enzyme histochemistry. In order to study enzymes it is necessary to quick freeze the tissue, because the enzymes will not remain active indefinitely. We test the tissue by putting it into a medium which contains all

the chemicals necessary for that enzyme's activity, as well as a dye which is bound to the tissue by the chemical activity of the enzyme. The greater the activity, the darker the stain becomes.

So that chemical activity is reflected as optical density?

Right. The advantage of histochemistry is that it results not only in some degree of quantification of the amount of chemical activity (by the darkness of the stain), but also the location of that particular activity within the cell.

How would you describe the objectives of your laboratory?

The overall purpose of our lab is to identify some of the metabolic changes that can be associated with physical activity (all kinds of activity). And we would like to relate these changes to changes that occur in muscular diseases. Then, perhaps, if we can put these two things together, we could use exercise in a rehabilitative manner. If we knew the specific adaptations that are needed to restore a diseased muscle, and we knew exactly the type of exercise that would induce this type of metabolic change, then we could formulate an exercise program to improve the person's condition. This program would probably not cure the disease, but at least improve the person's ability to perform daily chores. Related to this, we think that specific adaptations occur in specific types of exercise. We are just beginning a project to test the metabolic changes which occur as a result of exercise--not just running on a treadmill, but also weight lifting, isometric and isotonic exercises as well as running and jumping. We can test each of these types of exercise with the animals we are beginning to use for experimentation--the bush baby--a non-human primate.

How do you anticipate this primate will give you possibilities that you don't have in other experimental animals such as rats, guinea pigs and hamsters?

All you can do with rats and guinea pigs is train them to run on a treadmill. So you end up studying endurance-type activity. As a result we know quite a bit about some of the important changes that occur in muscle tissue with an endurance activity, but we don't know what happens with different types of exercise. The literature reports "the effects of exercise" and people have a tendency to consider all types of exercise as equivalent. We believe it is much more specific than that.

So a primary advantage of this higher type of experimental animal is its ability to be trained in various spe-

cific types of exercise allowing you to distinguish the results of each?

Right. And we are really interested to investigate not only whether specific changes may be induced by specific types of exercise, but what causes these changes. We can say that increased endurance-type exercise induces changes in the number of mitochondria within each muscle cell, but we don't know how this occurs. Is it because the cell runs out of oxygen? Or does the change in the amount and type of neural stimulation cause the change? We can't separate the metabolism of muscle cells from their neural control.

For example, we have found two types of fiber in muscle tissue, so called "red" fibers, associated with endurance-type activity and "white" fibers, associated with activity involving the strength of a single contraction -- weight lifting, for example. Various muscles have different proportions of red vs. white fibers. When a muscle which is predominantly "red" is surgically re-innervated with the nerve which had originally supplied a "white" muscle, that red muscle becomes white, and vice versa. So it may very well be that the number and type of impulses flowing through the nerve actually determine the metabolism within the muscle, although it appears difficult for many scientists to conceive of this. But then, not too long ago it was difficult for many scientists to imagine that the nerves secreted substances at the synapse which mediate the flow of impulses across that synapse, although this is now established.

Would you put these substances into the category of endocrine metabolites?

We can't really separate neural and endocrine control mechanisms, since nerves secrete specific hormones--epenephrine, for example--which fall into the category "endocrine".

Then in a sense you cannot dissociate your work either from the chemistry of learning (since you are trying to change muscle structure by changing activity) on one hand and from the metabolism of growth and cell replacement on the other (since there is a tendency for the body to reconstitute itself independent of exercise routine)?

Yes. Very probably all that exercise does is to alter the rate of synthesis or degradation of the proteins which constitute the enzymes that regulate cell metabolism. There is, in any case, a constant turnover of these proteins.

In a sense, the exercise you impose on your animals then, is like the destructive testing of some mechanism--a car for example--in which a certain part will be re-

placed every so many miles. If you run it faster, the change takes place in a shorter period of time, but at the same rate as far as the mechanism is concerned.

Yes, that may be exactly what happens. But perhaps when the muscle fiber is chronically stressed to a certain level, it will adapt to that level by building up a greater reserve concentration of enzymes within the cell. In this sense a muscle fiber which has adapted to a higher level of activity is "different" in composition from a fiber which has adapted to a less active state.

Another interesting point is the endocrinology. When a person is exposed to a stress -- we consider exercise a stress -- a certain amount of ACTH (adreno-cortico tropic hormone) would be released from the anterior pituitary. The overall effect of this hormone is to speed up the metabolism -- produce more energy. After he had adapted to that level of muscular activity, the same amount of exercise would not cause as much ACTH to be released. Thus, not only does the metabolism within muscle tissue respond to the influence of ACTH, but the mechanism by which this hormone is released somehow senses the level of activity to which the muscle fibers have adapted.

What are some of the most important ways muscle tissue can change as a result of exercise?

In endurance-type exercise, we are running the muscle to the point of fatigue--the point where it will no longer contract. We have found that this fatigue is a result of the muscle running out of ATP (adenosine tri-phosphate), the form in which glucose is stored in the cell. ATP is the "fuel" for muscle contractions. To produce ATP there must be sufficient glucose, sufficient oxygen, sufficient mitochondria and sufficient enzymes which utilize oxygen and glucose to produce ATP. The end product of "burning" the ATP is CO_2, so there must also be a sufficient mechanism to remove this from the cell and replenish the supplies of oxygen and glucose.

The adaptations as a result of chronic exercise are, as you would suspect: an increase in the capillarity of muscle fibers, which suggests more efficient blood flow to supply glucose and oxygen and to remove CO_2; an increase in the number of mitochondria; and an increase in the concentration of enzymes responsible for producing ATP. The result of these adaptations is more ATP--hence the ability to perform more exercise until the point of fatigue.

Having this change of internal equipment, does the chronically exercised muscle differ in other ways from unexercised muscle? Would there be a difference in strength? In reaction time?

We just completed a study of this a few months ago and as you might expect, endurance was increased as a result of the training program, but strength wasn't changed at all. The contractile properties were unchanged--the muscle was simply able to carry on its activity for a longer time.

That exercise program was quite specific for endurance. How about hypertrophy, or increased strength?

In swimming and running we have found no evidence of hypertrophy. To change the strength of a muscle, a high resistance overload is necessary. This causes changes in the contractile proteins which cause the contraction of the muscle.

Besides strength, are there any other qualities in the performance of the muscle which you could isolate so far as to be interested in them experimentally, such as the quality of contraction--the number of different types of contraction that can occur?

I would say that the changes we have noticed in the muscle are not really going to change the way a person integrates movement. I think such changes are localized in the central nervous system.

I would expect contraction time to be relatively constant and independent of the metabolic changes we have discussed. And the other types of behavioral changes which involve a difference in movement--the changes at different levels of maturity, different emotional states -- I am sure these result from changes in the central nervous system. These have not been adequately studied. I would love to be able to study such changes, but we know so little about the central nervous system, we can't even begin to approach such problems.

What would appear to be the key factors in initiating the changes in muscle tissue which you have studied?

We don't know. It could be lack of oxygen, or a change in ph, level of CO_2 in the cell, or the level of substrates or of any of the thousands of metabolites which are part of the cell's normal activity. Eventually it is going to have to be traced back to, and explained at, the level of DNA and RNA.

I see. Now we get into the interesting area of changes which take place in a cell after it is constituted. At some point the switch is somehow turned on that there needs to be a new cell or a new part of a cell synthesized. To what degree do the changes that have taken place in the cell up to that point affect the new material that is being put in?

Does skeletal muscle even reconstitute itself at the same frequency as other tissue?

Each protein has a specific half-life. The contractile proteins like actin or myocin have a half-life of about thirty days. In other proteins, I'm sure it's a matter of two or three days, perhaps even hours--others probably longer. In any case there is a constant turnover of protein.

I understood that skeletal muscle constituted the largest single mass in the body which existed from shortly after birth to death; that in other tissue cells died and were replaced by new ones, but that the same muscle cells persisted all through life. Is this view still accepted?

Well, one fellow has raised the possibility that whole cells are replaced in muscle tissue--although his suggestion is not a very likely one.

Would you say that as a general statement, as much of the vital machinery of the body is below the limit of the cell (within individual cells) as there is above cells in organs and larger structures?

Right, and I think the general concept is that most cells are pretty stable--that what they have they keep. The general assumptions about the nervous system--that it is quite stable, that it doesn't adapt, that metabolic activity is quite low, etc.--I think we will eventually discover to be false. The metabolic activity of the nervous system is much greater than was believed. For example the glia cells, supposedly had just a structural function as a base for the neurons. But there is good evidence to suggest that these cells also serve some important integral function in metabolic activity and perhaps other types of activity as well.

Have you any long-term plans to try some of your hypotheses in humans as well as animals? Obviously your main work has to be on animals, since you are removing whole sections of tissue and so on.

Yes, we would eventually like to make some studies on humans, but we haven't made any attempt yet because we're not ready. We are studying human muscle samples -- biopsies -- that we occasionally get. We are doing this to relate the metabolism and histochemistry of the human to rats and guinea pigs to see if we are in the same ball park. We are eventually going to relate the metabolic similarities and differences of the rat, guinea pig, mouse, bush baby, and human.

There are some studies in which I would really like to use humans in order to get at the primal aspects of the

fiber types that we mentioned. We have already found that in the rat and guinea pig, the red fiber is used preferentially for endurance-type activity. Our next step is to investigate whether the white fiber is used preferentially for activities involving strength. We are going to try to get at this problem with the bush baby for the reasons I mentioned earlier. Then we will be ready to see how this relates to fiber types in humans.

Thank you, Dr. Edgerton.

* * * * *

RUE