Oscar H. L. Bing *

CARDIAC CONTRACTION. III. EFECTS OF HYPOXIA ON THE MYOCARDIUM

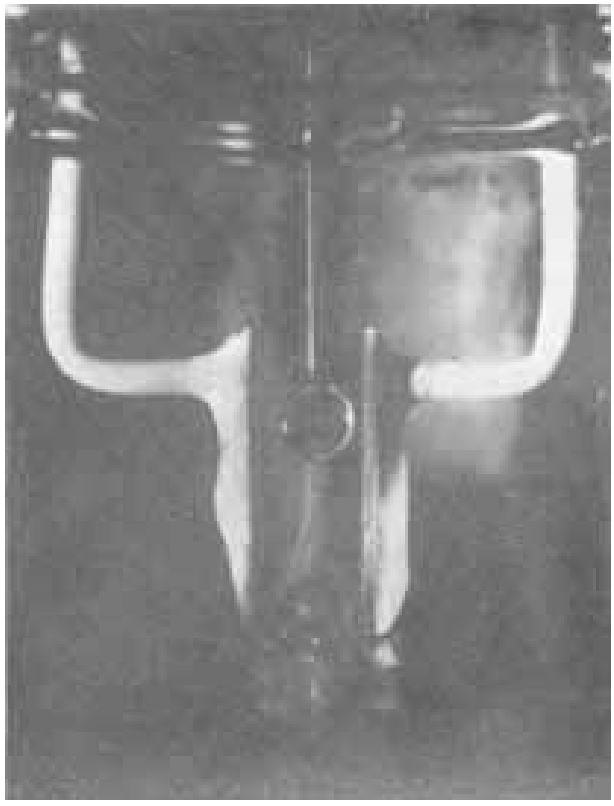
In the topics I have discussed earlier at these meetings, I have attempted to elucidate some of the complex events which occur during ischemia by studies of the relatively simple isolated muscle preparation.

We want to emphasize hawever, that hypoxia and ischemia are not identical processes. Hypoxia means decreased O_2 while with ischemia, in addition to oxygen deficiency, there is decreased presentation of other substracts and importantly, the accumulation of metabolites. Retention of metabolites, particularly acidosis has been considered by many to be a primary factor associated with deleterious changes to the ischemic myocardium. Thus, elucidating differences between ischemia and hypoxia and identifying the roles each of these processes plays, as well as their combined effect, is of considerable interest.

I would like to describe two experiments we have carried out to further define the role of bypoxia in the ischemic process. These experiments are relatively simple, but they may be the basis for new ideas and approaches toward understanding fundamental aspects of the ischemic process. For those of you who were not present at my other presentations, it is necessary that I briefly familiarize you with the isolated muscle preparation, also I would like to review fundamental aspects of the effects of hypoxia on cardiac muscle.

Rats are sacrificed, hearts are quickly removed and the papillary muscle from the left, ventricle dissected free and mounted in a chamber containing oxygenated krebs solution as shown in this slide.

This is a diagramatic representation of the muscle chamber. Muscles are stimulated at a rate of 12 per minute by parallel platinum electrodes. The spring clip on the tendon end of the muscle is connected to the lower arm of a low



inertia DC motor. The lower clips is attached to a semi conductor strain gauge transducer immersed in the bath.

Either the length or the tension of the preparation can be controlled by means of an electronic nervosystem controlled by a digital computer. Muscle contractions are recorded by recording length and force at a rate of one kilohertz. Quantitations error was less than 5 microns for length and 2O milligrams for force.

As example of the types of recordings we ob-

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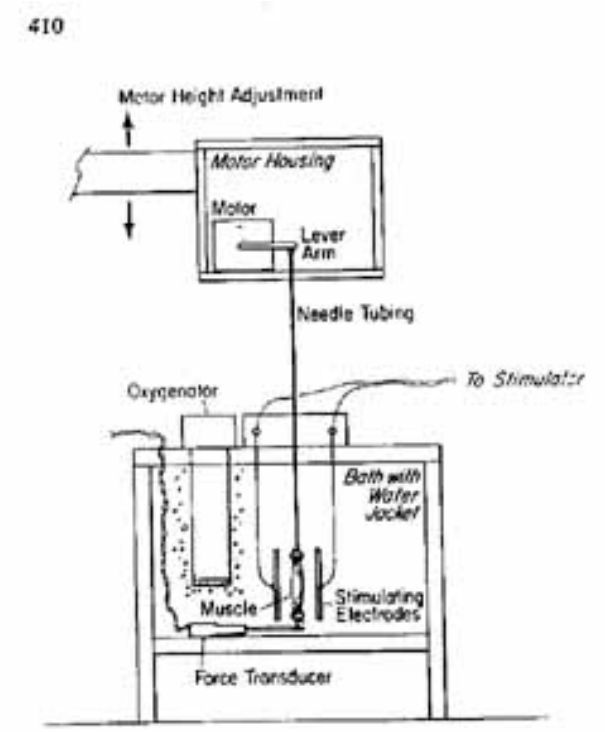
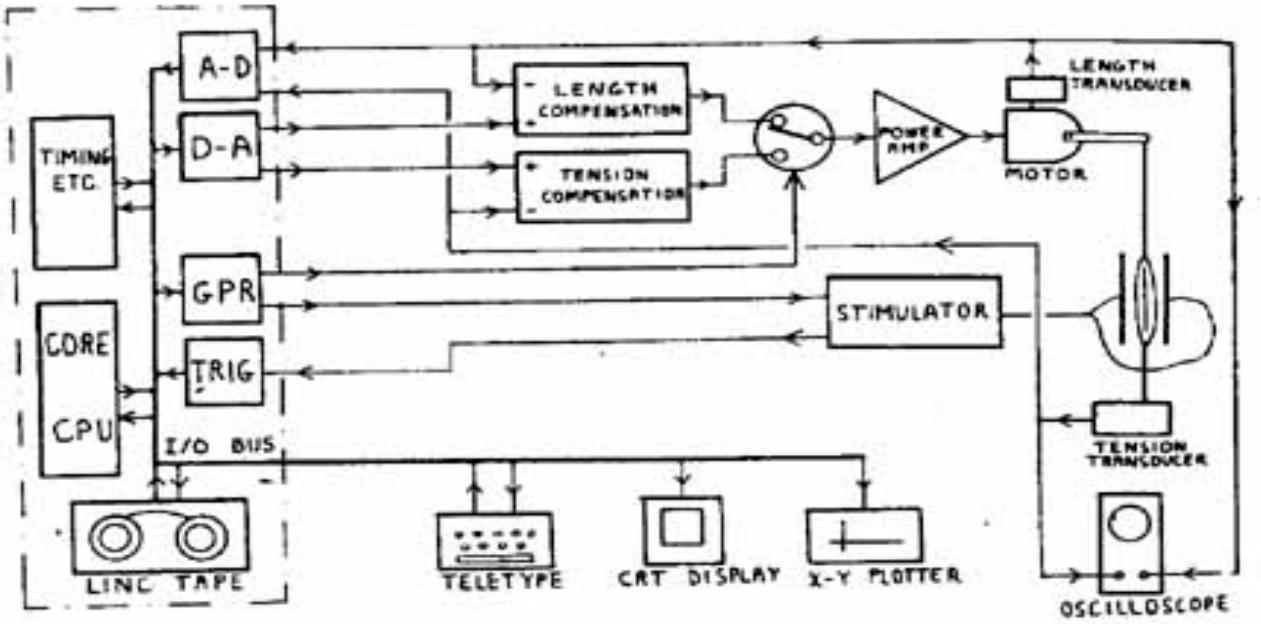
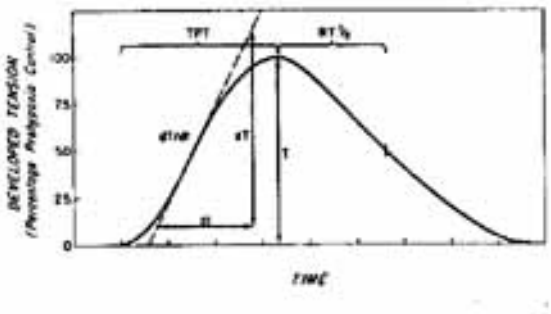


tain is shown in this slide. After stimulation, contraction rises to a peak value and then declines. Two processes govern the amplitude of contraction: 1) the intensity of contraction estimated from the maximum rate of use of tension and 2) the duration of the so called "active state" indexed by the time from the onset of tension to peak tension.

The effect of hypoxia is characterized first by a decrease in the duration of activity as measured by a decrease in TPT) followed by a later decrease in the intensity of activity (coradence max tdt/dt). During reoxygenation there is an early striking prolongation of mechanical activity followed by a gradual increase in the rate of rise of tension. These superimposed tracings are recorded at 5 minute intervals during hypoxia and reoxygenation. The earliest tracing during reoxygenation was recorded at 2 minutes.

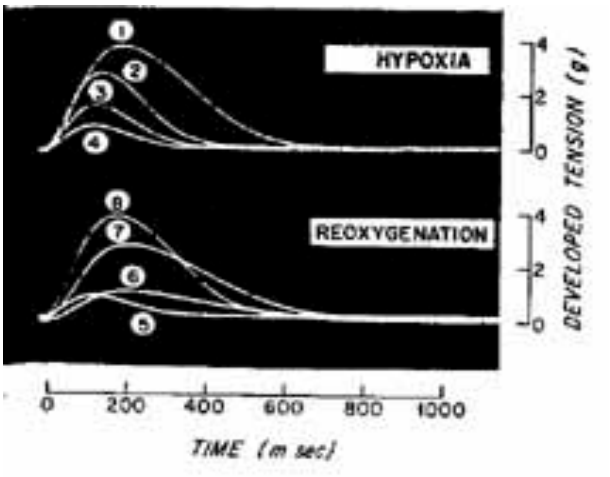
We have discussed another aspect of the hypoxia process: the appearance of rigor or contracture. This tracing was recorded at a much slower speed than the previous slide. In contrast to the gradual decline in peak active tension during hypoxia, there is a gradual but progressive increase in the baseline. This represents an increase in tension developed by the muscle in the passive, noncontracting state. If the ends of the muscle were not

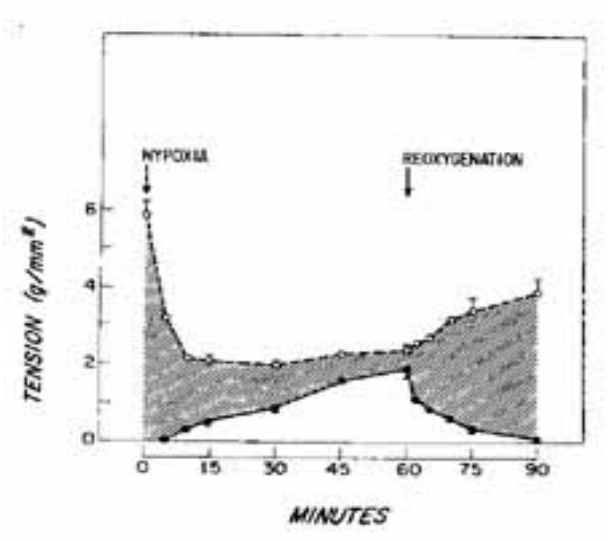


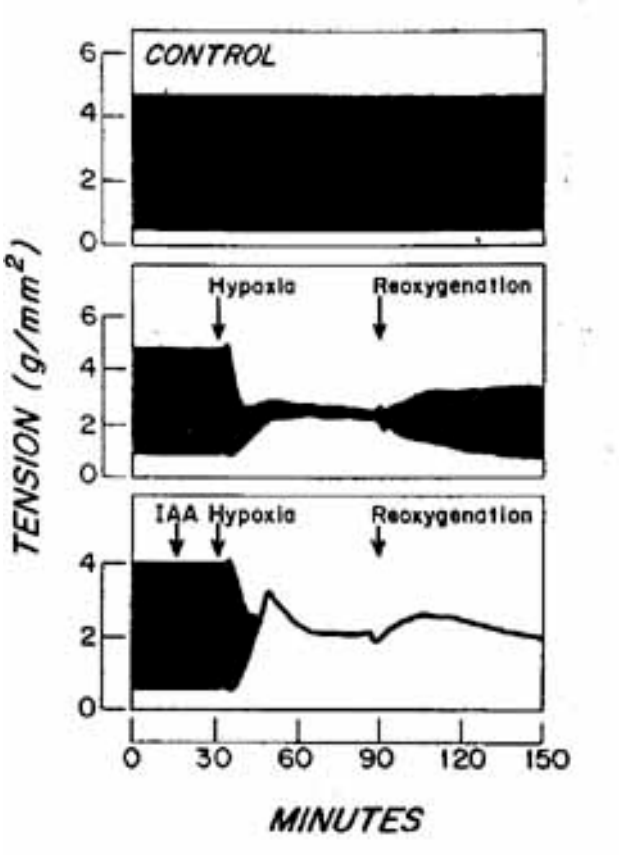


fixed, it would gradually shorten. These everts passive shortage or tendon increase describe the appearance of rigor or contracture in the muscle preparation. It is of interest that if hypoxia is not overly prolonged, both changes in active tension and rigor appears reversible with reoxygenation.

Cardiac muscle, similar to other tissues, must derive its energy from two sources: 1) oxidative processes involving Krebs-cycle activity, cellular respiration and oxydative phosphorylation, 2) the alternate, less efficient,







means for energy production is glycolysis. These facts can be clearly demonstrated by inhibiting respiration through oxygen removal and by inhibition of glycolysis with iodoacetate, na inhibitor of the glycolytic enzyme glyceraldlehyde 3 phosphate dehydrogenase.

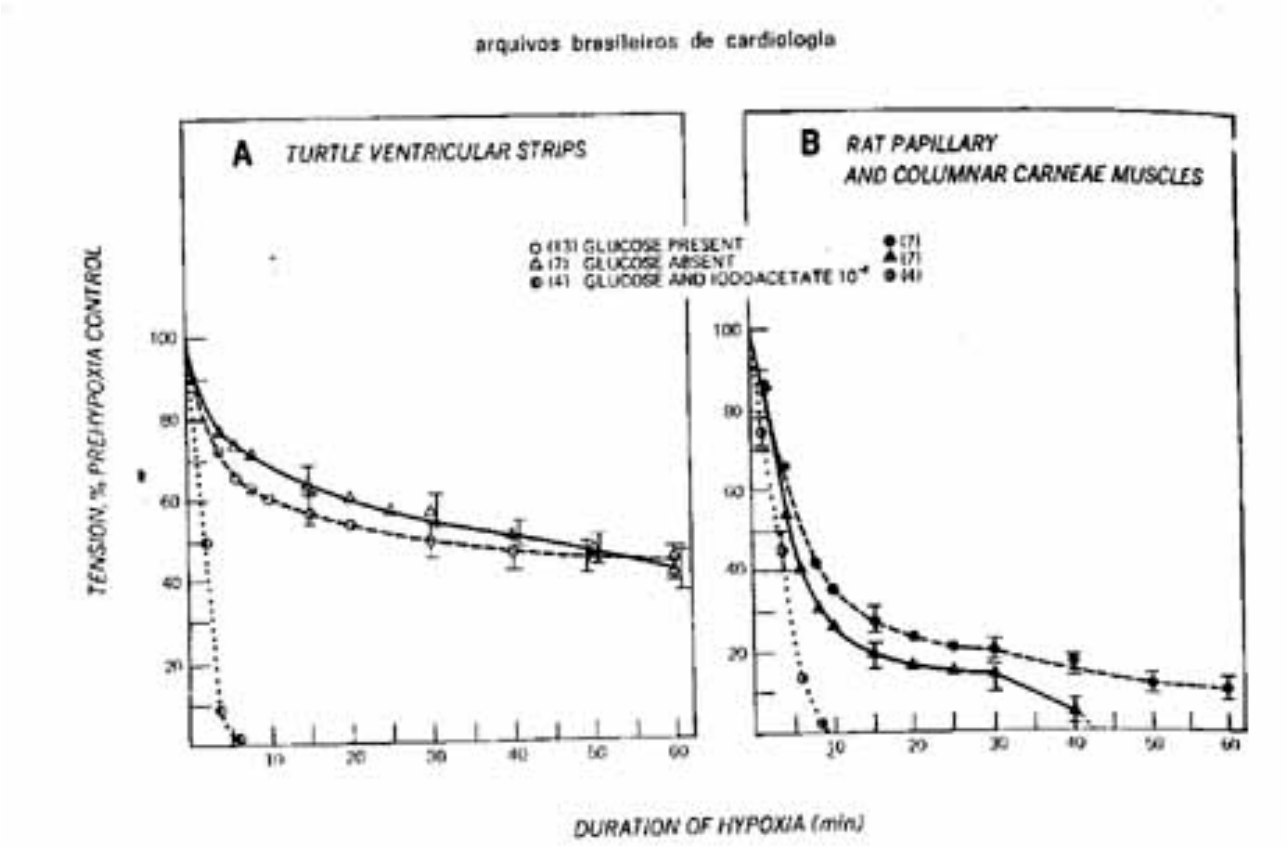
Under oxygenated conditions, stable mechanical performance is demonstrated on this slide. With the removal of oxygen, tension falls gradually towards zero. Note also the increase in resting tension or the development of contracture which was just described.

If glycolysis is blocked with iodoacetate under oxygenated conditions, no fall in mechanical activity is seen. This clearly demonstrates that, in the presence of oxygen, mechanical activity does not depend upon glycolysis. If hypoxia is produced when glycolysis is blocked with iodoacetate, however, mechanical activity falls abruptly as no longer any source for energy porduction is present. We can use these types of metabolic blockade experiments to examinen some interesting biological questions. For example, certain species, such as the turtle are notriously resistant to hypoxia. Is there a true difference between intrinsic tolerance to hypoxia, in these two species? In the intact animal the question of intrinsic differences in tolerance to hypoxia are complicated by hypothermia, hormonal factors, loading conditions and other variables.

If we obtain ventricular strips from turtle hearts and compare them with rat myocardium, we see that under similar experimental conditions turtle heart is indeed more tolerant to hypoxia. Glucose removal increases differences between rat and turtle suggesting that by adding carbohydrate we can make rat heart respond to hypoxia more like the turtle heart.

If we subject both preparations to hypoxia and glycolytic blockade, mechanical activity falls at a similar rate in both species. Since differences between species seen during hypoxia are abolished by glycolytic blockade, we can conclude that the increased performance during hypoxia demonstrated by turtle heart muscle is related to enhanced capacity for glycolysis.

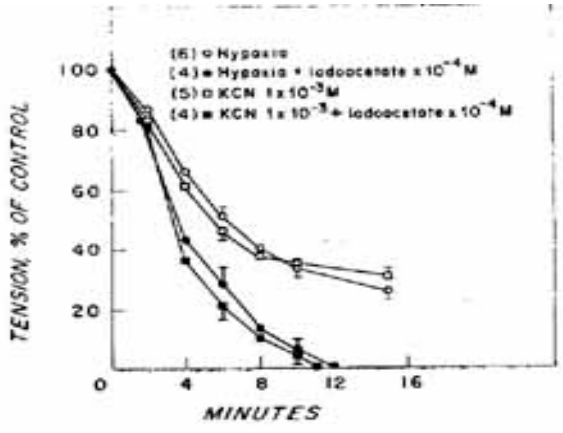
As prefaced in the beginning of this talk, we have utilized experiments with metabolic inhibitors to help us further understand the role of hypoxia in the ischemic process. In the next study we have attempted to define the mechanism for the abrupt early decline in mechanical performance which appears with ischemia. It has been recognized since 1935, when Tennant and Wiggers first described early bulging of ischemic myocardium, that failure of myocardial contraction is one of the earliest events that can be identified when focal myocardial ischemia is produced by coronary occlu-



sion. This change apparently occurs more rapidly than any known biochemical or metabolic event which has been identified as occurring in ischemic myocardium and its mechanism has not yet been satisfactorily explained.

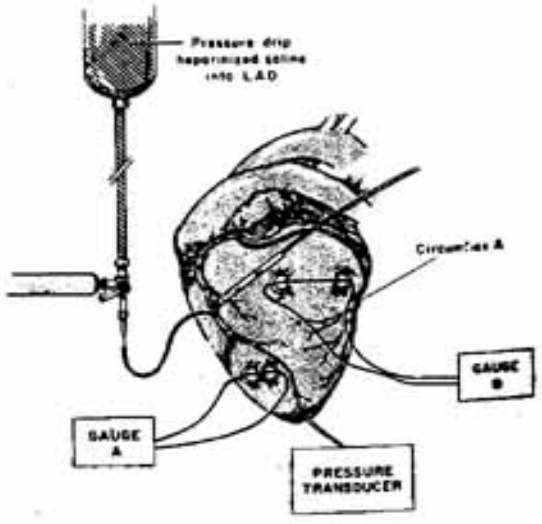
We used metabolic inhibitors in both isolated muscle studies and the intact heart to further define the mechanism for early loss of mechanical activity with ischemia. Our hypothesis was that hypoxia was responsible for the early loss of function and our goal was to compare the effects of hypoxia and ischemia on the function of a segment of myocardium.

Dur first objective was to define the similarity between hypoxia and the respiratory inhibitor cyanide in the isolated muscle preparation.



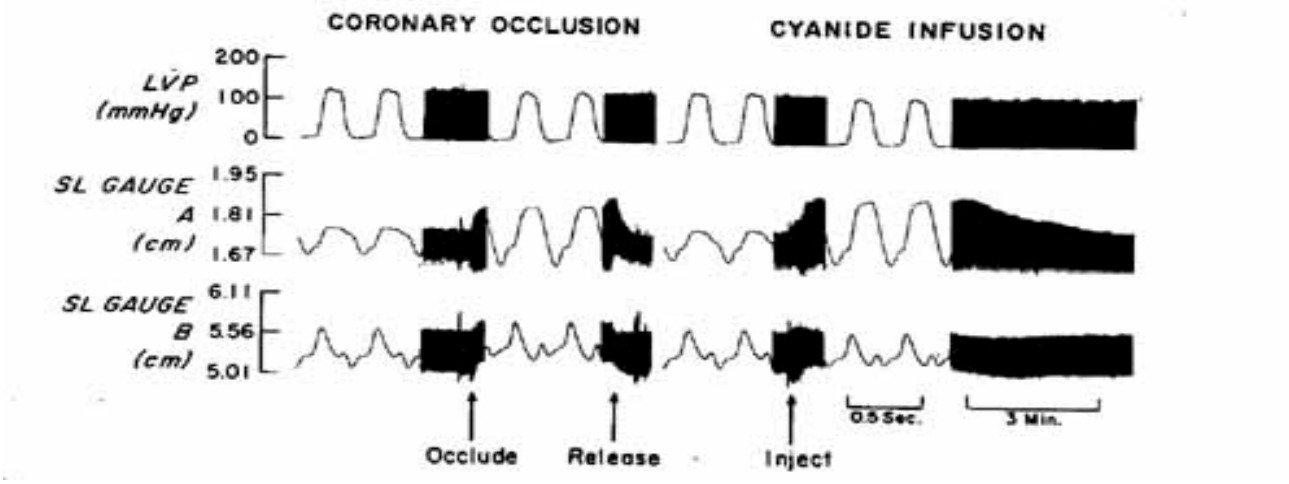
Hypoxia and Cyanide $1O^{-3}M$ were found to produce identical declines in contractile force during 15 minutes of hypoxia. When hypoxis was combined with glycolytic blockade and cyanide was similarly combined with glycolytic blockade the decline in force was again identical. On the basis of these studies, as well as other data, we felt we could substitute cyanide for hypoxia.

In the next study we canulated the left anterior descending coronary artery of open chest dog hearts with a number 1O polyethylene catheter. Segment length gauges recorded wall motion in the area to undergo ischemia or hypoxia (gauge A) while gauge B was located

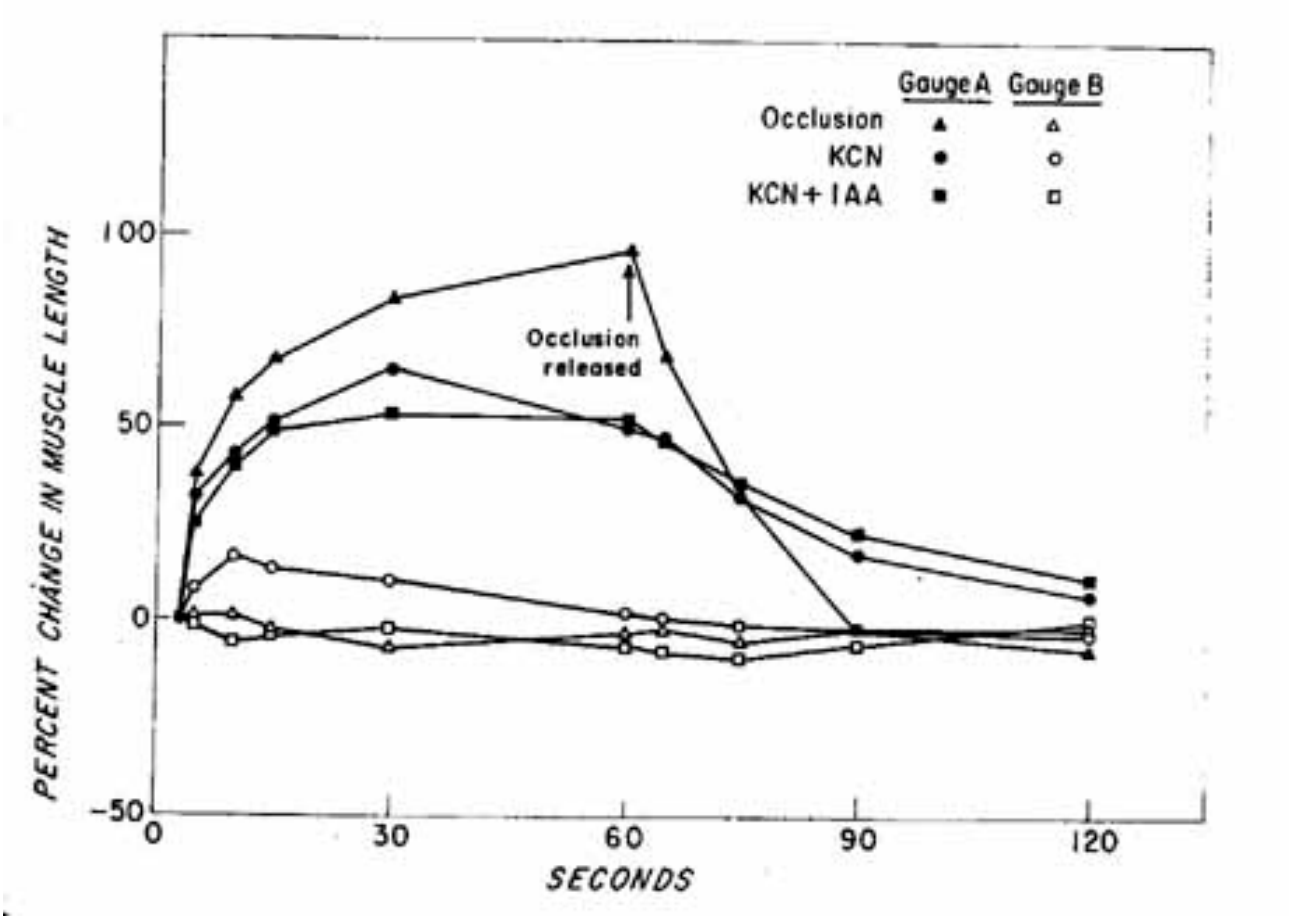


in the distribution of another vessel and served as control.

The next slide is an example of our results. The effects of coronary occlusion as compared



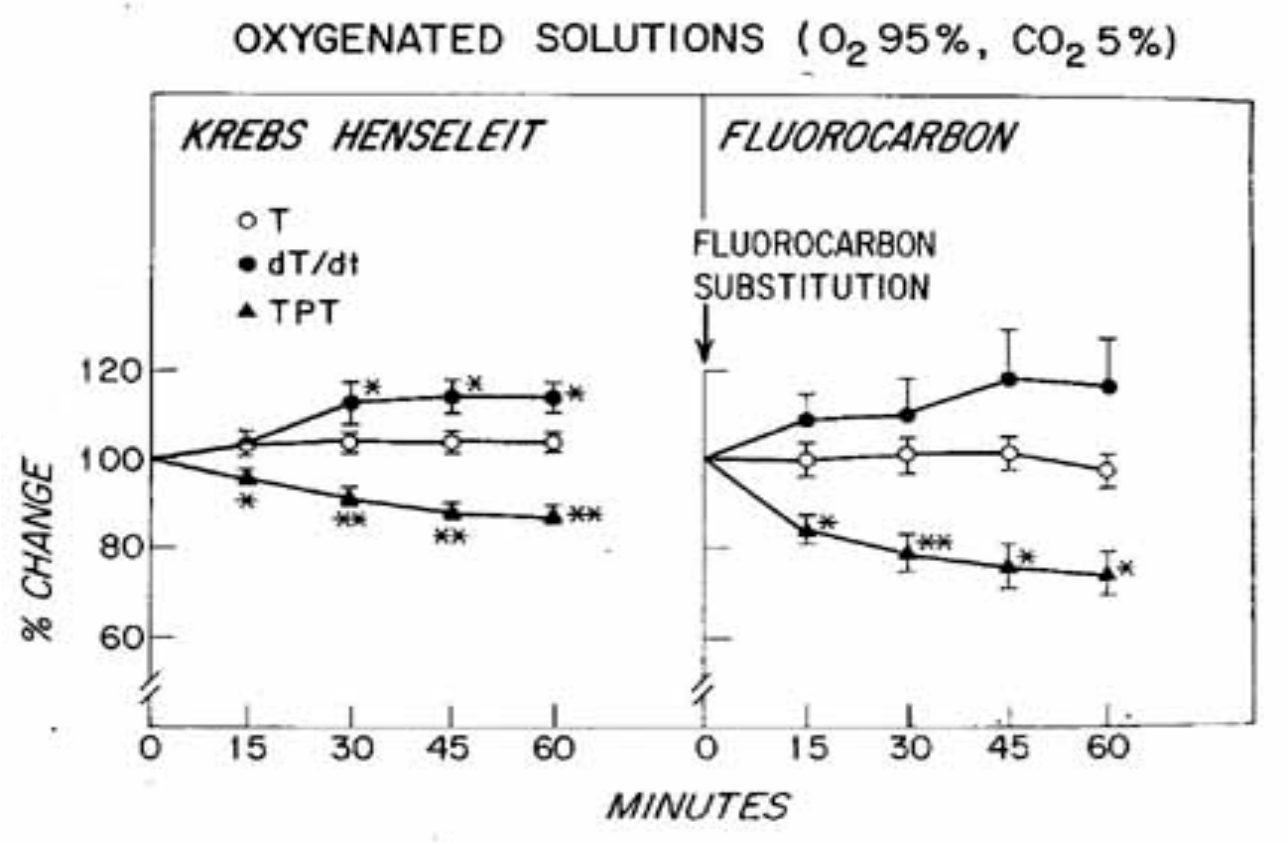
with cyanide administration. The major point to be made is that the time of onset of bulging occurs within seconds is identical with both ischemia and hypoxia.



This is more clearly illustrated in the next slide, which is a composite of eight experments. Upward deflections are a measure of systolic expension. This bulging of both ischemic and hypoxia myocardium appears within seconds after intervention. Findings thus suggest that the hypoxic component of the ischemic process appears responsible for the early systolic expansion of ischemic myocardium.

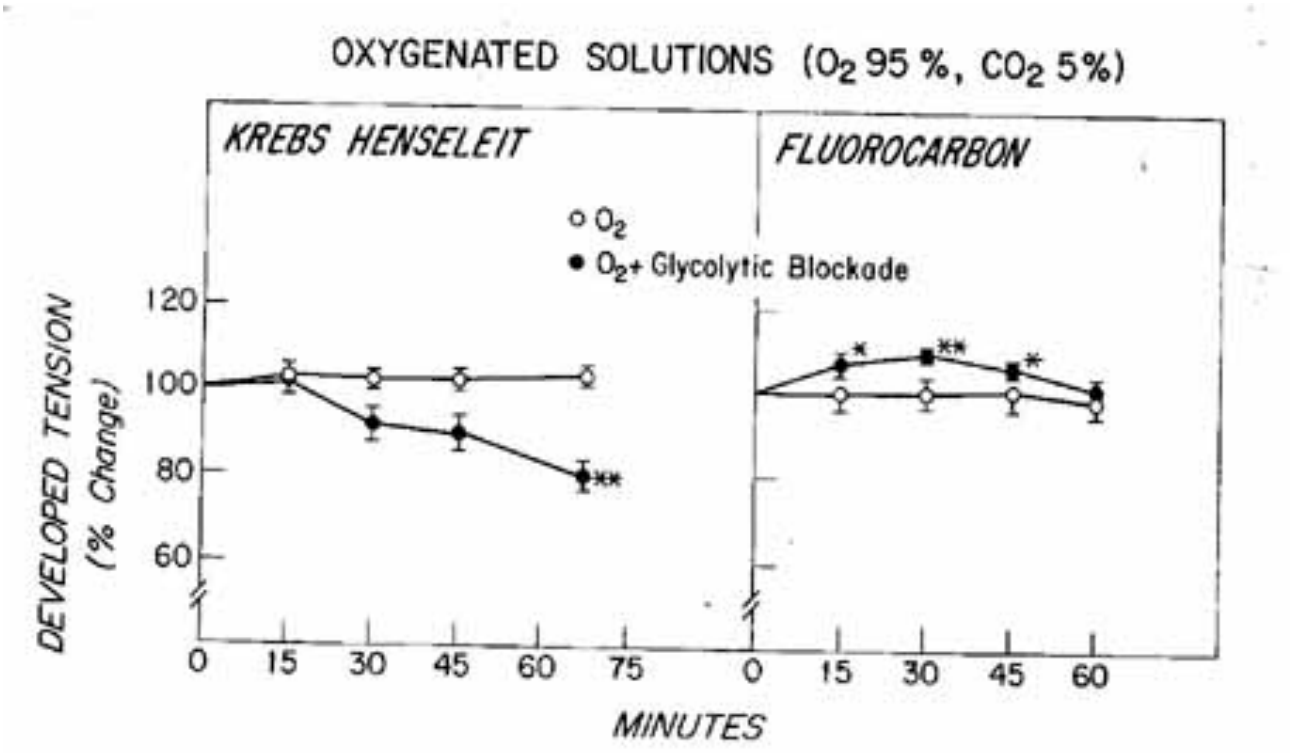
The mechanism responsible for abrupt myocardial mechanical failure after ischemia or hypoxia has not yet been identified. Since the myocardium remains excitable for many minutes after onset of ischemia, the early onset of mechanical dysfunction does not appear to have an electrophysiological basis. A number of studies all indicate that mechanical events occur too soon to be induced by changes in high energy phosphate stores. Katz and Hecht have speculated that a reduction of intracellular pH due to enhanced glycolysis during ischemia may alter the calcium troponin interaction, and lead directly to a loss of contractile activity. Recent work by Nayler et al has demonstrated that the fall in mechanical activity of ischemic and hypoxic heart muscle is associated relatively early with a decreased capability of superficially-located membrane binding sites to accumulate calcium. At this point it is unclear whether any of the mechanism can lead to mechanical changes which appear in 2-3 sec. The present experiments indicate that both cyanide and coronary occlusion induce equally prompt changes in local myocardial performance. Additional studies are indicated to further evaluate the mechanism by which cessation of cellular respiration induces the abrupt decline in myocardial mechanical activity.

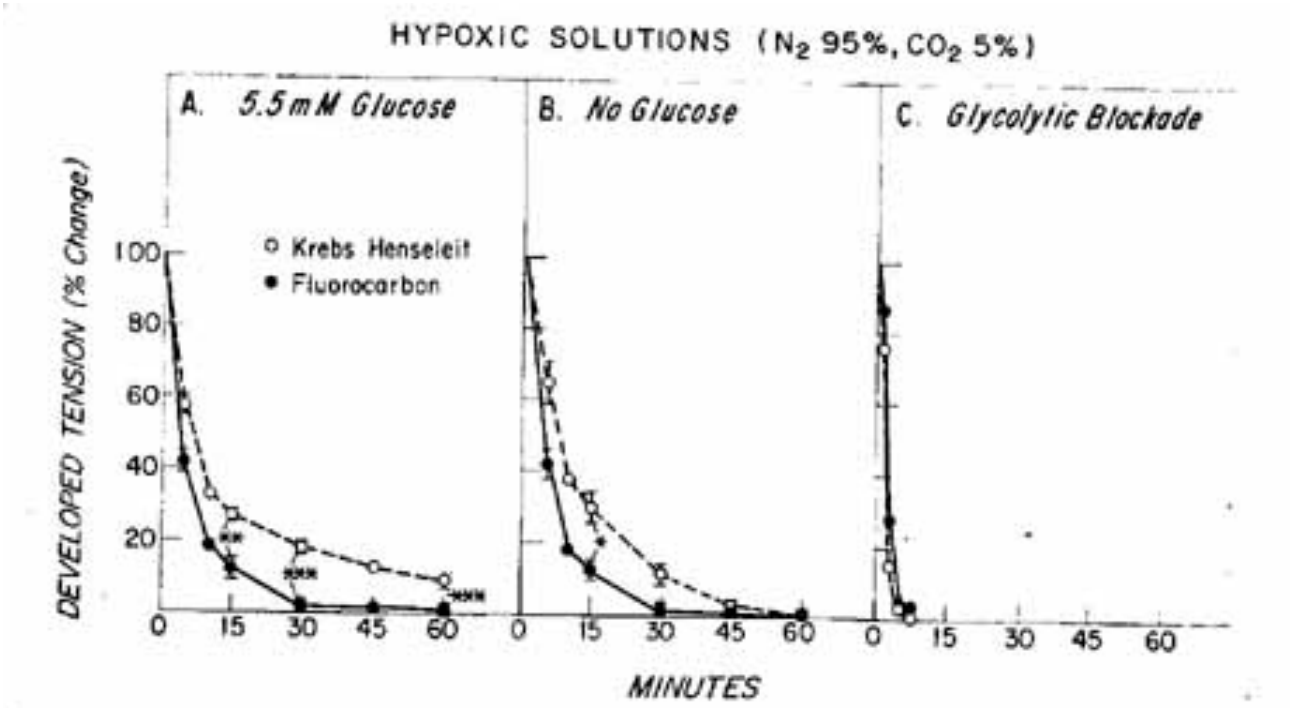
Finally, I would like to discuss some studies in which we have attempted to evaluate the later role of hypoxia on the ischemic process For these studies we have utilized Fluorocarbon, a biologically and chemically inert liquid in which carbon dioxide and oxygen are highly diffusible. Since fluorocarbon permits free diffusion of gasses while entrance and egress of substrates and etabolite are limited, we considered that fluorocarbon immersion may serve as a model of ischemia. By comparing cardiac performance is Krebs Henseleit and fluorocarbon solution we hoped to be able to further differentiate the effects of ischemia and hypoxia on the myocardium.



First, let us compare the performance of isolated cardiac muscle preparations in Krebs Henseleit and Fluorocarbon solutions under oxygenated conditions. Stable mechanical activity is seen in both Krebs and Fluorocarbon solutions for one hour. Thus, cardiac muscle is able to function in a relatively stable manner for a considerable period of time in a bath largely free of substrate and ions. It is likely that a small envelope of Krebs solution may surround the preparation. In addition, the preparation contains an interstical space and the remnants of a vascular system. Nevertheless, the volumes of these spaces are relatively small and it is perhaps surprising that the muscle preparation is able to maintain function in oxygenated fluorocarbon solution with little evidence of deterioration for as long as one hour.

When glycolysis is blocked with 1AA under oxygenated conditions, a slow decline in active tension is seen in Krebs solution but not in fluorocarbon. The reason for the difference is not clear, but the relatively stable performance of cardiac muscle preparations in the presence





lished observation that carbohydrates are not normally the major aerobic substrate for cardiac muscle.

The next slide demonstrates that with hypoxia active tension falls promptly in both Krebs and Fluorocarbon solutions. However, the fall in performance in fluorocarbon solution is more rapid and developed tension values are significantly lower than Krebs solution values after 15 minutes of hypoxia. When both aerobic and anaerobic sources of energy production are blocked by combined hypoxia and glycolytic blockade, active tension declines abruptly to zero within 10 minutes in both solutions. If glucose is removed from the Krebs solution differences between fluorocarbon and Krebs solution are decreased, suggesting that some of the differences are the result of substrate deprivation with fluorocarbon. Persisting differences, even when glucose is removed, suggest that additional factors such as metabolite retention or acidosis may play a role in the greater depression seen with fluorocarbon immersion.

Thus, like the isolated muscle preparation immersed in hypoxic fluorocarbon solution, the ischemic myocardium is subjected to the effects of substrate deficiency and metabolite accumulation in addition to being deprived of oxygen. We believe that experiments utilizing fluorocarbon in combination with metabolic blockade may be useful for further elucidating mechanisms for altered myocardial performance during ischemia.

In summary, we have reviewed the physiology of hypoxia on the intrinsic properties of cardiac muscle. Our studies suggest that oxygen deprivation plays an important role in terms of the early loss of contractile e activity following coronary occlusion or the bulging of ischemic myocardium. It also appears that the later effects of ischemia are due to factors in addition to hypoxia, such as substrate deprevation or metabolite accumulation.

Much work has yet to be done. Of particular importance is an elucidation of the factor or factors associated with irreversibility after myocardial ischemia. We believe that studies using the isolated muscle preparations may be useful in answering some of these important questions.