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The Oxygen Consumption Of Relaxed And Active Arterial Smooth Muscle

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THE OXYGEN CONSUMPTION OF RELAXED AND ACTIVE ARTERIAL SMOOTH MUSCLE

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ABSTRACT

By means of both a Warburg manometric technique and a polarographic technique measurements were made of the rate of oxygen uptake of isolated segments of dog femoral artery immersed in buffered physiological solution.

The respiratory rate of the relaxed unstretched arterial segment was found to be comparable to the values reported for other mammalian smooth muscles. When the arterial segment was stretched circumferentially the total rate of oxygen uptake increased to reach a maximum value equal to almost twice its original value in magnitude. The degrees of stretch were all in the physiological range. The cause of this increase in oxygen demand could only be speculated upon.

The effect of initial passive stretch on the mechanical responses of both isometrically and isotonically loaded arterial muscle was determined. Both types of response increased to a maximum value and then declined as the circumferential stretch of the arterial segment was progressively increased. In this respect the behaviour of arterial muscle is similar to that reported for a variety of other smooth, skeletal and cardiac muscles. This property of the arterial muscle may play an important part in the mechanisms responsible for the autoregulation of blood flow in the intact animal. The increase in the average rate or steady state rate of oxygen uptake of the active arterial muscle varied with the passive stretch in a manner which was quantitatively similar to

the changes occurring in the active tension maintained or the total external work performed by the arterial muscle (Fenn effect). The proportionality between the active mechanical and respiratory responses was quite high and yielded an average value for the efficiency of the isometrically loaded arterial muscle which was several orders of magnitude greater than that determined by other workers from the heat production of frog skeletal muscle which was also maintaining an active tension. No quantitative studies were made of the arterial muscle metabolism during the phase of tension development. The efficiency of the isotonically loaded arterial muscle with the respiration being employed as the criterion of its energy production was found to equal about 2% which is significantly smaller than the corresponding values reported for skeletal and cardiac muscles.

The nature of the loading of the arterial muscle significantly affected its oxidative requirements at the different values of passive stretch. In agreement with observations on skeletal muscle the peak isotonic contraction of arterial muscle consumed oxygen at a greater rate than did the peak isometric contraction.

The nature of the stimulus employed influenced the efficiency of the isometric contractions since direct electrical stimulation caused contractions which utilized oxygen at a smaller rate than did the mechanically equivalent noradrenaline contractions. No such pronounced difference in efficiency was noted when isotonic loading was used. No satisfactory reasons were arrived at for this apparent difference in efficiency despite the differential effect which temperature, pH, anoxia and different levels of sodium and potassium in the bathing solution have been shown to have on the drug and electrically-induced responses. It

still remains possible that the electrically-induced isometric contraction draws more of its energy from non-oxidative sources than does the noradrenaline-induced contraction.

Under the conditions of our experiments, the arterial muscle was shown to metabolize primarily carbohydrate since its respiratory quotient when it was either relaxed, stretched or contracted was relatively close to unity.

I. INTRODUCTION

There were several reasons for undertaking the study of the relation between the active mechanical responses of arterial muscle and its oxidative metabolism. The principal one was that although a considerable amount of work had been done on the resting oxygen consumption of this muscle, in only two instances that we have found, was there any attempt to investigate the dependence of its oxygen consumption on the passive tone of the muscle. Insofar as we are aware, no work had been done where the active development of tension or performance of work by arterial muscle was compared with its oxygen uptake. However, it had been shown that vascular smooth muscle does obtain a significant proportion of its basal energy requirements (up to 50%) by means of aerobic glycolysis. This fact, along with the possibility of shifts in the respiratory quotient and phosphorylation to oxidation ratio when the muscle is stimulated to contract, make it apparent that the oxygen consumption of the muscle is not the only indication of its energy metabolism. Nevertheless, we felt justified in devoting the entire research to the determination of the importance and efficiency of the contribution of oxidative processes to the energy metabolism of arterial muscle when it is exposed to various conditions of passive stretch and active tone.

A fairly comprehensive study has been done by Bulbring (1953) on the oxygen consumption of intestinal smooth muscle under various condi-

tions. It is not apparent that vascular smooth muscle should necessarily behave in a similar fashion since it is known that there are several differences between the many kinds of smooth muscle in their pharmacology and physiology, as well as their metabolism. In fact, at the beginning of the research it was even thought possible that vascular smooth muscle may operate like some molluscan smooth muscles which are thought to have a "catch" mechanism permitting a prolonged contraction to take place without a corresponding increase in the energy turnover of the muscle.

During the course of the research various methods of measuring the oxygen uptake were attempted on different preparations. Initially we attempted to obtain data using the isolated perfused ear of the rabbit, where vasomotor activity is very marked and the degree of active contraction of vascular smooth muscle may be measured quantitatively. Even with a closed circulatory perfusion system of minimal total volume, the arterial-venous differences from which the oxygen consumption could be calculated were too small to permit sufficient accuracy to be obtained with the polarographic technique that we used. In addition, the respiration of other cellular tissues such as the skin might be affected by pressor drugs, making it impossible to attribute the total increase in oxygen consumption to vascular smooth muscle alone.

The use of segments of arteries of the muscular type in a modified Warburg respirometer by Kosan and Burton (1966) yielded results which indicated the importance of initial stretch or tension on the mechanical and metabolic responses to drug stimulation. As a result, the Warburg respirometer was further modified so that the transmural pressure across the wall of the arterial segment could be recorded. The oxygen consumption of the segment was then determined as a function of its initial

tension and of the active tension it developed at different initial tensions when stimulated with norepinephrine or by direct electrical stimulation. In order to find out how the oxygen uptake was affected when the muscle was allowed to shorten and thus perform external work, the oxygen electrode was employed to record the changes in oxygen tension of a small volume of physiological salt solution containing the isotonically loaded arterial segment. The segment was once again stimulated to contract both electrically and by means of drugs.

Due to the interest in the effects of abnormal ionic environments on the responsiveness of arterial muscle, some experiments were performed with abnormally high and low concentration of potassium, sodium and hydrogen ions in the solution bathing the arterial segment. The influence of these different electrolytic media on the tension developed and the oxygen consumed during isometric contractions of the arterial muscle was investigated.

II. HISTORICAL REVIEW

A. Energetics of "Resting" Vascular Smooth Muscle

As pointed out by numerous investigators working with vascular muscle there is no definite transition from rest to activity of this muscle but rather, like most smooth muscles, it is always in a state of maintaining some spontaneous tone, and its energy metabolism is probably never operating at a "basal" level but rather at a reduced activity level. This has been shown to be the case even with isolated vascular muscle. Lundholm and Mohme-Lundholm (1960a) have shown that the addition of a nitrite vasodilator along with monoiodoacetic acid poisoning under anaerobic conditions is necessary to obtain a maximally relaxed state of this muscle. Nevertheless, for the sake of simplicity of expression, the terms "resting" or "relaxed" arterial muscle will be used when referring to muscle which has no detectable passive tension, and no drug or electrically-induced active tension.

Numerous measurements have been made of the metabolism of isolated resting arterial muscle where its oxygen consumption was used as the criterion of the energy demand. No measurements of the heat liberation of the resting muscle have yet been made, but some work has been done on the rate of splitting of high energy phosphate compounds and the energy thus supplied to the resting muscle. The substrates metabolized by the muscle under resting conditions and the enzymes and metabolic pathways involved in their breakdown to subsequently form high energy

TABLE 1

Oxygen Consumption of Resting Vascular Smooth Muscle

Investigator	Animal	Preparation	Q_{O_2} (µl/mg/hour) Wet Weight	Q_{O_2} (µl/mg/hour) Dry Weight
Briggs, Chernick and Chaikoff (1949)	rat	thoracic aorta		1.0
Krantz, Carr and Knapp (1951)	rat	thoracic aorta		1.0
Wertheimer and Ben-Tor (1961)	rat	aorta	0.1*	
Priest (1963)	rat	thoracic aorta stripped of its adventitia	0.29	
Malinow, Moguilevsky and Gerschenson (1964)	rat	thoracic aorta stripped of its adventitia		2.2
Michelazzi (1938)	rabbit	aorta		1.1
Costa, Weber and Antonini (1950)	rabbit	aorta		0.8
Furchgott (1955)	rabbit	aorta		1.0
Dury, Liegey and Dury (1957)	rabbit	aortic arch	0.09	
" " " " "	rabbit	descending aorta	0.07	
Whereat (1961)	rabbit	aorta	0.1*	

Fisher and Geller (1960)	rabbit	aorta	24-30	
Howard, Richardson, Smith and Patterson (1965)	hamster	mesenteric arterioles < 150μ diameter		1.05
" " " "		250-400μ diameter		0.58
" " " "	human	arterioles		
" " " "		65-175μ diameter		0.35
Kirk, Effersoe and Chiang (1954)	human	aortic intima		0.05
" " " " "	human	aortic media		0.05
" " " " "	human	aortic intima-media		0.04
" " " " "	dog	aortic intima		0.25
" " " " "	dog	aortic media		0.19
" " " " "	dog	aortic intima-media		0.20
Hiertonn (1952)	dog	aorta		0.24
" "	dog	peripheral arteries		0.31
Henderson and MacDougall (1956)	rat	aorta		2.4
" " " "	rabbit	aorta		1.6
" " " "	sheep	aorta		0.6
" " " "	pig	aorta		0.4
" " " "	ox	aorta		0.4
Lundholm and Mohme-Lundholm (1960)	cow	mesenteric artery		0.9
Rifkind and Munro (1963)	rat	aorta	1.4**	
" " " "	cockerel	aorta	0.29-0.50**	
" " " "	pullet	aorta	0.38-0.55**	

* Approximate calculated value

** Succinate was employed as substrate

compounds have also been looked into and will be described later.

1. Oxygen Consumption

That an adequate supply of oxygen is necessary for the maintenance of good inherent or spontaneous tone by vascular smooth muscle was shown a long time ago by Dale and Richards (1918) for the case of perfused vascular beds and by Rothlin (1920) for the isolated strips of large blood vessels. However, quantitative measurements of the actual oxygen demand by resting arterial muscle were not made until much later.

These quantitative studies of the oxygen consumption were generally made on slices of blood vessel wall, usually the aorta, from a variety of different sources. The results which have been obtained cover a broad range of values and are summarized in Table 1.

It is probable that aside from the influence of the size of the blood vessel and the size of the animal supplying it some of the differences in all these Q_{O_2} values were due to the different relative amounts of muscle tissue present in the various preparations and also perhaps to the differences in the time that elapsed between the isolation of the arterial tissue and the measurement of its respiration.

Additional scatter in the results from the different preparations may be due to differences in the proportion of the total energy production of resting arterial muscle which is supplied by respiration as compared with the other pathways which supply energy. By assuming that the total energy production of the arterial muscle was the sum of that provided through tissue respiration and aerobic glycolysis, Kirk, Effersoe and Chiang (1954) calculated from their measurements of the rate of oxygen uptake and rate of lactic acid production that the respiration of the human aortic samples accounted, on the average, for only 49%, and the

respiration of dog aortas for 61% of the total energy produced by the tissue. Sudhof (1950) and Lundholm and Mohme-Lundholm (1960a) have also shown that a significant degree of glycolysis takes place in resting arterial muscle under aerobic conditions. Since the contribution of this pathway may vary from one artery to another, it appears that the oxygen consumption alone cannot serve as a fully reliable criterion of the relative energy metabolism of the different types of resting arterial muscle tissue.

Other possible factors introducing variation in the above results quoted for the resting Q_{O_2} are the effects of storage, age, sex of the animal, as well as differences in the actual methods which were employed. Generally, the Warburg manometric technique was employed with the arterial tissue being in the form of either transverse slices, or as rings, or as longitudinally opened segments.

A comparison of these arterial muscle Q_{O_2} values with those from other resting muscles shows them to be significantly less than the cardiac and skeletal muscle values but comparable with most of the non-arterial smooth muscle Q_{O_2} values, which are located in the upper part of the range of values.

2. Substrates Metabolized by Resting Arterial Muscle

The effect of various oxidizable substances on the oxygen uptake of arterial tissue was investigated by Briggs, Chernick and Chaikoff (1949) who found that succinate elevated the oxygen consumption of rat thoracic aorta by 115% to 145% while lactate, pyruvate, acetate, α -ketoglutarate and citrate all stimulated the oxygen uptake by 20% to 40% above the value when no substrate whatsoever was present. Glucose, on the other hand, failed to increase the oxygen uptake but, as Barron and

Huggins (1946) have shown, substances which fail to increase the respiratory rate may, nevertheless, be utilized at appreciable rates. Dury et al. (1957) found that glucose actually slightly depressed the oxygen consumption of rabbit aortic arch but not of the descending aorta, while succinate elevated the oxygen uptake of both preparations by a factor of 5 to 6 times above their rates when in buffer alone.

Furchgott (1955) has referred to unpublished work where he found that glucose, pyruvate, acetate, butyrate and succinate were effective in restoring the response of substrate-depleted rabbit aortic strips to epinephrine and electrical stimulation. It has also been shown by Lundholm and Mohme-Lundholm (1962a) that addition of glucose to substrate-depleted bovine mesenteric arteries, under anaerobic conditions, increased the lactic acid production by 64%.

Measurement of the actual rate of glucose uptake, under aerobic conditions by resting aortic tissue, has been performed by Kirk et al. (1954) on human and dog aortas, and by Wertheimer and Ben-Tor (1961) on rat aortas. The mean values were found to equal 1.5 and 6.7 mg glucose utilized /g dry tissue/hour for the human and dog aortas, respectively, and 1.3 mg glucose /g wet tissue/hour for the rat aorta.

Determinations of the respiratory quotient of resting arterial muscle have been made by Costa, Weber and Antonini (1950) who obtained values ranging from 0.80 to 1.00 with rabbit aorta and by Kirk et al. (1954) who found the average RQ values of human and dog aortas to equal 0.91 and 0.99, respectively. On storage, the RQ tended to fall. The values on fresh tissue are consistent with the occurrence of nearly pure carbohydrate oxidation.

3. Enzymes and Metabolic Pathways

(a) Glycolysis

(i) Aerobic Glycolysis - Glycolysis, like the rate of oxygen uptake, is another frequently used index of tissue metabolism. Michelazzi (1938) determined the rate of aerobic glycolysis of resting rabbit aorta by the Warburg technique and found the number of mg lactic acid produced per g dry tissue per hour or the Q_g^O2 to range from 6.4 to 7.6. Sudhof (1950) measured the lactic acid production of segments of carotid arteries of cattle, from which Barrows and Chow (1959) and Lehninger (1959) calculated the Q_g^O2 to range from 1.2 to 1.3. Relatively low values were obtained for the lactic production of bovine mesenteric arteries by Lundholm and Mohme-Lundholm (1960a, 1962a) who found the wet weight Q_g^O2 in the absence of glucose to equal only 0.06 (or dry weight Q_g^O2 about 0.2) and to equal 0.12 (dry weight value about 0.4) in the presence of glucose under aerobic conditions.

Kirk et al. (1954) found the maximum initial Q_g^O2 values for human aortic samples to vary from 3.6 to 4.5, while the maximum initial value for dog aortas was 4.2. The mean Q_g^O2 values were equal to 2.5 in each case. They also found reasonably good agreement between the glucose utilization and lactic acid production in these tissues, so it is probable that the lactic acid formation measured in experiments of this kind is a result of the operation of the classic Embden-Meyerhof cycle. In addition, Kirk and Sorensen (1956) demonstrated the presence and measured the activity of aldolase, an enzyme within the glycolytic system, in human aortas. Evidence in support of glycolysis as the major pathway of glucose utilization in isolated rabbit aorta tissue when incubated in vitro was also obtained in a study by Mulcahy and Winegrad (1962). These workers

used C^{14}-labelled glucose to demonstrate that the major portion of the glucose uptake of the rabbit aorta could be accounted for by lactic acid production while oxidation to CO_2 and incorporation of glucose carbon into glycogen and total lipid, for progressively smaller fractions. The unpublished work referred to by Furchgott (1955) where he added various substrates and metabolic blocking agents also indicated the presence of the glycolytic pathway in rabbit aortic strips.

The relative importance of the glycolytic pathway in the metabolism of resting arterial muscle is apparent from the data of Kirk et al. (1954) which showed that glycolysis contributed an average of 51% of the total energy production by the intact intima-media preparation of the human aorta, and 39% of that produced by the preparation of dog aorta.

It is probable that in all these experiments the smooth muscle cells of the tunica media are the major source of this relatively high lactic acid production. This is somewhat extraordinary, for as Lehninger (1959) has mentioned, smooth muscle elsewhere in the body does not have a high rate of aerobic glycolysis even though it has a potential capacity for it. The fact that the supply of oxygen in the arterial wall is limited, as shown by Kirk and Laursen (1955a), may be responsible for the occurrence of aerobic glycolysis in arterial muscle.

(ii)Aerobic versus Anaerobic Glycolysis - Sudhof (1950) has found that the rate of lactic acid production by rings of beef arteries to be about 2 to 3 times as great under anaerobic as under aerobic conditions. In their experiments with bovine mesenteric arteries, Lundholm and Mohme-Lundholm (1960a, 1962a) have consistently observed a much larger ratio of about 10:1 between the rates of anaerobic and aerobic lactic acid production, respectively, both in the absence and presence of glu-

cose. These observations are not consistent with the results of experiments conducted by Kirk et al. (1954) on human and canine aorta since parallel determinations of the anaerobic and aerobic rates of glycolysis by these workers showed that the former was, on the average, only about 30% higher than the latter and hence no clear Pasteur effect was demonstrable. This result may have been due to the greater thickness of the aortic wall, so that even under aerobic conditions in the inner parts of the preparations, the oxygen tension was probably quite low with a consequent stimulation of glycolysis. It is, however, likely as pointed out by Lehninger (1959), that a substantial amount of aerobic glycolysis occurs even under physiological conditions in large arteries, since that tissue is vascularized only slightly, if at all, as shown by Woerner (1959).

Lehninger (1959) has also pointed out several significant consequences of this high aerobic glycolysis of arterial tissue in that it indicates some defect in the Pasteur effect which normally imposes a "braking" action on the rate of glycolysis to "match" it with the normal rate of oxidation of lactate and pyruvate via the Krebs cycle. A defect in the mechanism of the Pasteur effect may, in turn, involve the efficiency or the phosphorus: oxygen ratio of the oxidative phosphorylations associated with electron transport to oxygen in the mitochondria, since the relative concentrations of inorganic phosphate, ADP and ATP, in the cell, along with the partial pressure of oxygen, appear to be important factors controlling the rate of glycolysis as demonstrated by Gatt, Krimsky and Racker (1956). The high rate of formation and accumulation of lactic acid results in a relatively low pH near these cells which could be of extreme importance in controlling their metabolic activity and

physiological characteristics. Lehninger (1959) also considers glycolysis to be a suitable survival mechanism under anoxic conditions, provided that an ample supply of glucose is available. Sudhof (1950) has shown that the muscle cells present in the beef carotid artery are quite rich in glycogen.

(b) Citric Acid Cycle

The participation of the citric acid cycle in the metabolism of arterial tissue is suggested by the presence of several enzymes in the arterial wall which catalyze various steps of this cycle. Kirk and Laursen (1955b) have demonstrated dehydrogenase activity of human aortic tissue toward various intermediates of the citric acid cycle, while Laursen and Kirk (1955) and Sorensen and Kirk (1956) demonstrated the presence of two enzymes, aconitase and fumarase, in the arterial wall, which catalyze specific hydration and dehydration processes within the citric acid cycle. Kirk, Laursen and Schaus (1955) have shown the presence of succinic dehydrogenase in human aortic tissue. By measuring the oxidative response of aortic tissue to succinate and p - phenylenediamine, the presence of the succinic dehydrogenase and cytochrome oxidase enzyme systems was also shown by several workers including Maier and Haimovici (1957, 1958, 1965) and Daly and Gurpide (1959). Chang, Laursen and Kirk (1955) and Schaus, Kirk and Laursen (1955) have shown the presence of DPN and DPNase, and also the presence of riboflavin, FAD and FMN in human aortic tissue. Briggs et al. (1949) found that pyruvate and also the intermediates of the Krebs cycle, stimulated the respiration of intact rat aorta. Furchgott (1955) found that the same substances were also effective in restoring the contractile response of substrate-depleted strips of rabbit aorta to drug and electrical stimulation. In both these

studies with added substrates, the effects of various metabolic inhibitors were also tested and the results generally provided indirect evidence for the oxidation of carbohydrate in aortic tissue by way of the citric acid cycle. Although the information available is not sufficient to prove the occurrence of the Krebs cycle, it appears very likely that it is operative.

(c) Other Metabolic Pathways

Sbarra, Gilfillan and Bardawil (1960) obtained results with carbon 14-labelled substrates which suggested that guinea pig aorta has the necessary enzymes to metabolize glucose through the phosphogluconate or hexose monophosphate pathway. Using a similar technique, Mulcahy and Winegrad (1962) demonstrated that although the major portion of the glucose uptake of isolated rabbit aorta could be accounted for by lactate and CO_2 production, a detectable amount was incorporated into glycogen and total lipid. Sudhof (1950) has also demonstrated the formation of new glycogen in slices of beef carotid artery incubated in vitro with glucose under aerobic conditions. Synthesis of fatty acids, cholesterol and phospholipids by the aorta, in vitro and in vivo, has been demonstrated by numerous investigators including Chernick, Srere and Chaikoff (1949), Siperstein, Chaikoff and Chernick (1951), Werthessen, Milch, Redmond, Smith and Smith (1954), and Dury (1955a, 1955b). Furchgott (1955) refers to unpublished work from which he concluded that as well as a glycolytic pathway, a fatty acid oxidation system was active in aortic arterial muscle - both feeding "active acetate" into the Krebs cycle system. Zsoldos and Heinemann (1964) have also demonstrated lipolytic activity of the isolated rabbit aorta.

On the other hand, the high respiratory quotient (of 0.99) measured in vitro by Kirk et al. (1954) suggests that the rate of oxidation of fat

and also of protein is relatively limited in the arterial wall. In addition, Laborit and Brue (1962) concluded from their studies on the isolated rabbit aorta, that the arterial muscle has a predominant glycolysis-Krebs pathway, while the pathway of the pentose shunt was absent or of very little activity.

(d) High Energy Compounds Utilized

In their early studies on the metabolism of arterial muscle, Briggs et al. (1949) obtained data upon addition of fluoride which suggested that phosphorylated intermediates were involved in the metabolism of rat aorta. Since then, evidence has been presented by Krantz, Carr and Bryant (1951) and Krantz, Carr and Knapp (1951) which strongly suggested that the decomposition of ATP by arterial ATPase is the source of energy required by rabbit aorta for the maintenance of tonus and constriction. They showed that by interfering with the activity of the aortic ATPase, by administering organic nitrites and nitrates, the isolated vessel responded by relaxing. The role of ATP, and of certain muscle proteins in the maintenance of smooth muscle tonus and in initiation of its contraction, led to investigations of the presence of ATPase and its relationship to the contractile muscle proteins of arterial tissue. Banga and Nowotny (1951) have found large amounts of ATPase bound to actomyosin in human femoral arteries, and ATPase activity in the vascular tissue of various animals was also later demonstrated by Carr, Bell and Krantz (1952). It is likely that the enzyme activities found by these workers in blood vessels (especially in the muscular arteries) belong to the smooth muscle of the vessels.

The actual amount of high energy phosphates in arterial muscle has been measured by Furchgott and de Gubareff (unpublished data quoted by Furchgott (1955)) who obtained an average total corrected concentration of

ATP, ADP and CrP of 1.8 micromoles per gram wet weight. Lundholm and Mohme-Lundholm (1960a) have reported a very similar value with cow mesentric arteries. The content is still of the same order of magnitude as the values obtained with isolated rabbit stomach and uterine smooth muscles, but much lower than the values reported for skeletal or cardiac muscle. The possible significance of this relatively low content of preformed high energy phosphate compounds and of the magnitude of the changes in it, will be discussed later.

B. Metabolism of Actively Contracting Vascular Smooth Muscle

The investigation of the energy exchanges which occur when a muscle is developing tension or performing external work has only recently been extended to arterial muscle. Numerous studies of this nature have been conducted on other smooth muscles as well as on skeletal and cardiac muscles but because of space considerations, the conclusions from these studies will only be referred to, where necessary, in the Discussion section.

1. Biochemical Processes Providing the Energy for Active Contraction

(a) Oxidative Processes

Although some indirect information is available regarding the importance of oxygen for the contractile process of arterial muscle, we know of no direct measurements of the oxygen uptake which have been made during contraction. Hooker (1912), Dale and Richards (1918) and Rothlin (1920) demonstrated that the spontaneous tone of arterial muscle is dependent on an adequate supply of oxygen, while Furchgott (1952, 1955) has reported that the sensitivity of aortic arterial muscle to pressor drugs and the maximum contraction heights obtainable with them were considerably less

under anaerobic than under aerobic conditions, which may indicate that the energy production by anaerobic glycolysis is considerably less than that from aerobic oxidations. Coret and Hughes (1964) noted a similar difference between the drug-induced responses of oxygenated and hypoxic rabbit aorta. Smith and Coxe (1951), however, used pulmonary blood vessels isolated from dog, swine and cat and found no significant difference between the drug-induced contractile responses of these vessels when they were oxygenated and anoxic.

Krantz, Carr and Knapp (1951) investigated the effect of rapidly acting vasodilators of the nitrite-nitrate series on the oxygen uptake of rat aorta and found that glyceryl trinitrate at therapeutic levels significantly inhibited the oxygen uptake of the tissue. Since this compound also interferes with the ATPase activity of arterial tissue as has been shown by Krantz, Carr and Bryant (1951), the authors suggested that through the action of this vasodilator ATP breakdown is inhibited, so that the chemical energy necessary for the maintenance of tone and constriction is not provided. It follows that if the aerobic metabolism supplied energy for the resynthesis of ATP, it would be reduced when the rate of splitting of the ATP is reduced.

Lundholm and Mohme-Lundholm (1960a) determined separately the oxygen consumption and tone (actually the change in length at constant load) of a series of bovine mesenteric arteries under conditions where the muscle was dependent on its own energy reserves, and as these decreased so did its energy production. A concurrent fall of tone and oxygen uptake was observed similar to that found by Kirk, Effersoe and Chiang (1954) in experiments on human and canine aorta.

(b) Glycolysis

(i) Aerobic Glycolysis - It has been demonstrated by Lundholm and Mohme-Lundholm (1960a) that with substrate depletion of bovine arterial muscle the tone decreased in a manner which was parallel to the decrease in the lactic acid production and glycogenolysis of the muscle under both aerobic and anaerobic conditions, the decrease being more rapid in the latter case. It was also found by Lundholm and Mohme-Lundholm (1963a) that although increased glycogen breakdown was associated with electrically-induced contractions of arterial muscle it was not found with the equivalent contractions caused by adrenaline under aerobic conditions. In a study reported by Lundholm and Mohme-Lundholm (1962a) it was shown that adrenaline stimulated the glycolysis and that glycolysis-inhibiting substances and other metabolic inhibitors such as dinitrophenol blocked the contractility of vascular muscle under aerobic conditions and in the presence of glucose to such an extent that it was estimated that up to 50% of the total energy required for these contractions could have been obtained from glycolytic reactions. They mention that this fact along with the appreciable variations in the phosphorylation to oxidation ratio which may occur make it evident that oxygen uptake alone of arterial muscle is not a reliable indicator of its energy metabolism.

(ii) Anaerobic Glycolysis - Most of the studies on the metabolism of active arterial muscle have been performed under anaerobic conditions. Thus the studies of Lundholm and Mohme-Lundholm (1960a, 1962a) demonstrated that, under these conditions, the tone of bovine mesenteric arteries varied in a manner parallel to the lactic acid production and glycogenolysis of this tissue. Most of the lactic acid production was attributed to the breakdown of glycogen. Tone increasing agents, such as histamine and bar-

ium ions, influenced the carbohydrate metabolism only during the initial contraction phase with the elevated tone level then being maintained without affecting the energy metabolism. However, inhibition of the energy metabolism was reflected in a decreased tone even under the influence of these agents. From this the authors concluded that the variations in the energy production of the muscle induced the changes in tone.

Further evidence to this effect was presented by Lundholm and Mohme-Lundholm (1963b) and Beviz and Mohme-Lundholm (1965) in that despite the quantitative and temporal correlation of the lactic acid production to the contraction, their data also suggested that the metabolic change was not necessarily secondary to the contraction, as it is in striated muscle. The possible significance of this coincident stimulation of the contractile mechanism and the metabolism was discussed by the authors from the point of view that tonus changes of smooth muscle apparently reflect varying degrees of activity rather than transition from a resting to an active state. Thus even the "basal" energy production is utilized to some extent by the muscle for the maintenance of a certain level of activity.

By using the lactic acid production under anaerobic conditions as the index of the energy metabolism Lundholm and Mohme-Lundholm (1962a) demonstrated that the drug-induced isotonic contraction of arterial muscle showed both a quantitative and a temporal correlation to the increase in the lactic acid production. Glycolysis-inhibiting substances blocked the contractility. Later, Lundholm and Mohme-Lundholm (1965) also found that the rate of energy demand of bovine arterial muscle was always greater in order to attain a certain tension level or degree of shortening than to maintain it. The energy demand was also found to be appreciably greater

for the isometric than for the isotonic contractions during both phases of the contraction. The metabolism of the adrenaline-induced isometric contractions was elevated three to five-fold concomitant with the rise of tension but amounted to about only 60% when the tension had reached maximal level. Hence, the energy metabolism of arterial muscle is highly dependent both on the nature of loading used on the muscle and on which phase of the contraction is being examined.

The above authors noted that the tension-production rate of energy consumption appeared to be of similar magnitude for different types of muscle, whereas the tension-maintenance rate of energy consumption was appreciably lower for vascular smooth muscle than for frog striated muscle. Their explanation of this effect assumed that the initial elevation of tension after adrenaline probably reflected the degree to which the arterial muscle was in an "active state" which elicited a commensurate stimulation of the metabolism. They suggested that the phase of maintenance of constant tension following the initial phase could have been independent of this "active state" and have been due to a more passive "tonic" mechanism but one which still demanded some energy. Somewhat the same idea has been proposed by Laszt (1960) from studying the potassium-induced contractions of cattle arterial muscle.

(c) Substrates Utilized by Contracting Arterial Muscle

The only relevant data available on this topic is that mentioned by Furchgott (1955) in his review article on arterial muscle. He mentions having found that the contractile response of substrate-depleted rabbit aortic strips to stimulation by epinephrine and direct electrical stimulation was restored by the addition of glucose, pyruvate, acetate, butyrate and succinate to the oxygenated Krebs solution. These results, along with

other results on the inhibiting action of such metabolic blocking agents as iodoacetic acid, glyceraldehyde and fluoroacetate indicated that there is both a glycolytic pathway as well as a fatty acid oxidation system in active vascular smooth muscle, feeding "active acetate" into the Krebs cycle system.

(d) Utilization of High Energy Phosphate Compounds

Studies on bovine arterial muscle by Lundholm and Mohme-Lundholm (1960a, 1962a) demonstrated that this muscle contained only about one-seventh of the preformed high energy compound content of frog striated muscle but consumed, for a maximum contraction, about fifty times as much energy. Although the total content of high energy phosphate compounds was unchanged when the arterial muscle was stimulated with tone-increasing drugs such as adrenaline, the results suggested that the ATP content rose due to the stimulation of the carbohydrate metabolism whereas the CrP content fell since some was utilized by the contracting muscle. It was shown that the muscle's preformed high-energy phosphate compounds could suffice for only a fraction of a total maximum contraction, and probably for this reason, arterial muscle is far more dependent than striated muscle upon a continuous energy production for the contractile processes. Later, Lundholm and Mohme-Lundholm (1962b, 1965) calculated that the preformed high energy phosphate compounds in mesenteric arteries were exhausted after only 6 to 7 minutes under basal anaerobic conditions. More quantitative measurements of the changes in the high energy phosphate content of isolated bovine mesenteric arteries during adrenaline-induced isometric contractions were made by Beviz, Lundholm, Mohme-Lundholm and Vamos (1965). One minute after the addition of adrenaline, when the rise of tension was one-half maximal, the content of ATP and CrP had fallen by

about one-half the amount when the elevation of tension was maximal at the end of seven minutes. Although the maximal tension was maintained for sixty minutes, only the CrP content of the tissue was slightly reduced at the end of this period while the ATP content was at control levels.

2. Effect of Initial Length or Tension on the Resting Metabolism, and on the Drug and Electrically-induced Isometric and Isotonic Responses

(a) Effect on the Resting Metabolism

No direct observations have been made, that we are aware of, of the influence of the length of arterial muscle on its resting metabolism.

(b) Effect on Isometric Responses

Ducret (1931) demonstrated that the isometric contraction of arterial rings from a variety of species, when stimulated with epinephrine at different initial lengths displayed the same behaviour as smooth muscles generally by increasing to a maximum value and then falling off with further stretch. He obtained for the epinephrine contraction of mesenteric arteries a maximal stress increment of 800 g/cm^2 at the optimal initial length. Fischer (1944) has calculated similar values for other types of smooth muscles and although these values were much lower than those for striated muscles their reproducibility was very good. The results of Alexander (1954) on dog thoracic aorta also suggested that the active tension developed on adrenaline stimulation depended on the initial length of the muscle. A detailed study of the length-tension relationships of arterial strips was carried out by Speden (1958, 1959, 1960) who found that the norepinephrine-induced isometric contractions increased to a maximum and then decreased when the initial length or resting tension of the

strip was increased over a wide range. In a further attempt to apply these results to a possible mechanism of myogenic autoregulation, Sparks and Bohr (1962) measured the isometric tension developed in response to epinephrine and electrical stimulation by helical strips cut from small branches of the dog mesenteric artery. Both types of stimuli gave responses which increased until a certain optimal length was reached, after which the response decreased with further stretch of the strip. By studying the response of the quickly stretched strip at different initial tensions but at constant initial length, they suggested that the length was more important than the passive tension in influencing the active response.

(c) Effect on Isotonic Responses

Isotonic loading of the arterial muscle by Wallace and Speden (1959) showed that the external work performed by the arterial strip in response to norepinephrine stimulation at first increased to a maximum and then fell off as the load was increased further. Bevan (1960) using epinephrine to stimulate rabbit aortic strips at different loads observed qualitatively much the same thing.

The results of these isotonic experiments are in good qualitative agreement with those previously reported for isolated skeletal muscle by Doi (1920), isolated cardiac muscle by Patterson, Piper and Starling (1914), isolated uterine smooth muscle by Csapo (1954) and for intact nictitating membrane smooth muscle by Hampel (1933).

3. Effect of Drug and Electrical Stimulation of Arterial Muscle

(a) Catecholamine Stimulation

(i) Mechanical Effects - As mentioned by Furchgott (1955) in his review article on the pharmacology of arterial muscle, the smooth muscles of different blood vessels may vary considerably in their sensitivity to catecholamines. Thus, in some vascular beds epinephrine also produces vasodilation at low and vasoconstriction at higher concentrations, while with norepinephrine this dual effect is much less prominent. The ratio of the potency of norepinephrine to epinephrine in producing contraction of vascular muscle also varies somewhat with the vascular bed or blood vessel being studied. Furchgott found with spiral strips of rabbit aorta that norepinephrine was a slightly more potent contracting agent, but if allowance was made for the masked relaxing action of epinephrine at low concentrations, then the two appeared to be of about equal potency. The time-characteristics of contraction of spirally cut strips of rabbit aorta on addition, and of relaxation after washout, as well as the concentration-activity curves of epinephrine have been reported by Furchgott and Bhadrakom (1953) and Bevan (1960).

It has been demonstrated by Smith and Coxe (1951) that epinephrine causes vasoconstriction of isolated surviving pulmonary arteries of the dog, cat, swine and man. Other large blood vessels were tested by Dodd and Daniel (1960) who found that epinephrine produced contraction in all of the preparations tested which included rabbit, cat, rat and dog aorta and human and dog femoral artery. Their results indicated that the femoral artery contracts at a faster rate and attains a slightly greater

tension than the aorta. They attributed this to the greater proportion of smooth muscle in the femoral artery than in the aorta and to the more rapid rate of diffusion of the drug into the thinner artery. The maximum rate of relaxation was about 1/3 of the maximum rate of contraction which agrees with similar findings by other workers. They also demonstrated that the data obtained in one species of animal on one type of tissue cannot be transferred without specific proof of its validity to date obtained in other species, or even in closely related tissues of the same species. Rondell and Gross (1960) have made a similar observation.

On the other hand, Bevan (1961) found that the vascular muscle of the isolated rabbit thoracic and abdominal aorta and femoral and pulmonary arteries all have identical slopes of the dose - response curves for the two amines, epinephrine and norepinephrine. All of the preparations were found to be more sensitive to norepinephrine than to epinephrine.

Speden (1960) has investigated the effect of different concentrations of noradrenaline on the response of strips of sheep mesenteric artery. A stable and maintained isometric response was obtained at higher drug concentration e.g. of 10^{-6} to 10^{-5} g/ml, while with the lower drug concentrations, the response was more variable, being either maintained, not maintained, or inducing rhythmic contractile activity. Hinke and Wilson (1962a), however, found that 5×10^{-7} g/ml norepinephrine produced submaximal but strong and consistent responses in segments of isolated rat tail artery.

Using rabbit aortic strips, Bevan (1960) found that up to fairly high concentrations of epinephrine (two to three times the median effective dose) the rate of contraction was controlled by the rate of diffus-

ion of epinephrine to its receptor site. In comparison to this, the rate of reaction of the drug with its receptors is fast. His analysis also suggested that a diffusion barrier exists between the extracellular space and the adrenergic receptors and that the diffusion is activated.

The effect of catecholamine stimulation on the responses of isolated small arteries or arterioles has been studied by comparatively few workers. Bohr, Goulet and Taquini (1961) stimulated smooth muscle strips from small dog and rabbit resistance vessels with epinephrine and norepinephrine. Smooth muscle from renal resistance vessels was slightly more sensitive to epinephrine than that from mesenteric vessels while that from lung and brain resistance vessels was extremely refractory to this agent. This pattern of responsiveness was duplicated for norepinephrine except that the concentration required was two to three times greater than that for epinephrine. In comparison, the aortic smooth muscle was consistently more sensitive to epinephrine than the smooth muscle from either the renal or mesenteric resistance vessels. By perfusing the resistance vessels of an isolated dog mesenteric artery preparation at physiological pressures, Rogers, Atkinson and Long (1965) also found that the sensitivity to epinephrine was about three times greater than that for norepinephrine.

The nature of the response of coronary vessels to catecholamine stimulation appears to be affected by the size of the artery as shown by Zuberbuhler and Bohr (1965) in that the small coronary arteries isolated from dog were generally relaxed but in some cases a contractile response of the large vessels to catecholamines was observed.

The shortening process involved in the isotonic contractions of

rabbit aortic strips in response to epinephrine and norepinephrine was investigated by Brodie, Bohr and Smit (1959) who found that it had a dual character, consisting of a fast (F-) and a slow (S-) component. Low concentrations of epinephrine and norepinephrine activated the F- component more than the S- component. After Dibenamine blockade, norepinephrine in high concentrations caused a separation of the F- and S- components by imposing a period of relaxation between the two. The authors postulated that Dibenamine may exert this effect by altering the ratio of available receptors which activate the two components.

(ii) Metabolic Effects - Lundholm, Mohme-Lundholm and co-workers have carried out a series of studies on the effects of catecholamines on the metabolism of arterial muscle. Lundholm and Mohme-Lundholm (1960b) confirmed Sudhof's (1950) finding that the constrictor effect of adrenaline on vascular muscle was associated with stimulation of its carbohydrate metabolism. They went on to demonstrate that this effect was not secondary to the muscle contraction but involved a separate mechanism which could be activated by adrenaline either separately or along with the contractile mechanism. Noradrenaline was observed to have a much weaker effect than adrenaline in stimulating the carbohydrate metabolism of the muscle and its subsequent lactic acid production, a fact which they thought may explain its predominantly vasoconstrictor action.

Lundholm and Mohme-Lundholm (1962a) found that adrenaline stimulated glycolysis in vascular muscle even under aerobic conditions. Previous experiments by them on arterial muscle failed to detect any glycogenolytic effect of adrenaline so it was postulated that the increased content of hexosephosphates observed by Beviz and Mohme-Lundholm (1964) after the addition of the drug as being due to a precursor which was

either a polysaccharide of low molecular weight or an intermediate of the pentose shunt. These changes in metabolite concentrations along with those of the high energy phosphate compounds were found in other studies by Lundholm and Mohme-Lundholm (1926b, 1963a) and by Beviz and Mohme-Lundholm (1965) to be due to adrenaline stimulating two types of receptors in arterial muscle: α-receptors linked with arterial muscle contraction and hydrolysis of ATP and CrP, and β-receptors associated with relaxation, synthesis of ATP and CrP and stimulation of the carbohydrate metabolism. Thus only some of the metabolic changes were direct results of the contractile process while some were due to the stimulation of the carbohydrate metabolism by adrenaline via a direct mechanism while some were due to both actions. The effects exerted by noradrenaline probably differ qualitatively from those of adrenaline in that the former drug stimulates primarily the α-receptors. That the β-receptors may be activated by noradrenaline was suggested by the results of Mohme-Lundholm (1956) who reported a relaxing and lactic acid forming effect with high concentrations of noradrenaline which was detectable but considerably less than that found for adrenaline in the bovine tracheal smooth muscle preparation that she used.

The above property of adrenaline accounts for its reversal effect on smooth muscle. In addition, when adrenaline stimulates both the contractile mechanism and the carbohydrate metabolism and the former effect predominates then the tone, of course, increases rather than decreases. Even when the contractile mechanism is stimulated, however, the concomitant lactic acid production has an inhibitory effect since the stimulatory effect of adrenaline on smooth muscle has been shown to be enhanced in alkaline and attenuated in acid solution by Burget and Visscher (1927) and

Tobian, Martin and Eilers (1959).

(b) Direct Electrical Stimulation of Arterial Muscle

(i) Mechanical Effects - Relatively little work has been done on the processes involved in producing contraction of arterial muscle with electrical stimulation. Meyer (1906), in the first paper on the behaviour of arterial rings, reported that they contracted in response to single strong inductorium shocks. Bevan (1962) employed a single condenser discharge of 180 volts to obtain a pure direct response of the isolated rabbit pulmonary artery which was unaffected by a supramaximal dose of yohimbine hydrochloride. An initial fast contraction was obtained which started almost immediately after stimulation and was followed after a short pause by a slower contraction which reached a maximum a few minutes later. Relaxation was slow and usually complete within thirty minutes. However, like many other smooth muscle preparations, arterial rings or strips generally respond much more vigorously to short periods of five to ten seconds of stimulation with a faradic or alternating current as shown by the work of Kosuge (1934) and Furchgott (1952) than they do to single shocks.

Furchgott (1952) has reported that the direct application of an alternating current for about ten seconds to spirally cut strips of rabbit aorta, caused a relatively fast contraction during current flow, followed after cessation of current by a further slower contraction and a prolonged slow relaxation. He postulated that the contraction after current flow was probably due to the liberation of an epinephrine-like substance in the artery during electrical stimulation, since A.C. current, after Dibenamine, produced only the relatively fast contraction, followed immediately by a faster relaxation than occurred without Dibenamine. This

finding was later confirmed by Gillis and Yates (1960) who proposed that the slow, secondary contraction following the direct electrical stimulus was due to the liberation of norepinephrine since pretreatment of the animals with reserpine, which depletes the vessel wall norepinephrine, abolished the secondary contraction and only the immediate rapid response was observed. In addition, Schmiterlow (1948) has shown that arterial walls do contain measurable quantities of epinephrine-like material as well as histamine and acetylcholine.

Good consistent responses of a variety of arterial strips have also been obtained by a number of workers by using low voltage alternating current stimulation for fairly long periods of time (0.5-15 seconds). Leonard (1957) found that the contraction continued for about one or two more minutes after the termination of the stimulus and that relaxation was not complete for ten to thirty minutes when the tension developed was 80% of maximum. It was found that greater tensions could be obtained by progressively increasing the voltage but then the relaxation times became inconveniently long. His muscle preparation also exhibited the staircase phenomenon, a step-wise increase in tension on repeated stimulation with a period of rest allowed between stimulations. This effect was attributed to a progressive loss of muscle fiber potassium.

Sparks and Bohr (1962) have employed helically cut strips of the wall of small branches of the dog mesenteric artery and found that the maximum active tension developed with stimulation by low voltage alternating current was of the same approximate magnitude as that produced by the addition of epinephrine of 10^{-7} g/ml final bath concentration. Similar stimulation has also been used by Waugh (1962) to obtain consistent responses of his segments of dog intestinal artery.

Square wave pulses of 10 milliseconds duration, 5-15 volts amplitude, at 20 pulses per second for a 15 second period have been used by Rondell and Gross (1960) to stimulate rabbit aorta, renal, mesenteric and carotid arteries, as well as rat aorta. It was found that repetitive stimulations, at ten minute intervals, produced contractions of comparable tension without measurable change in the base-line tonus.

(ii) Electrical Effects - It has been observed by Roddie (1962) that direct electrical stimulation of isolated segments of turtle aorta gave rise to a conducted action potential which travelled along the vessel at about 0.5 mm/sec for a distance of about 2 cm from the point of stimulation. Monnier (1944) has obtained a value of 2 mm/sec for the speed of conduction of excitation in isolated muscular mesenteric arteries of cattle. A preparation similar to the one which we have used, the dog femoral artery, has been employed by Barr (1959) who recorded contractions and complex action potentials in response to direct electrical stimulation. The possibility that conduction and contraction are not necessarily consecutive processes is suggested by some observations of Monnier (1943) who demonstrated that conduction can occur without contraction of parts of the isolated artery through which conduction has taken place.

The above results are at variance with those of Burnstock and Prosser (1960) who found that electrical stimulation of pig carotid artery and renal vein did not initiate propagated action potentials in their smooth muscle. This result may have been due to the relatively high intercellular spacing in their muscle tissues.

Some data which strongly suggests that direct electrical stimulation of vascular smooth muscle may not exert its effect through local nerve nets has been obtained by Fulton and Lutz (1942). They observed

that direct electrical stimulation of arterioles of the frog retrolingual membrane caused strong constrictions even though the nerves were blocked with cocaine. In addition, Bevan (1962) has found that pulmonary artery preparations from reserpine-pretreated rabbits did not respond to sympathetic nerve stimulation but still retained normal contractility to direct electrical stimulation. Some data provided by Roddie (1962) also suggested that nerves were not necessary for the conduction of action potentials produced by direct electrical stimulation of isolated segments of turtle aorta.

(iii) Metabolic Effects - The changes occurring in the metabolism of arterial muscle being stimulated with direct electrical stimulation have not to our knowledge been looked into, with the exception of a study made by Lundholm and Mohme-Lundholm (1963a). They found that electrical stimulation of the isolated bovine mesenteric artery with A.C. current at 50 cycles per second and 15 volts amplitude for three to four minutes in the presence of the bathing solution, was accompanied by appreciable glycogenolysis and concomitant contraction under aerobic conditions but no glycogenolysis was observed under anaerobic conditions.

(c) Comparison of Drug and Electrically-induced Responses

Despite the many similarities, some differences have also been observed between the responses which were produced by catecholamines and those which were produced by direct electrical stimulation of arterial muscle. These include the effect of the membrane potential on the two types of stimulation and the changes in the potential which are produced by them, their differential effect on the muscle metabolism and the influence of temperature on the two types of response.

(i) Membrane Effects - Several studies have yielded findings which

favour the dissociation between the contraction of arterial muscle and changes in its membrane potential when catecholamine but not when electrical stimulation is used. Thus, Barr (1961) and Waugh (1962) demonstrated that vascular smooth muscle, in the presence of depolarizing concentrations of external potassium ion, still exhibited strong responses to catecholamine but not to electrical stimulation. Also in partial disagreement with general ionic theory is the data obtained by Headings, Bohr and Rondell (1960) and Headings and Rondell (1962) which indicated that net potassium efflux and hence perhaps membrane depolarization are not essential events in the contraction of arterial muscle in response to catecholamine stimulation but, on the other hand, are associated with the electrically-induced contractions. Direct electrical measurements of the membrane potential of the vascular smooth muscle cells of rabbit pulmonary artery were made by Su, Bevan and Ursillo (1964) who could not detect any change in the potential when the artery was stimulated to contract with norepinephrine.

However, depolarization and/or the normal changes in sodium or potassium fluxes during catecholamine-induced contractions of arterial muscle have been reported by Tobian and Fox (1956), Barr (1959), Keatinge (1964) and Friedman and Friedman (1964). Although no comparative studies have been done on the effect of abnormal ionic media on the catecholamine and electrically-induced responses of arterial muscle, Leonard (1957) and Dickinson (1960) have found the electrically-induced responses in abnormal potassium environments to be the reverse of those observed by numerous investigators with catecholamine stimulation of arterial muscle. So it is possible that in some of the above studies, although no quantitative differences between the ion fluxes with catechol-

amine and those usually obtained with electrical stimulation were apparent, qualitative differences could have been present.

(ii) Metabolic Effects - Some differences in the carbohydrate metabolism of mesenteric arterial muscle which has been stimulated to contract with adrenaline and with direct electrical stimulation have been reported by Lundholm and Mohme-Lundholm (1963a). They found that increased glycogen breakdown was associated with the electrically-induced isotonic contractions but not with the equivalent contractions caused by adrenaline under aerobic conditions. It was also found that glycogenolysis and lactic acid production did not correlate very well so that substantial amounts of lactic acid could have been formed from intermediate products of the carbohydrate metabolism. Hence the metabolic efficiency of the electrically-induced contraction in terms of the number of moles of high energy phosphate formed per mole of lactic acid may not necessarily be greater than that of the adrenaline-induced contraction. Under anaerobic conditions neither type of stimulation was accompanied by glycogenolysis although the lactic acid production was increased so that the correlation between the lactic acid production and muscle contraction was generally far more evident than that between glycogenolysis and contraction.

(iii) Temperature Effects - It has been demonstrated by Keatinge (1964) that at the low temperature of 5°C the contractions produced by electrical stimulation of spiral strips of sheep carotid artery were almost as large as at 35°C while no response was produced by adrenaline and noradrenaline stimulation at this low temperature. These contractions were physiological and not due to denaturation. At the higher temperature the high voltage direct electrical stimulation produced isotonic responses which were roughly equivalent to those produced by the catecholamines.

This result leads us to suggest that the electrical stimulus acts through or on later stages of the contractile sequence than do the catecholamines and that the reactions which are associated with electrical stimulation are probably less affected by immediate large changes in the energy production of the tissue.

Despite these possible differences the two types of stimulation have been shown to have an interaction effect. Thus, Barr (1959) has observed that the tension developed and the spike activity of strips of dog femoral arteries in response to direct electrical stimulation were increased by the previous addition of epinephrine.

4. Proportionality between the Mechanical and Metabolic Responses

(a) Isometric Contractions of Arterial Muscle

By measuring the lactic acid production under anaerobic conditions, Lundholm and Mohme-Lundholm (1965) noted that during the development of tension by the bovine arterial muscle stimulated to contract with adrenaline, the metabolism was elevated 3- to 5-fold. When the tension had reached maximal level, the metabolic elevation was much more moderate and equal to about 60%. During this phase of constant maximum tension, it was found by Beviz, Lundholm, Mohme-Lundholm and Vamos (1965) that only the CrP content was still reduced while the ATP content was at control levels. The total hydrolysis of ATP and CrP during the rise of tension yielded an isometric coefficient (or $\frac{PL}{H}$ quotient) of 9.5 which is of similar magnitude to that found from the heat production of striated and smooth muscles. The coefficient is reduced to 4.2 when the possible resynthesis of these high energy compounds, which may occur during the period of contraction, is taken into account.

(b) Isotonic Contractions of Arterial Muscle

Although isotonic contractions of arterial muscle have been shown to require energy in the studies of Lundholm and Mohme-Lundholm (1960a, 1962a, 1965) it has also been consistently shown by them that the metabolism was elevated only during the phase of shortening or increasing tension and returned to resting levels when the tension of the muscle had reached a fairly constant level. The increase in the lactic acid production of the bovine mesenteric artery when it was stimulated to contract showed both a quantitative and a temporal correlation to the degree of contraction in terms of the degree of shortening of the muscle strip but no demonstrable correlation could be found to exist between the work performed and the lactic acid production. However, only very light and very heavy loads were used, with the extra lactic acid production upon contraction being in both cases very low. In fact, it was calculated that in these experiments the external work accounted for only 0.6% of the total increase in energy production accompanying contraction which would make any changes in the energy production with different loads quite small and hence difficult to detect.

(c) Comparison of the Metabolic Responses of Isotonic and Isometric Contractions

The lactic acid production under anaerobic conditions was used as an index of the energy metabolism in order to compare the energy demands of the bovine mesenteric artery under both isometric and isotonic conditions in a study by Lundholm and Mohme-Lundholm (1965). It was found that the energy metabolism was appreciably greater for the isometric than for the isotonic contractions, both during the increasing tension phase and during the maintenance of constant tension. The large disparity in terms

of energy metabolism, between the two types of contraction could not be attributed to differences in their time pattern or duration, or to the degree of activation or the number of activated muscle fibres, or to differences in the initial lengths of the muscle preparations. The authors suggested, however, that a maximal isotonic contraction from the fully relaxed state of the muscle would give, theoretically at least, a metabolic increase somewhat exceeding that for isometric contraction. In addition, the isotonically-loaded muscle was usually only lightly loaded in order to observe easily the large changes in length upon stimulation, so that the isotonic responses even more so than the isometric responses, occurred at applied loads which were far removed from the optimal values.

III. CONCLUSIONS AND REASONS FOR THE PRESENT INVESTIGATION

As was shown, most of the measurements of the "basal" Q_{O_2} of arterial muscle were performed on aortic tissue and very few have been done on the more muscular arteries. Generally, the agreement between the aortic Q_{O_2} values was very good despite the very low values obtained, which tend to make accurate measurements difficult to obtain. Since, in our studies, we intended measuring the increment in the Q_{O_2}, if any, of a femoral arterial segment as a function of its passive stretch and active tension, it was necessary to have some idea of the magnitude and stability of its "basal" Q_{O_2}.

The dependence of the resting oxidative metabolism on the initial length or passive tension has been shown for a variety of muscles but not for arterial muscle. The influence of these same parameters on the isometric and isotonic mechanical responses of arterial muscle has been demonstrated but, again, the corresponding changes in the oxygen demand have not been investigated. More information on the dependence of the mechanical and metabolic responses of vascular smooth muscle on its initial length or tension would also throw more light on the nature of the mechanisms involved in the autoregulation of blood flow.

The proportionality between the tension developed and the energy liberated, measured as either the heat produced or oxygen consumed by the isometrically contracting muscle, has been demonstrated for a number of

skeletal, cardiac and smooth muscles. By considering the rate of lactic acid production and the changes in the content of high energy phosphates, the same proportionality has been shown to exist for arterial muscle under anaerobic conditions.

That extra energy is mobilized in proportion to the work performed by the muscle, i.e. the Fenn effect, has been demonstrated for skeletal, cardiac and smooth muscles, but no such correlation was found in the one study performed by Lundholm and Mohme-Lundholm (1962a) on vascular muscle under anaerobic conditions where the lactic acid production was used as the criterion of its energy metabolism.

Comparison of the magnitude of the energy liberated by skeletal muscle under isometric and isotonic conditions has proven to be useful in developing mechanical models of the contractile system and in determining the physical and physiological characteristics of its various components. Generally, most of the work done shows the energy demand of the isotonic contraction to be less than that of the isometric contraction at light loads and greater at heavier loads. In the single study reported by Lundholm and Mohme-Lundholm (1965), arterial muscle under anaerobic conditions was found to utilize much less energy during the isotonic contraction relative to the isometric contraction. No systematic study of the oxygen consumed by arterial muscle under various isometric and isotonic loads has been performed which would throw more light on its behaviour than the few experiments mentioned above.

Changes in the rate of oxygen consumption may not always reflect changes in energy production because of possible accompanying changes in metabolic pathways. A change in metabolic pattern can be detected by continuously measuring the respiratory quotient (RQ) of the muscle.

Although only a few reports on cardiac and smooth muscles have indicated that the increase in respiration with chemical stimuli may be significantly greater than with electrical stimuli, this result would not be entirely unexpected in view of the qualitatively different effects which temperature, drugs, and abnormal ionic environments have been reported to have on the two modes of stimulation. For this reason, among others, it was decided to determine the effects of anoxia, temperature, pH and of abnormal levels of sodium and potassium in the bathing solution on the respiratory and mechanical responses produced by the two types of stimuli. These studies, however, do not form part of this thesis.

As a result it was attempted to evaluate the magnitude and efficiency of the oxidative processes in arterial muscle which has been stimulated to contract at different values of passive tension. It was also intended to investigate the influence of the type of stimulus (norepinephrine and electrical) and of the type of loading used (isometric and isotonic) on the oxygen uptake of the contracted arterial muscle.

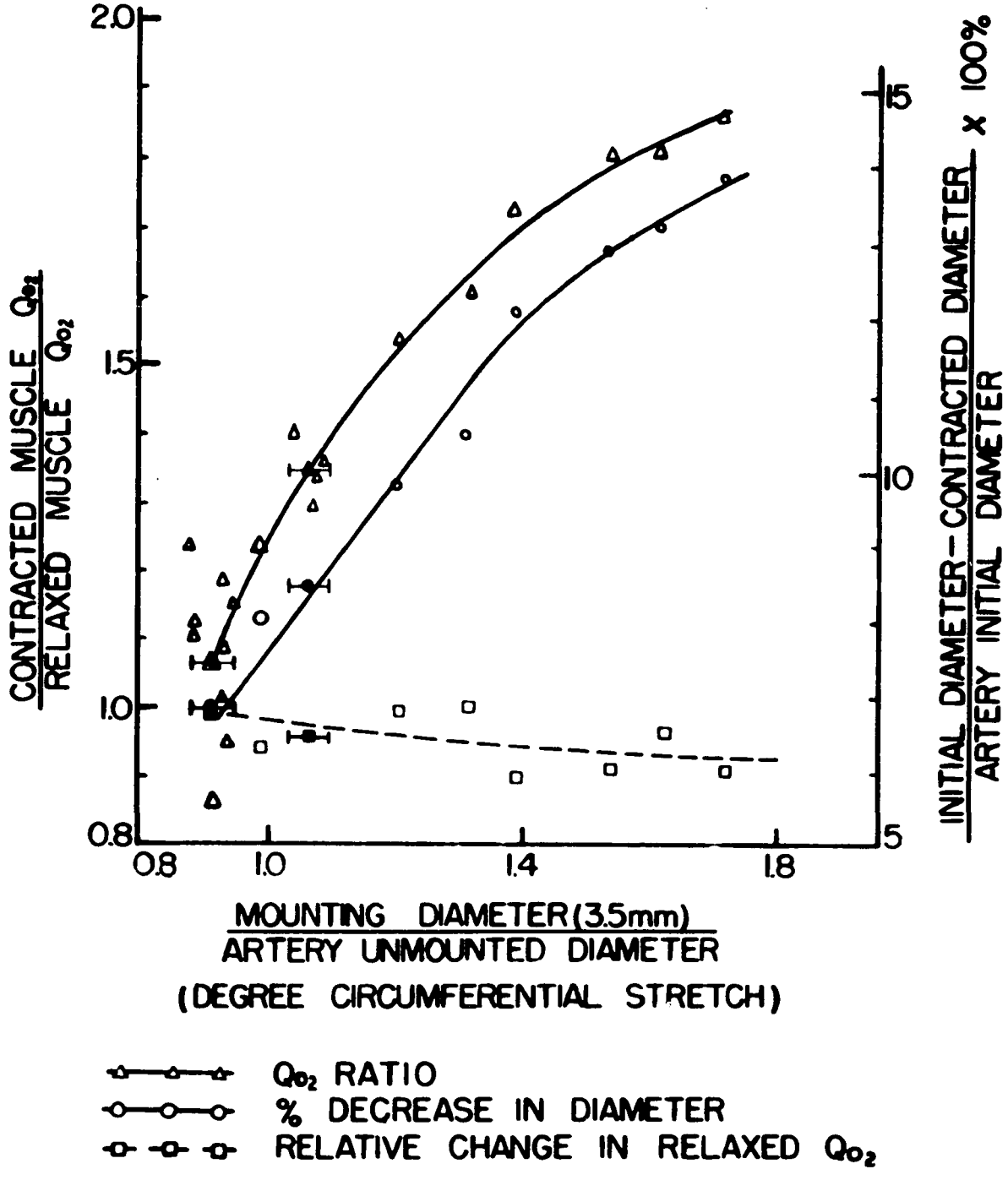
IV. PRELIMINARY FINDINGS

In an initial series of experiments, the results of some of which have been reported by Kosan and Burton (1966), the "basal" Q_{O_2} and "basal" RQ of resting arterial muscle and the changes which take place in these parameters over short and long periods of time (hours) were determined. The stability of these reference values of the oxidative metabolism of arterial muscle was good enough to permit measurements to be made of the oxygen consumption of contracted arterial segments when they were stimulated with epinephrine or norepinephrine.

A modified Warburg constant-volume technique was used to measure the "activity" oxygen consumption with the index of the degree of contraction being the relative decrease in the diameter of the segment upon stimulation. The major finding of this study turned out to be the strong effect exerted by the initial stretch of the muscle on its active mechanical and respiratory responses to drug stimulation. The relevant results which have been obtained illustrating this effect have been reproduced in Figure 1. It is apparent that the criteria of both the degree of initial stretch and of the degree of active contraction are only qualitative in nature but the effect of the former parameter on the behaviour of the latter still stands out. Since other studies on skeletal, cardiac and smooth muscle have also shown a strong dependence of the metabolism of muscle and of its active responses on its initial length, it was thought

FIGURE I

The effect of passive stretch on the active responses of adventitia-free arterial segments as illustrated by the results from preliminary experiments. When the circumferential stretch of the artery wall is increased, the relative decrease in diameter and the relative increase in the oxygen consumption of the contracted artery both increase in a parallel fashion. Same concentration of epinephrine (5×10^{-7} g/ml) was used to stimulate each preparation. With stretch relatively little change is observed in the relaxed (non-stimulated) muscle Q_{O_2}. Solid triangles, dots and squares represent corresponding average values over the intervals indicated by their horizontal bars.


2.0
1.5
1.0
0.8
0.8
1.0
1.4
1.8
15
10
5
CONTRACTED MUSCLE Q_{O_2} / RELAXED MUSCLE Q_{O_2}
INITIAL DIAMETER − CONTRACTED DIAMETER / ARTERY INITIAL DIAMETER x 100%
MOUNTING DIAMETER (3.5mm) / ARTERY UNMOUNTED DIAMETER
(DEGREE CIRCUMFERENTIAL STRETCH)
Q_{O_2} RATIO
% DECREASE IN DIAMETER
RELATIVE CHANGE IN RELAXED Q_{O_2}

worthwhile to investigate the above effects in a more quantitative and thorough fashion.

V. METHODS

The first experimental arrangement described by Kosan and Burton (1966) was further modified to give quantitative values for the passive pressures and the active pressures developed across the wall of the arterial segment. The experiments performed with this modified Warburg technique were then repeated by means of another technique which was quite different in that it employed a polarograph for the continuous recording of the rate of oxygen uptake by the arterial segment.

Both techniques permitted the measurement of the oxygen consumption of the segment both when it was stretched initially by different amounts, and also, when the stretched segment was stimulated to contract isometrically. The polarographic technique also permitted measurements of the rate of oxygen uptake to be made when the segment was contracting isotonically. In both cases the segment could be stimulated either with drugs or with direct electrical stimulation.

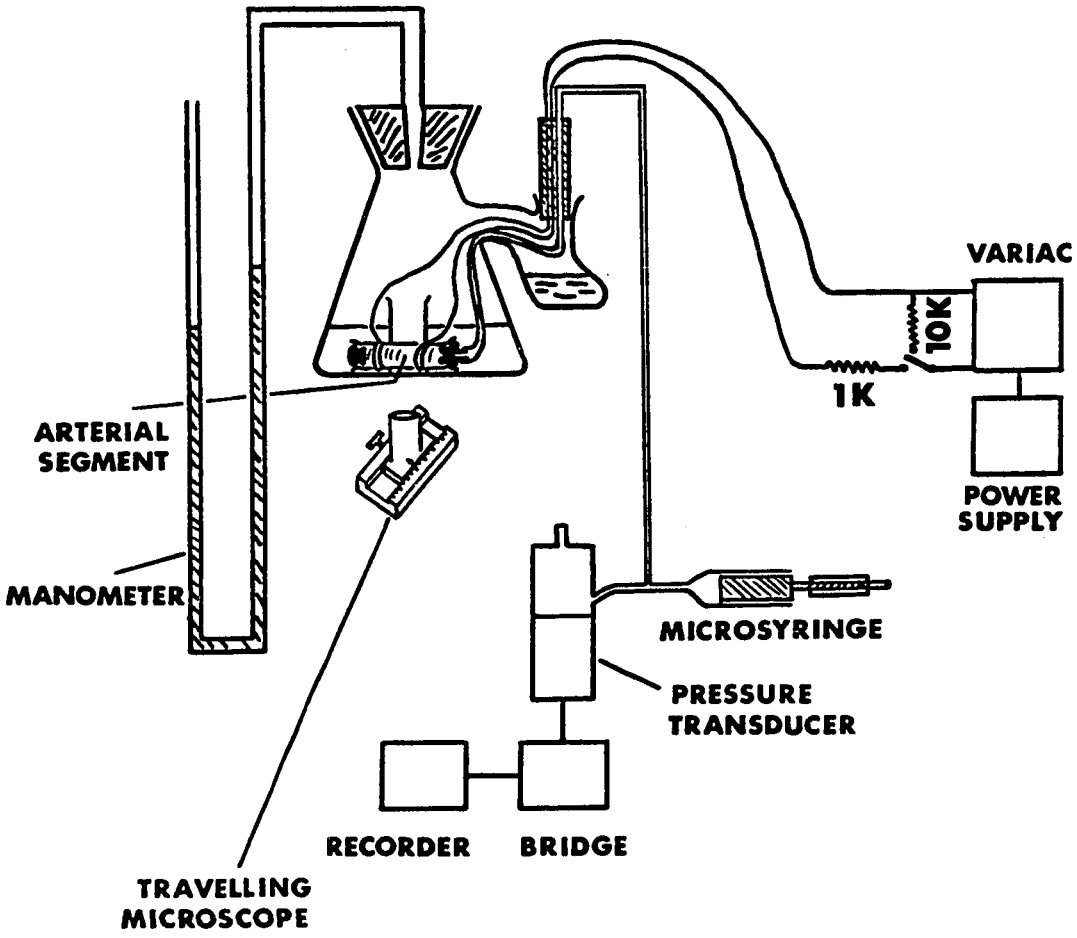
Once again, segments of arteries from the muscular dog femoral artery were used in all of these studies since smooth muscle makes up a large part of their total weight and the elastin and collagen fibres which make up most of the rest of the weight are acellular and presumably do not consume oxygen.

A. Arterial Segment Preparations

The segment of femoral artery, about 3 cm in length, was isolated

FIGURE 2

A schematic diagram of the Warburg apparatus as modified to permit the measurement of the transmural pressure changes across the wall of the arterial segment upon drug or direct electrical stimulation. To allow space for the long arterial support and the coiled tubing and electrical leads, 30 ml Warburg reaction flasks were used.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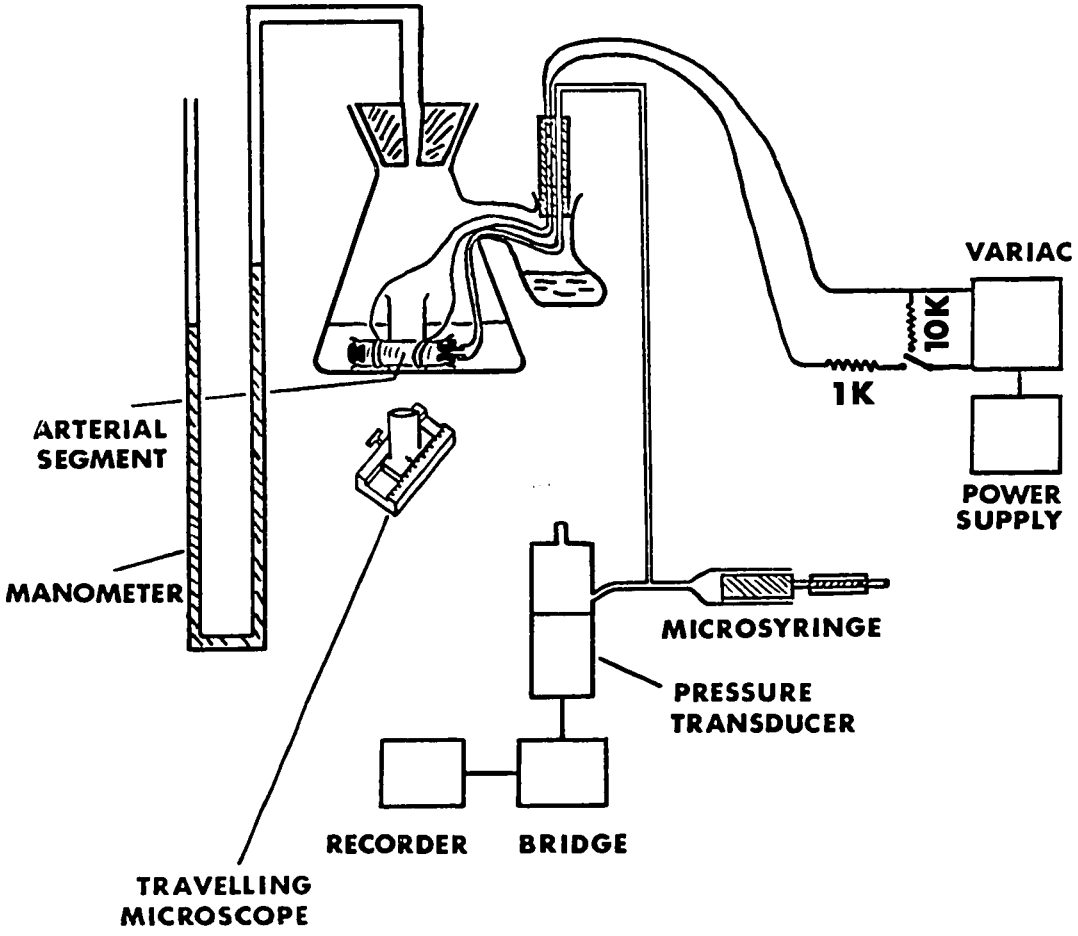
ARTERIAL SEGMENT
MANOMETER
MICROSYRINGE
PRESSURE TRANSDUCER
RECORDER
BRIDGE
TRAVELLING MICROSCOPE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MICROSYRINGE
PRESSURE TRANSDUCER
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TRAVELLING MICROSCOPE

from nembutal-anaesthetized dogs of a variety of breeds, ages and sex. Immediately after isolation, the segment was placed in a buffered physiological salt solution containing glucose (modified Ringer solution). This solution was described previously by Kosan and Burton (1966), (see Appendix for composition). With many of the segments, the outer layer of fibrous tissue, the tunica adventitia, was then gently stripped off.

It was found that with those segments which had their tunica adventitia intact, leakage of fluid across the wall of the segment became significant at transmural pressures usually above 50 mm Hg, while with those segments which had their adventitia stripped off, the leakage was very significant at very low pressures. This was the case even when the segments were free of branches. To prevent or minimize this leakage, a thin-walled segment of rubber tubing was drawn through the adventitia-free arterial segment and was tied to the support so as to make a loose, waterproof lining for the stretched arterial segment. The diameter of the rubber tubing was equal to 3.0 mm. Since no such tubing was commercially available it was made by ourselves, and a brief description of the process used is given in the Appendix. This tubing was very distensible and so did not absorb much of the total pressure drop across the two segments. In most cases, the leakage of fluid now presented no problem.

B. Apparatus and Procedures - Warburg Technique (Method A)

The general arrangement of the equipment with this modified Warburg technique is shown in Figure 2. The bath was made of plastic to allow easy observation of the segment. A travelling microscope was placed under the bath to permit the measurement of the outer diameter of

the arterial segment. This enabled us to convert the transmural pressures to the corresponding values of wall tension. Shaking of the flasks was accomplished with a modified Warburg linkage.

1. Mounting of the Arterial Segment

The primary difference between this physical set-up and the previous one used and described by Kosan and Burton (1966) was in the design of the stainless steel bracket to which the arterial segment was tied. The stainless steel annuli were replaced by a solid steel plug at one end of the arterial support and an annulus with a small nipple extending outwards at the other end. The distance between the annuli equalled 18 mm while their outside diameter equalled 3.0 mm. This support was mounted on the centre well of a large 30 ml Warburg reaction flask as shown in Figure 2. One end of the adventitia-intact segment was tied to the plug and with the segment stretched about 40% of its initial length to compensate for the retraction upon excision, the free end was then tied to the annulus. Thus the mounted length was close to the *in vivo* length. All the tying was performed with both the segment and the support immersed in warm modified Ringer solution so as to minimize the possibility of trapping any air or oxygen bubbles within the segment. A length of polyethylene tubing (PE 10), also filled with modified Ringer solution, was tied to the nipple and then the support and its attachments were lowered into the flask and secured to the centre well.

2. Transmural Pressure Measurement

As shown in Figure 2, the polyethylene tubing from the arterial segment was, in turn, connected to a microsyringe and a Statham pressure transducer. After bubbles of oxygen were eliminated from this closed system, the fluid pressure was increased by adjusting the microsyringe

and recorded by means of the transducer output. If the connecting tubes are distensible then the volume of the artery will decrease during a contraction because the total fluid inside is constant. Therefore the distensibility of the different components of the system was measured and the volume decrease of the arterial segment upon contraction is estimated to be less than 0.2% in the range of tensions studied. The device used and the results obtained are given in detail in the Appendix. In view of these results, it appears that the contraction of the artery upon stimulation was essentially isometric in nature.

Because the manometry was by the constant pressure method, the external pressure in the artery was always atmospheric.

3. Stimulation of the Arterial Segment

The norepinephrine was obtained as 4 ml ampoules of Levophed bitartrate brand of levarterenol U.S.P. 0.2% solution (= 0.1% base) Winthrop Laboratories of Canada, Ltd. The drug was diluted with modified Ringer-glucose solution to prevent any dilution of the medium when the drug is added. When norepinephrine stimulation was used, 0.5 ml of this drug solution was added to the sidearm of the flask.

For electrical stimulation two stainless steel rings were pulled over the arterial segment before it was tied to the annulus. These rings were connected by insulated platinum leads to the voltage source. To maintain good contact between the rings and the segments, from the largest to the smallest arteries, rings were used which had an inner diameter of 2.0 mm. The two insulated platinum leads along with the polyethylene tubing all passed through a hole which was drilled straight through the sidearm plug of the reaction flask and sealed at its lower end with epoxy resin.

(A sufficient amount of slack tubing and wire was left below the plug to allow the arterial segment with its support to be handled beyond the mouth of the Warburg flask when the sidearm plug was seated in the sidearm. While the arterial support, with its attached segment, leads and tubing was being lowered into the flask, the slack in the tubing and electrical leads was moved out of the way by withdrawing the sidearm plug at the same rate that the support was being lowered into the flask. This prevented damage to the tubing and leads while the support was seated and fastened into position. The sidearm plug was then slowly replaced, allowing the tubing and leads to coil in the major compartment of the reaction flask.)

The two electrical leads were connected to a Variac autotransformer fed from a Sorensen constant power supply. The stimulation was controlled manually by the switch shown in Figure 2. In the "stimulus-off" position the Variac current passed through the 10 K load resistor. The voltage drop across the 1 K series resistor divided by its resistance gave the total current through the arterial tissue and through the fluid bathing it.

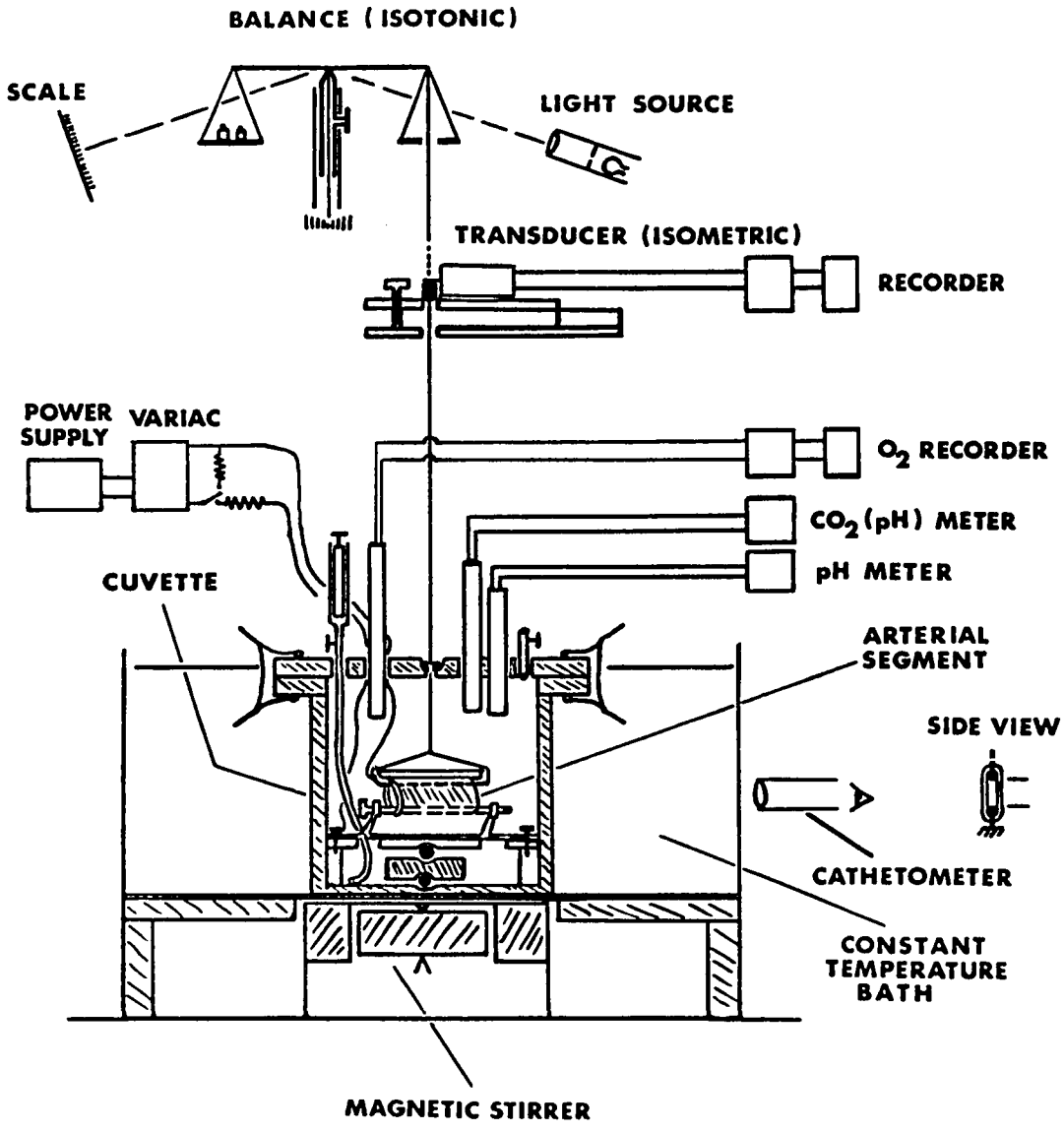
It was found that for a given strength and duration of the electrical stimulus the maximum mechanical response was obtained when the distance between the ring electrodes was equal to 1.0 cm, each being located 0.5 cm from the centre of the segment. For this reason, this distance was maintained at 1.0 cm throughout this series of experiments.

4. Protocol for Oxygen Uptake Measurements

With the arterial segment tied to its support, 10 ml of the modified Ringer solution was added to the main compartment of the flask as this was sufficient to cover the entire arterial segment when no

FIGURE 3

A schematic diagram of the apparatus used for the measurement of the isotonic work and isometric tension of arterial segments using electrodes to monitor changesin the pH, oxygen and carbon dioxide concentrations of the cuvette solution. It was possible to stimulate the segments both with drugs and electrically with this set-up.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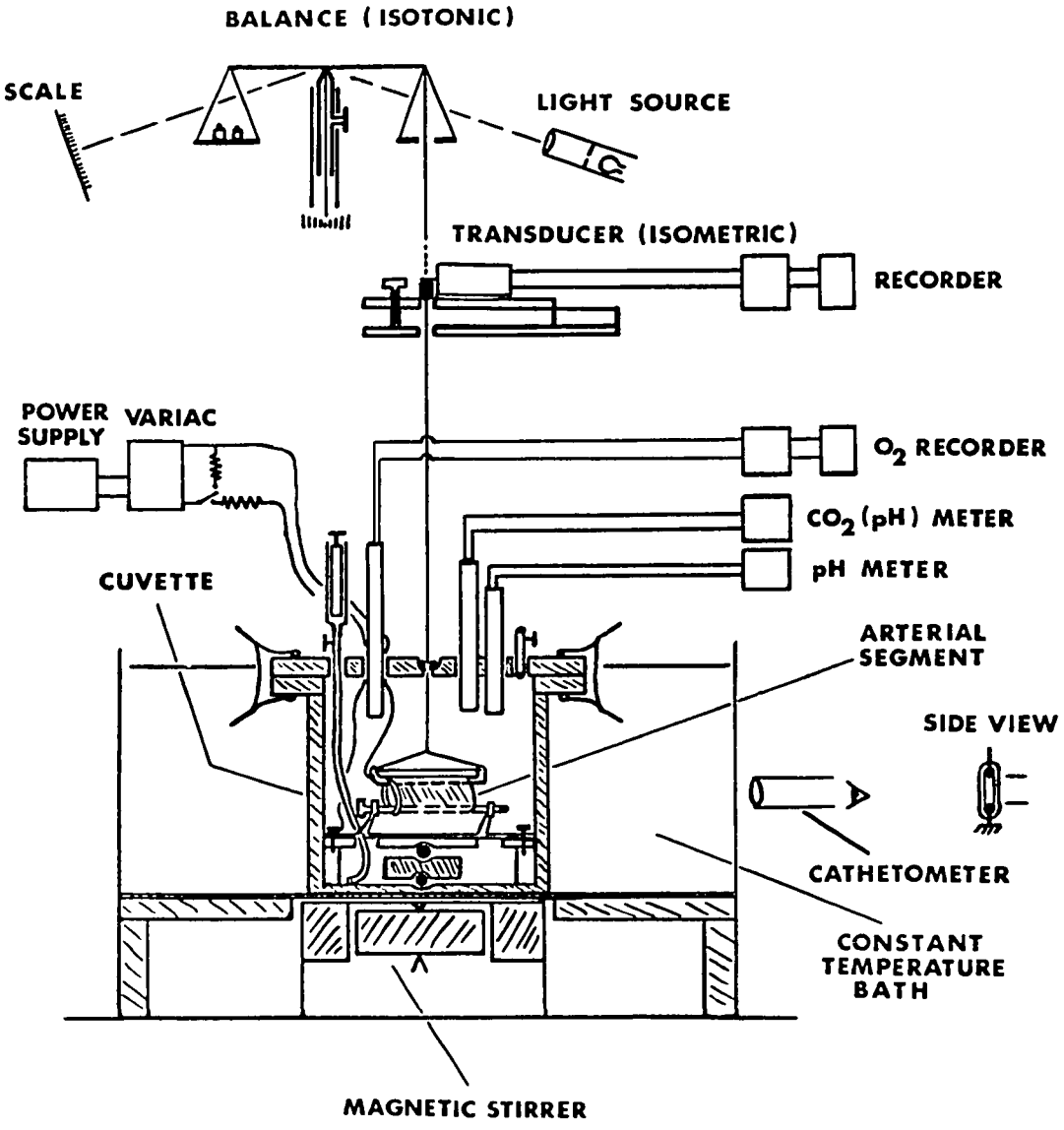
SCALE
LIGHT SOURCE
TRANSDUCER (ISOMETRIC)
RECORDER
POWER SUPPLY
VARIAC
O_2 RECORDER
CO_2 (pH) METER
pH METER
CUVETTE
ARTERIAL SEGMENT
SIDE VIEW
CATHETOMETER
CONSTANT TEMPERATURE BATH
MAGNETIC STIRRER


BALANCE (ISOTONIC)
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TRANSDUCER (ISOMETRIC)
RECORDER
POWER SUPPLY
VARIAC
O_2 RECORDER
CO_2 (pH) METER
pH METER
CUVETTE
ARTERIAL SEGMENT
SIDE VIEW
CATHETOMETER
CONSTANT TEMPERATURE BATH
MAGNETIC STIRRER

agitation was taking place. To absorb the CO_2 that was produced, 0.2 ml of 20% potassium hydroxide was added to the centre well. For drug stimulation, 0.5 ml of the norepinephrine solution was next added to the sidearm. The flask was gassed through the sidearm with preheated 100% oxygen for 3 to 4 minutes. The thermobarometric control flask contained a mounted rubber segment in place of the artery. The two flasks were shaken in a bath maintained at 37°C. All readings of oxygen consumption were made under conditions of constant gas pressure (atmospheric pressure). (This obviates correcting for volume changes of the segment.) Thermobarometer corrections, though small, were always made.

C. Polarographic Technique - Method B

A second experimental arrangement using a different method of mounting the arterial segment was developed which allowed study of both isotonic and isometric contractions and easy measurement of the thickness of the adventitia-free arterial segment. The shape of the mount also made direct electrical stimulation of the segment an improvement over the Warburg method.

1. Description of Mount

The device that met the requirements is shown in Figure 3. The mount for the arterial segment consisted of a U-shaped stainless steel bracket and two stainless steel rods. The bracket was secured to a horizontal baffle plate in the cuvette so that its two arms, which were about 2 cm apart, extended upwards for about 0.5 cm. Since a threaded annulus was soldered to the top of each arm, a steel rod could be passed through these annuli and the lumen of the arterial segment in order to secure the lower end of the segment. A second steel rod, connected by a wire to the force measuring equipment, served to stretch the segment vertically.

2. Electrical Stimulation of the Arterial Segment

Two platinum leads (0.005" diameter) were connected to the stainless steel rods which served to stretch the arterial segment so that, in effect, one lead made electrical contact with the lower part of the segment, the other lead with the upper part of the segment. These two insulated leads were led out of the cuvette containing the arterial segment and connected to a Variac autotransformer fed from a regulated constant power supply. As before, a switch was used to feed the current through a load resistor until it was needed to stimulate the arterial segment. The switch was operated manually. Also, another resistor, placed in series with the arterial segment, permitted measurement of the total current that was being drawn during stimulation.

3. Measurement of the Thickness of the Arterial Segment

A cathetometer with a graduated reticule, placed as shown in Figure 3, permitted measurements to be made of both the arterial wall thickness and also of any shortening of the segment.

The thickness of the segment wall was measured at two points located midway between the two rods stretching the segment. The thickness readings were compared with that obtained for the diameter of the lower mounting rod, which had been measured with a micrometer. From their ratio the thickness was computed in cm. Because the boundaries of the segment in the bathing solution were ill-defined, this method was not considered sufficiently accurate for absolute values, but adequate for obtaining the relative thickness variations under load.

In order to arrive at a more accurate value for the thickness of the unstretched segment an additional procedure was carried out at the end of each experiment. Before drying the segment to determine its dry

weight, a few rings were cut out of the segment at various positions along its length. Each of these rings was placed under a low power microscope and its unstressed thickness measured. To do this, the ring was allowed to dry, and hence shrink, while under observation, until it appeared to be physiologically moist, somewhat glistening. At this time, several thickness readings were quickly made on each ring by means of a calibrated reticule in the objective lens. The average of the readings made on three rings gave the absolute value for the thickness of the unstretched arterial segment.

To obtain the final actual thickness values of the segment at the different loads, all of the cathetometer thickness measurements were normalized relative to the thickness at zero load, and then multiplied by the absolute value for the thickness of the unstretched segment determined with the microscope. The resultant values were used to express the passive load on the segment and its active isometric response in units of stress, i.e., grams force per square centimeter of cross-sectional area. Expressing the results in units of stress makes the data more meaningful and easier to compare with other data.

4. Force Measurements

(a) Isotonic Loading

Isotonic loading of the segment was achieved by employing the ordinary balance shown in Figure 3. One pan of the balance was modified so that the wire stretching the segment could be passed through a hole in the centre of the pan and be fastened to the hook at the end of the balance arm. The pan was suspended from this same hook. Weights were added to the other pan in the usual way. The vertical rod supporting the horizontal balance beam could be elevated and locked in any position up

to 1/2" above its lowest or resting position. The long needle pointer was used to indicate the balanced position and also any large change from this position.

For finer measurements of the degree of unbalance and, therefore, of the amount of shortening of the contracting segment, an optical lever was used. A small mirror glued underneath the bracket supporting the needle pointer reflected a beam of collimated light onto a distant blackboard. Any movement of the arterial segment was magnified about 30 times.

To set up an isotonic load on the arterial segment, one end of the segment was rigidly fastened while a wire connected to the other end was fastened to the hook on the balance arm. Weights were added to the left hand pan until an approximate balance was obtained. The entire balance was then elevated by rotating each of its three levelling screws the same amount until just a little slack remained in the arterial segment. The balance was then relevelled and rebalanced. The desired initial load was then added to the left hand pan. The centre rod on which the balance arm pivoted was then slowly raised until balance was again attained. The centre rod was locked in position. The weight just added was, therefore, equal to the load applied to the arterial segment. To avoid over-stretching of the segment, the movement of the balance arm was limited to a small angle from the horizontal until the new balance point was reached. In order to avoid hysteresis effects, the weights were added in increasing quantities only. To compensate for the change in length of the segment at constant load (creep), the centre rod was raised in order to restore balance about 10-15 minutes after the initial balancing was completed.

The condition of zero load on the arterial segment was checked at

the end of each experiment and usually the weight necessary to just balance the total weight of the wire, steel rod and segment was not significantly different from that at the beginning of the experiment. The large moment of inertia of this arrangement is of no consequence in these measurements as the rate of shortening is less than 1.0 mm per minute.

(b) Isometric Loading

To record the tension developed by the isometrically contracted arterial segment, the wire fastened to the upper end of the segment was connected to a mechano-electronic transducer (RCA No. 5734). A detailed description of the construction and characteristics of this transducer is given by Brecher (1959).

The transducer circuit was such that the recorder input voltage, with zero load on the lever, could be adjusted to equal zero and with the maximum experimental load of 100 grams on the lever, a full scale deflection of 10 millivolts could be obtained. The calibration of the transducer was checked at the end of each experiment. Further details are given in the Appendix.

In order to increase the effective load on the arterial segment to the desired value, the plastic strip supporting the transducer was elevated by slowly rotating the small brass screw shown in Figure 3. Due to stress relaxation of the arterial tissue the load usually had to be readjusted by a significant amount about 15 minutes after the initial adjustment.

5. Cuvette for Mounting the Arterial Segment

Some of the advantages of this particular set-up are the ease of maintaining a constant medium, and of stimulating the arterial segment with drugs. Continuous monitoring of the oxygen and carbon dioxide

concentrations and pH levels in the medium is also possible as well as isotonic loading of the segment since no shaking of the cuvette or of its contents is necessary with this arrangement.

(a) General Description

The equipment that met the requirements is shown schematically in Figure 3. The large transparent plastic box contains a thermoregulator, heating element, stirrer and a water bath which was maintained at 37°C ± 0.1°C. Located in the centre of this tank is the circular plastic cuvette which contains the mounted arterial segment, its bathing solution, electrodes to monitor the oxygen, carbon dioxide and pH levels, and a stirring bar which was held in place by a plastic baffle plate.

A thick plastic disc served as the cover plate for the cuvette and contained openings to accomodate each of the electrodes, two openings fitted with valves which were used for solution replacement and to eliminate gas bubbles from the solution, and a funnel-shaped central opening which contained mercury and thus formed a gas tight seal around the wire stretching the arterial segment. Two large spring clamps were used to clamp the cover plate to the broad rim of the cuvette after some Dow Corning vacuum grease was applied to the two surfaces.

(b) Stirring

Rapid stirring of the cuvette solution was essential in order to prevent oxygen gradients and, therefore, erroneous readings of the average oxygen concentration. The stirring was accomplished by means of a plastic coated stirring bar within the cuvette which was activatedby a magnetic stirrer located underneath the big plastic tank. The speed of the stirrer was gradually increased until the stirring bar rotated as fast as possible without "skipping" or changing its speed in any way.

Since the oxygen electrode was very sensitive to changes in the stirring rate, the stirrer was supplied with power from a constant power supply.

(c) Solution Change

Since the maximum change in the oxygen concentration of the cuvette solution that was allowed with this set-up was -2% (from about 99% to 97%), it was necessary to replace the cuvette solution with fresh solution quite often during any one experiment. To do this, a 100 ml syringe was fitted into the valve on the cover plate which was connected at its lower end to some polyethylene tubing. This tubing extended to the bottom of the cuvette. With the second valve also open, the 50 ml of cuvette solution was quickly withdrawn by means of the syringe. In the same way, the cuvette could be quickly filled with freshly oxygenated solution. During the filling period, the cuvette was agitated manually to release any bubbles adhering to the segment, stirring bar etc. The cuvette was also tilted in various directions for the same reason.

(d) Drug Stimulation

Norepinephrine stimulation of the arterial segment was performed by using a 5 ml syringe which was fitted into the valve connected to the polyethylene tubing. The syringe contained 0.5 ml of the heated and oxygenated norepinephrine solution. About 4 ml of the cuvette solution was drawn into the syringe before the drug together with the solution were injected into the cuvette. This withdrawal and reinjection of 4 ml of solution was quickly repeated two more times to ensure that all of the norepinephrine actually went beyond the polyethylene tubing and into the cuvette solution, as well as to speed up its dispersion throughout the solution.

6. Electrodes

Changes in the oxygen, carbon dioxide and pH levels of the cuvette

solution were determined by means of electrodes which were fitted into the cuvette cover plate by means of openings provided for this purpose. Each of these openings was threaded over most of its depth to receive a long, hollow, brass screw. The electrode was lowered through the opening in the brass screw so that when the screw was turned, its base forced a rubber washer to expand against the casing of the electrode, holding it quite rigid.

(a) Oxygen Electrode

The Clark oxygen electrode was used to monitor changes in the concentration of oxygen gas in the bathing solution. The theory underlying the operation of this electrode, together with its auxiliary circuitry and some of the common problems it presents, have been described extensively by Clark (1956), Severinghaus and Bradley (1958) and Severinghaus (1959). The electrode consisted of a silver chloride anode and a platinum cathode separated by a solution of saturated potassium chloride. A thin polyethylene membrane, permeable to gases, separated the electrode and its electrolyte from the surrounding medium. A polarizing voltage of 0.68 volts was applied across the electrodes. Any oxygen present in the surrounding medium diffuses through the membrane and is reduced at the platinum cathode causing a flow of current between the two electrodes. The current flow was recorded as a voltage across a fixed load resistor. A schematic diagram of the circuit that was used to supply the polarizing voltage to the electrodes and also to suppress a part of the output signal so that small changes in oxygen concentration could be recorded on a 10 millivolt full scale recorder is shown in the Appendix.

The value of the load resistor was chosen so that a change in the oxygen concentration of -2% (from about 99% to 97%) was equivalent to

10.0 millivolts. It is these voltage changes which were actually recorded and converted to the corresponding changes in the oxygen concentration.

The response of the electrode in this system can be seen from the records. There is a delay of about two to three minutes before a doubling of the rate of oxygen consumption was actually recorded as such (Figure 17). Since the oxygen concentration in our experiments changed rather slowly with time, the delay of the electrode itself in registering these changes may be negligible. The rate of stirring of the solution within the cuvette was adjusted at a maximum possible value so as to minimize the total delay time by minimizing the time required for equilibration of the oxygen gas throughout the entire solution. The rate of oxygen consumption by the electrode itself was too small (at most equal to 1 µl/hour or about 2% of the arterial consumption) to warrant correction.

The detailed procedures that were involved in calibrating the oxygen electrode and in translating a change in the oxygen concentration caused by the respiring segment to its equivalent Q_{O_2} value are given in the Appendix.

(b) Carbon Dioxide Electrode

The carbon dioxide electrode was supplied by the National Welding Equipment Company (Medical Equipment Division, San Francisco, California). It is a slightly improved version of the one first described by Stow, Baer and Randall (1957) and is essentially a pH glass electrode arranged so as to measure the pH of a very thin film of aqueous sodium bicarbonate solution which is separated from the sampling solution by a Teflon membrane. This membrane is permeable to carbon dioxide gas molecules but not to ions that might alter the pH. The response time of this electrode was quite long, of the order of 1/2 to 2 minutes, but it was still comparable to the

time required for uniform distribution of oxygen, and therefore, presumably of carbon dioxide, throughout the cuvette solution; thus it was not a severely limiting factor. The electrode was connected to a high input impedance pH meter (Beckman Model 76, Expanded Scale). By means of the expanded scale, voltages could be measured to ± 0.5 millivolts, which was equivalent to a change in the carbon dioxide concentration of about ± 0.015%.

Due to some difficulties encountered with this electrode and which are mentioned in the Appendix, the carbon dioxide electrode output was not recorded but read at intervals. Usually, consistent behaviour of the electrode was obtained only in the latter part of a run when the carbon dioxide concentration was equivalent to a pCO_2 of 4 mm Hg or more.

The detailed procedures that were involved in calibrating the carbon dioxide electrode and in translating a change in the carbon dioxide concentration caused by the respiring segment to the equivalent Q_{CO_2} value are given in the Appendix.

(c) pH Electrode

The pH changes in the cuvette solution were measured by means of a Beckman combination pH electrode on the Beckman Model 76 pH meter, the same meter used with the CO_2 electrode. Since the calibration for pH interfered with the carbon dioxide or voltage calibration, only one of the two parameters was measured during any one experiment.

7. Mounting Protocol

Mounting of the arterial segment to the support was performed outside of the cuvette in a bath of modified Ringer solution. The adventitia-free arterial segment, 1.8 cm in length, was held fast to the support by a steel rod which was passed through one of the annuli, on through the lumen

of the arterial segment and was eventually threaded tightly into the annulus at the other end. A second steel rod, with slightly upturned ends, was then passed through the lumen of the segment so that both ends extended beyond the ends of the segment by an equal amount. One end of the long piece of stainless steel wire (0.005" diameter) or heavy suture thread was passed through the small holes located near the upturned ends of the rod. The short end of the wire or thread was then securely tied to the longer end which was passed through the hole located in the centre of the cuvette cover plate. The mounted segment was then manoeuvered into the cuvette so that the support rested on the baffle plate to which it was rigidly fastened. The two platinum leads used for electrical stimulation of the segment were led out of the cuvette through the opening provided for the oxygen electrode in the cuvette cover. The cover plate was lowered and clamped onto the cuvette rim. The cuvette was then filled with oxygenated modified Ringer solution. When the bubbles were eliminated and with fluid seeping out of the electrode and centre openings, the brass screws were tightened to fix all of the electrodes rigidly into position. A small amount of mercury was added to the funnel-shaped centre opening and both valves closed. The wire stretching the segment was attached to either the isotonic balance or the isometric transducer and the stirring bar activated so that changes in the oxygen concentration of the cuvette solution could be recorded.

8. Measurement of the Wet and Dry Weight of the Segment

The wet weight of each segment was measured at the end of the experiment before the thickness measurements were made. The fluid was gently squeezed out of the segment and both the internal and external surfaces of the segment were allowed to dry on the balance pan for about

five minutes. At this time, when the segment glistened just noticeably and the decrease in segment weight with time was not too rapid, the weight of the segment was recorded.

After the thickness measurements were completed the dry weight of the segment was determined by heating all of the pieces making up the original segment at 50°C for 48 hours, after which time no change in the weight could be detected with still further heating of the segment.

D. Effect of Passive Stretch on the Oxygen Uptake

The aim of this series of experiments was to measure the dependence of the rate of oxygen uptake (Q_{O_2}) on the passive tension of the arterial muscle.

1. Warburg Technique

Using the Warburg technique the procedure was as follows. At zero mm Hg transmural pressure, the oxygen consumption was measured over 20 minutes. This average Q_{O_2} was termed the "basal" Q_{O_2} and it was measured again at the end of each run. At each desired passive tension, the oxygen consumption over 10 minutes was measured, and the average Q_{O_2} computed. Usually three adjustments of the applied pressure were made in order to compensate for stress relaxation of the segment and to obtain the steady transmural pressure desired.

The bathing solution was changed about every half hour to minimize possible lactic acid accumulation, pH changes, and depletion of glucose. The wet and dry weights were measured for each segment at the end of the experiment.

Three adventitia-intact and three adventitia-free segments were used in the above manner. For the latter, the rubber segment lined the

preparation. For the adventitia-intact segments the pressure was varied from 0 to 250 mm Hg in steps of 50 mm Hg. The range for the adventitia-free segments was from 0 to 200 mm Hg because the segment began to balloon at the high pressure.

2. Polarographic Technique

Because the later Warburg experiments showed that the Q_{O_2} dependence on passive stretch was not affected by a previous series of contractions, the data for this dependence in the polarographic studies was obtained from the studies on isometric contraction.

E. Dependence upon Initial Stress or Tension of Respiration and Isometric Tension in Drug-induced Contractions

The aim of this series of experiments was to determine the effect of varying the degree of initial passive stretch of the arterial segment on its mechanical and respiratory responses to a given drug stimulus.

1. Choice of Drug Level

Preliminary Warburg experiments on both adventitia-intact and adventitia-free segments showed that a strong but submaximal response to norepinephrine stimulation was produced with a concentration of 5×10^{-7} g base/ml. This concentration is also in the physiological range. The time required for complete relaxation of the segment, after washout of the drug, was also conveniently small and equal to only 10 to 20 minutes. In order to obtain the same magnitude of response and relaxation time in the polarographic set-up, it was necessary to reduce the concentration of norepinephrine to 5×10^{-8} g/ml.

2. Control Experiments - Effect of Time and Previous Stimulation

In order to test the effect of time and previous stimulation with

norepinephrine on the mechanical and respiratory responses of the arterial segment in the Warburg set-up, segments were stimulated three times in succession at each of two different initial transmural pressures. Since it was known from previous work that the responses to norepinephrine increased with increased initial stretch, the two initial transmural pressures were arbitrarily chosen at 50 and 150 mm Hg for the adventitia-intact segments and 25 and 50 mm Hg for the adventitia-free segments. In the polarographic set-up, adventitia-free segments were stimulated up to seven times in succession with 5×10^{-7} and 5×10^{-8} g/ml norepinephrine when loaded with 40 g or with a passive stress of about 600 g/cm^2. These experiments were necessary in order to show that norepinephrine, at these concentrations, could be used several times on the same segment and yield responses with little variation between them.

3. Protocol for the Main Experiment

In order to determine the effect of initial stretch on the activity respiration and isometric tension of both adventitia-free and adventitia-intact segments when they are stimulated with norepinephrine, the general procedure in both the Warburg and polarographic methods was as follows. The oxygen uptake of the relaxed segment was measured at various values of passive pressure or passive stress. At each of these progressively increasing values of passive pressure or stress, norepinephrine was added and the oxygen uptake monitored for 5-10 minutes at the new value of steady pressure or stress. The norepinephrine was washed out and the oxygen uptake and pressure measured when the relaxation of the segment was completed.

The range of passive pressure values for the adventitia-intact segments was from 0 to 250 mm Hg in steps of 50 mm Hg, and for the

adventitia-free segments from 0 to about 150 mm Hg in steps of about 25 mm Hg. In each case, the stimulating agent was 5×10^{-7} g/ml norepinephrine. The range of passive stress values in the poloragraphic experiments was from 0 to about 1500 g/cm^2 in steps of about 250 g/cm^2. At the end of each experiment the oxygen uptake of the unstretched, relaxed segment was measured over 10 minutes. The total time required to complete an experiment usually was from 5 1/2 to 7 1/2 hours.

Certain differences existed between the protocol of the Warburg and polarograph experiments. In the Warburg experiments, measurements were made of the diameter of the segment at each passive pressure so that the passive and active pressure values could be converted to their corresponding values of wall tension. Measurements of the thickness of the segment in the polarograph experiments enabled us to translate the load or force values to their equivalent values in units of stress. Due to the limited change which was allowed to take place in the oxygen concentration of the cuvette solution, the duration over which oxygen uptake measurements could be taken in the polarograph experiments was from 5 to 10 minutes, while in the Warburg experiments it was from 10 to 20 minutes. The washing out of old solution and replacement with fresh solution took about 3 to 4 minutes with both the Warburg and polarographic techniques but this operation had to be performed twice as frequently with the polarographic technique.

F. Dependence upon Initial Stress or Tension of Respiration and Isometric Tension in Electrically-induced Contractions

1. Choice of Stimulus

Various types of direct electrical stimulation were tried. It was

found with both the Warburg and polarographic techniques that stimulation with ordinary alternating current, 60 cycles per second, 25-40 volts in amplitude, applied for 30 seconds, with the bath surrounding the segment, not only gave mechanical responses of reasonable magnitude and duration, but, equally important in our experiments, electrolysis was never observed at the electrodes either. With the Warburg method, shaking of the flasks was interrupted during the 30 second stimulation period.

The strength and duration of the electrical stimuli were chosen so that the magnitude of the electrically-induced mechanical responses matched those produced by stimulation with norepinephrine. By stimulating several segments both electrically and with norepinephrine in the Warburg set-up it was found that a 40 volt stimulus applied for 30 seconds gave mechanical responses equivalent in magnitude to those produced by stimulation of the same segments with 5×10^{-7} g/ml norepinephrine. Repeating the procedure with the polarograph set-up showed that 30 volts applied for 30 seconds gave mechanical responses closely approximating in magnitude those produced by 5×10^{-8} g/ml norepinephrine on the same segments. The duration of the maximum amplitude of the electrically-induced mechanical response was sufficient to permit the measurement of the corresponding steady state oxygen consumption of the contracted segment. The time required for complete spontaneous relaxation of the segment was conveniently low and equal to about 15 minutes. Only adventitia-free segments were stimulated electrically.

2. Control Experiments - Effect of Time and Previous Stimulation

In order to test the effect of time and previous electrical stimulation on the mechanical and respiratory responses of the arterial segment in the Warburg set-up, segments were stimulated three times in

succession at each of three different initial transmural pressures. The three initial pressures employed were 10, 50 and 100 mm Hg. The oxygen uptake of the contracted segment was measured over a three to five minute interval of maximum mechanical response. With the polarographic set-up, segments were stimulated electrically up to eight times in succession when loaded optimally with about 600 g/cm^2 passive stress.

3. Protocol for the Main Experiment

The general procedure in both the Warburg and polarographic experiments for determining the effect of initial stretch on the activity respiration and electrically-induced active tension was as follows. As in the norepinephrine experiments the oxygen uptake was measured at various values of passive pressure or stress, the segment was stimulated electrically and the oxygen uptake of the contracted segment recorded over 4-5 minutes. After the spontaneous relaxation of the segment was completed, the oxygen uptake and passive pressure of the stretched segment were measured over 5-10 minutes. In the Warburg experiments the pressure was progressively increased from 0 to about 140 mm Hg in steps of about 20 mm Hg. In one experiment only, the passive pressure was also decreased in steps, from its highest to its lowest value, the segment stimulated electrically at each pressure and the responses recorded.

In the polarographic experiments the range of passive stress values extended from 0 to about 1200 g/cm^2 in steps of about 200 g/cm^2. At the end of each experiment the oxygen uptake of the unstretched, relaxed segment was measured over 10 minutes. The total time required to complete an experiment usually was from 5 to 7 hours.

In the Warburg experiments, measurements of the diameter were made at each of the different pressure values while in the polarographic ex-

periments measurements of the thickness of the segment wall were made instead. Otherwise, the differences between the two techniques were the same as those already outlined in the norepinephrine experiments.

G. Drug and Electrically-induced Isotonic Contractions

1. Choice of Stimulus

Since isotonic loading of the segment was possible only with the polarographic arrangement, throughout this series the concentration of norepinephrine used was 5×10^{-8} g/ml and the electrical stimulus was always 30 volts applied for 30 seconds.

2. Control Experiments - Effect of Time and Previous Stimulation

With norepinephrine stimulation, the segments were stimulated up to four times in succession at two different values of passive stress, 200 and 1000 g/cm^2. With electrical stimulation the segments were stimulated up to four times in succession at a passive stress of 500 g/cm^2.

3. Effect of Initial Stress on the Norepinephrine and Electrically-induced Isotonic Responses

(a) Protocol

The general procedure followed was very similar to that of the isometric experiments. The oxygen uptake of the segment was measured at various values of passive stress. At each of these progressively greater values of passive stress the segment was stimulated either with norepinephrine or electrically and the oxygen uptake monitored for as long as any shortening of the segment was detectable. Measurements of the shortening of the segment were made manually about once every 30-60 seconds. The total shortening of each contraction was always noted. With drug stimulation, when a constant shortened length was attained, the drug was washed

out allowing the segment to relax. With electrical stimulation, relaxation of the segment was spontaneous. When the relaxation was complete, the oxygen uptake was measured over 5-10 minutes.

In the norepinephrine experiments the range of passive stress values extended from about 300 to 1800 g/cm^2 in steps of about 300 g/cm^2 while in the electrical experiments the stress values ranged from about 250 to 1500 g/cm^2 in steps of about 250 g/cm^2. After each loading of the segment, from 10-15 minutes had to be allowed for the creep of the segment or its change in length at constant load to disappear. At the end of each experiment the oxygen uptake and new resting length of the unstretched, relaxed segment were measured. Measurements of the thickness of the segment were made in the usual way. The average experiment lasted about seven hours.

H. Carbon Dioxide Production and pH Changes during Contraction

In all of the isometric and isotonic experiments both when norepinephrine and direct electrical stimulation were employed, measurements were made of either the CO_2 produced or of changes occurring in the pH of the solution. Readings of the carbon dioxide concentration were taken intermittently when the initial carbon dioxide concentration exceeded 0.5% and were continued until it was almost 2%. Below the 0.5% concentration level, the electrode output was very variable. These readings were taken about once every 2 minutes for up to 10 minutes while the segment was relaxed and unloaded, when it was stretched, and also, in several cases, when it was maximally contracted. This electrode, along with the oxygen and pH electrodes, was always recalibrated at the end of each experiment as well as at the beginning.

The pH electrode output was also read intermittently to determine the upper and lower limits of the solution pH while the segment was relaxed, stretched or contracted. Readings were taken just after fresh solution was added and just before it was to be replaced, with checks in between.

VI. RESULTS

A. Basal Respiration and Stability of the Preparation

Except for instances which will be mentioned later the respiration of the resting unstretched segment remained remarkably steady for as long as eight hours. This was found to be the case with both the Warburg and polarograph experiments provided that the bathing solution was replaced with fresh solution quite frequently. This constancy of the "basal" Q_{O_2} over extended periods of time permitted several measurements of the effects of stretch, stimulation etc. to be made on any one segment over the same prolonged period of time.

By employing the procedures described in the Appendix, the absolute values of the average "basal" Q_{O_2} of all the adventitia-intact segments that were used was found to equal 0.37 µl/mg wet weight/hour ± 0.04 (SEM). Similarly, the average "basal" Q_{O_2} of the adventitia-free segments was found to equal 0.56 µl/mg wet weight/hour ± 0.02 (SEM) in the Warburg experiments and 0.53 µl/mg wet weight/hour ± 0.02 (SEM) in the polarograph experiments. The individual values of "basal" Q_{O_2} in the various experiments are given in Tables 3, 5, 7, 10, 11, 14 and 15.

B. Effect of Stretch on the Oxygen Uptake of Arterial Segments

1. Results and Definitions

With increased stretch, the total oxygen uptake of the segments increased steadily at first with transmural pressure, after which it

FIGURE 4

The effect of stretch alone on the respiration of arterial segments.

(a) Top Left

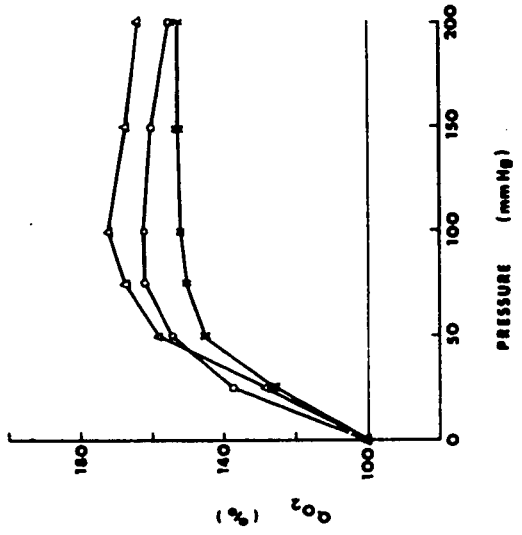
The effect of different transmural pressures on the ("basal" + "tension") Q_{O_2} values of three adventitia-intact arterial segments (Warburg method).

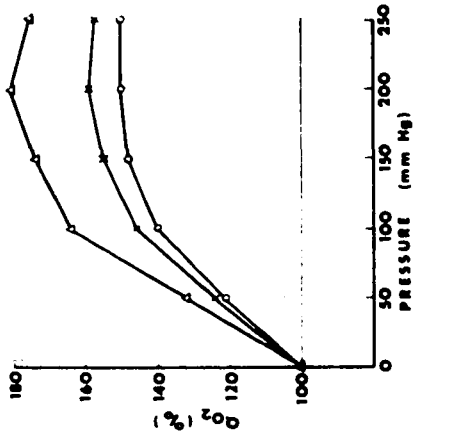
(b) Top Right

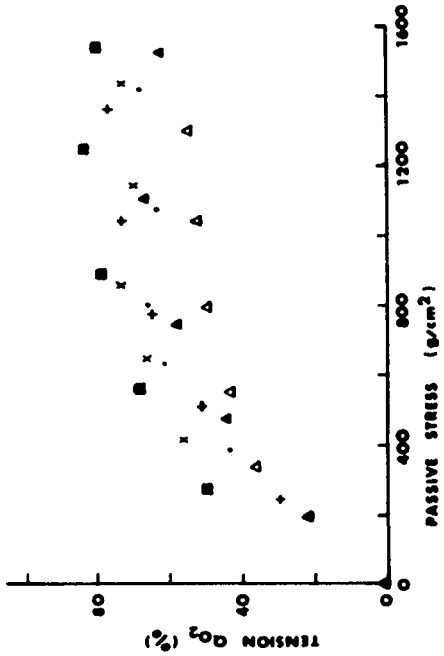
The effect of different transmural pressures on the ("basal" + "tension") Q_{O_2} values of three adventitia-free arterial segments (Warburg method).

(c) Bottom

The effect of passive stress on the "tension" Q_{O_2} values of six adventitia-free arterial segments. In each case, the "tension" Q_{O_2} values of each segment are expressed as a percentage of the initial "basal" Q_{O_2} value of that segment (polarographic method).


Q_{O_2} (%)
180
140
100
0
50
100
150
200
PRESSURE (mmHg)


Q_{O_2} (%)
180
160
140
120
100
0
50
100
150
200
250
PRESSURE (mm Hg)


TENSION Q_{O_2} (%)
80
40
0
0
400
800
1200
1600
PASSIVE STRESS (g/cm²)

levelled off or sometimes decreased. This increment in the Q_{O_2} of arterial muscle, due to passive stretch or tension, henceforth to be called the "tension Q_{O_2}", was obtained at each value of passive transmural pressure by substracting the Q_{O_2} at zero mm Hg pressure from the total Q_{O_2} obtained at each of the higher passive pressures. The Q_{O_2} of the muscle at zero mm Hg pressure will be referred to, from now on, as the "basal Q_{O_2}". Hence, the total Q_{O_2} of stretched muscles equals the sum of two components, the "basal" Q_{O_2} plus the "tension" Q_{O_2} at that particular value of passive stretch. Figure 4 illustrates for adventitia-intact segments the change in Q_{O_2} with passive pressure. The Q_{O_2} is expressed as a percentage of the "basal" Q_{O_2} for that particular arterial segment. Expressed in this way, the maximum value of the "tension" Q_{O_2} for the three segments investigated, ranged from 48% to 81%. It was assumed that the "basal" Q_{O_2} determined at the beginning of the experiment remained constant at that value throughout the experiment. Actually, however, the "basal" Q_{O_2} determined at the completion of each experiment was found to be from 6% to 13% greater than the initial "basal" Q_{O_2} value. This was probably due to the increase in the final diameter of the arterial segment at zero mm Hg transmural pressure which was, in turn, a result of the high degree of stretching to which the segment was exposed. Thus the segment usually suffered some irreversible stretch and a corresponding increase in its "basal" Q_{O_2}. The initial "basal" Q_{O_2} was always considered as the reference value throughout the experiment.

All the results of this series of experiments are illustrated in Figure 4. The pressure at which the maximum Q_{O_2} was first attained was greater for the adventitia-intact than for the adventitia-free segments because of differences in their wall structure and extensibility. For

FIGURE 5

The effect of time and previous stimulation on the isometric responses of arterial segments to stimulation by norepinephrine.

(a) Top Left

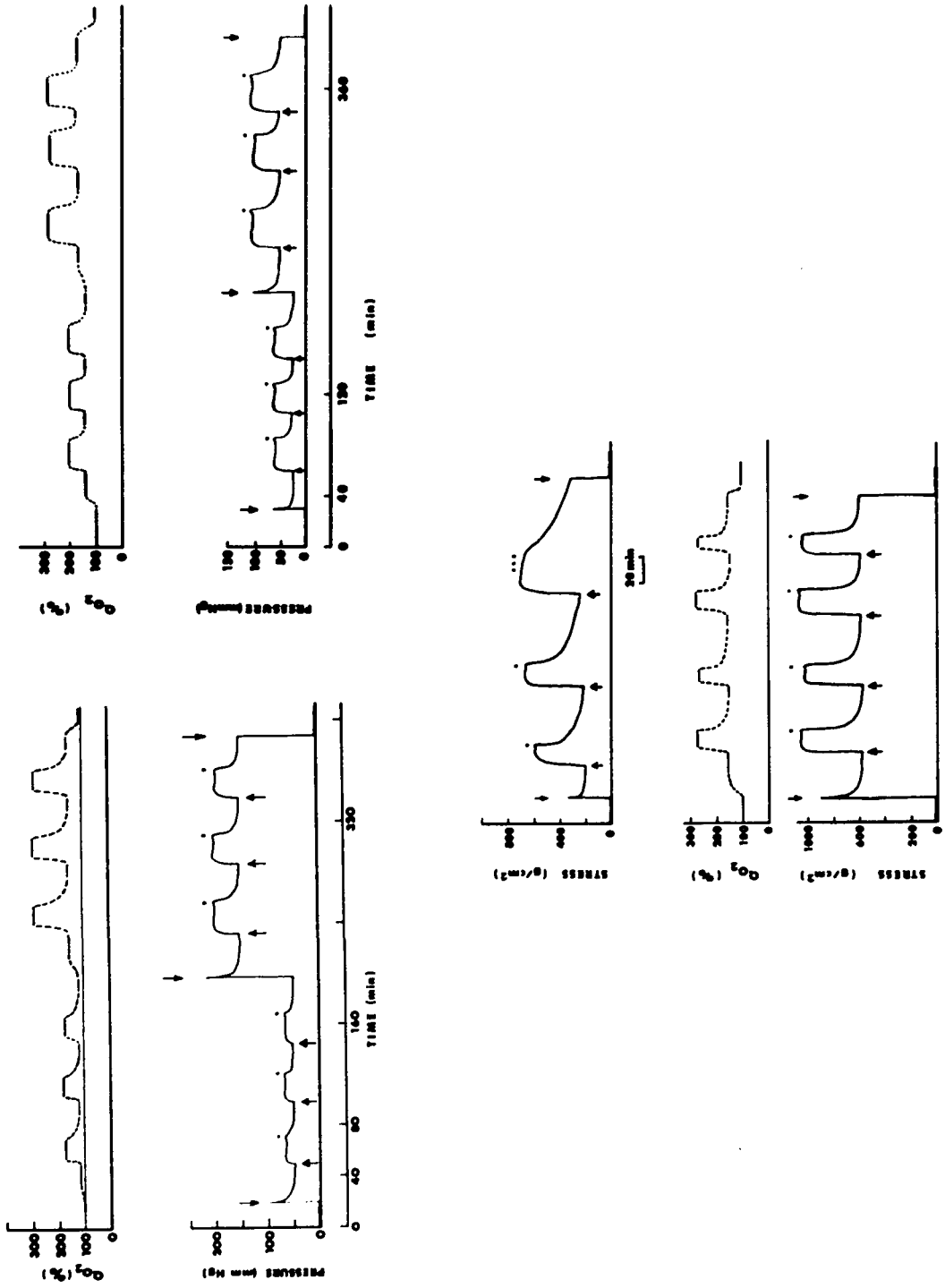
The response of an adventitia-intact segment stimulated with 5×10^{-7} g/ml norepinephrine at two different values of passive pressure (Warburg method). The passive pressure was adjusted manually at each of the inverted arrows, while norepinephrine was added and washed out at each of the upright arrows and solid circles, respectively. Note the stress relaxation of the segment after each increase in its transmural pressure. All of the Q_{O_2} measurements have been expressed as a percentage of the initial "basal" Q_{O_2} of the segment, and are shown plotted as solid horizontal lines. The dashed lines have been interpolated solely for the purpose of continuity. The initial "basal" Q_{O_2} is represented by the thin horizontal line at the 100% level.

(b) Top Right

The response of an adventitia-free arterial segment stimulated with 5×10^{-7} g/ml norepinephrine at two different values of passive pressure (Warburg method).

(c) Bottom

The response of an adventitia-free arterial segment. In the polarographic method the intimal surface of these adventitia-free arterial segments was exposed to the drug. The uppermost curve shows the effect of adding 5×10^{-7} g/ml and the two lower curves the effect of adding 5×10^{-8} g/ml norepinephrine to two separate arterial segments. An approximately optimal initial stress was used with the lower drug concentration.


Q_{O_2} (%)
PRESSURE (mm Hg)
TIME (min)
STRESS (g/cm²)
30 min

the adventitia-free segments the maximum "tension" Q_{O_2} ranged from 53%-73% with the Warburg method and from 55%-84% of the "basal" Q_{O_2} with the polarographic method. With the polarographic data the passive load on the segment has been expressed in units of stress.

C. Effect of Initial Stretch on the Isometric Responses of Arterial Segments with Norepinephrine Stimulation

1. Control Experiments - Effect of Time and Previous Stimulation

As shown in Figures 5a and 5b, the use of 5×10^{-7} g/ml norepinephrine in the Warburg experiments gave mechanical and oxidative responses which were constant up to at least 6 hours. Therefore, this concentration of drug and time between contractions are suitable for use in the main experiment.

As shown in Figure 5c, when 5×10^{-7} g/ml norepinephrine was used to stimulate the stretched arterial segment in the polarographic set-up, a considerable amount of time had to be allowed for the segment to relax completely. After the second stimulus, this time was increased further. When stimulated a third time, this effect became very marked with the segment being far from fully relaxed one hour after the drug had been completely washed out. In addition, the magnitude of the response was lower than the previous two responses. Similar results were obtained with a second segment stimulated with 5×10^{-7} g/ml norepinephrine. When the concentration of the norepinephrine was reduced by a factor of 10, from 5×10^{-7} to 5×10^{-8} g/ml, both the mechanical and respiratory responses were not significantly different from one another when stimulation was repeated 4 or 7 times. Only 15 to 20 minutes were required for the segment to relax completely and this time did not appear to get greater with

FIGURE 6

Typical isometric responses of arterial segments to stimulation by norepinephrine at different values of passive stretch.

The passive pressure or stress was adjusted manually at each of the inverted arrows and norepinephrine was added and washed out at each of the upright arrows and solid circles, respectively. All of the Q_{O_2} values are expressed as a percentage of the initial "basal" Q_{O_2} of the segment which is represented by the thin solid horizontal line at the 100% level. All of the average Q_{O_2} values of the relaxed, stretched and contracted segment are represented by the solid horizontal lines with the dashed lines joining them being only rough interpolations drawn in for the sake of continuity. Note the slight increase in the "basal" Q_{O_2} at the end of the experiment.

(a) Top Left

The responses of an adventitia-intact segment stimulated with 5×10^{-7} g/ml norepinephrine. (Warburg method).

(b) Bottom Left

Responses of an adventitia-free segment stimulated with 5×10^{-7} g/ml norepinephrine. (Warburg method).

(c) Right

Responses of an adventitia-free segment stimulated with 5×10^{-8} g/ml norepinephrine. (Polarographic method). All of the passive and active load values have been converted to units of stress.

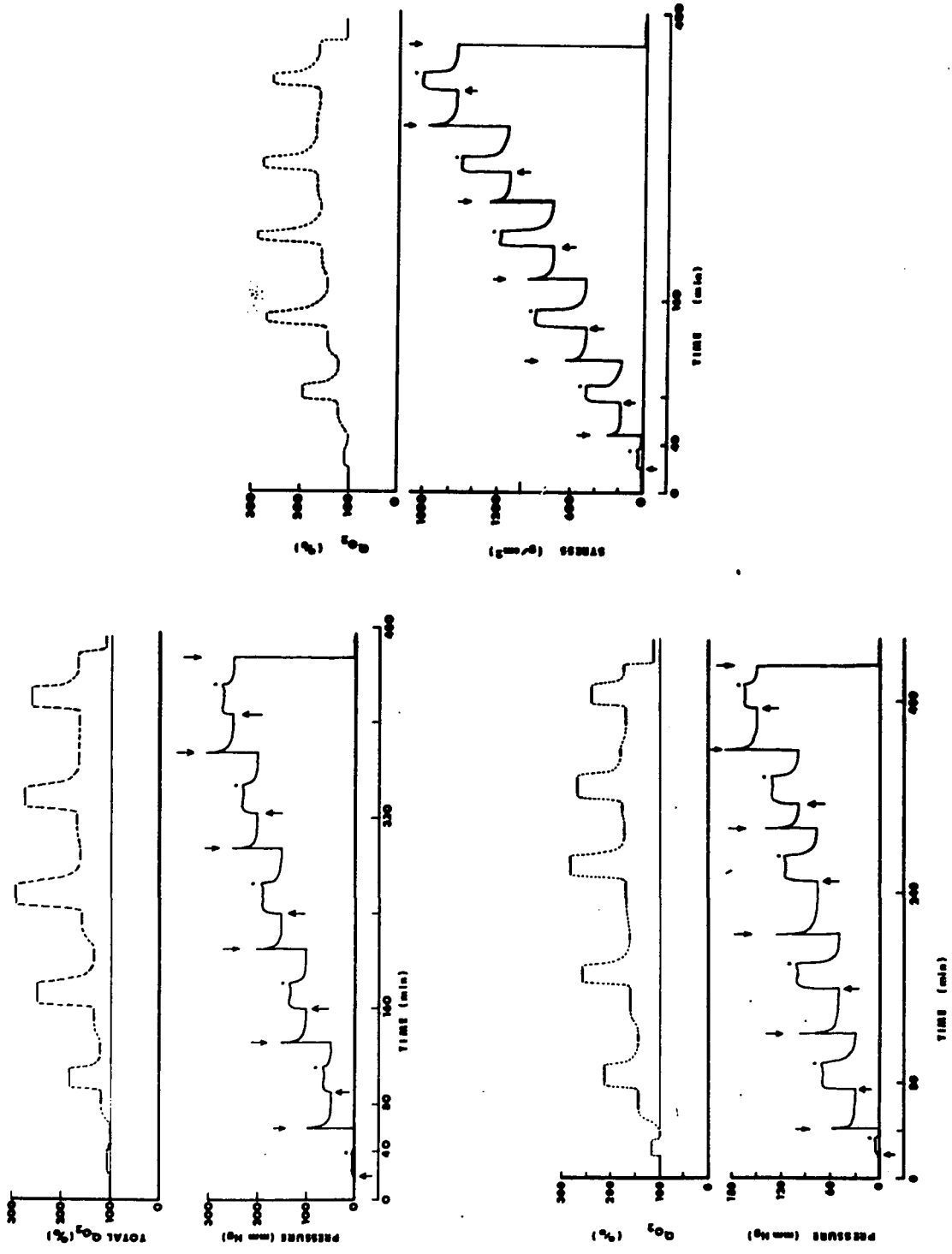


TABLE 2

Effect of Initial Pressure on the Active Responses of an Adventitia-intact Arterial Segment with Norepinephrine Stimulation

Passive Pressure	Basal Q_{O_2}		(Basal+Tension) Q_{O_2}	Tension Q_{O_2}	(Basal+Tension +Activity) Q_{O_2}	Activity Q_{O_2}	Total Pressure	Active Pressure
mm Hg	µl/mg /hour	%	%	%	%	%	mm Hg	mm Hg
0	0.32	100	100	0	107	7	3	3
50			118	18	183	65	64	14
100			133	33	247	114	132	32
150			158	58	290	132	189	39
200			167	67	273	106	231	31
250			163	63	260	97	271	21
0	0.35	111						

TABLE 3

Norepinephrine-induced Isometric Responses of Adventitia-intact Arterial Segments

Artery No.	Passive Pressure	Basal Q_{O_2}		Activ. Q_{O_2}	Normalized Activ. Q_{O_2}	Active Press.	Normalized Active Pressure
	mm Hg	µl/mg /hour	%	%	%	mm Hg	mm Hg
1	0	0.32	100	7	6	3	3
	50			65	59	14	13
	100			114	103	32	30
	150			132	119	39	36
	200			106	96	31	29
	250			97	88	21	19
2	0	0.43	100	10	11	1	1.5
	50			61	68	15.5	21.5
	100			104	115	26	36
	150			107	119	25	34
	200			91	101	17	24
	250			72	80	10	14
3	0	0.29	100	14	14	5	4.5
	50			66.5	67	22	18.5
	100			97	98	38	31.5
	150			111	112	43	36
	200			118	119	39	32
	250			108	109	24.5	20.5

Average Maximum Activity Q_{O_2} = 119 %±5.9 % (SEM)

Average Maximum Active Pressure = 36 mm Hg ± 4.2 (SEM)

Average Basal Q_{O_2} = 0.35 µl/mg/hour ± 0.03 (SEM)

TABLE 4

Effect of Initial Tension on the Active Responses of an Adventitia-free Arterial Segment with Norepinephrine Stimulation — Method A

Passive Pressure	Radius of Segment	Passive Tension	Basal Q_{O_2}		(Bas.+Ten.) Q_{O_2}	Tension Q_{O_2}	(Bas.+ Ten. +Act.) Q_{O_2}	Activity Q_{O_2}	Total Press.	Active Press.	Active Tension
mm Hg	cm	g/cm	µl/mg /hour	%	%	%	%	%	mm Hg	mm Hg	g/cm
0	0.16	0	0.44	100	100	0	116.8	16.8	5.1	5.1	1.1
30	0.21	8.6			144	44	215.0	71.0	70.4	40.4	11.5
50	0.25	17.2			163	63	261.5	98.5	101.5	51.5	17.5
75	0.27	27.6			172	72	284.0	112.0	114.5	39.5	14.5
100	0.30	40.8			178	78	268.7	90.7	131.6	31.6	12.9
150	0.33	67.4			173	73	241.3	68.3	165.4	15.4	6.9

TABLE 5

Norepinephrine-induced Isometric Responses of Adventitia-free Arterial Segments — Method A

Artery No.	Passive Tension	Basal Q_{O_2}		Activity Q_{O_2}	Normalized Activity Q_{O_2}	Active Tension	Normalized Active Tension
	g/cm	µl/mg /hour	%	%	%	g/cm	g/cm
1	0.0	0.55	100	14.3	13	0.76	0.6
	6.4			91.5	83	9.2	7.3
	13.9			115.0	104	14.8	11.7
	26.2			139.0	126	20.6	16.3
	41.1			123.0	112	16.7	13.2
	57.6			92.6	84	11.1	8.8
2	0.0	0.44	100	16.8	19	1.07	1.0
	8.6			71.0	80	11.5	10.7
	17.2			98.5	111	17.5	16.3
	27.6			112.0	126	14.5	13.5
	40.8			90.7	102	12.9	12.0
	67.4			68.3	77	6.87	6.4
3	0.0	0.68	100	6.7	7	1.5	2.1
	11.2			55.1	57.4	7.9	11.4
	23.5			92.0	96	11.3	16.3
	40.7			121.0	126	9.65	13.9
	55.0			105.7	110	8.2	11.8
	74.3			70.0	73	4.3	6.2

4	0.0	0.48	100	10.2	10	1.15	1.2
	12.5			82.2	81	9.0	9.4
	30.0			122.0	120	15.6	16.3
	44.5			128.0	126	12.0	12.5
	62.3			109.0	107.4	9.86	10.3
	77.7			95.8	94.2	6.8	7.1
5	0.0	0.63	100	11.4	15	0.7	0.8
	4.9			32.8	43	5.0	5.9
	17.0			71.6	94	11.3	13.3
	38.3			96.0	126	13.9	16.3
	54.0			78.5	103	11.2	13.1
	70.6			52.0	68	7.5	8.8
6	0.0	0.69	100	6.3	5	0.46	0.4
	7.5			77.7	62	6.3	5.5
	27.7			137.0	109	18.7	16.3
	35.5			158.0	126	17.7	15.4
	49.3			143.0	114	14.6	12.7
	65.2			96.5	77	10.1	8.8

Average Maximum Activity Q_{O_2} = 126 % ± 8.0 % (SEM)

Average Maximum Active Tension = 16.3 g/cm ± 1.3 (SEM)

Average Basal Q_{O_2} = 0.58 µl/mg/hour ± 0.04 (SEM)

FIGURE 7

The effect of different values of passive stretch on the isometric responses of arterial segments stimulated to contract with norepinephrine.

All of the Q_{O_2} values have been expressed as a percentage of the initial "basal" Q_{O_2} value of the segment. The ("basal" + "tension") Q_{O_2} and the ("basal" + "tension" + "activity") Q_{O_2} values are represented by the triangles and the X'es, respectively. The active pressure or stress is represented by the dashed lines.

(a) Top Left

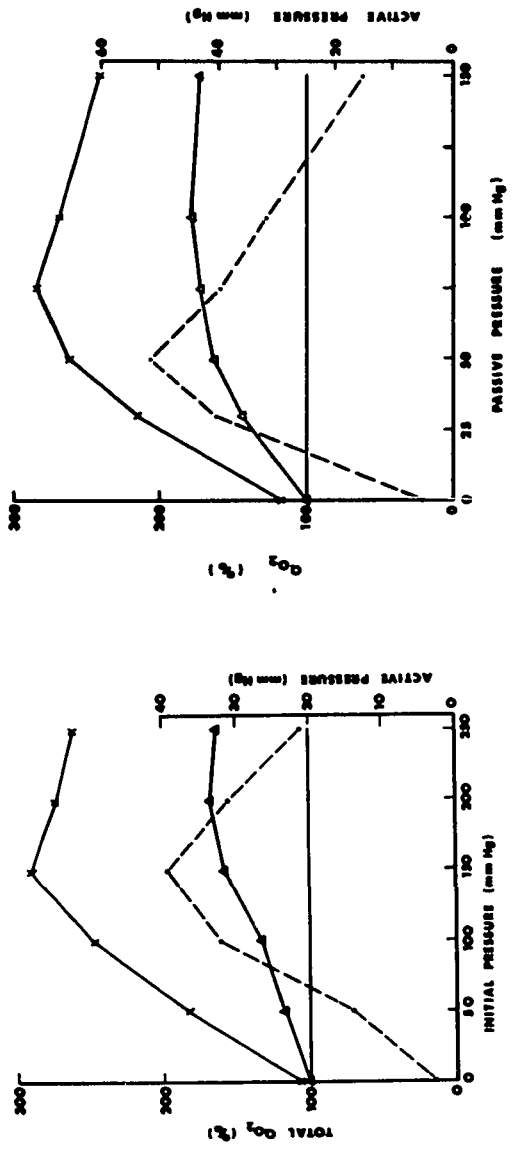
Adventitia-intact segment; 5×10^{-7} g/ml norepinephrine; Warburg method.

(b) Top Right

Adventitia-free segment; 5×10^{-7} g/ml norepinephrine; Warburg method.

(c) Bottom

Adventitia-free segment; 5×10^{-8} g/ml norepinephrine; polarographic method.


ACTIVE PRESSURE (mm Hg)
PASSIVE PRESSURE (mm Hg)
Q_{O_2} (%)
TOTAL O_2 (%)
INITIAL PRESSURE (mm Hg)

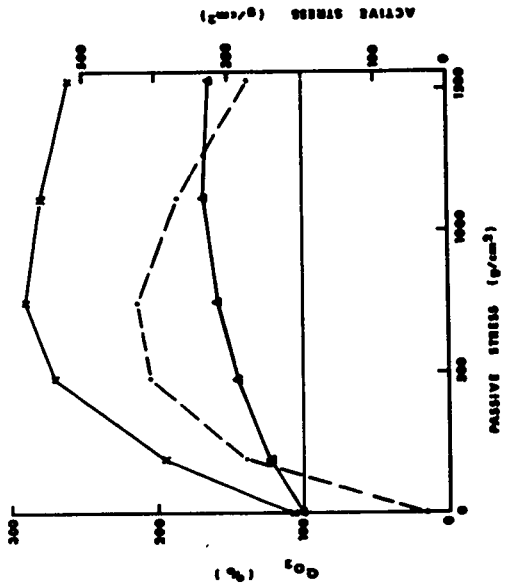
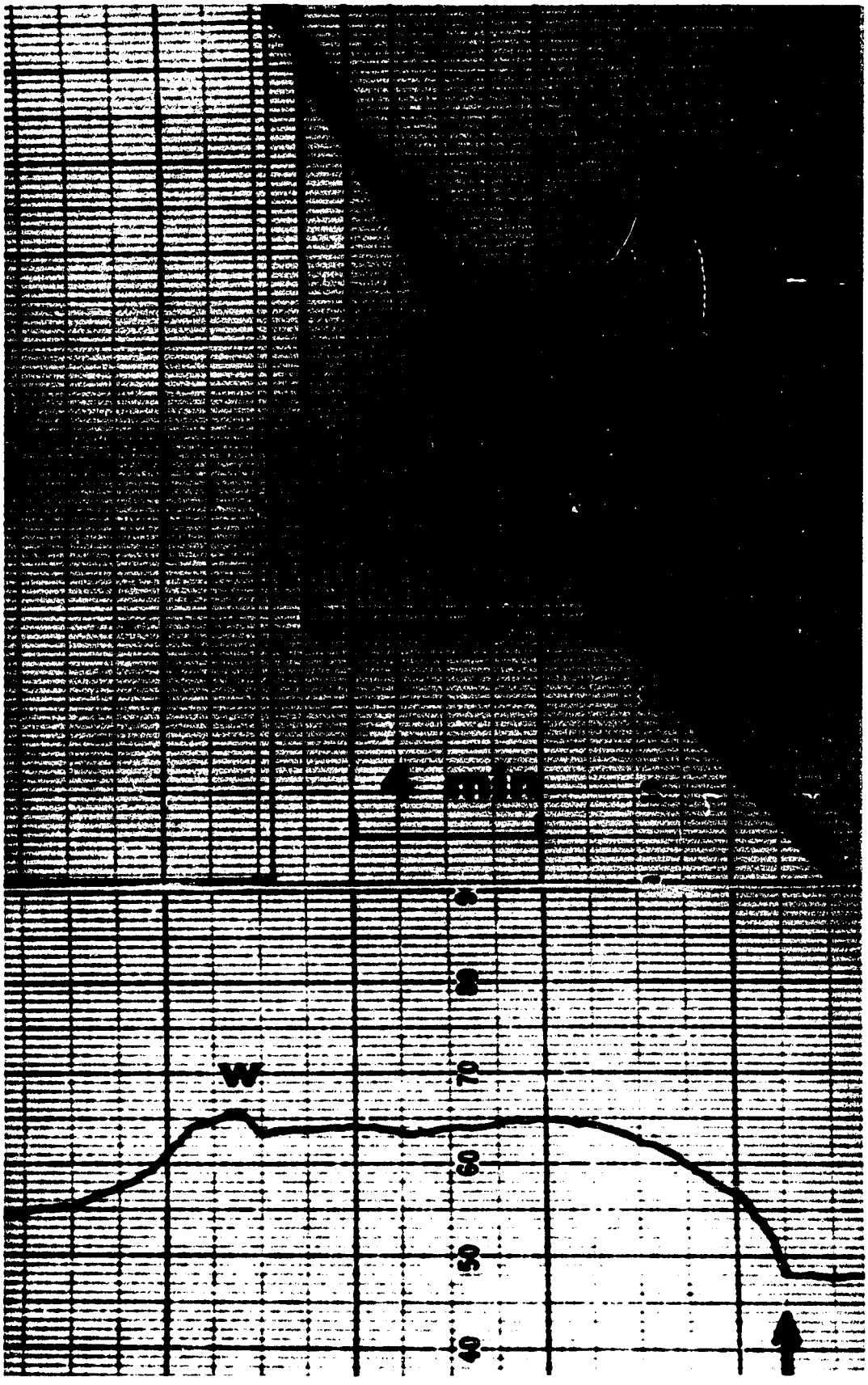
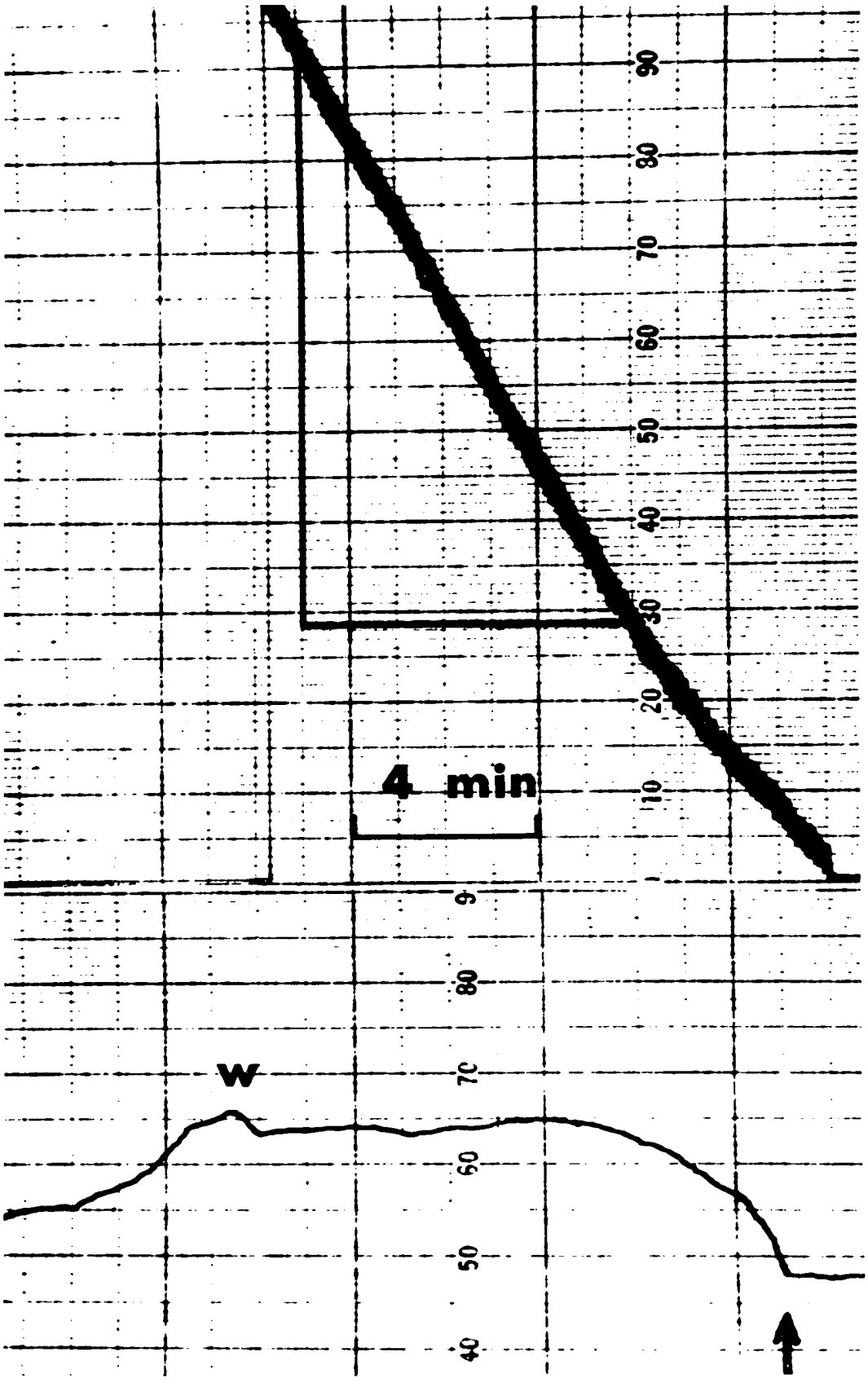

ACTIVE STRESS (g/cm²)
PASSIVE STRESS (g/cm²)
Q_{O_2} (%)

FIGURE 8

Experimental records of the typical isometric responses of an arterial segment to stimulation by norepinephrine (5×10^{-8} g/ml).

Both records have to be read from right to left. The lower record shows the mechanical response of the segment when norepinephrine was added at the arrow and washed out at the "W". The ordinate scale is 1 gram/division and is linear up to full scale of 100 grams. The upper record represents the decrease in the concentration of oxygen with time in the cuvette solution or the amount of oxygen taken up by the segment. The slope of the tracing gives the rate of oxygen uptake. The full scale of 100 divisions represents a change in the oxygen concentration of -2%, i.e., from 100% at zero to 98% oxygen at full scale. Both the active force and "activity" Q_{O_2} fluctuated relatively little during the period of measurement which was equal to seven minutes in this example.


W
70
60
50
40


4 min
W

subsequent stimulation. Both segments were stimulated at the rate of once every hour, so that the duration of the experiments was in one case 4 hours, and in the other, about 7 hours.

2. Results from Warburg Method

Some typical results that were obtained using adventitia-intact and adventitia-free segments are shown in Figures 6a and 6b. Table 2 shows in detail for an adventitia-intact segment how the results are analyzed into "basal" Q_{O_2}, "tension" Q_{O_2} (see page 72 for definition) and "activity" Q_{O_2}. The "activity" Q_{O_2} is the increment in the Q_{O_2} occurring during contraction. Similarly the active pressure is the increment in pressure produced by the isometric contraction. Table 3 summarizes the results on three adventitia-intact segments.

Table 4 shows the detailed results for an adventitia-free segment in the Warburg method, while Table 5 shows a summary of all such results. Active tension is the increment in tension produced during isometric contraction. The passive and active tensions were calculated from their corresponding pressures by using Laplace's Law which relates the tangential force per centimeter of length T in a cylinder of radius r to the hydrostatic pressure P by the equation $T = P \cdot r$. The behaviour with increasing passive stretch of the active pressure or tension and of the three components making up the total Q_{O_2} of the contracted segments is shown in Figures 7a and 7b.

3. Results from Polarographic Method

Some typical results obtained using adventitia-free segments with the polarographic method are shown in Figure 6c. The sample trace shown in Figure 8 shows the steady rate at which oxygen was consumed during contraction and how the Q_{O_2} was measured in relation to the time course of

TABLE 6

Effect of Initial Stress on the Isometric Responses of an Adventitia-free Arterial Segment with Norepinephrine Stimulation — Method B

Load	Wall Thick.	Initial Stress	Basal $\dot{Q}_{O_2}$		(Bas.+Ten.) $\dot{Q}_{O_2}$	Tension $\dot{Q}_{O_2}$	(Bas.+Ten.+Act.) $\dot{Q}_{O_2}$	Activity $\dot{Q}_{O_2}$	Total Force	Active Force	Active Stress
g	cm	g/cm^2	µl/mg /hour	%	%	%	%	%	g	g	g/cm^2
0	0.018	0	0.44	100	100	0	107	7	2.0	2.0	31
11	0.016	191			122	22	194	72	26.9	15.9	276
24	0.014	477			144	44	270	126	44.6	20.6	408
35	0.013	748			158	58	289	131	55.0	20.0	427
48	0.012	1110			167	67	279	112	64.0	16.0	371
66	0.012	1530			163	63	259	96	77.9	11.9	275

TABLE 7

Norepinephrine-induced Isometric Responses of Adventitia-free Arterial Segments — Method B

Artery No.	Passive Stress	Basal Q_{O_2}		Tension Q_{O_2}	Activity Q_{O_2}	Normalized Activity Q_{O_2}	Active Stress	Normalized Active Stress
	g/cm^2	µl/mg /hour	%	%	%	%	g/cm^2	g/cm^2
1	0	0.44	100	0	7	6	31	28.5
	191			22	72	63	276	252
	477			44	126	110.5	408	373
	748			58	131	115	427	391
	1110			67	112	98	371	340
	1530			63	96	84	275	252
2	0	0.39	100	0	5	4	59	52
	274			49	75	59	389	344
	568			68	134	105	442	391
	895			79	147	115	396	350
	1250			84	129	101	289	255
	1545			80	86	67	173	153
3	0	0.58	100	0	7	7.5	42	43
	340			36.5	82	83	314	325
	555			43	97	99	378	391
	798			49.5	113	115	344	356
	1042			52.5	92	94	294	304
	1304			55	75	76	226	233

4	0	0.65	100	0	9	11	29	33
	384			43	77	90	229	262
	632			61	98	115	308	352
	800			66	93	109	342	391
	1079			64	73	86	294	337
	1420			68	61	71	206	236
5	0	0.49	100	0	14	13	33	32
	245			29	79	76	297	288
	512			51	107	103	403	391
	780			65	119	115	360	349
	1047			73	102	99	305	296
	1365			77	82	79	265	257
6	0	0.34	100	0	18	23	76	84
	412			56	69	90	275	304
	650			67	82	106	354	391
	861			74	89	115	331	366
	1149			71	69	89	266	294
	1440			74	50	64	188	208

Average Maximum Activity Q_{O_2} = 115 % ± 7.9 % (SEM)

Average Maximum Active Stress = 391 g/cm^2 ± 14.9 (SEM)

Average Basal Q_{O_2} = 0.48 µl/mg/hour ± 0.05 (SEM)

the norepinephrine-induced contraction. Table 6 shows the detailed results for an adventitia-free segment, while Table 7 shows a summary of all such results. Active stress equals the force, in grams, developed by the segment during contraction divided by the effective cross-sectional area of the segment in cm^2. The passive stress was also calculated from the passive load on the segment. A clearer presentation of the behaviour of the active stress and of the three components of the total Q_{O_2} with passive stretch is shown in Figure 7c. The active stress and total Q_{O_2} both increase to a maximum and then decrease with increasing passive stretch. The maximum value of the total Q_{O_2} of the contracted segment is about three times the basal value.

4. Treatment of the Data

In order to get some idea of the degree of scatter of all the active responses at any one value of passive stress, the responses were normalized as follows. Each active mechanical or respiratory response was first normalized relative to the maximum value obtained for that particular segment and then all such values were multiplied by the average maximum response of all the segments in that series. The maximum response was chosen as the reference point since it represents the best criterion that we have of the amount and responsiveness of the active tissue present in each segment. The "activity" Q_{O_2} values of each segment were expressed as a percentage of its initial "basal" Q_{O_2} in order to minimize scatter due to intersegmental differences in the basal respiration. The resultant "activity" Q_{O_2} values were normalized. The values of passive pressure, tension or stress were not treated in any way.

This normalized data is shown plotted against the passive stretch for the adventitia-intact and adventitia-free segments and for both the

FIGURE 9

The normalized isometric responses of arterial segments stimulated with norepinephrine as a function of the passive stretch.

The "activity" Q_{O_2} values of each segment are expressed as a percentage of the initial "basal" Q_{O_2} of that segment. All "activity" Q_{O_2} values were normalized relative to the maximum value for that segment and then multiplied by the average maximum "activity" Q_{O_2} of all the segments. The resultant values were plotted. The same procedure was followed with the absolute values of the active pressure, tension or stress of each segment. The solid horizontal line at the top of each set of curves represents the corresponding average maximum response of all the segments. The same symbol is used to represent the responses of a given segment in both sets of curves.

(a) Top Left

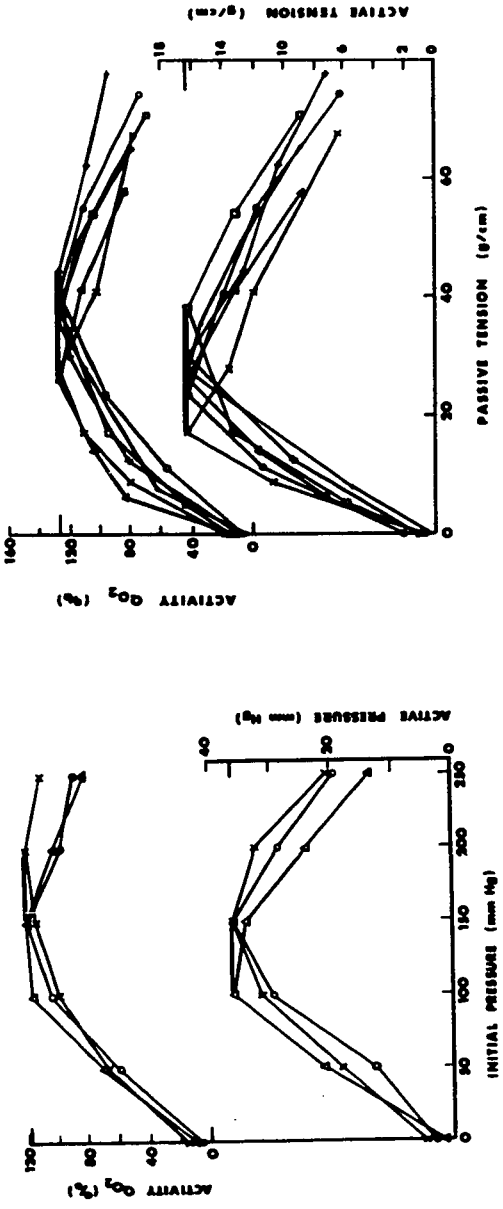
Adventitia-intact segments; 5×10^{-7} g/ml norepinephrine; Warburg method.

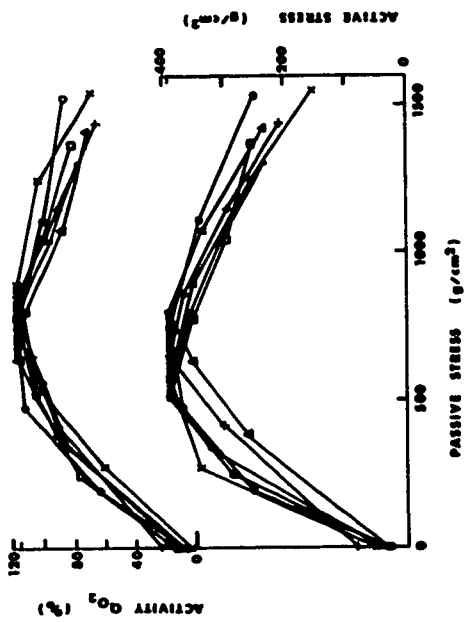
(b) Top Right

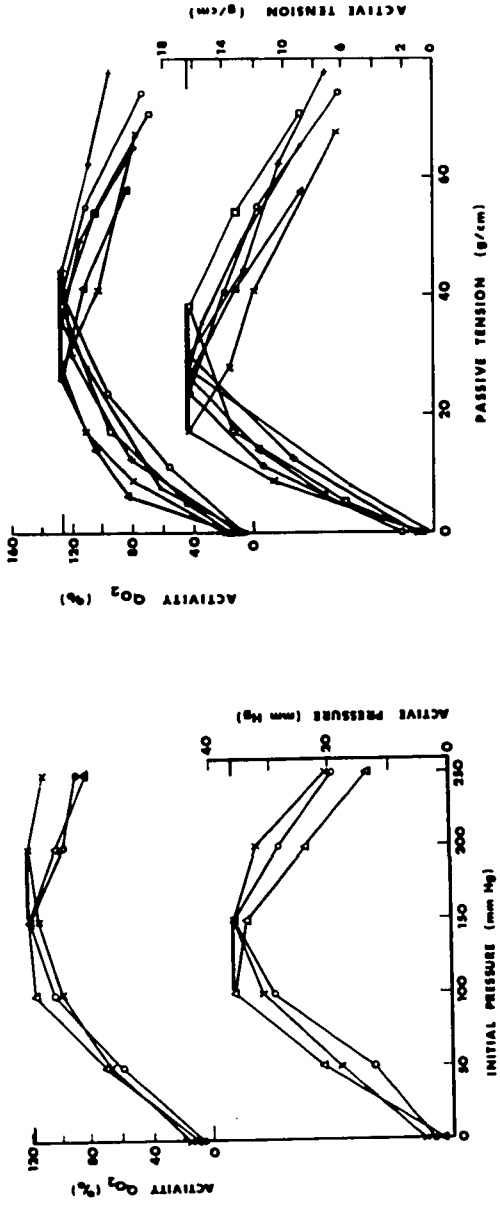
Adventitia-free segments; 5×10^{-7} g/ml norepinephrine; Warburg method.

(c) Bottom

Adventitia-free segments; 5×10^{-8} g/ml norepinephrine; polarographic method.


ACTIVE TENSION (g/cm)
PASSIVE TENSION (g/cm)
ACTIVITY Q_{O_2} (%)
ACTIVE PRESSURE (mm Hg)
INITIAL PRESSURE (mm Hg)
ACTIVITY Q_{O_2} (%)


ACTIVE STRESS (g/cm²)
PASSIVE STRESS (g/cm²)
ACTIVITY Q_{O_2} (%)


ACTIVITY Q_{O_2} (%)
ACTIVE TENSION (g/cm)
PASSIVE TENSION (g/cm)
ACTIVE PRESSURE (mm Hg)
INITIAL PRESSURE (mm Hg)

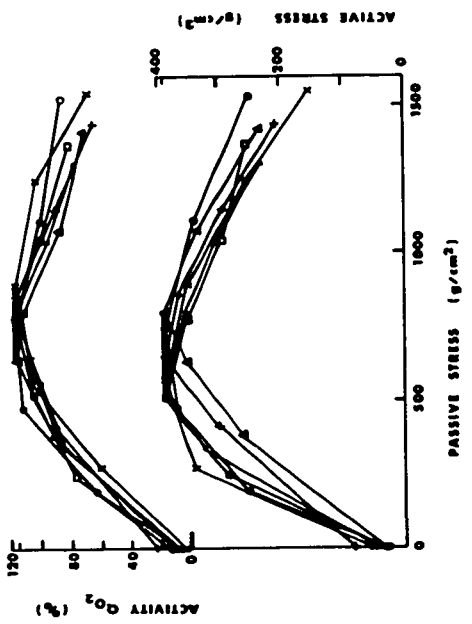

ACTIVITY Q_{O_2} (%)
ACTIVE STRESS (g/cm²)
PASSIVE STRESS (g/cm²)

FIGURE 10

The variation of the "activity" Q_{O_2} of adventitia-free arterial segments with their active tension when stimulated with norepinephrine (5×10^{-7} g/ml). Results obtained with Warburg technique (Method A).

The "activity" Q_{O_2} values of each segment are expressed as a percentage of the initial "basal" Q_{O_2} value of that segment. The solid line represents the regression line of the norepinephrine data with the dashed lines drawn parallel to it representing plus and minus one standard error of the estimate. The coefficient of correlation between the two parameters equals 0.89 ± 0.08 (SE) while the regression line has the equation $y = 6.8x + 16.9$.

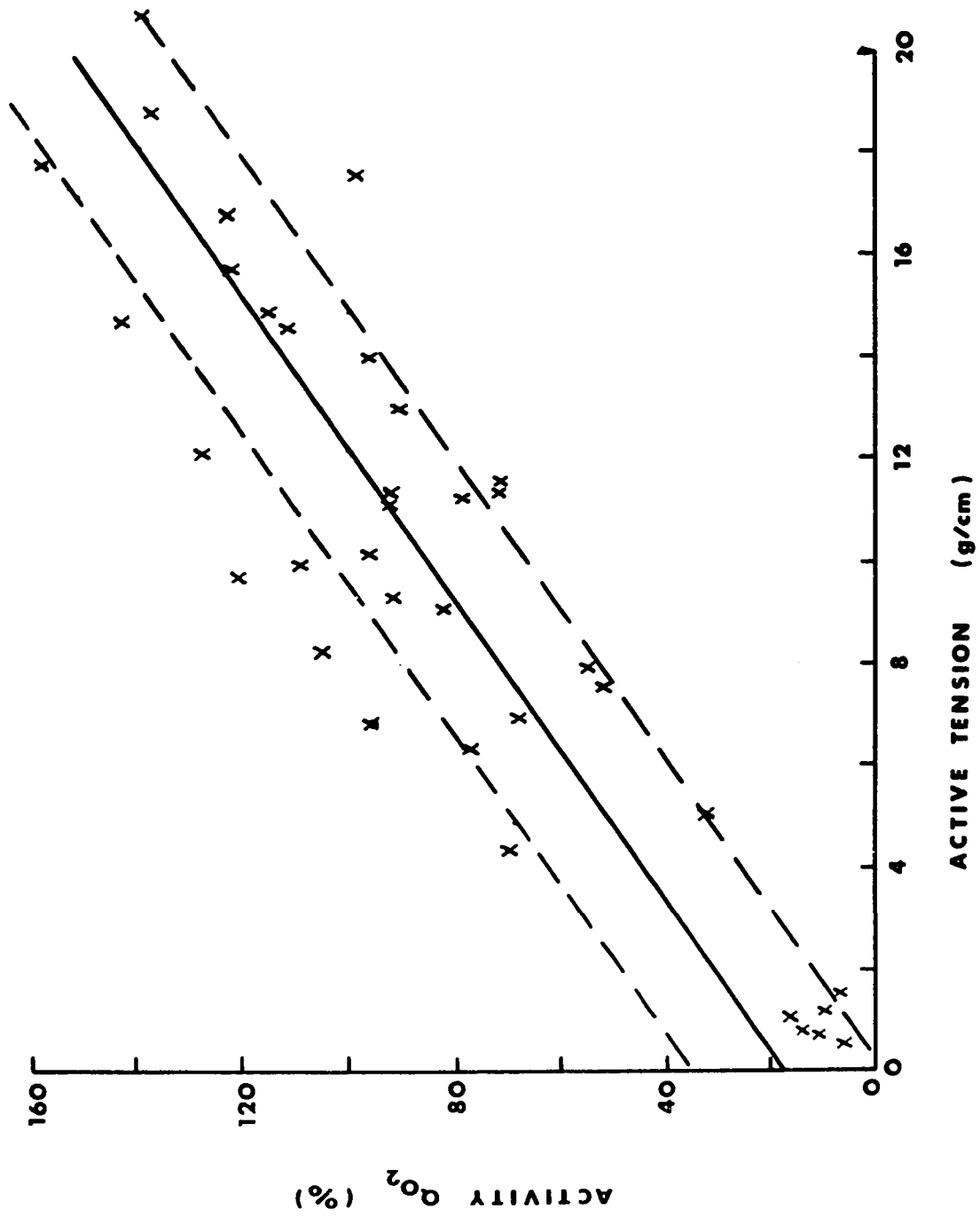
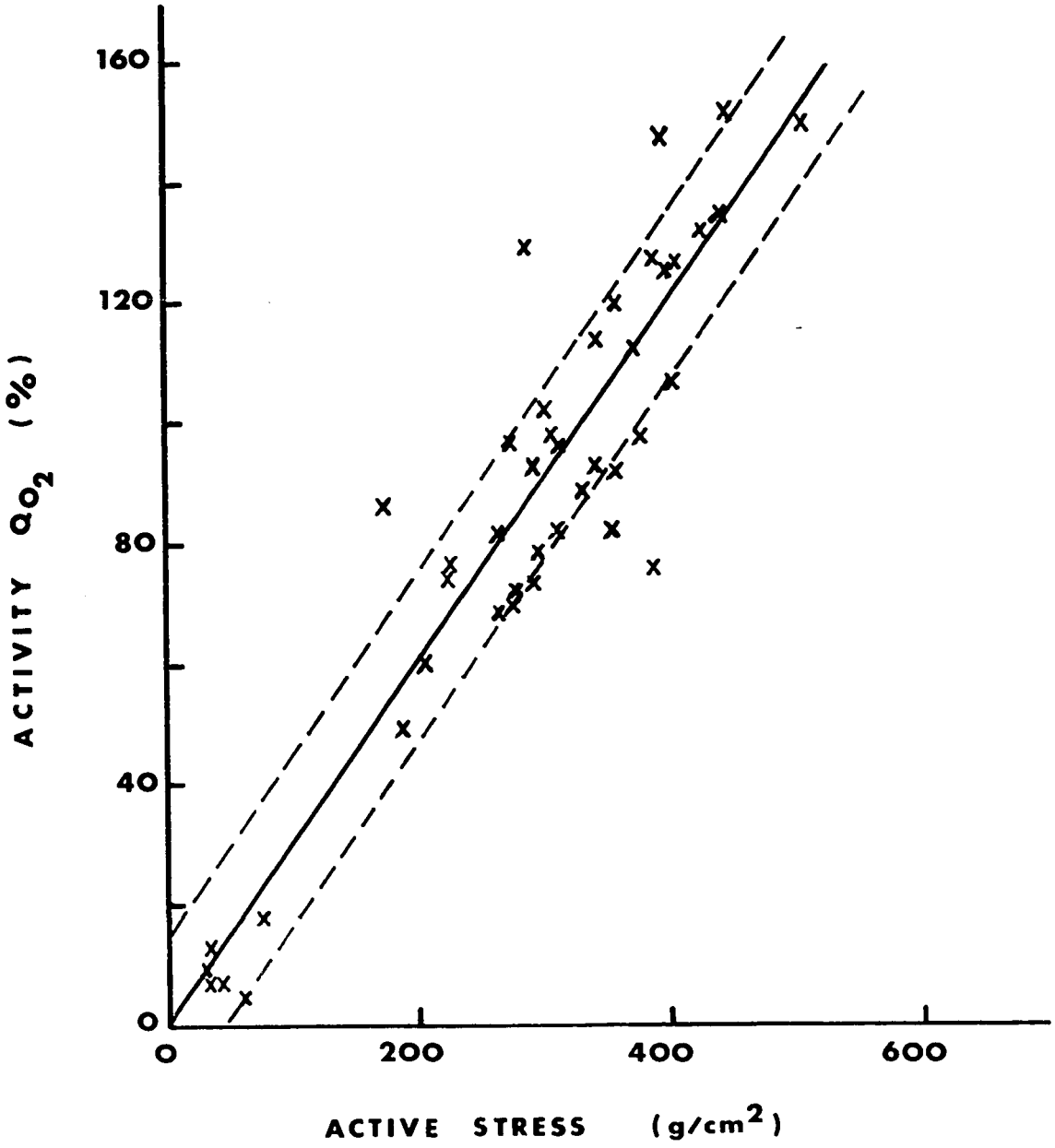

ACTIVITY Q_{O_2} (%)
160
120
80
40
0
0
4
8
12
16
20
ACTIVE TENSION (g/cm)

FIGURE 11

The variation of the "activity" Q_{O_2} of adventitia-free arterial segments with their active stress when stimulated with norepinephrine (5×10^{-8} g/ml). Results obtained with polarographic technique (Method B).

The solid line represents the regression line of all the data with the dashed lines drawn on either side of it and parallel to it representing plus and minus one standard error of the estimate. The coefficient of correlation between the two parameters equals 0.93 ± 0.06 (SE) while the regression line has the equation $y = 0.30x + 0.21$.


160
120
80
40
0
0
200
400
600
ACTIVITY Q_{O_2} (%)
ACTIVE STRESS (g/cm^2)

Warburg and polarographic experiments in Figure 9. The actual data is listed in Tables 3, 5 and 7. The average maximum values are indicated by the solid horizontal bars at the top of each set of curves. The actual average maximum values and their standard error of the mean (SEM) are also given in Tables 3, 5, and 7. The three average maximum mechanical and respiratory values obtained with the two different preparations and techniques are not significantly different from one another within one SEM.

Comparison of curve shapes shows no distinct differences between adventitia-intact and adventitia-free segments or with the different methods that were employed. Despite the scatter still present in the data both active responses behave in a parallel manner - increasing to a maximum value with increasing passive stretch and then decreasing with still further stretch. The active mechanical response generally increases more steeply up to its maximum than does the activity respiration and also decreases equally steeply beyond this maximum so that the mechanical but not the respiration curve is relatively symmetrical. A smaller degree of passive stretch is necessary for the attainment of the maximum mechanical than for the maximum respiratory response. The optimal mechanical and respiratory responses occurred at values of passive stretch which were in the physiological range and extended from 100-200 mm Hg for the adventitia-intact segments, from 20-40 g/cm for the adventitia-free segments in the Warburg method and from about 500-900 g/cm^2 for the segments in the polarographic experiments.

The line of least squares or the regression line of the "activity" Q_{O_2} on the active tension and active stress is shown in Figures 10 and 11, respectively. All of the data is in its original unnormalized form. The

FIGURE 12

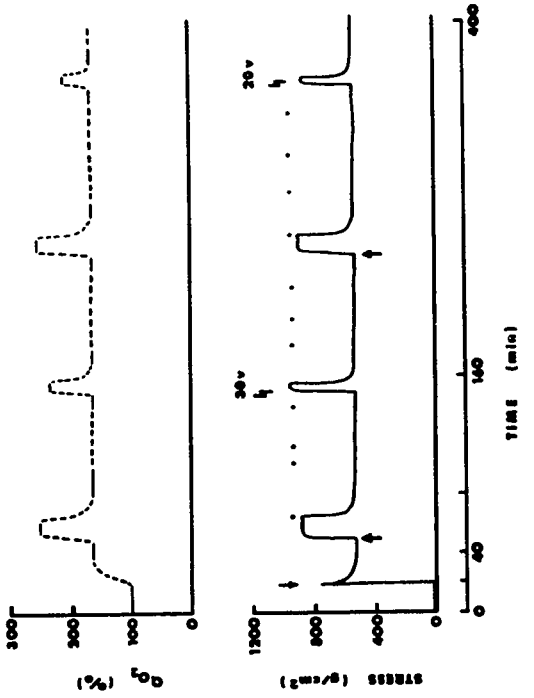
Norepinephrine and direct electrical stimulation of isometrically-loaded arterial segments.

(a) Left

Method A - Norepinephrine (5×10^{-7} g/ml) was added at the upright arrows and an electrical stimulus applied at each of the thunderbolts. The bathing solution was changed at the solid black circles.

(b) Right

Method B - The passive stress was kept constant at an approximate optimal value. Norepinephrine (5×10^{-8} g/ml) was added at each of the upright arrows while an electrical stimulus was applied for 30 seconds with the voltage as indicated at each of the thunderbolts. The steady state Q_{O_2} values measured were all expressed as a percentage of the initial "basal" Q_{O_2} value and are shown plotted as solid horizontal lines with the dashed lines joining them being drawn in just to preserve continuity. Note the difference in the rate of oxygen uptake between the two types of contraction.


O_{O_2} (%)
300
200
100
0
STRESS (g/cm²)
1200
800
400
0
20v
30v
40v
0
40
140
400
TIME (min)

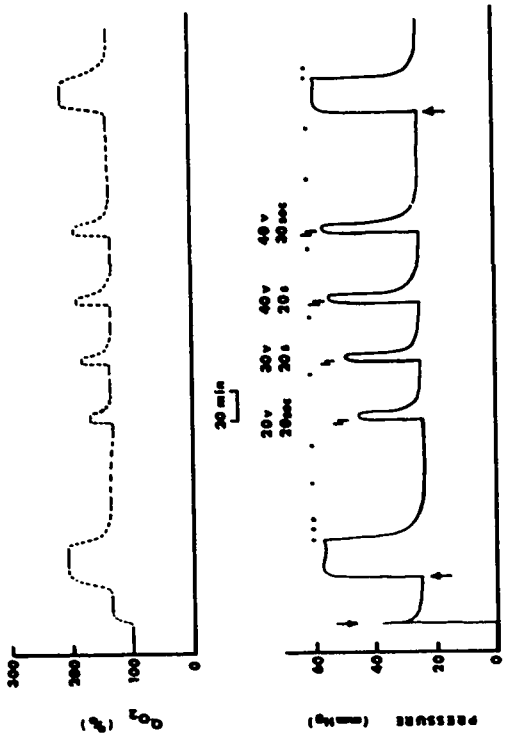

O_{O_2} (%)
300
200
100
0
20 min
20v 30sec
30v 20s
40v 20s
40v 30sec
PRESSURE (mmHg)
60
40
20
0

FIGURE 13

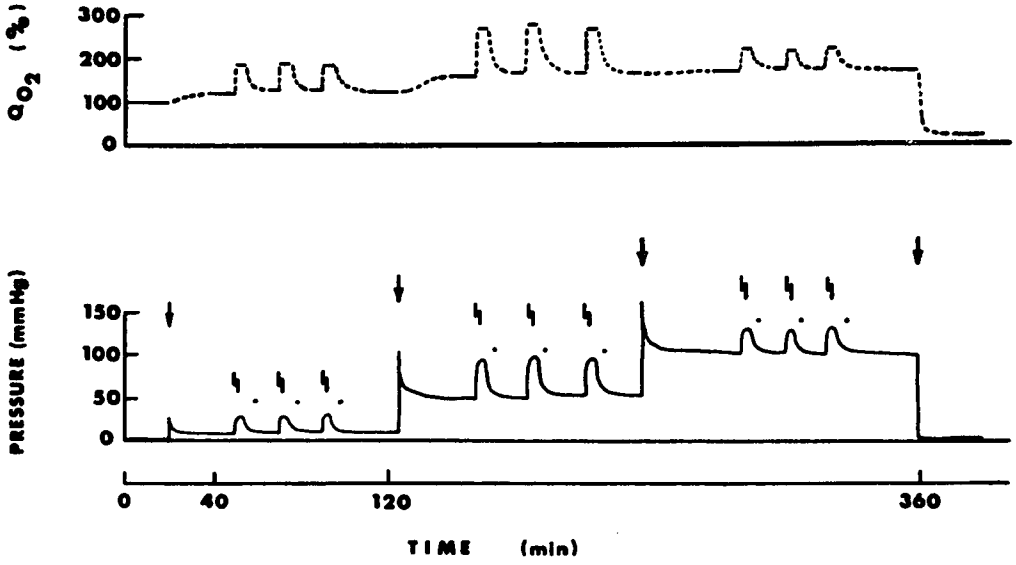
The effect of time and previous stimulation on the isometric responses of adventitia-free arterial segments stimulated electrically.

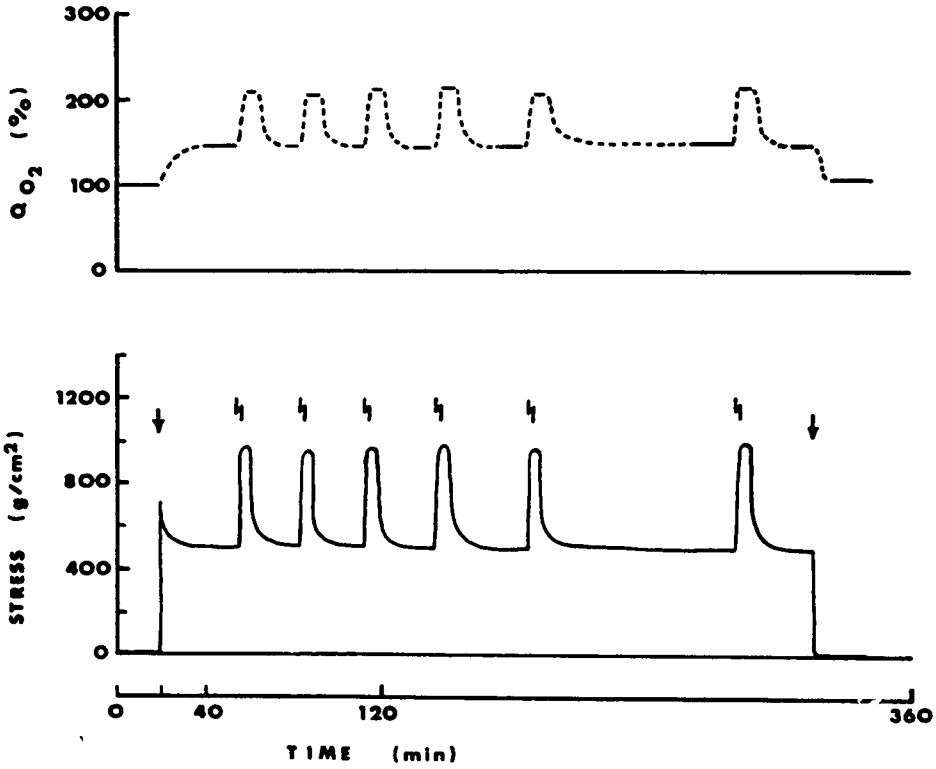
(a) Top

Method A - Three different values of initial passive pressure were employed in the example shown. The segment was stimulated electrically (40 volts for 30 seconds) at each of the thunderbolts. The bathing solution was replaced with fresh solution at each of the solid circles. The maximum value of the mechanical response was maintained for only about three to four minutes and it was during this interval that measurements of the rate of activity oxygen uptake were made.

(b) Bottom

Method B - The passive stress was kept constant at an approximate optimal value. The electrical stimulus (30 volts for 30 seconds) was applied at each of the thunderbolts. The steady state Q_{O_2} values expressed as a percentage of the initial "basal" Q_{O_2} value, are represented by the solid horizontal lines and the roughly interpolated Q_{O_2} values by the dashed lines.


Q_{O_2} (%)
300
200
100
0
PRESSURE (mmHg)
150
100
50
0
0
40
120
360
TIME (min)


Q_{O_2} (%)
300
200
100
0
STRESS (g/cm²)
1200
800
400
0
0
40
120
360
TIME (min)

high coefficient of correlation between the two parameters indicates that they are linearly related. Most of the scatter in the data is due to the difference in asymmetry between the "activity" Q_{O_2} and active tension or stress curves when they are plotted against the passive stretch, the "activity" Q_{O_2} curves being more asymmetrical. The fact that for a given segment the two maxima often did not occur at the same passive stress also introduced some scatter into the data.

D. Dependence upon Initial Stress or Tension of Respiration and Isometric Tension in Electrically-induced Contractions

1. Choice of Electrical Stimulus

Stimulation of the arterial segment with 40 volts AC for 30 seconds in the Warburg arrangement and 30 volts for 30 seconds in the polarographic set-up yielded mechanical responses which were comparable in magnitude to those obtained by stimulation with 5×10^{-7} and 5×10^{-8} g/ml norepinephrine in the two cases, respectively. As shown in Figure 12 the shape of the response curves with the two types of stimulation were quite different. The short duration of the maximum electrically-induced response was sufficient, however, to permit measurements to be made of the increase in the average or steady state rate of oxygen uptake occurring during maximal contraction. The "activity" Q_{O_2} during this interval was significantly smaller for the electrical contraction than for the mechanically-equivalent norepinephrine contraction.

2. Control Experiments - Effect of Time and Previous Electrical Stimulation

As shown in a sample Warburg experiment illustrated in Figure 13a, the three mechanical and respiratory responses obtained at any one of the

FIGURE 14

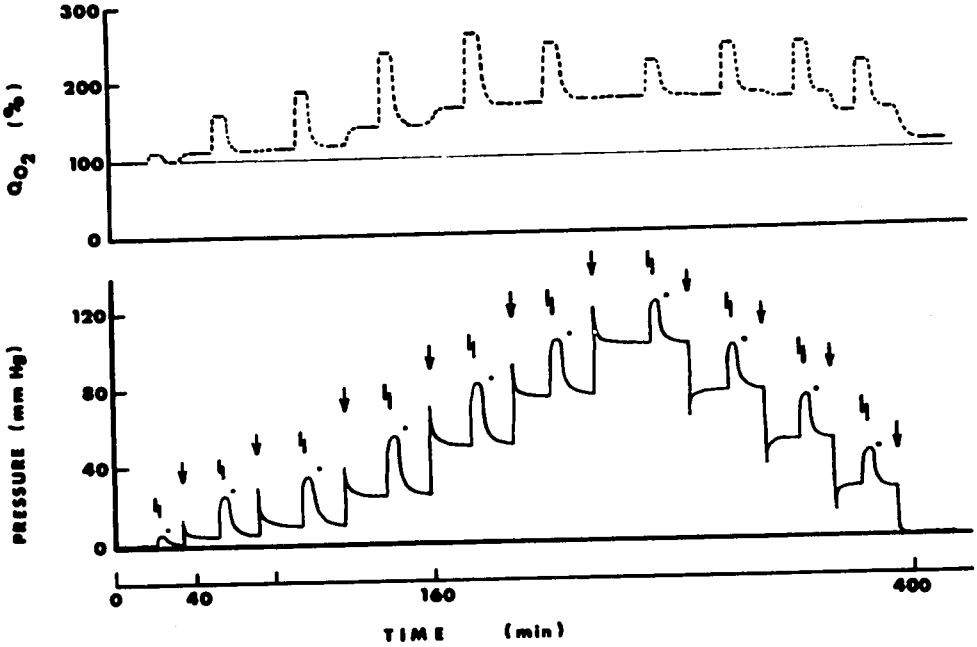
The effect of different values of initial passive stretch on the isometric responses of adventitia-free arterial segments to direct electrical stimulation.

(a) Top

Method A - The passive pressure was increased step by step up to a maximum value and then decreased to zero again in fewer steps. The inverted arrows, thunderbolts and solid circles represent manual adjustments of the transmural pressure, application of the electrical stimulus, and replacement of the bathing solution, respectively. The "basal" Q_{O_2} at the end of the long experiment was slightly elevated above its initial value.

(b) Bottom

Method B - The passive stress was adjusted manually at each of the inverted arrows and the arterial segment was stimulated electrically (30 volts for 30 seconds) at each of the thunderbolts. All of the steady state Q_{O_2} values measured were expressed as a percentage of the initial "basal" Q_{O_2} value of the segment and are shown plotted as solid horizontal lines. The dashed lines joining them have been only roughly interpolated for the sake of continuity.


300
200
100
0
Q_{O_2} (%)
120
80
40
0
PRESSURE (mm Hg)
0
40
160
400
TIME (min)

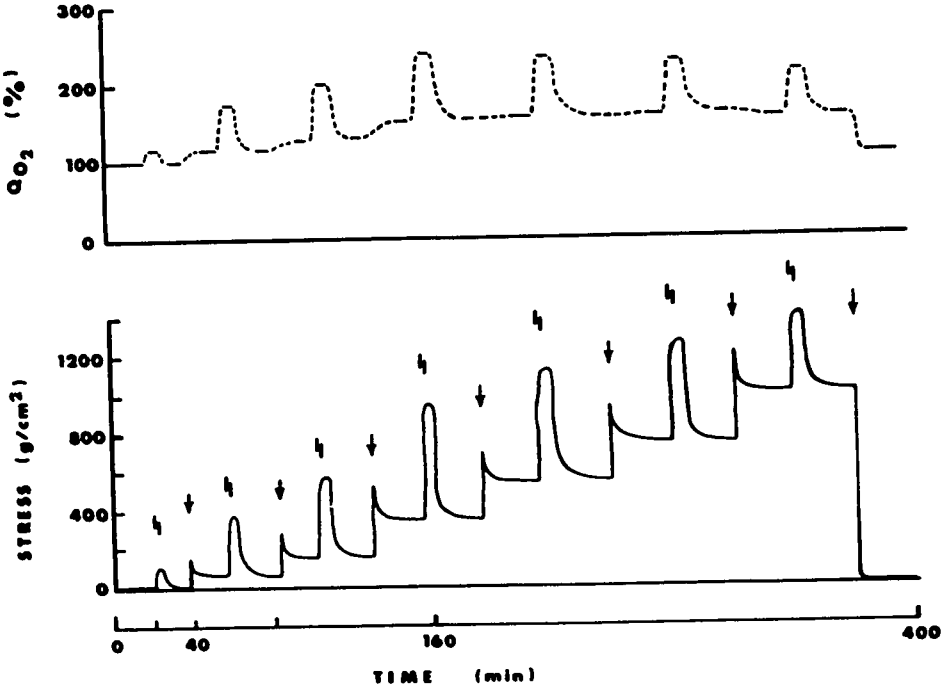

300
200
100
0
Q_{O_2} (%)
1200
800
400
0
STRESS (g/cm²)
0
40
160
400
TIME (min)

TABLE 8

Effect of Initial Tension on the Active Responses of an Adventitia-free Arterial Segment with Direct Electrical Stimulation — Method A

Passive Pressure	Radius of Segment	Passive Tension	Basal Q_{O_2}		(Bas.+ Ten.) Q_{O_2}	Tension Q_{O_2}	(Bas.+ Ten. + Act.) Q_{O_2}	Activity Q_{O_2}	Total Press.	Active Press.	Active Tension
mm Hg	cm	g/cm	µl/mg /hour	%	%	%	%	%	mm Hg	mm Hg	g/cm
0	0.16	0	0.56	100	100	0	111	11	6	6	1.3
5	0.18	1.2			112	12	160	48	26	21	5.1
10	0.20	2.7			120	20	188	68	35	25	6.8
25	0.22	7.6			140	40	236	96	55	30	9.1
50	0.27	18.5			165	65	258	93	82	32	11.8
75	0.32	32.6			169	69	245	76	104	29	12.6
100	0.34	45.7			173	73	220	47	123	23	10.5
75	0.33	33.5			172	72	238	66	100	25	11.1
50	0.30	20.1			170	70	239	69	73	23	9.3
25	0.26	8.7			148	48	214	66	44	19	6.6

TABLE 9

Effect of Initial Stress on the Isometric Responses of an Adventitia-free Arterial Segment with Direct Electrical Stimulation — Method B

Load g	Wall Thick. cm	Initial Stress g/cm^2	Basal $\mu l/mg/hour$	Q_{O_2} %	(Bas.+Ten.) Q_{O_2} %	Tension Q_{O_2} %	(Bas.+Ten.+Act.) Q_{O_2} %	Activity Q_{O_2} %	Total Force g	Active Force g	Active Stress g/cm^2
0	0.020	0	0.62	100	100	0	116	16	8	8	111
5	0.018	77			116	16	174	58	25	20	308
10	0.017	164			130	30	200	70	35	25	410
20	0.016	350			151	51	237	86	54	34	590
30	0.0155	540			155	55	231	76	62	32	575
40	0.015	740			158	58	226	68	68	28	520
50	0.014	995			156	56	212.5	56.5	70	20	400

TABLE 10

Electrically-induced Isometric Responses of Adventitia-free Arterial Segments — Method A

Artery No.	Passive Tension	Basal Q_{O_2}		Activity Q_{O_2}	Normalized Activity Q_{O_2}	Active Tension	Normal. Active Tension
	g/cm	µl/mg /hour	%	%	%	g/cm	g/cm
1	0	0.56	100	11.0	11.9	1.3	1.6
	1.2			48.0	52.0	5.1	6.1
	2.7			68.0	73.8	6.8	8.2
	7.6			96.0	104.0	9.1	10.9
	18.5			93.0	101.0	11.8	14.1
	32.6			76.0	82.5	12.6	15.1
	45.7			47.0	51.0	10.5	12.6
2	0	0.47	100	15.5	14.1	1.6	1.5
	3.5			35.0	31.9	7.8	7.3
	10.5			69.0	63.0	13.6	12.7
	19.2			95.5	87.2	16.2	15.1
	25.6			114.0	104.0	15.0	14.0
	35.0			77.0	70.3	13.3	12.4
	45.2			50.0	45.6	11.6	10.8
3	0	0.62	100	7.5	10.1	1.1	1.4
	9.3			48.0	64.8	8.6	10.9
	19.5			77.0	104.0	10.4	13.1
	28.2			70.5	95.2	11.9	15.1
	40.0			49.0	66.1	9.7	12.3
	54.0			27.5	37.1	6.6	8.3
4	0	0.59	100	12.0	9.7	2.0	1.5
	1.9			39.5	31.9	8.8	6.8
	8.9			87.5	70.5	15.3	11.7
	23.5			129.0	104.0	18.0	13.8
	36.3			111.0	89.5	19.7	15.1
	50.4			77.0	62.1	16.5	12.6
	63.0			43.0	34.7	12.9	9.9

Average Maximum Activity Q_{O_2} = 104 % ± 9.8 % (SEM)

Average Maximum Active Tension = 15.1 g/cm ± 1.6 (SEM)

Average Basal Q_{O_2} = 0.56 µl/mg/hour ± 0.03 (SEM)

TABLE 11

Electrically-induced Isometric Responses of Adventitia-free Arterial Segments — Method B

Artery No.	Passive Stress	Basal Q_{O_2}		Activity Q_{O_2}	Normalized Activity Q_{O_2}	Active Stress	Normalized Active Stress
	g/cm^2	µl/mg /hour	%	%	%	g/cm^2	g/cm^2
1	0	0.62	100	16	13.8	111	99.5
	77			58	50	308	276
	164			70	60	410	368
	350			86	74	590	530
	540			76	65.5	575	516
	740			68	58.5	520	467
	995			56.5	49	400	359
2	0	0.37	100	5	4	52	49
	121			39	35	322	301
	280			64	56	475	445
	478			79	70	567	530
	655			84	74	495	462
	980			63	56	409	382
3	0	0.60	100	21	23	92	95
	81			36	40	225	232
	155			54	60	364	375
	386			62	68.5	515	530
	577			67	74	432	445
	812			55	61	327	336

4	0	0.43	100	9.5	14	72	84
	95			24	36	228	267
	188			42	63.5	362	424
	406			49	74	453	530
	637			37	56	395	462
	870			25	38	315	369
5	0	0.56	100	10	11	35	44
	148			40	47	236	294
	294			61	70.5	425	530
	522			64	74	365	455
	805			48	55.5	284	354
	1090			32	37	190	237
6	0	0.58	100	18	14	64	54
	96			37.5	30	288	243
	222			71	56.5	486	410
	389			93	74	557	470
	565			83.5	66.5	628	530
	833			59.5	47	455	384

Average Maximum Activity Q_{O_2} = 74 % ± 6 % (SEM)

Average Maximum Active Stress = 530 g/cm^2 ± 30 (SEM)

Average Basal Q_{O_2} = 0.53 µl/mg/hour ± 0.04 (SEM)

FIGURE 15

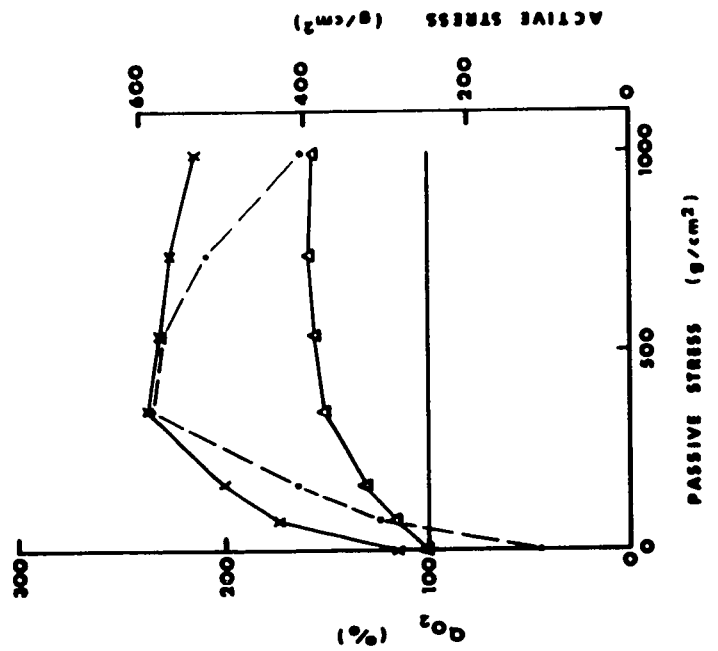
The effect of different values of initial stretch on the active responses of arterial segments to direct electrical stimulation.

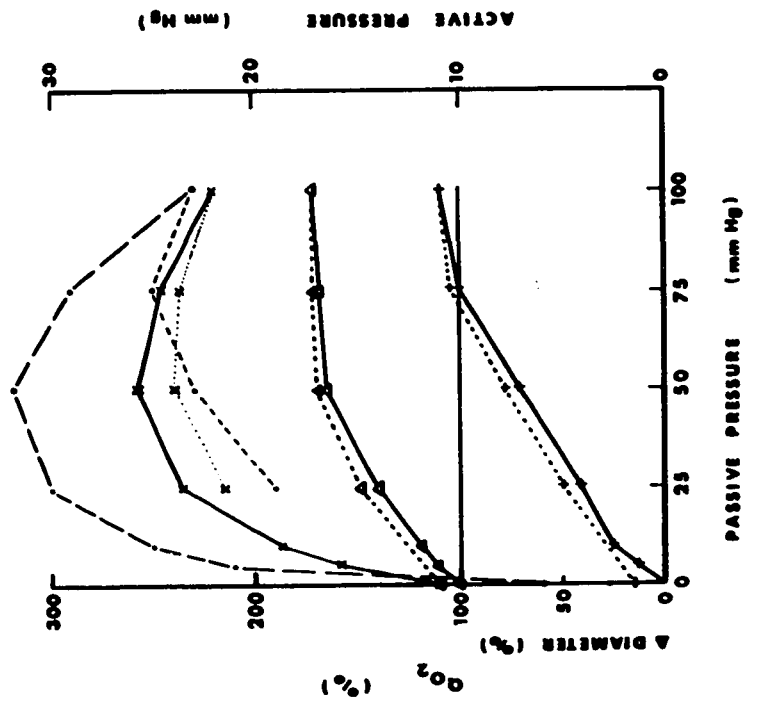
(a) Left

Method A - All Q_{O_2} values are expressed as a percentage of the initial "basal" Q_{O_2} value which was assumed to remain constant at 100% and is represented by the solid horizontal line. The lines connecting the triangles and X'es represent the percentage values of the ("basal" + "tension") and ("basal" + "tension" + "activity") Q_{O_2} values, respectively. The heavy dashed line joining the solid circles represents the absolute value of the active pressure while that joining the crosses represents the percentage change in the diameter of the segment which occurs with stretch. The lightly dashed or dotted lines in each case represent the effect of decreasing the passive pressure on that particular parameter. Hysteresis of all the parameters is apparent especially in the case of the active pressure which shifts its peak value to a higher passive pressure with decreasing stretch of the segment. The descending or dashed portions of the ("basal" + "tension") Q_{O_2} curve and that representing the change in diameter behave in a quantitatively similar fashion.

(b) Right

Method B - The same symbols have been used to represent the same parameters as in Method A.


ACTIVE STRESS (g/cm²)
600
400
200
0
1000
500
0
PASSIVE STRESS (g/cm²)
300
200
100
0
Q_{O_2} (%)


ACTIVE PRESSURE (mm Hg)
30
20
10
0
100
75
50
25
0
PASSIVE PRESSURE (mm Hg)
Δ DIAMETER (%)
300
200
100
50
0
Q_{O_2} (%)

three initial passive pressures were almost identical in shape and magnitude. Thus, over the range of passive pressures that we are interested in, the effect of previous electrical stimulation was not significant for up to at least 5 1/2 hours. For a typical polarographic experiment, Figure 13b shows little change between successive responses for up to 5 hours. Therefore, these electrical stimuli and times between contractions are suitable for use in the main experiment.

3. Results from Main Experiments

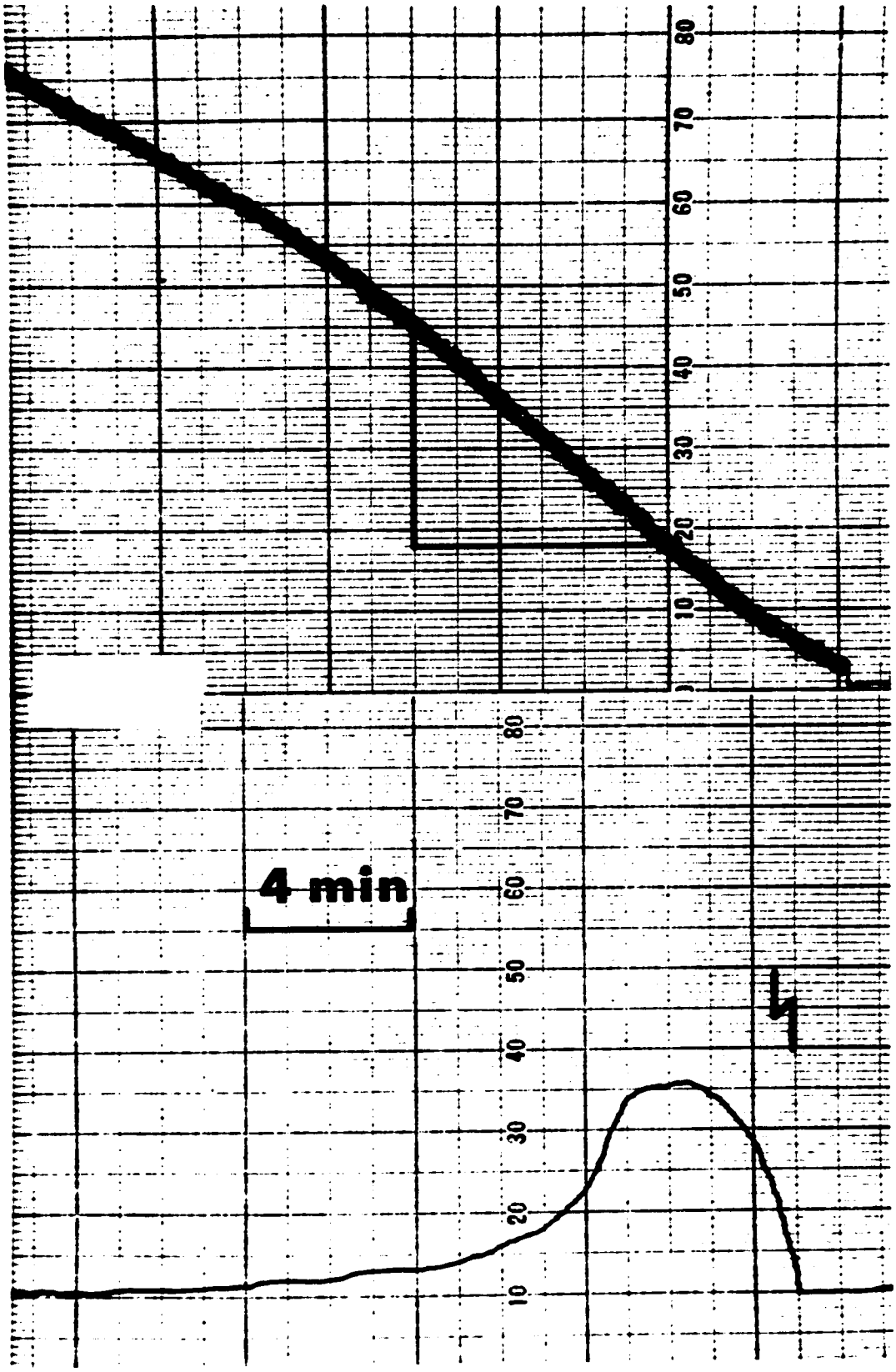
Some typical results for adventitia-free segments using the Warburg method are shown in Figure 14a, and for the polarographic method in Figure 14b. As shown in detail in Tables 8 and 9 for the Warburg and polarographic data, respectively, the results were analyzed into the "basal", "tension" and "activity" Q_{O_2} values as well as the active tension or stress values in exactly the same manner as were the norepinephrine results. A summary of all such Warburg and polarographic results is given in Tables 10 and 11. The active pressure or stress and total Q_{O_2} of the contracted arterial segment both increase to a maximum value and then decline as the passive stretch is increased as is shown in the examples in Figures 15a and 15b.

The example from the Warburg experiments shown in Figures 14a and 15a also illustrates what happened when the passive pressure was decreased in steps from its highest value. Both the active pressure and total Q_{O_2} attain new maxima which are smaller and occur at higher values of passive stretch than those maxima obtained with increasing stretch of the segment. The changes in the diameter of the segment are parallel to those of the "tension" Q_{O_2} during both increasing and decreasing stretch. Hysteresis of each parameter is also evident.

FIGURE 16

Experimental records from the polarograph experiments of the isometric responses of a typical arterial segment when stimulated electrically (30 volts for 30 seconds).

Both records have to be read from right to left. The lower record demonstrates the mechanical response of the segment when it was stimulated electrically at the thunderbolt. Each small vertical division is equivalent to one gram force. Although the maximum active force was maintained for only about two minutes before spontaneously decreasing to zero, the rate of oxygen uptake, as shown in the upper record, after the initial delay period of about three minutes, remained constant at a higher value for at least six minutes even when the arterial segment was quite relaxed. The spontaneous decrease in the rate of oxygen uptake to its pre-stimulation value is also shown. The tangent of the triangle shown was used to calculate the steady state Q_{O_2} of the contracted segment. Each small division in the upper record is equivalent to a change in the oxygen concentration in the cuvette solution of 0.02%.


80
70
60
50
40
30
20
10
80
70
60
50
40
30
20
10
4 min

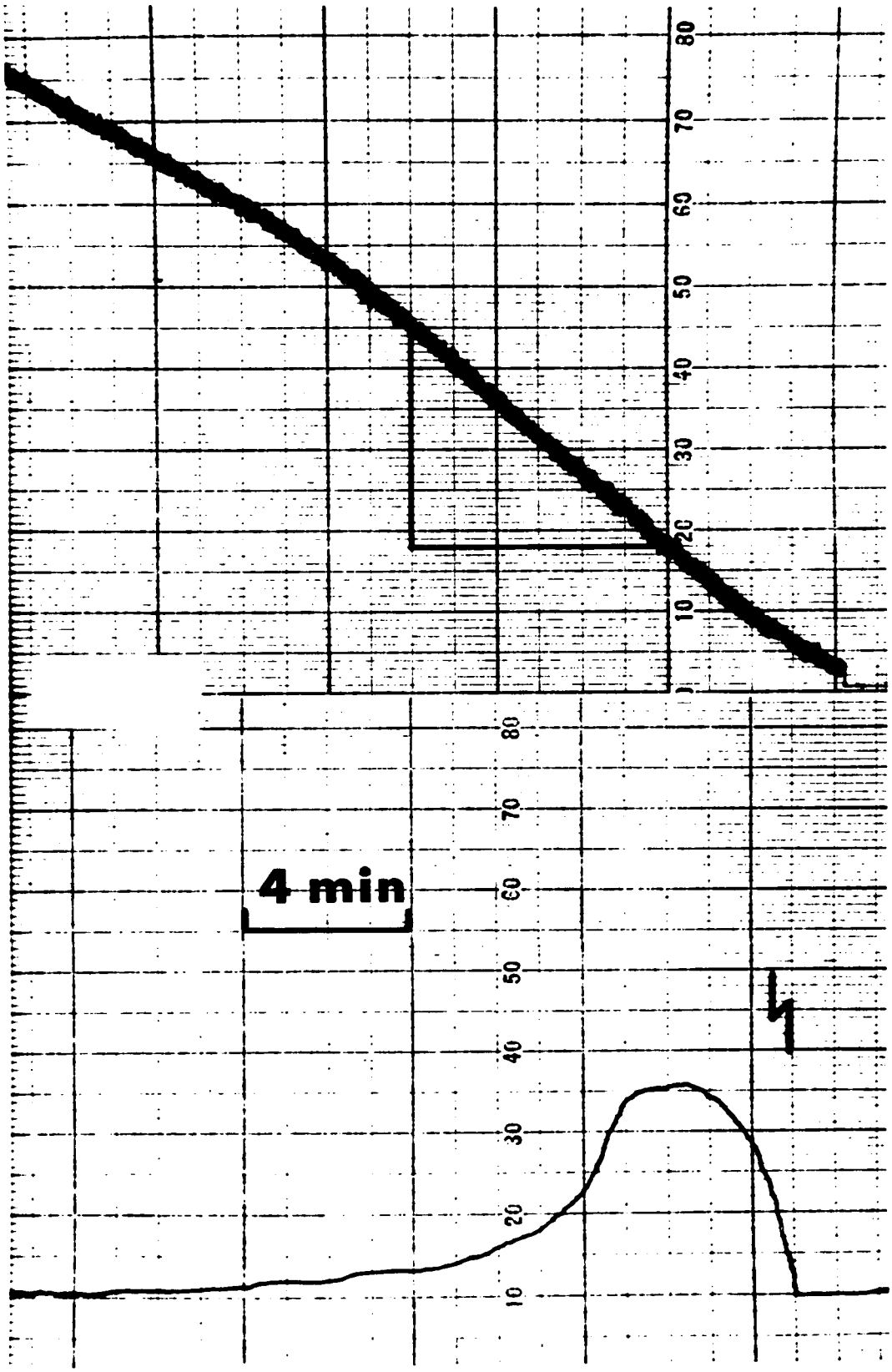
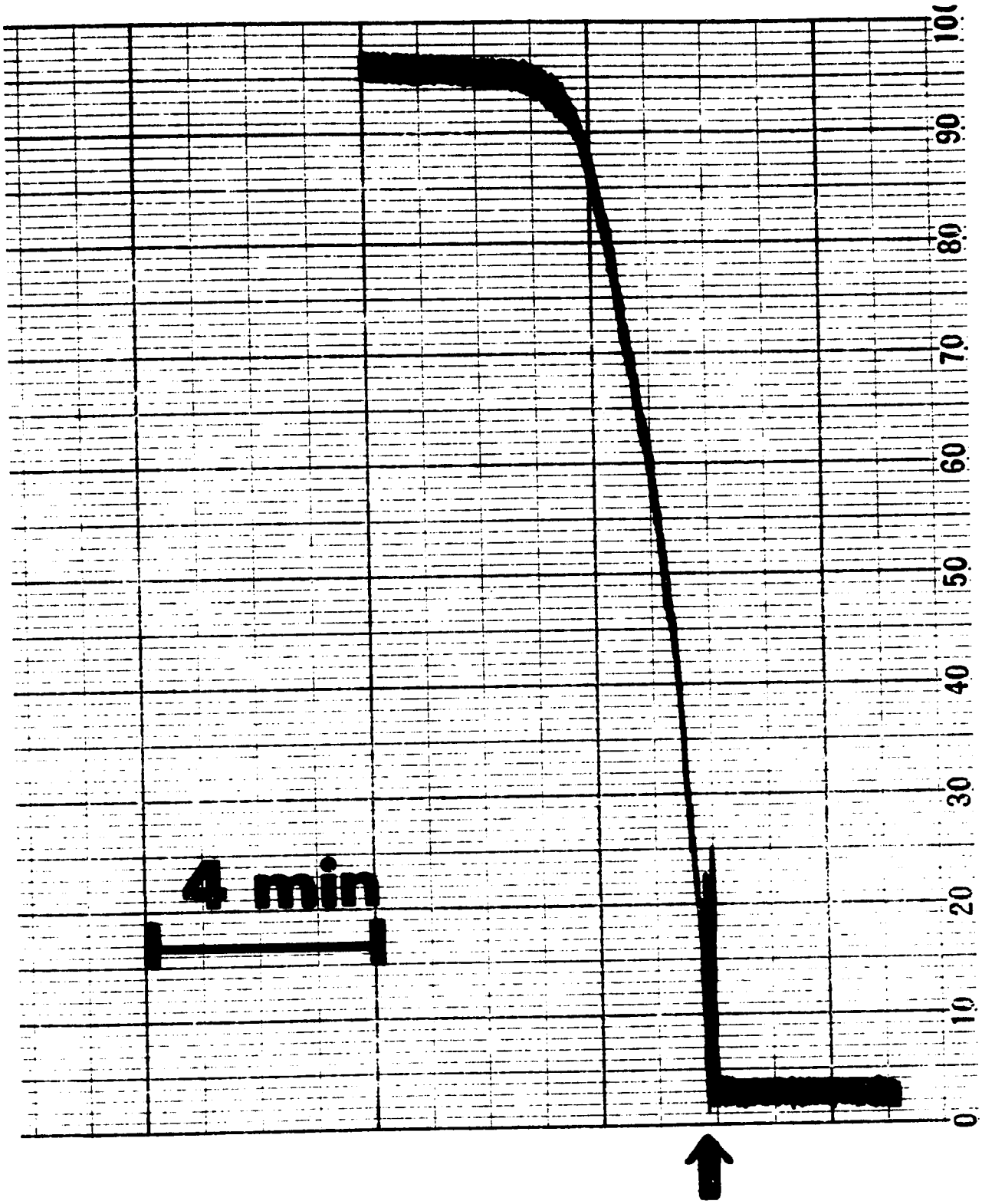

4 min

FIGURE 17

Control trace showing the delay of the oxygen-recording system.

The trace is read from right to left. Nitrogen-saturated modified Ringer solution (2.0 ml) was added at the arrow to the 50 ml of oxygenated modified Ringer solution in the cuvette. Theoretically, this procedure should cause a final change in the average oxygen concentration of the cuvette solution of -2%. The nitrogen-saturated solution was slowly injected over 20 seconds and immediately afterwards, 2 ml of oxygen-saturated solution was slowly injected through the same valve in order to ensure that all of the former solution actually reached the cuvette solution. This injection procedure also resulted in most of the nitrogen-saturated solution's being at the bottom of the cuvette. The change in reading at the electrode is thus the result of the efficiency of the stirrer and the response time of the electrode. The ordinate scale of 100 divisions represents a change in the oxygen concentration of 2%, the ordinate value increasing with decreasing oxygen concentration. Assuming an exponential increase in the output as obtained on the trace, and the rise time from 10% to 90% of the total expected change, gives a time constant for the delay of our system of about one minute.


4 min
0
10
20
30
40
50
60
70
80
90

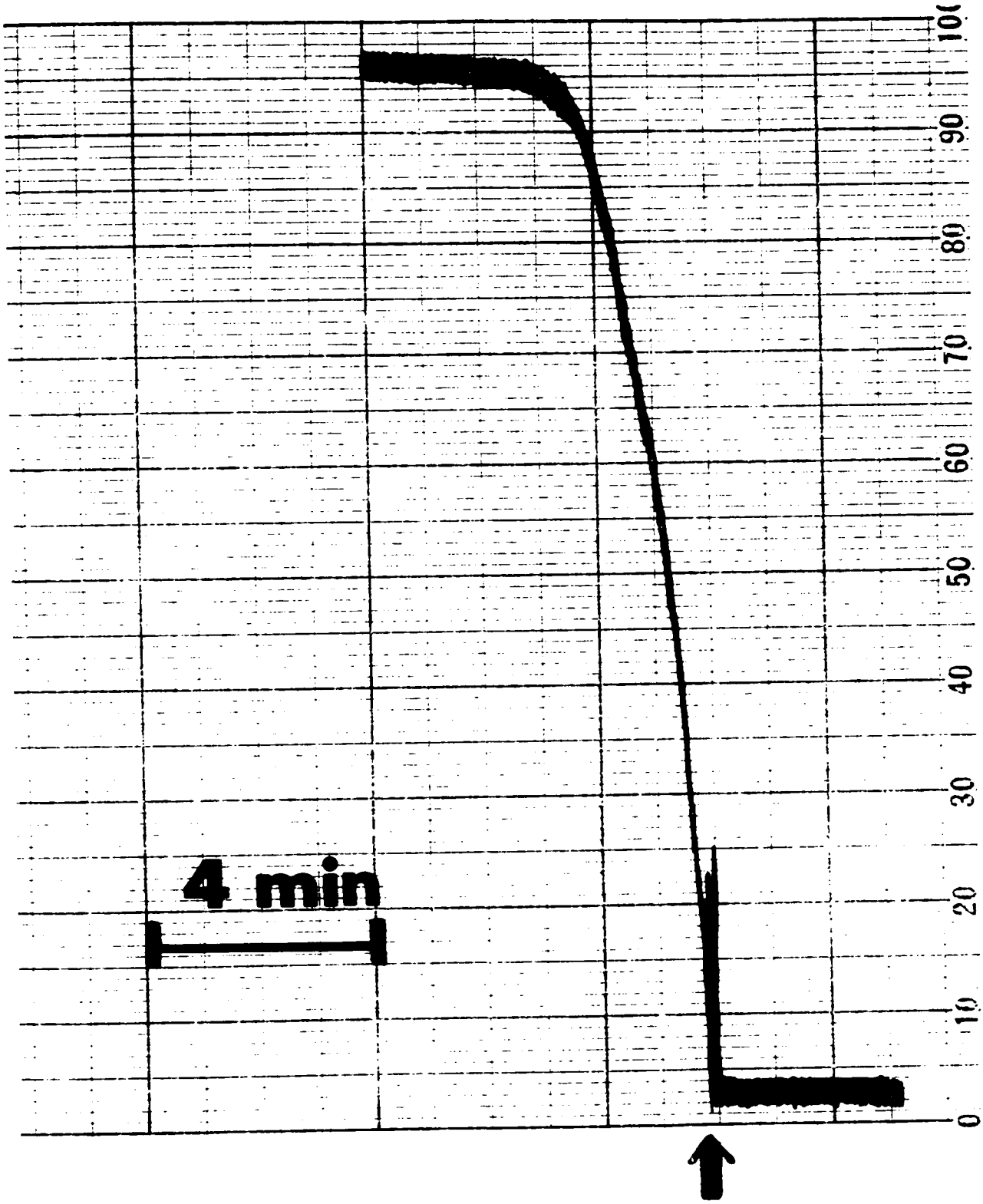

4 min
0
10
20
30
40
50
60
70
80
90
100

FIGURE 18

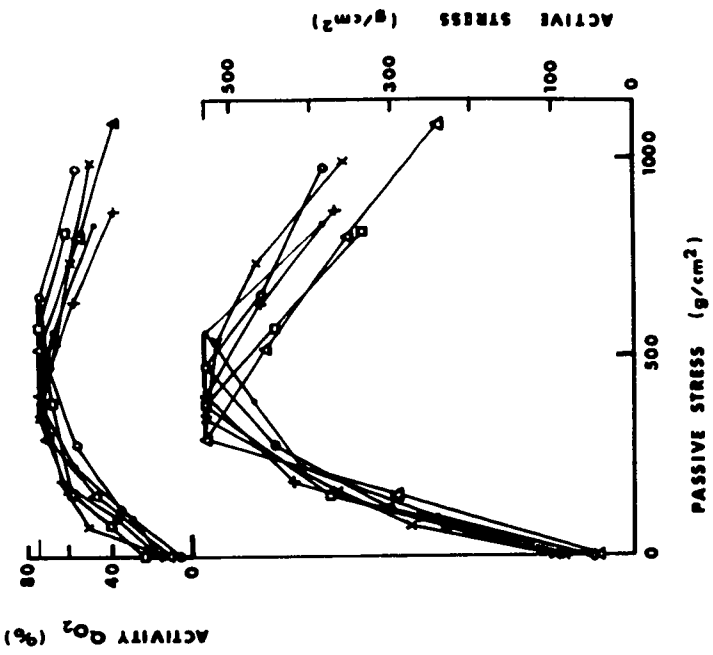
The normalized active responses of adventitia-free arterial segments, which were stimulated electrically, as a function of the passive stretch.

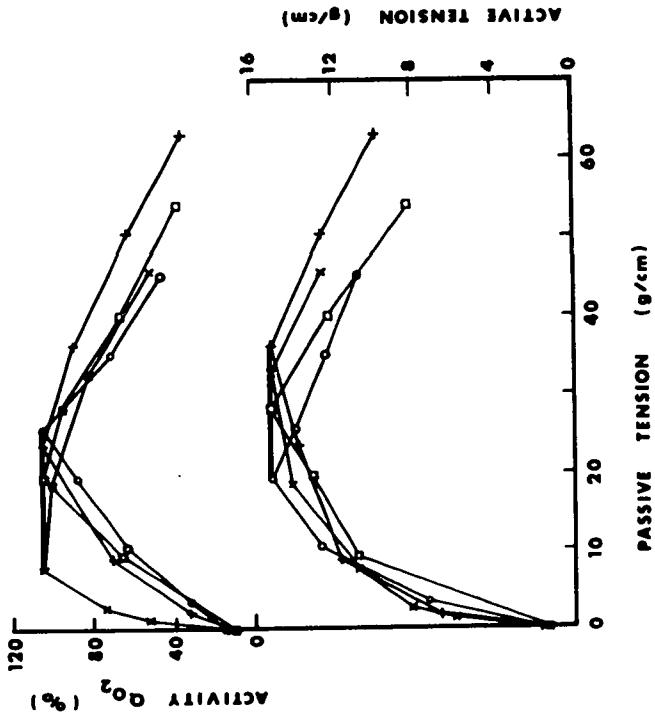
(a) Left

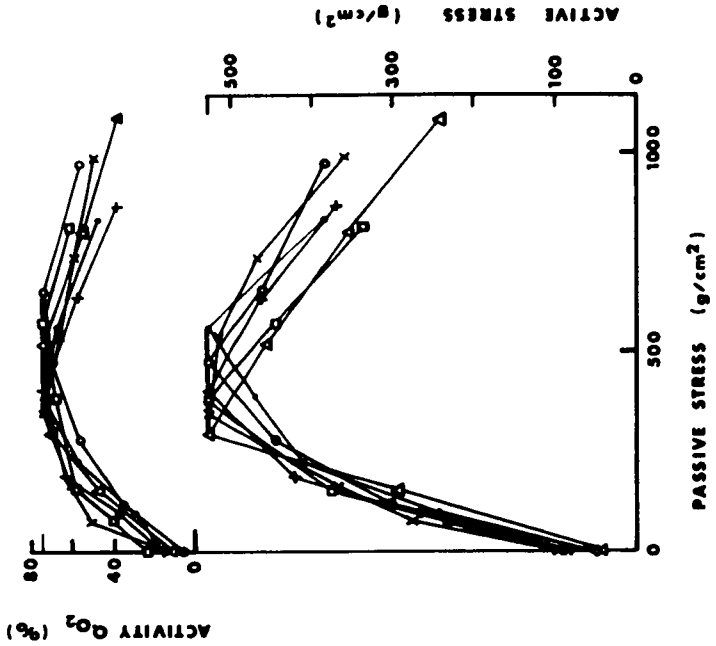
Method A - All of the "activity" Q_{O_2} and active tension values of each segment were normalized relative to their respective maximum values and the results multiplied by the corresponding average maximum value of all four segments. These values are shown plotted. The same symbol is used to represent the responses of a given segment in both sets of curves. In this series of experiments it is seen that the maximum "activity" Q_{O_2} values generally occur at lower values of passive tension than the corresponding maximum values of active tension.

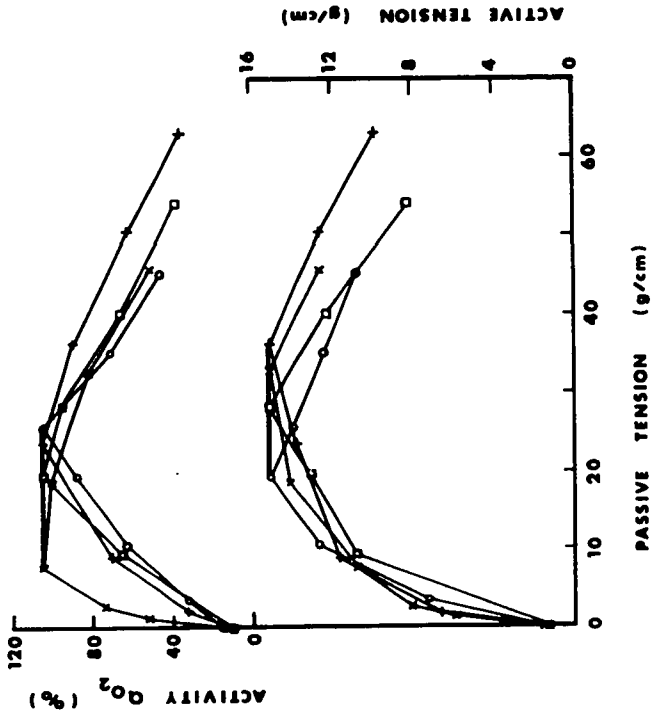
(b) Right

Method B - In this series of experiments the maximum "activity" Q_{O_2} values generally occur at higher values of passive stress than the corresponding maximum values of active stress.


ACTIVE STRESS (g/cm²)
500
300
100
0
1000
500
0
PASSIVE STRESS (g/cm²)
80
40
0
ACTIVITY Q_{O_2} (%)


ACTIVE TENSION (g/cm)
16
12
8
4
0
60
40
20
10
0
PASSIVE TENSION (g/cm)
120
80
40
0
ACTIVITY Q_{O_2} (%)


ACTIVITY Q_{O_2} (%)
0
40
80
PASSIVE STRESS (g/cm²)
0
500
1000
ACTIVE STRESS (g/cm²)
0
100
300
500


ACTIVITY Q_{O_2} (%)
0
40
80
120
PASSIVE TENSION (g/cm)
0
10
20
40
60
ACTIVE TENSION (g/cm)
0
4
8
12
16

The sample trace from the polarographic experiments shown in Figure 16 illustrates that despite the short duration of the maximum mechanical response with the electrical stimulus (about 3 minutes), oxygen is consumed at a steady rate for about 6 minutes. This steady rate was used to calculate the Q_{O_2} of the contracted segment. The delay of about two minutes between the attainment of maximum mechanical response and the steady rate of oxygen uptake may be a biological delay but may also be explained by the delay of our oxygen recording system. In support of this possibility, Figure 17, a trace from a control experiment, shows that about 3 minutes are required for an isolated change in the oxygen content of the cuvette solution to be registered as a new steady oxygen concentration.

4. Treatment of Data

Conversion of the raw Warburg data to units of tension and the polarographic data to units of stress was performed as before. Normalization of the active tension, stress and "activity" Q_{O_2} data was then performed for the same reasons and in the same manner as were the norepinephrine results. These normalized data are shown plotted against the passive stretch in Figure 18 and is also listed in Tables 10 and 11 together with the average maximum responses. The values of passive stretch are all absolute values.

Although the average maximum active tension and active stress of the Warburg and polarographic series, respectively, are not significantly different within one SEM, the average maximum "activity" Q_{O_2} of the polarographic experiments is significantly smaller. With each set of data the mechanical and respiratory responses increase in a parallel fashion to reach a maximum value with increasing passive stretch and then decrease

FIGURE 19

The variation of the "activity" Q_{O_2} of adventitia-free arterial segments with their active tension when stimulated electrically (40 volts for 30 seconds). Results obtained with Warburg technique (Method A).

The "activity" Q_{O_2} values of each segment are expressed as a percentage of the initial "basal" Q_{O_2} of that segment. The solid line represents the regression line of all the data with the dashed lines drawn parallel to it representing plus and minus one standard error of the estimate. The coefficient of correlation between the two parameters equals 0.86 ± 0.10 (SE) while the regression line has the equation $y = 5.54x + 4.97$.

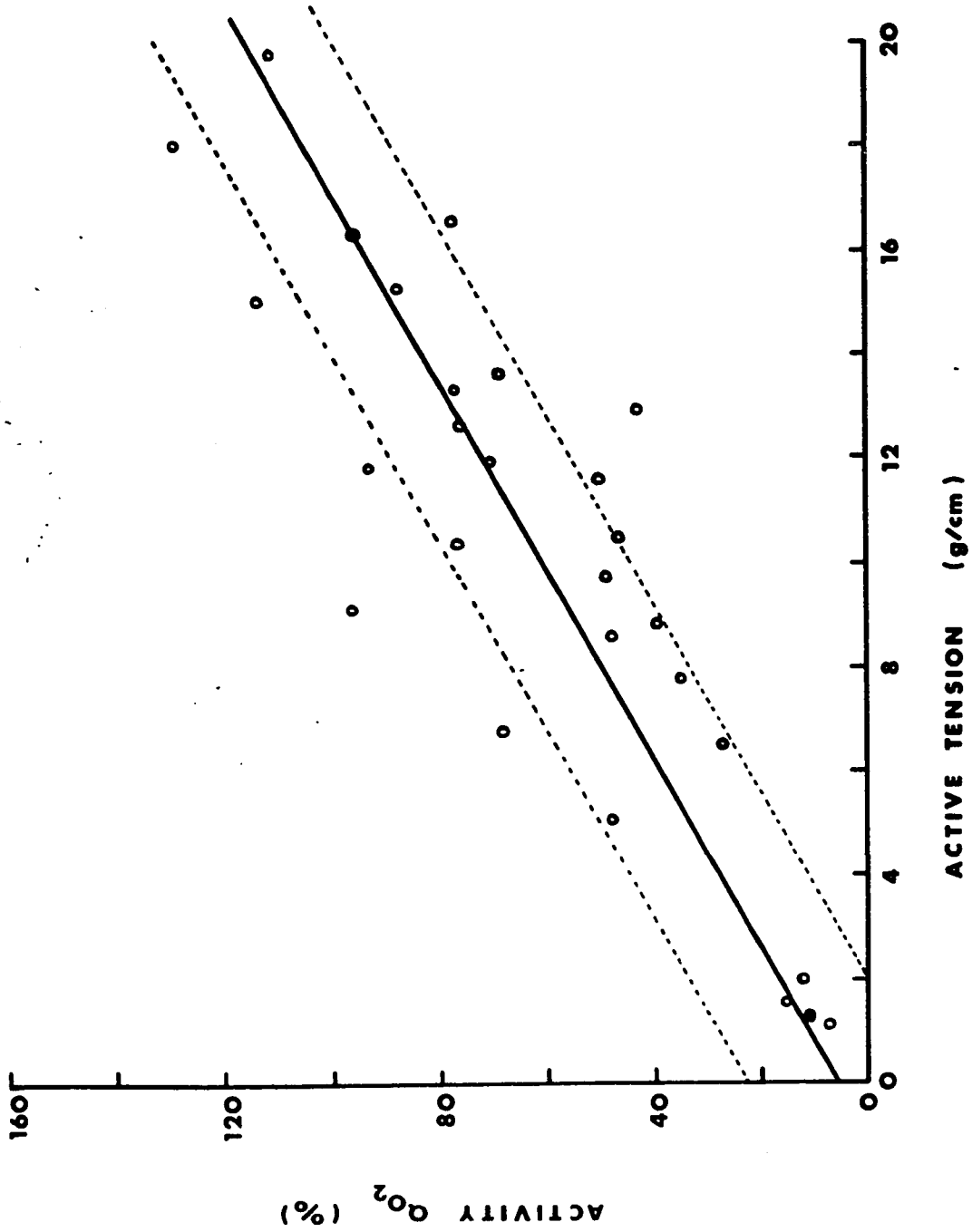

ACTIVITY Q_{O_2} (%)
160
120
80
40
0
0
4
8
12
16
20
ACTIVE TENSION (g/cm)

FIGURE 20

The variation of the "activity" Q_{O_2} of adventitia-free arterial segments with their active stress when stimulated electrically (30 volts for 30 seconds). Results obtained with the polarographic technique (Method B).

The solid line represents the regression line of all the data with the dashed lines drawn on either side of it and parallel to it representing plus and minus one standard error of the estimate. The coefficient of correlation between the two parameters equals 0.94 ± 0.05 (SE) while the regression line has the equation $y = 0.14x + 2.39$.

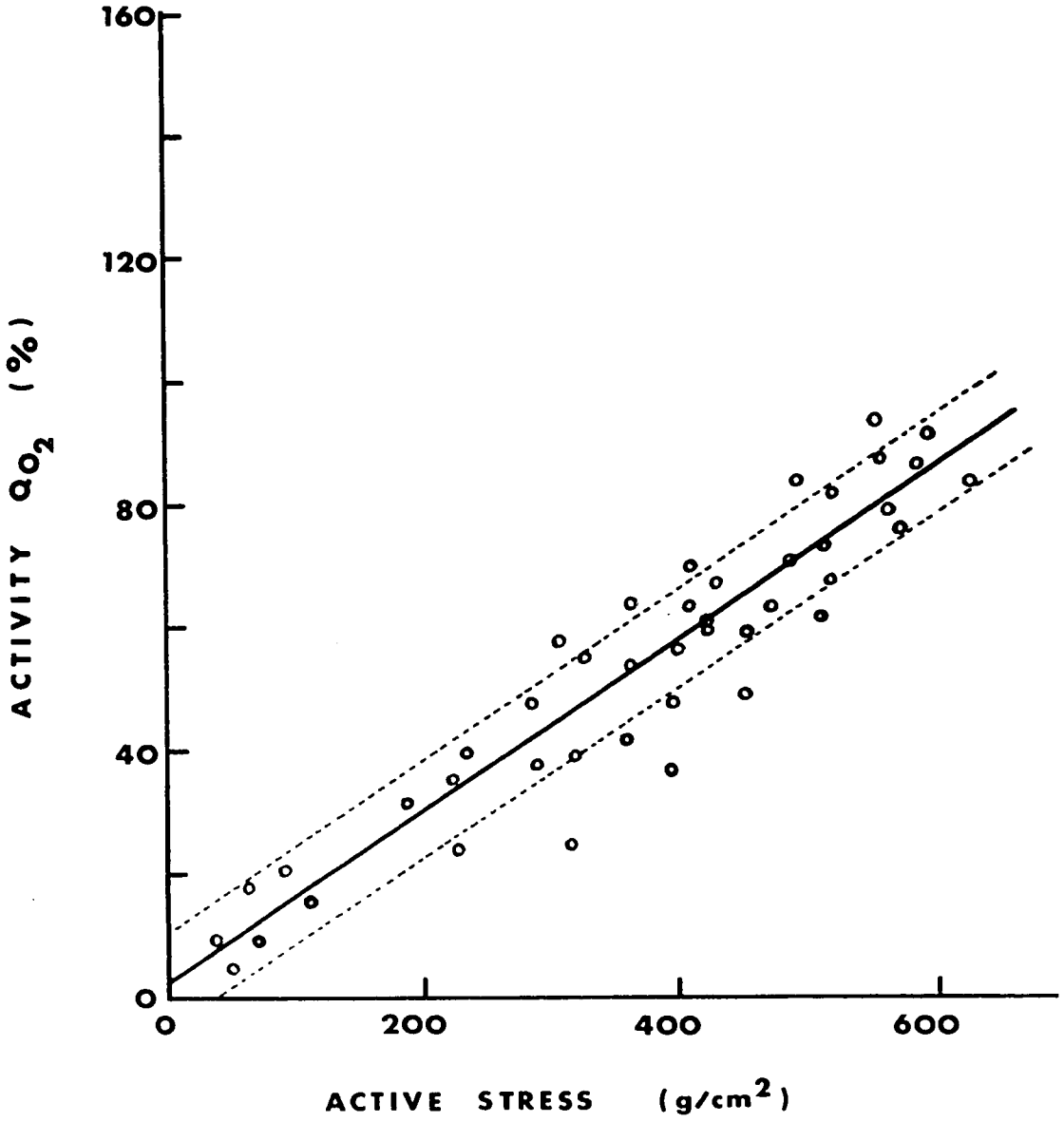

160
120
80
40
0
ACTIVITY Q_{O_2} (%)
0
200
400
600
ACTIVE STRESS (g/cm^2)

FIGURE 21

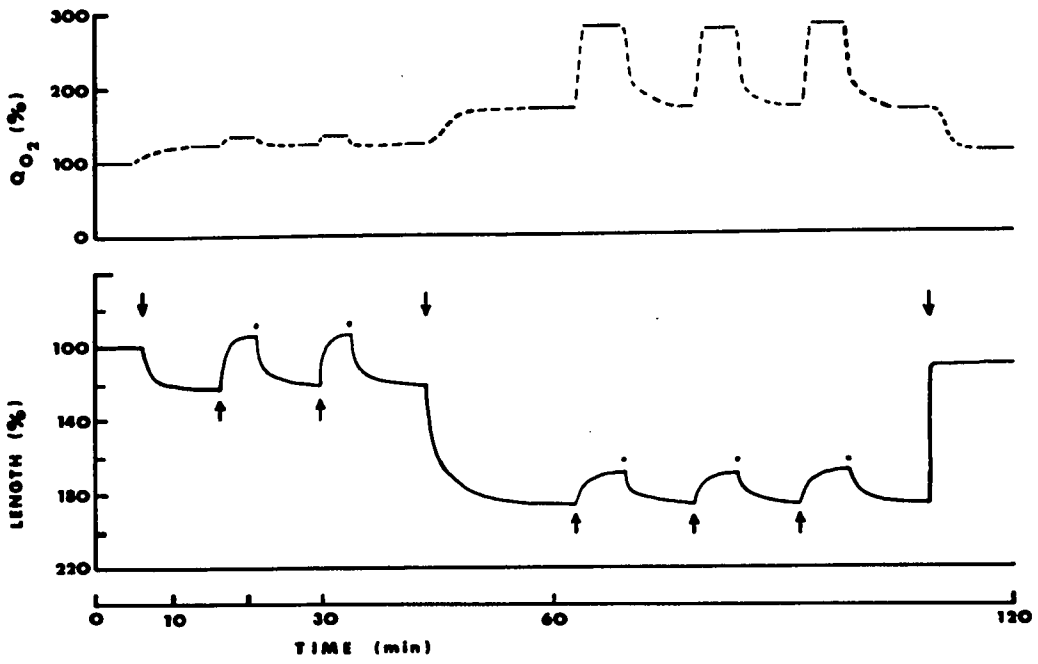
The effect of time and previous stimulation on the isotonic responses of arterial segments.

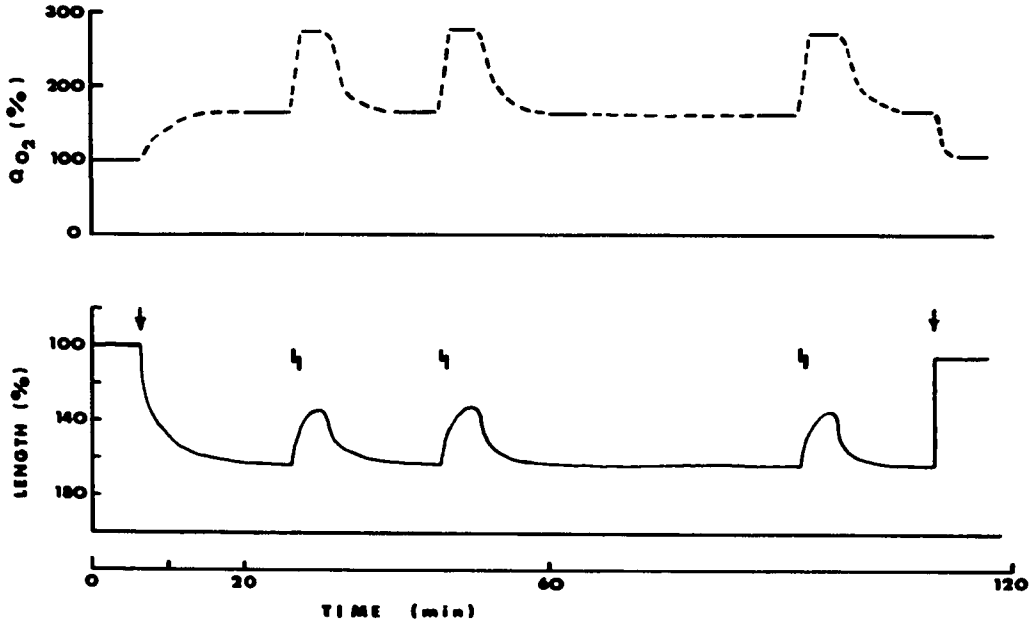
(a) Top

The results obtained by stimulation with norepinephrine (5×10^{-8} g/ml) at two different values of passive load. The vertical length of the segment is expressed as a percentage of its initial value at zero load. The passive load or stress was adjusted manually at each of the inverted arrows while norepinephrine was added and washed out at each of the upright arrows and solid circles, respectively. The steady state Q_{O_2} values are all expressed as a percentage of the initial "basal" Q_{O_2} value and are represented by the solid horizontal lines which have been roughly connected together by the dashed lines for the sake of continuity.

(b) Bottom

A direct electrical stimulus (30 volts for 30 seconds) was applied at each of the thunderbolts.


300
200
100
0
a_{O_2} (%)
100
140
180
220
LENGTH (%)
0
10
30
60
120
TIME (min)


300
200
100
0
a_{O_2} (%)
100
140
180
LENGTH (%)
0
20
60
120
TIME (min)

with still further stretch. With the Warburg data the "activity" Q_{O_2} generally reached its maximum at a smaller value of passive stretch than did the active tension, while in the polarographic series the more common opposite tendency was observed. The decline of the two active responses beyond their maxima was equally steep for the Warburg curves but with the polarographic curves the "activity" Q_{O_2} decreased less steeply than the active stress. The maximum active responses occurred in the physiological range of passive stretch which was comparable for the two techniques employed but the optimal stretch was less with electrical than with norepinephrine stimulation.

The variation of the unnormalized "activity" Q_{O_2} values with their corresponding unnormalized active tension and active stress values is shown in Figures 19 and 20, respectively. Each of the "activity" Q_{O_2} values is still expressed as a percentage of the "basal" Q_{O_2}. The high coefficient of correlation between the two active responses observed with both techniques suggests that they are linearly related.

E. Effect of Initial Stretch on the Isotonic Responses of Arterial Segments with Norepinephrine and Direct Electrical Stimulation

1. Control Experiments - Effect of Time and Previous Stimulation

As shown in Figures 21a and 21b and in two other similar but longer experiments, either norepinephrine or direct electrical stimulation of the isotonically-loaded arterial segments gave mechanical responses or changes in length which changed little in shape or in magnitude up to at least four hours. The steady rate of oxygen uptake measured during the shortening process also changed little.

FIGURE 22

The effect of passive stress on the isotonic responses of arterial segments to stimulation by norepinephrine and direct electrical stimulation.

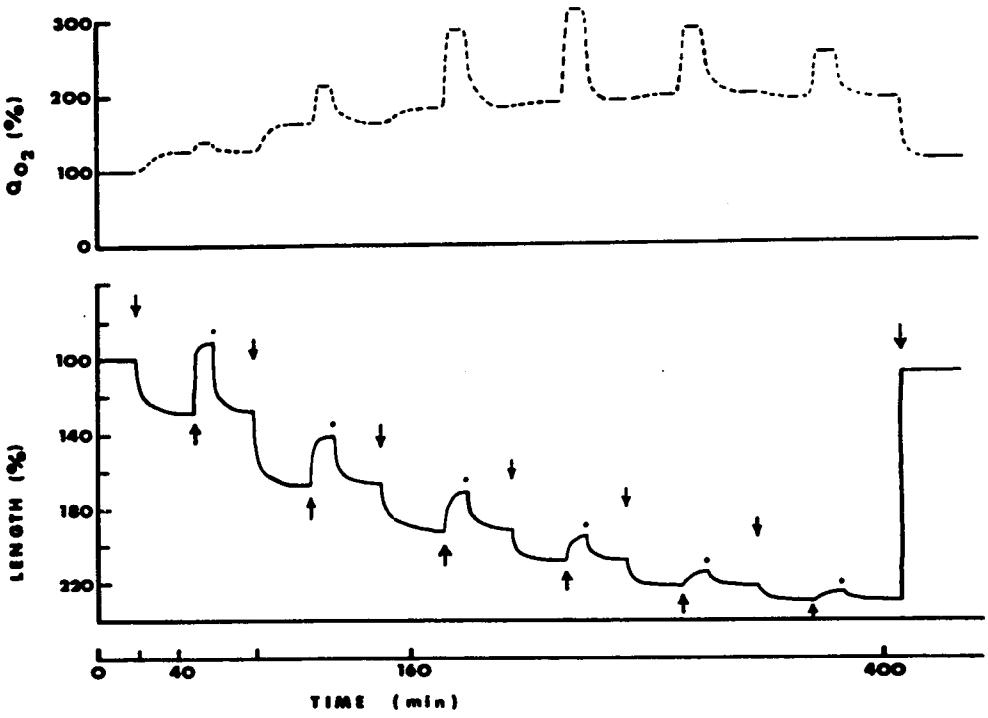
(a) Top

The results obtained by stimulation of an arterial segment with 5 x 10^{-8} g/ml norepinephrine. The mechanical responses have been expressed as percentage changes in the vertical length of the segment relative to the initial vertical length of the unloaded segment. The load was changed at each of the inverted arrows and the norepinephrine was added and washed out at the upright arrows and solid circles, respectively. The steady state Q_{O_2} values are all expressed as a percentage of the initial "basal" Q_{O_2} value and are represented by the solid horizontal lines drawn before, during and after each contraction with the dashed lines joining them being only very approximate.

Note the direct relationship which exists between the speed of shortening of the segment and its degree of shortening. If the delay in the oxygen-recording system is taken into account, measurements of the Q_{O_2} of the contracted segment were made only while the segment was shortening or performing work.

(b) Bottom

Direct electrical stimulation (30 volts for 30 seconds) was applied at each of the thunderbolts.


300
200
100
0
a_{O_2} (%)
100
140
180
220
LENGTH (%)
0
40
160
400
TIME (min)

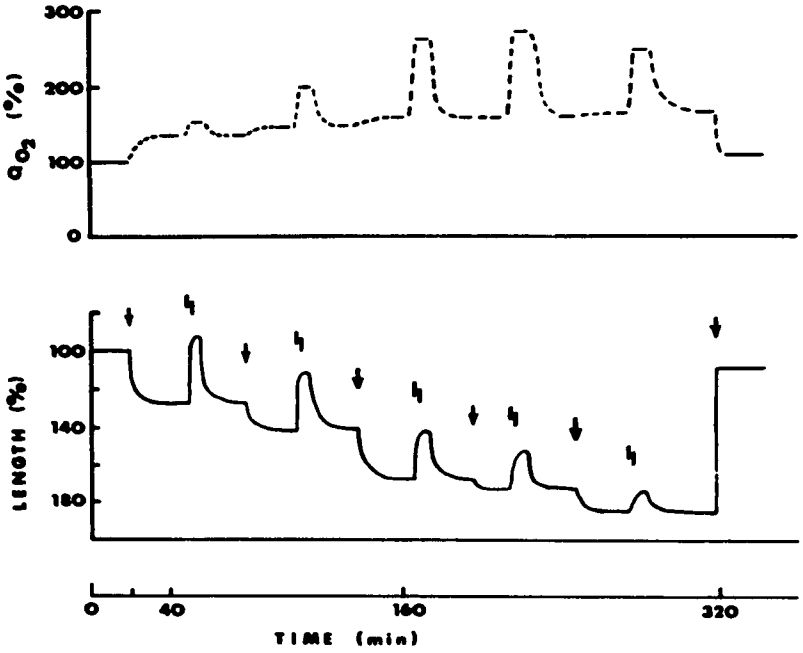

300
200
100
0
a_{O_2} (%)
100
140
180
LENGTH (%)
0
40
160
320
TIME (min)

TABLE 12

Effect of Initial Stress on the Isotonic Responses of an Arterial Segment with Norepinephrine Stimulation

Load	Wall Thick.	Initial Stress	Basal Q_{O_2}		Tension Q_{O_2}	Activity Q_{O_2}	Total Short.	Work Perf.	Stretched Length	Maximally Shortened Length	"Correct." Tension Q_{O_2}	"Correct." Activity Q_{O_2}
g	cm	g/cm^2	µl/mg /hour	%	%	%	cm	g·cm	%*	%*	%	%
0	0.019	0	0.55	100	0	–	–	–	100	–	–	–
10	0.016	174			27	12	0.140	1.40	128	92	0	39
32	0.014	635			63	49	0.096	3.08	167	142	43	69
44	0.0125	978			81	103	0.076	3.35	192	172	68	116
54	0.0115	1305			89	121	0.054	2.92	208	195	84	126
64	0.011	1620			95	89	0.031	1.98	222	214	95	89
68	0.010	1890			89	61	0.018	1.22	230	225	89	61

* Expressed as a percentage of the Initial Unstretched Length

TABLE 13

Effect of Initial Stress on the Isotonic Responses of an Arterial Segment with Electrical Stimulation

Load	Wall Thick.	Initial Stress	Basal Q_{O_2}		Tension Q_{O_2}	Activity Q_{O_2}	Total Short.	Work Perf.	Stretched Length	Maximally Shortened Length	"Correct." Tension Q_{O_2}	"Correct." Activity Q_{O_2}
g	cm	g/cm^2	µl/mg /hour	%	%	%	cm	g·cm	%*	%*	%	%
0	0.022	0	0.59	100	0	—	—	—	100	—	—	—
20	0.019	295			36	18	0.15	3.00	128	92	0	54
35	0.0175	556			48	51	0.13	4.55	142	111	13	86
50	0.0165	841			60	102	0.11	5.50	168	142	48	114
60	0.016	1042			60	112	0.085	5.10	173	153	53	119
75	0.015	1390			64	85	0.05	3.75	185	173	60	89

* Expressed as a percentage of the Initial Unstretched Length

FIGURE 23

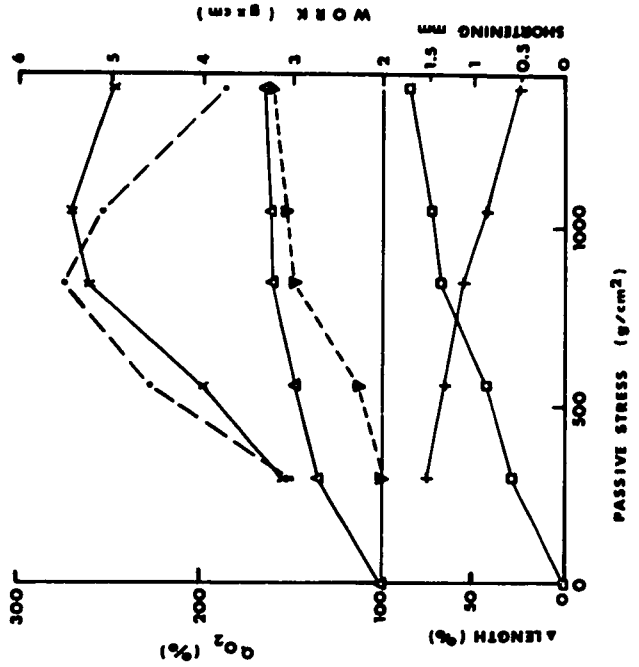
The typical isotonic responses of arterial segments to stimulation by norepinephrine and electrical means as a function of the initial passive stress.

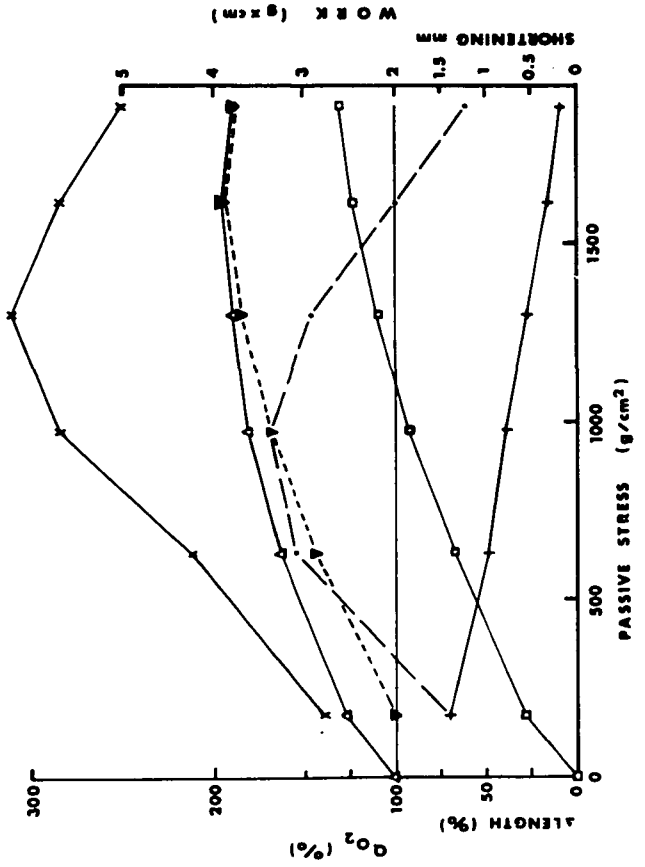
(a) Left

The results obtained by stimulation of an arterial segment with 5×10^{-8} g/ml norepinephrine. All steady state Q_{O_2} values are expressed as a percentage of the initial "basal" Q_{O_2} value which is represented by the solid horizontal line at the 100% level. The ("basal" + "tension") Q_{O_2} and ("basal" + "tension" + "activity") Q_{O_2} values are represented by the lines joining the hollow triangles and the X'es, respectively. The dashed lines joining the inverted solid triangles represent the changes in the ("basal" + "corrected tension") Q_{O_2} values of the segment. The dashed lines joining the solid circles represent the absolute values of the external work performed by the segment while the solid lines joining the squares and crosses represent the percentage change in the vertical length of the stretched segment and its amount of shortening upon contraction, respectively.

(b) Right

The results obtained by direct electrical stimulation of an arterial segment. The symbols represent the same paramenters as described above.


WORK (g×cm)
SHORTENING mm
PASSIVE STRESS (g/cm²)
ΔLENGTH (%)
Q_{O_2} (%)


WORK (g×cm)
SHORTENING mm
PASSIVE STRESS (g/cm²)
ΔLENGTH (%)
Q_{O_2} (%)

2. Results of Main Experiments

Some typical results obtained in these two series of experiments are shown in Figures 22a and 22b. Tables 12 and 13 give in detail the breakdown of the Q_{O_2} into its three components, the passive load, and total shortening of the segment at each passive load. Since it was decided to calculate only the total external work performed by the segment during each contraction and correlate this with the Q_{O_2}, it was only necessary to multiply the load in grams by the total shortening in centimeters to get the total work. It is seen that the external work and total Q_{O_2} values both increase to a maximum and then decline as the passive stretch is increased.

Also shown in Tables 12 and 13 are some "corrected" "tension" Q_{O_2} values. Although the "tension" Q_{O_2} values behave with stretch in the same quantitative manner as those determined using isometric loading, in these isotonic experiments the possibility must be considered that the "tension" Q_{O_2} changes in proportion to the changes in length of the contracted segment. This dependence of the "tension" Q_{O_2} on the length rather than on the passive stress is supported by data presented previously and by the curves shown in Figure 23 which illustrate a parallel relationship between the two parameters. These parallel curves were used to calculate a maximum correction for the "tension" Q_{O_2} values of these contracted segments. At each passive stress, the "corrected" "tension" Q_{O_2} was considered equal to the "tension" Q_{O_2} of the maximally shortened vertical length of the contracted segment at that passive stress. The resultant values are shown plotted in Figure 23 and are also listed in Tables 12 and 13. At the lower passive stress values where the degree of shortening is greatest, the "corrected" "tension" Q_{O_2} values were significantly smaller

TABLE 14

Norepinephrine-induced Isotonic Responses of Arterial Segments

Artery No.	Passive Stress g/cm^2	Basal Q_{O_2} $\mu l/mg/hour$	Basal Q_{O_2} %	Activity Q_{O_2} %	Normalized Activity Q_{O_2} %	Work g·cm	Normalized Work g·cm
1	174	0.55	100	12	13	1.40	1.44
	635			49	52	3.08	3.17
	978			103	110	3.35	3.45
	1305			121	129	2.92	3.01
	1620			89	95	1.98	2.04
	1890			61	65	1.22	1.26
2	227	0.36	100	27	31	1.22	1.51
	610			74	84.5	2.45	3.04
	861			113	129	2.78	3.45
	1115			105	120	2.15	2.67
	1333			92	105	1.40	1.74
	1550			75.5	86	0.55	0.68
3	348	0.47	100	26.5	23	1.74	1.59
	707			73	64.5	3.30	3.02
	920			137	121	3.77	3.45
	1152			146	129	3.25	2.98
	1410			127.5	112.5	2.30	2.10
	1640			99	87.5	1.15	1.05
4	363	0.41	100	21.5	17	1.51	1.08
	645			70	55	3.73	2.67
	830			116	91	4.50	3.22
	1048			165	129	4.81	3.45
	1300			143	112	3.14	2.25
	1590			124	97	1.90	1.36
5	290	0.46	100	43.5	57	1.88	2.54
	710			94	122.5	2.55	3.45
	1005			99	129	2.40	3.25
	1190			95	124	2.20	2.97
	1395			87	113	1.70	2.30
	1655			80	104	1.05	1.42

Average Maximum Activity Q_{O_2} = 129 % ± 11 % (SEM)

Average Maximum Work = 3.45 g·cm ± 0.36 (SEM)

Average Basal Q_{O_2} = 0.45 $\mu l/mg/hour$ ± 0.03 (SEM)

TABLE 15

Electrically-induced Isotonic Responses of Arterial Segments

Artery No.	Passive Stress g/cm^2	Basal Q_{O_2} $\mu l/mg/hour$	Basal Q_{O_2} %	Activity Q_{O_2} %	Normalized Activity Q_{O_2} %	Work g·cm	Normalized Work g·cm
1	295	0.59	100	18	15	3.00	2.57
	556			51	44	4.55	3.89
	841			102	87	5.50	4.70
	1042			112	96	5.10	4.36
	1390			85	73	3.75	3.21
2	340	0.63	100	22	23	2.33	2.52
	510			45	47	3.75	4.11
	665			83	87	4.30	4.70
	960			92	96	4.15	4.55
	1230			65	68	3.05	3.34
	1540			38	40	2.10	2.30
3	175	0.40	100	13	9.5	2.15	1.69
	310			31	23	4.45	3.49
	490			69	51	5.90	4.62
	700			124	91	6.00	4.70
	940			131	96	5.75	4.50
	1305			99	72.5	4.35	3.41
4	160	0.53	100	7	10	1.80	2.23
	470			40	56	3.65	4.52
	730			69	96	3.80	4.70
	1080			51	71	2.75	3.41
	1360			38	53	1.60	1.98
5	240	0.56	100	14	18	2.55	2.92
	485			60	78	4.10	4.70
	765			74	96	3.70	4.25
	990			56	73	3.15	3.61
	1220			47.5	61.5	2.35	2.70
	1530			42	54.5	1.55	1.78

Average Maximum Activity Q_{O_2} = 96 % ± 10 % (SEM)

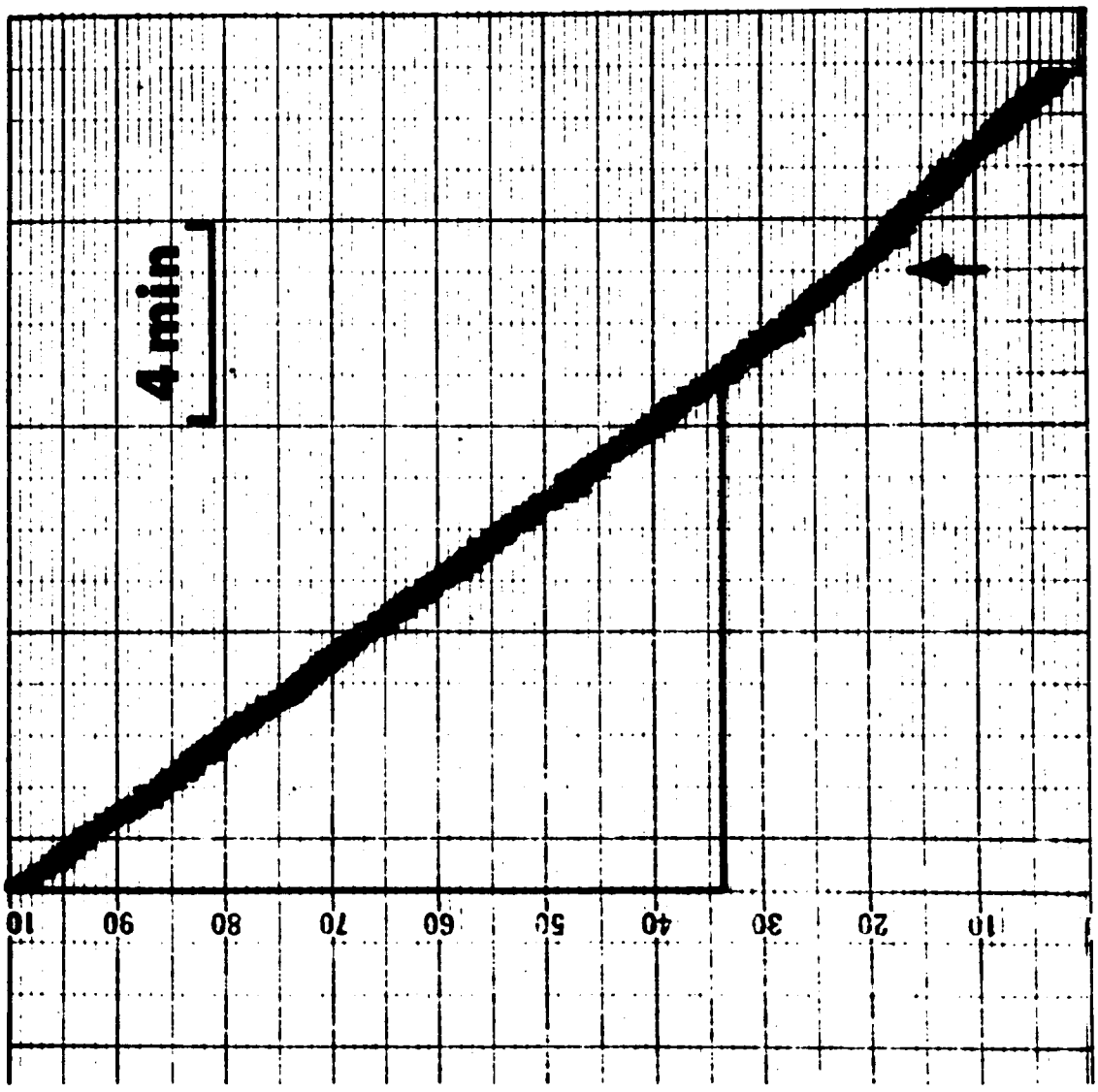
Average Maximum Work = 4.74 g·cm ± 0.38 (SEM)

Average Basal Q_{O_2} = 0.54 $\mu l/mg/hour$ ± 0.04 (SEM)

FIGURE 24

A typical experimental record showing the oxygen consumed during a norepinephrine-induced isotonic contraction of an arterial segment.

The record, when read from the right to the left, shown that about two minutes after the addition of norepinephrine at the arrow, a new and greater steady rate of oxygen consumption was established which remained constant for as long as the muscle was shortening. Although it is not shown in this record, a few minutes after shortening was complete, the rate of oxygen uptake decreased to a noticeably different value at which it remained until the drug was washed out. In this example the segment was heavily loaded and continued to shorten almost to the end of the scale so that the tangent of the triangle drawn was used to calculate the rate of oxygen uptake of the contracting segment. The bottom of the record represents an oxygen concentration of about 99.5% and the top a concentration of 97.5% oxygen. The thickness of the tracing varied somewhat from experiment to experiment and was probably due to the stirrer causing pressure variations inside the cuvette which in turn caused the output of the electrode to fluctuate around a mean value.


4 min
10
20
30
40
50
60
70
80
90
10

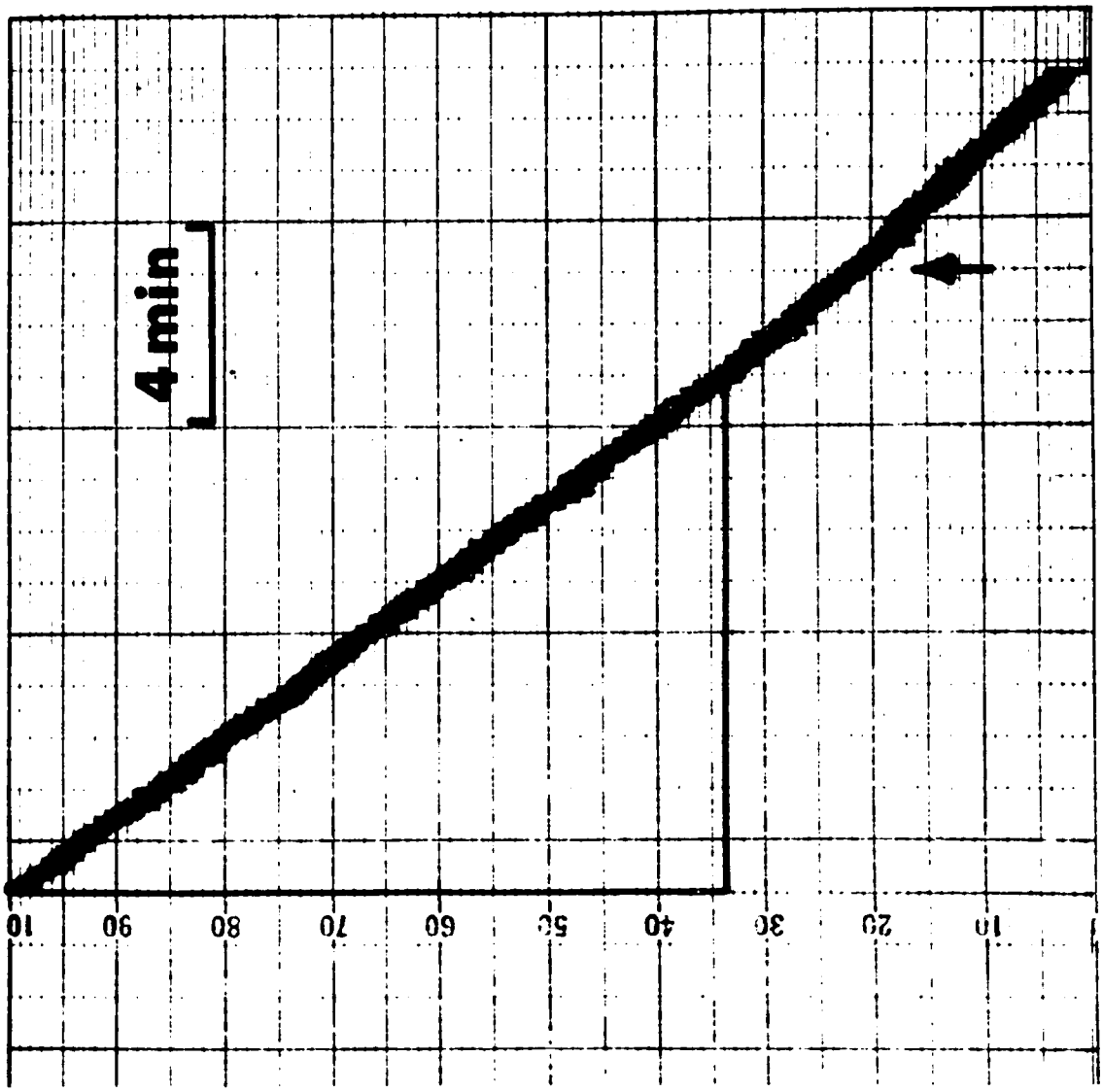
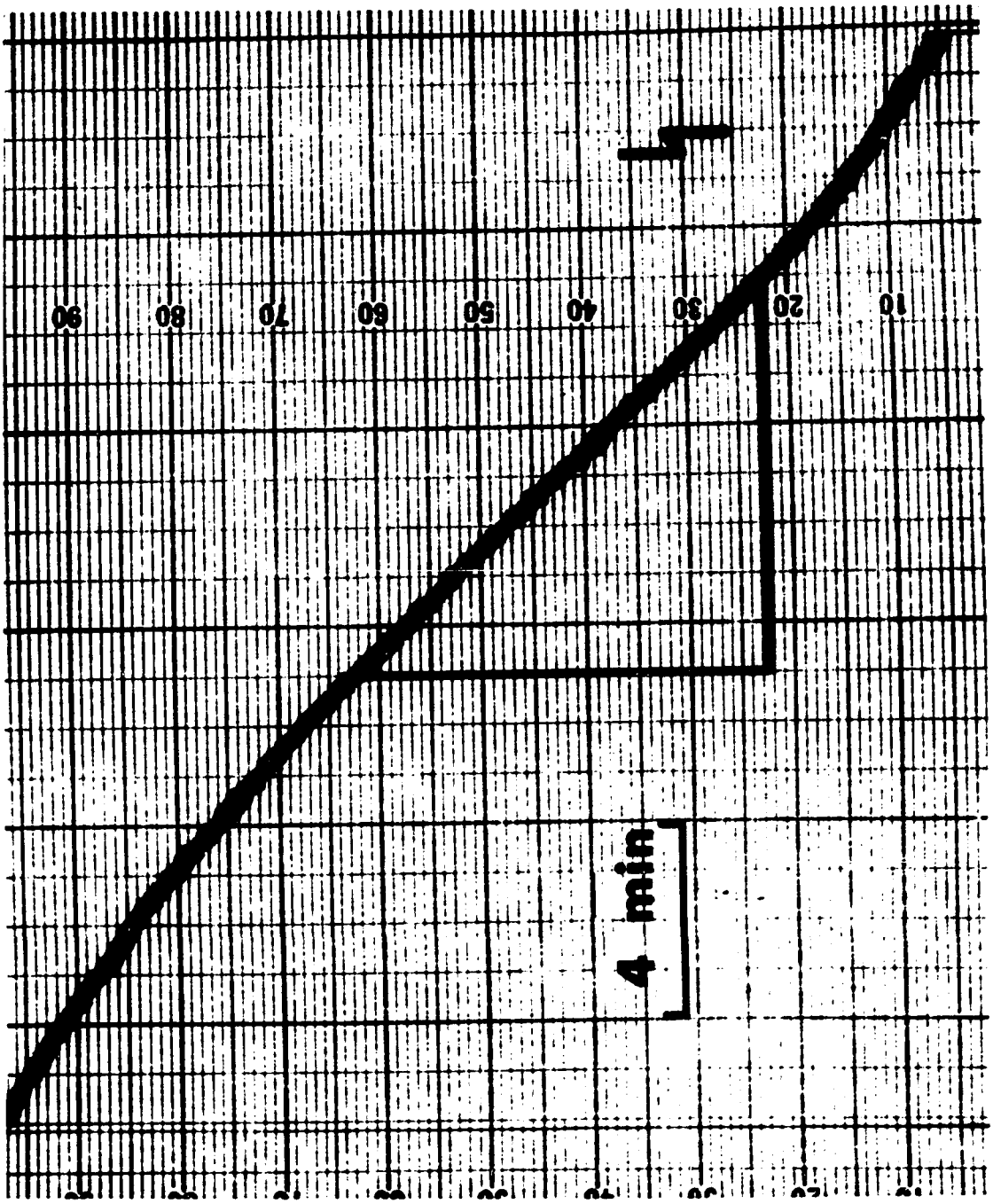

4min
10
20
30
40
50
60
70
80
90
100

FIGURE 25

A typical experimental record showing the oxygen consumption of an arterial segment during an electrically-induced isotonic contraction.

By reading the record from right to left it is seen that about two minutes after the application of the direct electrical stimulus at the thunderbolt, the slope of the tracing or the rate of oxygen uptake stops increasing and is maintained constant at the higher level, in this case, for at least eight minutes before it starts decreasing to its prestimulation level. The segment had actually ceased shortening about two minutes earlier so that it is probable that the delay in the oxygen record relative to the mechanical readings is about two minutes both with increasing and decreasing rates of oxygen uptake. The time required for complete equilibration of the oxygen throughout the cuvette solution is probably the major reason for this delay between the two records. The tangent of the triangle shown was used to calculate the Q_{O_2} of the contracted segment usually by simply expressing it as a percentage of the slope of the tracing depicting the initial "basal" respiration of the segment.


10
20
30
40
50
60
70
80
90
4 min

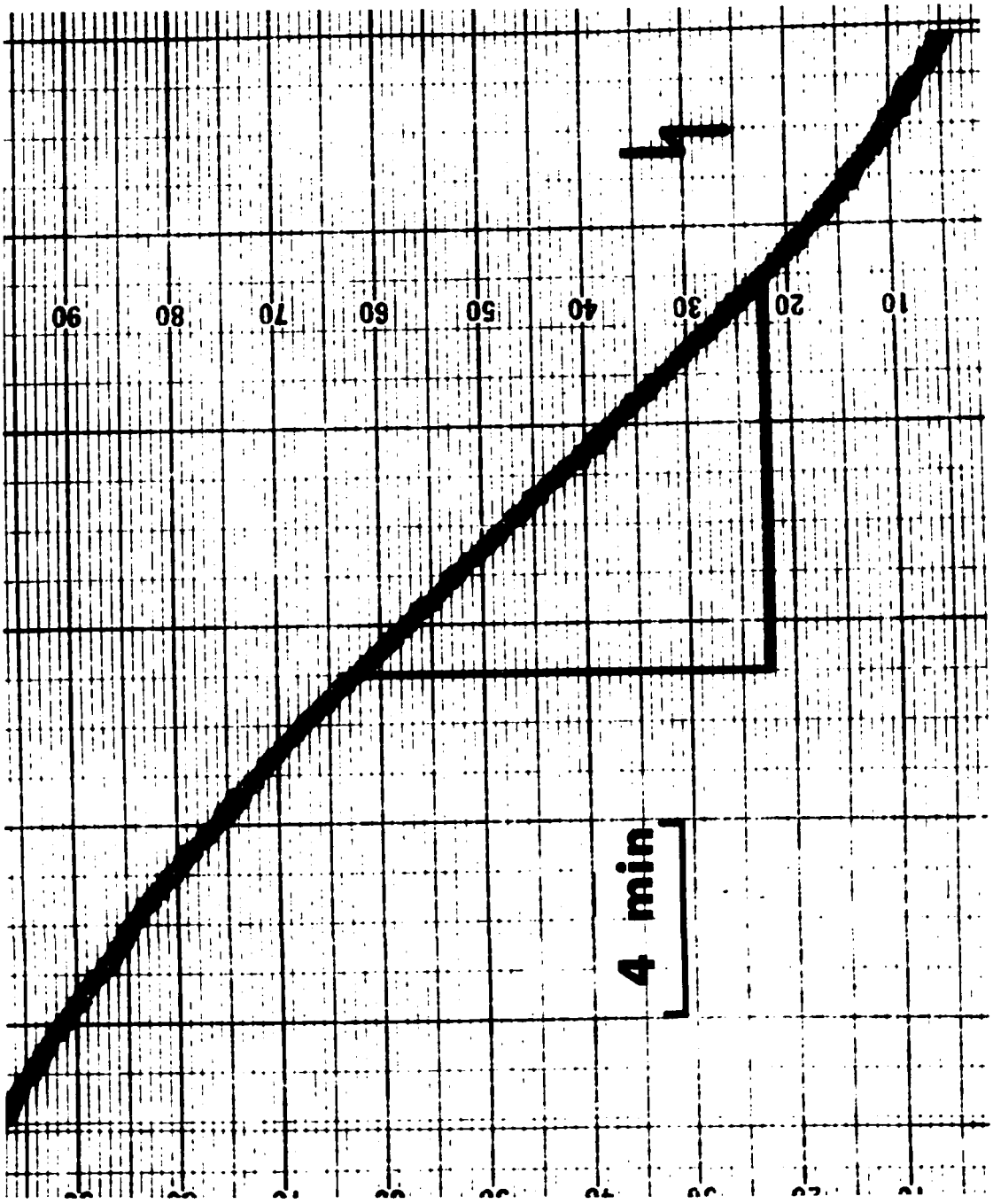

10
20
30
40
50
60
70
80
90
4 min

FIGURE 26

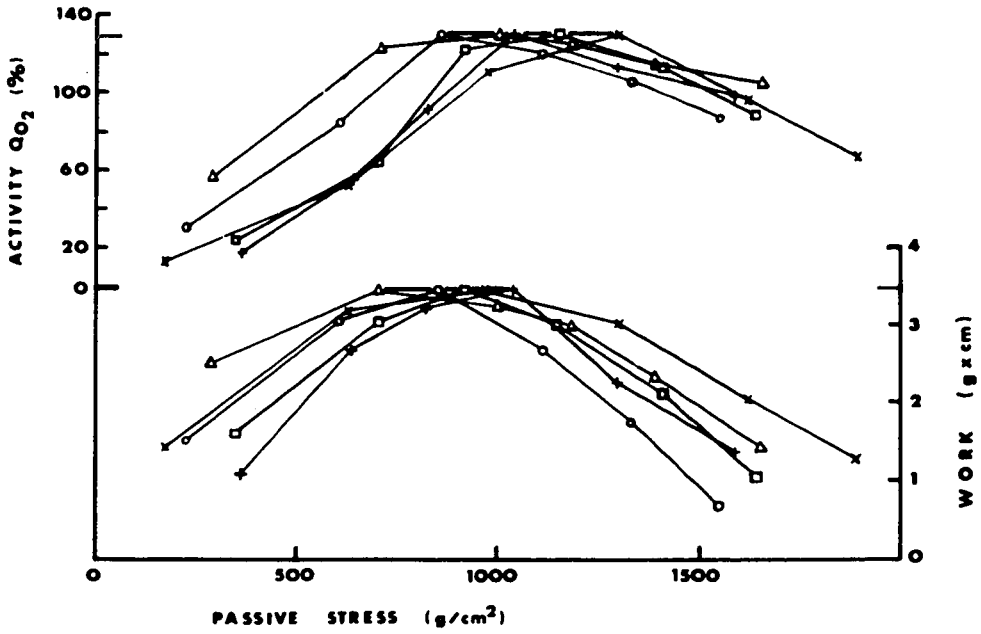
The effect of initial passive stress on the normalized isotonic responses of arterial segments to stimulation by norepinephrine and electrical means.

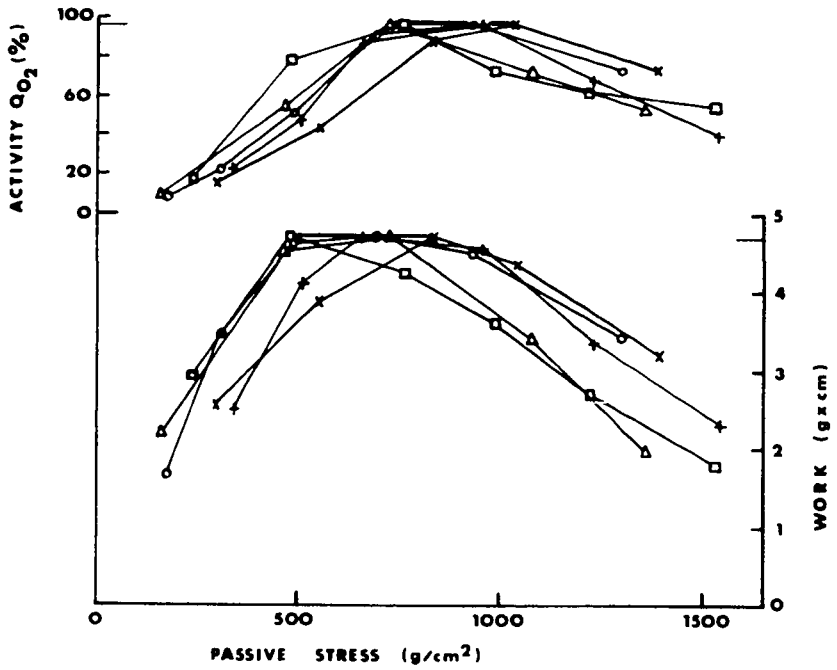
(a) Top

The results obtained by stimulation of the arterial segments with 5×10^{-8} g/ml norepinephrine. The active responses of each segment were normalized relative to their own maximum values before being multiplied by the average maximum of all the segments. The solid horizontal line at the top of each set of curves represents this average maximum value. The "activity" Q_{O_2} values of each segment that were normalized were first expressed as a percentage of the initial "basal" Q_{O_2} value for that segment. The same symbol was used to represent both the active mechanical and respiratory responses of each segment.

(b) Bottom

The results obtained by direct electrical stimulation of the arterial segments.


ACTIVITY Q_{O_2} (%)
140
100
60
20
0
WORK (g x cm)
4
3
2
1
0
0
500
1000
1500
PASSIVE STRESS (g/cm^2)


ACTIVITY Q_{O_2} (%)
100
60
20
0
WORK (g x cm)
5
4
3
2
1
0
0
500
1000
1500
PASSIVE STRESS (g/cm^2)

than the uncorrected measured values, but this difference became insignificant at the highest passive stresses. The curve is very approximate at the lowest passive stress value since the segments often shortened to a length which was less than their initial length and so the "corrected" "tension" Q_{O_2} values would actually be negative in value which would be pointless, so in these cases they were made equal to zero.

Since the total Q_{O_2} is a constant at a given passive stress, any decrease in the "tension" Q_{O_2} is reflected in a corresponding increase in the "activity" Q_{O_2} at that passive stress. These "corrected" "activity" Q_{O_2} values are also listed in the sample data in Tables 12 and 13. The effect of this estimated and arbitrary correction on the magnitude of the isotonic "activity" Q_{O_2} values is only illustrated here and in the future all "tension" Q_{O_2} and "activity" Q_{O_2} values will be of the uncorrected variety. A summary of all the results obtained in this series of experiments is listed in Tables 14 and 15.

The steady rate at which oxygen is consumed during the shortening process is shown in the sample traces in Figures 24 and 25. Even though the segment began shortening immediately after stimulation, there was, as usual, a delay of a few minutes before the steady rate was reached and also before it disappeared once the shortening had ceased. The traces also indicate how the steady state Q_{O_2} of the contracted segment was calculated.

3. Treatment of the Data

Normalization of all the "activity" Q_{O_2} and work values was performed for both the norepinephrine and electrical data in the usual way. The results are plotted against the passive stress in Figure 26 and are listed in Tables 14 and 15. Even with the comparatively high degree of

FIGURE 27

Variation of the "activity" Q_{O_2} of arterial segments with the external work that they performed when stimulated with norepinephrine (5×10^{-8} g/ml).

The "activity" Q_{O_2} values of each segment are expressed as a percentage of the initial "basal" Q_{O_2} value for that segment and are not corrected for possible changes which might occur in the "tension" Q_{O_2} during shortening. The solid line represents the regression line of the data with the dashed lines representing plus and minus one standard error of the estimate. The coefficient of correlation between the two parameters equals only 0.57 ± 0.16 (SE) while the regression line has the equation $y = 21.1x + 38.8$.

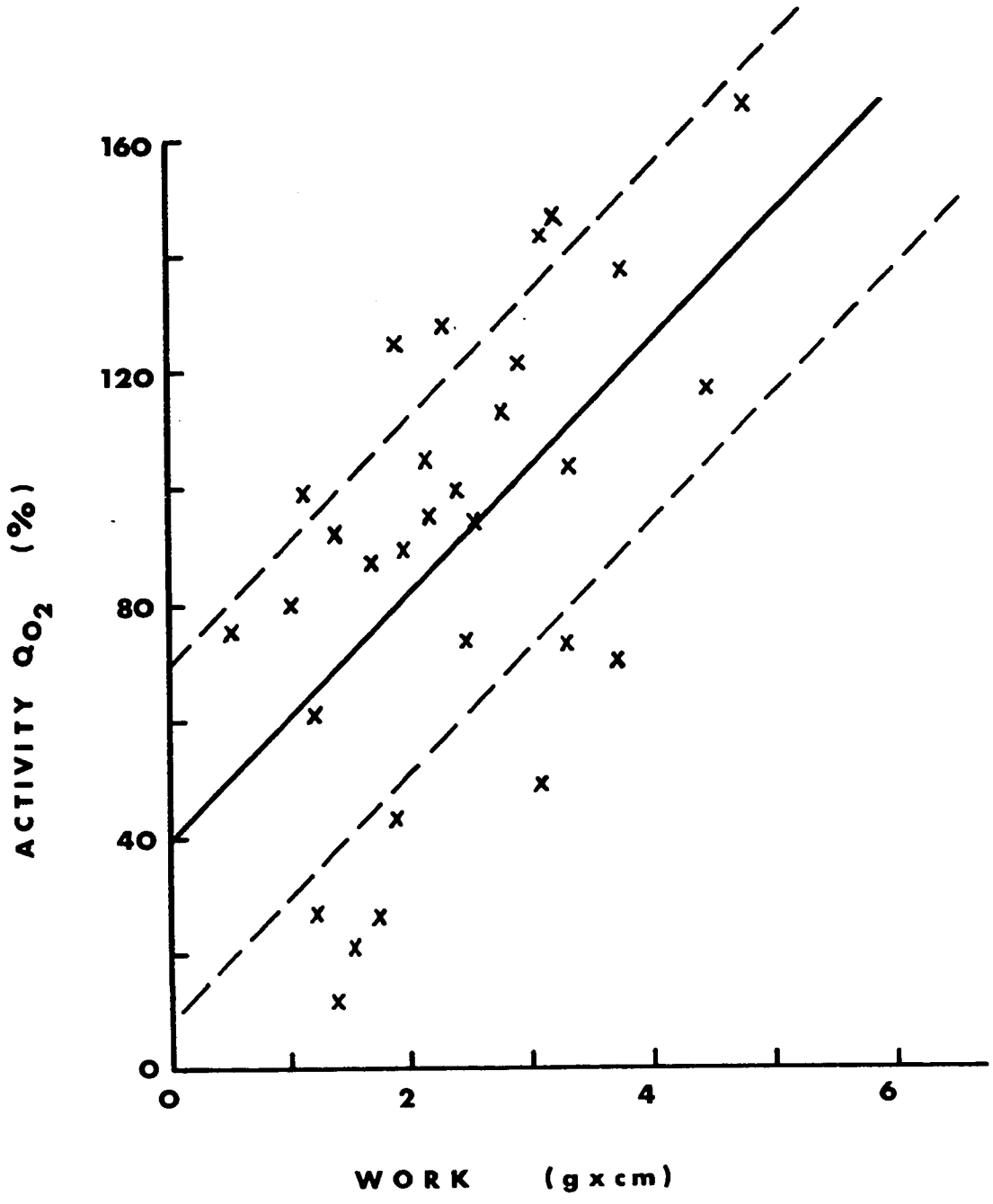

160
120
80
40
0
0
2
4
6
ACTIVITY Q_{O_2} (%)
WORK (g x cm)

FIGURE 28

Variation of the "activity" Q_{O_2} of arterial segments with the external work that they performed when stimulated electrically (30 volts for 30 seconds).

The "activity" Q_{O_2} values are not corrected for possible changes which might occur in the "tension" Q_{O_2} during the shortening process. The solid line represents the regression line of the data while the dashed lines represent plus and minus one standard error of the estimate. The coefficient of correlation between the two parameters equals 0.78 ± 0.12 (SE) while the regression line has the equation $y = 20.2x - 12.8$.

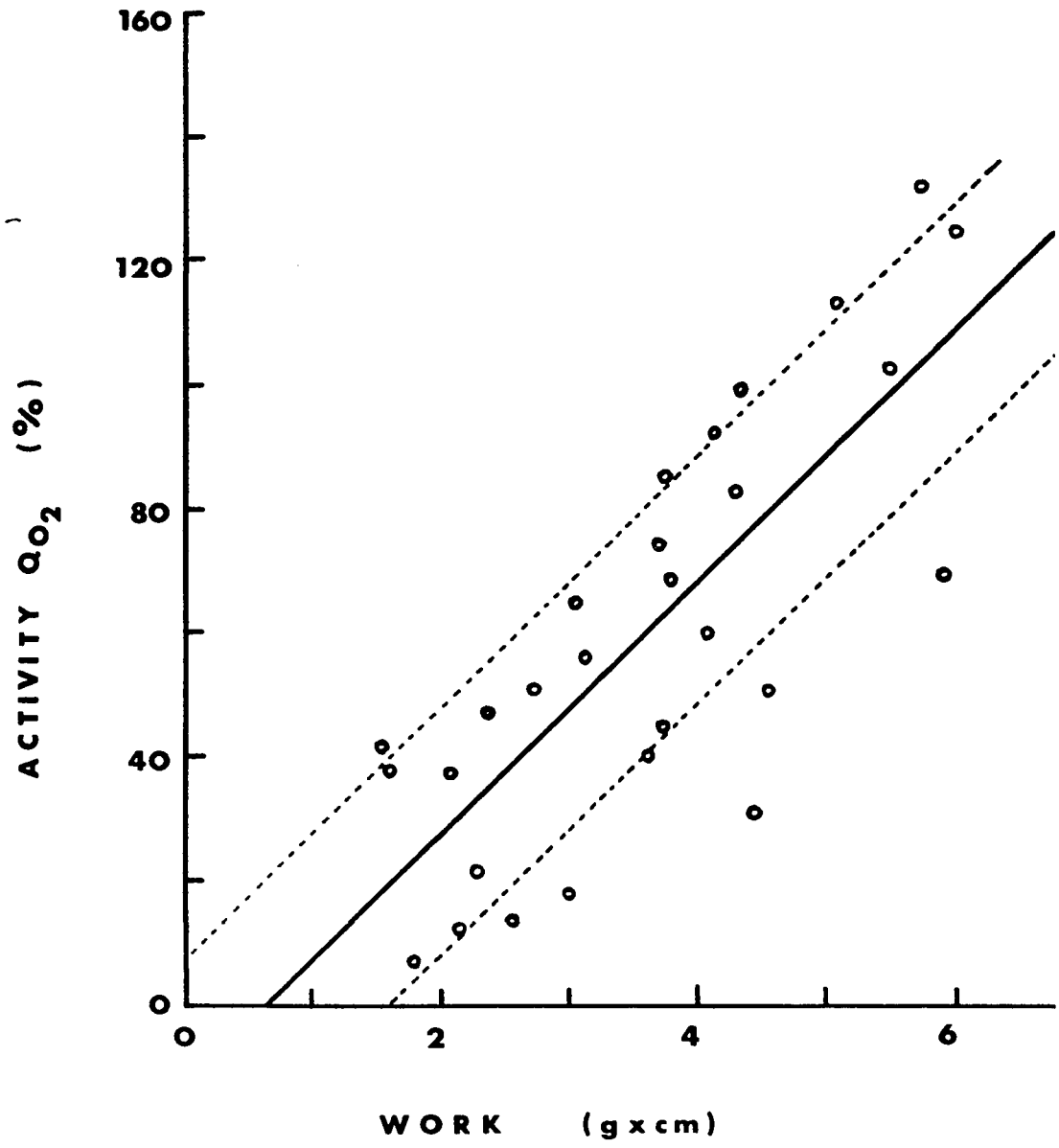

160
120
80
40
0
ACTIVITY Q_{O_2} (%)
0
2
4
6
WORK (g x cm)

TABLE 16

The Carbon Dioxide Production and R.Q. of Relaxed, Stretched and Isometrically Contracted Arterial Segments Stimulated with Norepinephrine

Artery No.	Basal Q_{CO_2} µl/mg /hour	Basal Q_{O_2} µl/mg /hour	Basal R.Q.	Tension Q_{CO_2} % *	Tension Q_{CO_2} µl/mg /hour	Tension Q_{O_2} % **	Tension Q_{O_2} µl/mg /hour	Tension R.Q.	Activity Q_{CO_2} % *	Activity Q_{CO_2} µl/mg /hour	Activity Q_{O_2} % **	Activity Q_{O_2} µl/mg /hour	Activity R.Q.
1	0.41	0.44	0.93	39	0.16	44	0.19	0.85	71	0.29	72	0.32	0.90
				63.5	0.26	63	0.28	0.94	123	0.505	126	0.555	0.91
									115	0.47	112	0.49	0.96
3	0.60	0.58	1.04	47	0.28	49.5	0.29	0.98	98	0.59	97	0.565	1.04
				48	0.29	52.5	0.305	0.95	108	0.65	113	0.655	0.99
				55	0.33	55	0.32	1.03					
5	0.47	0.49	0.96	28	0.13	29	0.14	0.93	13	0.06	13.5	0.07	0.89
				46	0.215	51	0.25	0.86	107	0.505	106.5	0.52	0.97
				68	0.32	77	0.38	0.84	113	0.53	119	0.58	0.91
									81	0.38	82	0.40	0.96
Average R.Q. Values			0.98 ± 0.03 (SEM)					0.92 ± 0.02 (SEM)					0.95 ± 0.02 (SEM)

* Expressed as a percentage of the Basal Q_{CO_2}

** Expressed as a percentage of the Basal Q_{O_2}

scatter still present in the data, it is apparent that as the passive stress is increased, both the external work and the "activity" Q_{O_2} increase to reach a maximum value after which they decline, the external work declining more steeply than the "activity" Q_{O_2}. With both types of stimulation a greater degree of passive stretch was necessary for the attainment of the maximum "activity" Q_{O_2} than for the maximum external work. These values of passive stretch were greater for the norepinephrine than for the electrical stimulus but were all within the physiological range. Comparing the norepinephrine and electrical data further, it is seen that the former has a significantly greater average maximum "activity" Q_{O_2} value but a smaller average maximum work value than the latter.

All of the uncorrected unnormalized "activity" Q_{O_2} data is shown plotted against the corresponding unnormalized external work data in Figure 27 for the norepinephrine experiments and in Figure 28 for the electrical experiments. The "activity" Q_{O_2} values of each segment are still expressed as a percentage of the "basal" Q_{O_2}. The coefficient of correlation between the two parameters is lower than in the isometric experiments but is still significantly different from zero, suggesting that the parameters are linearly related.

F. Carbon Dioxide Production and pH Changes

1. Isometric Experiments - Norepinephrine Stimulation

Using the polarographic technique the carbon dioxide production of three segments was measured. Table 16 summarizes the results that were obtained and the RQ values calculated. The carbon dioxide production of the relaxed, unloaded segment or its "basal" Q_{CO_2} divided by its

"basal" Q_{O_2} yielded the "basal" respiratory quotient for the segment, while the "tension" Q_{CO_2} divided by the corresponding "tension" Q_{O_2} gave the "tension" respiratory quotient. Similarly, the increment in the carbon dioxide production of the segment occurring during contraction or its "activity" Q_{CO_2} divided by its "activity" Q_{O_2} gave what was referred to as its "activity" respiratory quotient or RQ. All of the Q_{CO_2} and Q_{O_2} values used in calculating the various RQ values are expressed as the number of µl gas (at STP) produced or consumed per mg wet weight of muscle per hour.

The pH was recorded manually during three other experiments, and appeared to decrease at a steady rate which was probably primarily dependent on the magnitude of the carbon dioxide production of the segment. The time course of the carbon dioxide production during contraction of the segment, although more difficult to record than that of the oxygen consumption, was qualitatively not unlike the latter when the different delays of the two electrodes were taken into account. The variation in these pH rates from one segment to another, under otherwise identical conditions, was often of the order of ± 50%, so that the effect of different types of stimulation on the changes of pH when using different segments could not be effectively determined. For a 2% change in the oxygen concentration of the cuvette solution there was a drop in its pH of 0.03 to 0.06 pH units.

It was consistently observed that as the arterial segment consumed oxygen, it produced an approximately equivalent amount of carbon dioxide gas so that when the oxygen concentration had dropped by 2%, the carbon dioxide concentration had increased by about 2%. However, no change was observed in the rate of oxygen consumption by the relaxed or stretched or

contracted segment when no carbon dioxide was present as compared to when its concentration was about 2%. Since previous procedures have shown that the calibration of the oxygen and carbon dioxide electrodes was unaffected by the 2% increase in carbon dioxide and the 2% decrease in oxygen, respectively, it appears that the actual oxygen uptake by the segment is unaffected by either the small decrease in the oxygen concentration itself or by the corresponding increase in the carbon dioxide concentration up to a maximum of 2%. Accompanying this 2% increase in the carbon dioxide concentration of the cuvette solution was a drop in its pH of 0.03 to 0.06 pH units. Despite these combined changes in the carbon dioxide concentration and the pH in the environment of the arterial segment, its "basal", "tension" and "activity" oxygen consumption values remained constant. However, in order to minimize any effect of the lowered pH on the developed mechanical response of the segment, the latter was stimulated only when bathed in relatively fresh solution of pH 7.4.

No corrections were found to be necessary for the retention of carbon dioxide by the phosphate buffer. Since the solubility of carbon dioxide in the buffered Ringer-glucose solution as measured with the CO_2 electrode was found to be just slightly less than that in distilled water, it must be that the electrode, in effect, senses the presence of that gas which is retained by the phosphate buffer. The retention of carbon dioxide is also highly dependent on the pH of the solution but as the latter was allowed a maximum drop of only 0.03 to 0.06 pH units, the retention of the carbon dioxide, among other things, could not have changed very much.

2. Isometric Experiments - Electrical Stimulation

The results that were obtained for two segments and the corres-

TABLE 17

The Carbon Dioxide Production and R.Q. of Relaxed, Stretched and Isometrically Contracted Arterial Segments Stimulated Electrically

Artery No.	Basal Q_{CO_2} µl/mg /hour	Basal Q_{O_2} µl/mg /hour	Basal R.Q.	Tension Q_{CO_2} % *	Tension Q_{CO_2} µl/mg /hour	Tension Q_{O_2} % **	Tension Q_{O_2} µl/mg /hour	Tension R.Q.	Activity Q_{CO_2} % *	Activity Q_{CO_2} µl/mg /hour	Activity Q_{O_2} % **	Activity Q_{O_2} µl/mg /hour	Activity R.Q.
1	0.60	0.62	0.97	17.5	0.105	17	0.11	0.96	56	0.335	58	0.36	0.93
				48	0.29	43	0.27	1.07	87.5	0.525	86	0.53	0.99
				61	0.365	59	0.37	0.99	73	0.44	76	0.47	0.93
									67	0.40	68	0.42	0.95
5	0.52	0.56	0.93	23	0.12	23	0.13	0.93	38	0.20	40	0.23	0.88
				48	0.25	50	0.28	0.89	63.5	0.33	64	0.36	0.91
									30	0.155	32	0.18	0.86
Average R.Q. Values			0.95 ± 0.01 (SEM)					0.97 ± 0.03 (SEM)					0.92 ± 0.02 (SEM)

* Expressed as a percentage of the Basal Q_{CO_2}

** Expressed as a percentage of the Basal Q_{O_2}

TABLE 18

The Carbon Dioxide Production and R.Q. of Isotonically Contracting Arterial Segments Stimulated with Norepinephrine

Artery No.	Basal Q_{CO_2} µl/mg /hour	Basal Q_{O_2} µl/mg /hour	Basal R.Q.	Tension Q_{CO_2} % *	Tension Q_{CO_2} µl/mg /hour	Tension Q_{O_2} % **	Tension Q_{O_2} µl/mg /hour	Tension R.Q.	Activity Q_{CO_2} % *	Activity Q_{CO_2} µl/mg /hour	Activity Q_{O_2} % **	Activity Q_{O_2} µl/mg /hour	Activity R.Q.
2	0.31	0.36	0.87	45	0.14	47	0.17	0.84	84	0.26	74	0.27	0.98
				61	0.19	56	0.20	0.93	139	0.43	113	0.41	1.06
				64.5	0.20	64	0.23	0.88	126	0.39	105	0.38	1.02
3	0.485	0.47	1.03	58	0.28	63	0.30	0.94	68	0.33	73	0.34	0.97
				62	0.30	74	0.35	0.86	133	0.645	137	0.645	1.00
				68	0.33	78	0.37	0.90	99	0.48	99	0.465	1.03
Average R.Q. Values			0.95 ± 0.06 (SEM)					0.89 ± 0.01 (SEM)					1.01 ± 0.01 (SEM)

* Expressed as a percentage of the Basal Q_{CO_2}

** Expressed as a percentage of the Basal Q_{O_2}

TABLE 19

The Carbon Dioxide Production and R.Q. of Isotonically Contracting Arterial Segments Stimulated Electrically

Artery No.	Basal Q_{CO_2} µl/mg /hour	Basal Q_{O_2} µl/mg /hour	Basal R.Q.	Tension Q_{CO_2} % *	Tension Q_{CO_2} µl/mg /hour	Tension Q_{O_2} %**	Tension Q_{O_2} µl/mg /hour	Tension R.Q.	Activity Q_{CO_2} % *	Activity Q_{CO_2} µl/mg /hour	Activity Q_{O_2} %**	Activity Q_{O_2} µl/mg /hour	Activity R.Q.
2	0.58	0.63	0.92	46.5	0.27	48	0.30	0.89	44	0.255	45	0.28	0.91
				52	0.30	54	0.34	0.89	84.5	0.49	92	0.58	0.84
				69	0.40	66	0.42	0.95					
4	0.47	0.53	0.88	45	0.21	39	0.21	0.99	40	0.19	40	0.21	0.91
				62	0.29	57	0.30	0.97	83	0.39	69	0.37	1.05
									55	0.26	51	0.27	0.96
									38	0.18	38	0.20	0.92
Average R.Q. Values			0.90 ± 0.01 (SEM)					0.94 ± 0.02 (SEM)					0.93 ± 0.02 (SEM)

* Expressed as a percentage of the Basal Q_{CO_2}

** Expressed as a percentage of the Basal Q_{O_2}

ponding RQ calculations are summarized in Table 17. The pH was recorded in three of the other experiments and again, it was found to decrease at a rate which was dependent on the rate of oxygen consumption, and hence, on the rate of carbon dioxide production of the segment. The maximum drop in the pH of the cuvette solution was about 0.04 pH units.

3. Isotonic Experiments - Norepinephrine Stimulation

Measurements of the carbon dioxide production were made on two segments. Table 18 shows all such results and the calculated RQ values. The pH changes were monitored in the three other experiments of this series. The maximum decrease in the pH from its initial value of 7.40 was a drop of 0.05 pH units which paralleled a 2% drop in the oxygen concentration of the cuvette solution.

4. Isotonic Experiments - Electrical Stimulation

The carbon dioxide data obtained on two segments of this series is listed in Table 19. The changes in pH were monitored in two experiments. Its rate of fall during active shortening of the segment was not significantly different from that observed in the norepinephrine experiments. The maximum drop in the pH that was observed during a 2% drop in the oxygen concentration was from 7.40 to 7.33, i.e. a drop of 0.07 pH units.

VII. DISCUSSION

A. Technical Considerations

The limitations of the equipment used in Methods A and B as well as some relevant technical considerations and reasons for discontinuing some of the experiments are discussed in the Appendix.

B. "Basal" Q_{O_2} Values of Adventitia-intact and Adventitia-free Segments

A comparison of the "basal" Q_{O_2} values of the adventitia-intact and adventitia-free segments shows that the latter have absolute values which are about 35% to 55% greater than the absolute values of the former. This result roughly corresponds with the fraction of the intact femoral artery which we found to consist of muscular media and fibrous adventitia on a wet weight basis. We found, by rather crude dissection, that the tunica adventitia made up an average of 32% of the wet weight of the intact segment so that, theoretically, stripping off the adventitia should increase the "basal" Q_{O_2} by about 47%. The agreement between the expected difference in the respiration of the adventitia-intact and adventitia-free segments and the actual observed difference makes it improbable that the stripping procedure injured the arterial muscle tissue to such an extent as to significantly affect its basal respiration.

The average value of the "basal" Q_{O_2} that was determined for the adventitia-free segments by the polarographic and modified Warburg techniques was slightly but not significantly less than the average

values previously published for the modified Warburg technique by Kosan and Burton (1966). A possible explanation may be that about one-half of the segments used in the preliminary experiments were circumferentially stretched to a variable extent. According to the present study this treatment would have increased their Q_{O_2} values significantly above their unstretched "basal" values.

All of these values are still somewhat greater than comparable results reported by other investigators whose values have ranged from 0.1 to 0.5 µl/mg wet weight/hour (see Table 1). Some possible causes of our higher "basal" Q_{O_2} are: 1) In our experiments there was no control on the quality of the dogs. Despite this factor the SEM of these results is small, about ±7% (67 segments). Therefore the variation between dogs is not very significant. 2) Does the anaesthetic raise the "basal" Q_{O_2}? Howard et al. (1965) found that pentobarbital anaesthesia had no significant effect on the basal respiration of hamster arterioles and venules. Therefore, the anaesthetic probably did not significantly influence the "basal" Q_{O_2} of our arterial segments. 3) Do we expect the femoral artery to have the same Q_{O_2} as the aortic tissue used by other workers? We know that on a weight to weight basis the femoral artery has more smooth muscle and that stripping off the adventitial layer accentuates this (compare adventitia-intact and adventitia-free "basal" Q_{O_2} values). 4) We may be underestimating the wet weight of our preparations. This is not likely since our wet weight/dry weight ratio of 4 is comparable to that reported by Peterson (1962) for a variety of peripheral arteries. 5) Effect of diffusion: although diffusion of oxygen may have limited the basal respiration of the thicker aortic preparations used in other studies this effect

was not significant in our experiments since both the Warburg and polarographic methods give the same average "basal" Q_{O_2}. Also, no significant correlation was found to exist between the thickness of the unstretched arterial wall of our segments and their "basal" Q_{O_2}. Therefore, the supply of oxygen to our preparation is definitely adequate.

Therefore, there appear to be no systematic errors in the determination of the "basal" respiration. The "basal" Q_{O_2} values reported here are as soundly based as any that have been published.

It is difficult to estimate how close our "basal" Q_{O_2} values are to those of femoral arterial tissue *in vivo*. The presence of the adventitial coat, as our data indicates, depresses the "basal" Q_{O_2} by about 40% while the application of a passive pressure of 100 mm Hg tends to almost double the rate of respiration of the segment so that the resultant respiration of the adventitia-intact segment which is stretched to a physiological extent is somewhat greater than our average "basal" Q_{O_2} values. The actual result *in vivo* is further influenced by the longitudinal stretch of the artery and differences in its blood or oxygen and nutritional supplies. Approximately sixty to ninety minutes elapsed between the time the segment was removed from the dog and the time when the first measurements were made on it. During the interval the segment was continuously bathed in physiological salt solution. If metabolic changes did occur there is no way in which their extent can be evaluated. Because of this, extrapolation to the Q_{O_2} *in vivo* is uncertain.

In the majority of cases, the "basal" respiration of the segment at 37°C remained unchanged over a period of several hours. The stability

of this preparation is noteworthy and deserves to be exploited further. Is this stability a characteristic of slowly metabolizing tissues?

C. Tension Q_{O_2} Values of Adventitia-intact and Adventitia-free Segments

In this respect results very similar to our own have been obtained by several other workers using different kinds of muscles. Thus, Evans (1923) has observed that resting cat intestinal muscle increased its oxygen usage significantly when stretched up to a certain limit, beyond which the usage was reduced again. In our experiments, however, the decrease in the uptake at high passive tensions was quite slight. Essentially similar results have been obtained by Evans and Hill (1914) for the heat production of frog skeletal muscle and by Evans and Matsouka (1915) and Meyerhof, Gemmill and Benetato (1933) for the oxygen uptake of cardiac and skeletal muscles as well as by Feng (1932) for both the heat production and oxygen uptake of resting frog skeletal muscle at different degrees of stretch. More recently, a similar dependence of oxygen uptake on stretch has been reported by Bulbring (1953) for guinea pig intestinal smooth muscle, by Lee (1960) for isolated cat papillary muscle and by Whalen (1960) and Whalen, Collins and Berry (1962) for cardiac and skeletal muscles, respectively.

The maximum values of the "tension" Q_{O_2} of the adventitia-intact segments ranged from 50% to 80% of the "basal" Q_{O_2} while the corresponding maxima of the adventitia-free segments ranged from 53% to 73%. It is to be expected that these two sets of "tension" Q_{O_2} maxima would not be much different when each is expressed as a percentage of its own "basal" Q_{O_2}, provided that there is no limitation of oxygen in the thicker adventitia-intact segment.

That the thickness of the segment is not a factor in determining the "basal" Q_{O_2} or the maximum "tension" Q_{O_2} is shown by the quantitatively similar data that have been obtained with different adventitia-intact segments whose unstretched thicknesses differed by as much as 80%. This "tension" Q_{O_2} (actually the "tension" Q_{O_2}/"basal" Q_{O_2} ratio) was found to be independent of the thickness over the range of thicknesses encountered. This could result if both the absolute "tension" Q_{O_2} and "basal" Q_{O_2} are dependent on the thickness in the same way. The increase in the Q_{O_2} which occurs with increase in the passive tension is accompanied by a thinning of the wall of the arterial segment. The progressive decrease in thickness shortens the diffusion path for oxygen and therefore may increase the partial pressure of oxygen in the arterial wall and hence the Q_{O_2} as well. Due to the faster diffusion which occurs as the wall thins down, increased stretch could also lead to a decrease in the concentration of some metabolic product which inhibits respiration. The application of the diffusion equations of Warburg (1923) and of Hill (1929) to our experimental conditions gives no support to the existence of a lowered pO_2 in the unstretched wall because of diffusion limitation. Therefore there is no need to suppose that the Q_{O_2} of arterial tissue increases with stretch due to an increase in its pO_2. Likewise, there seems to be no grounds for thinking that CO_2 or lactate accumulate to levels known to affect the Q_{O_2}. Thus there is no strong reason for thinking that the "tension" Q_{O_2} merely results from mechanical thinning of the wall of the arterial segment.

It was also noticed that the adventitia-intact segments required higher passive pressures to reach their maximum "tension" Q_{O_2} value com-

pared to the adventitia-free segments which reached their maximal values at passive pressures which were 50 to 100 mm Hg less. The extensibility of smooth muscle is so much higher than that of the primarily collagenous adventitia, that it may be that the presence of the adventitial sheath prevents the smooth muscle from absorbing the full effect of the higher applied pressures with the "tension" Q_{O_2} of the muscle behaving accordingly. It is assumed that the smooth muscle is the major respiring element in the arterial wall and that relatively small amounts of it are found in the adventitia as compared to the media.

A comparison of some of the results obtained using the polarographic technique with those obtained using the Warburg technique shows that the optimal passive stress range of 700 to 1100 g/cm^2 is equivalent to an optimal passive tension range of about 21 to 29 g/cm and this compares favourably with the range of passive tension values of 15 to 30 g/cm at which the maximum "tension" Q_{O_2} values were observed in the Warburg experiments. The corresponding values of the maximum "tension" Q_{O_2} ranged from 55% to 84% and from 53% to 73% with the two techniques and hence also compare well. A quantitative study by Lee (1960) on isolated cat papillary muscle yielded data which is close to our own. He obtained an average value of the maximum "tension" Q_{O_2} which equalled 93% of the "basal" resting Q_{O_2} at a passive stress of 1200 g/cm^2.

As the passive stretch on the segment was increased the "tension" Q_{O_2} often decreased somewhat after attaining its maximum value. It is not likely that this decrease was due to damage to the muscle segment by overstretching it, since the "basal" Q_{O_2} remained just slightly above its initial value and the muscle responded readily to stimulation. In

fact, with the electrically stimulated segment which exhibited hysteresis when its passive pressure was progressively decreased, the contractile responses once again increased to a maximum before falling off. Thus, it is difficult to see how slow stretching of the arterial muscle could "reversibly damage" the muscle cell membrane or contractile protein to such an extent as to significantly influence the "tension" Q_{O_2}.

D. Possible Causes of the "Tension" Q_{O_2}

Although no specific reasons for the existence of the "tension" Q_{O_2} have, to date, been proposed and confirmed experimentally, several general possibilities have been mentioned which might explain this phenomenon. Whalen (1960) has suggested that the metabolism of stretched heart muscle may be elevated as preparation for a forthcoming contraction or that the energy of maintenance of a passive tension is itself greater than the metabolism of the resting unstretched muscle.

Bulbring (1953) has proposed that the increased "surface energy" of stretched intestinal muscle is responsible for its elevated oxygen uptake but has not specifically defined what the "surface energy" itself is. It may include an effect described by Freeman-Narrod and Goodford (1962) who found that the rate of exchange of cations across the cell membrane of intestinal smooth muscle cells increased with increasing stretch. It is possible that this higher exchange rate requires additional energy and that respiratory processes in the muscle cells are called upon to supply some of it. However, it is not known whether the same events occur in stretched arterial muscle, let alone if they are responsible for its increased oxygen uptake.

It could also be possible that the increased uptake of oxygen with stretch of the muscle may merely reflect some change in the actual efficiency of utilization of the oxygen but this is pure speculation.

E. Effect of Time and Previous Stimulation on the Active Responses

With both the adventitia-intact and adventitia-free segments, the submaximal dose of norepinephrine of 5×10^{-7} g/ml used in the Warburg set-up gave successive mechanical and respiratory responses which were virtually identical. No staircase of summation phenomena or changes in sensitivity with time as observed by Leonard (1957) and Furchgott and Bhadrakom (1953) on aortic strips were apparent in our experiments. The time required for complete relaxation, after washout of the drug and before stimulation could be repeated, was only 10 to 20 minutes. The contractile response was fairly large, being equal to about 70% of the maximal value. The time over which the muscle segment could be made to respond consistently to this stimulus extended up to seven hours with many, but not all, of the segments. The behaviour of the oxygen uptake of the segment was generally as consistent as the mechanical response causing it. Although the oxygen consumption of the contracted segment could not be recorded continuously in the Warburg experiments, the average consumption by the contracted segment was always significantly greater than its resting oxygen consumption.

It was observed that the concentration of norepinephrine used in the polarographic experiments of 5×10^{-8} g/ml gave responses which were not dependent on previous stimulation if a period of relaxation of about 15 minutes was allowed after the drug was washed out. When the concentration used was 5×10^{-7} g/ml of norepinephrine this was

not the case, even if the period of time allowed for relaxation was doubled.

Hinke and Wilson (1962a) have reported that catecholamine stimulation of the intimal surface of the artery results in a response equivalent in magnitude to that obtained with a five to ten times greater drug concentration when the latter is applied to the adventitial surface only. In the modified Warburg technique, only the adventitial surface was directly stimulated by the drug and may be the reason for the lower sensitivity of the segment to the norepinephrine. That the same effect was observed with direct electrical stimulation of the segment may be due to the slow release of norepinephrine from the arterial wall with this type of stimulation as has been shown by Gillis and Yates (1960).

The effect of time and previous direct electrical stimulation on subsequent responses was the same as that observed in the norepinephrine experiments, i.e., no significant effect of time existed for as long as seven hours in both the isometric and isotonic experiments. Since no change was noticed in the magnitude or shape of the electrically-induced mechanical responses of a given segment after even six previous stimulations it appears that relatively little norepinephrine is released by each electrical stimulus compared to the total amount of the hormone stored in the arterial wall. On the other hand, the slow component of the mechanical response could have been produced by means other than norepinephrine being released from the arterial wall which would eliminate the depletion problem.

It was noticed in all of these experiments that the rate of oxygen uptake remained constant for as long as the tension developed was

roughly constant. When the isometric or isotonic response of the segment began to decrease, either spontaneously or not, the oxygen consumption diminished at about the same rate so that when relaxation was complete, the oxygen uptake was equal to its prestimulation value, indicating that there is little difference in the time course of the mechanical and metabolic processes.

An explanation of the close temporal relationship between contraction and energy production in arterial muscle may be found in the observation of Lundholm and Mohme-Lundholm (1965) that the energy from the splitting of preformed high energy phosphates was far from sufficient for a maximal isometric contraction of vascular muscle, which indicates that the energy has to be supplied continuously. This circumstance could account for the more intimate relationship between contraction and energy production in smooth than in striated muscle since it has also been estimated by Lundholm and Mohme-Lundholm (1965) that, in comparison, frog striated muscle consumeswith a maximal contraction only about 3% of its known preformed high-energy phosphate compounds.

In all of these experiments it appears that stripping the adventitial coat off of the otherwise intact section of blood vessel wall was probably a less traumatic procedure than the cutting of helical strips performed by other workers, for neither nitrate pretreatment nor protracted equilibration was found to be necessary for inducing a steady base-line or establishing consistent responses in the majority of segments. Furchgott and Bhadrakom (1953) have found helical strips of aorta to be much poorer in this respect.

Such consistency in the spontaneous and active tone of arterial muscle is somewhat surprising in view of the changes in ionic distribu-

tion which are known to take place in tissues incubated in vitro for any length of time in hours. Our experiments lasted up to seven to nine hours so it is probable that some of this ionic unbalance must have been present during the major portion of each experiment and yet no significant differences were noted during the course of most of the experiments in the values of the spontaneous and active tone. Their magnitudes varied no more than what would be expected over a short time interval and there was no consistent direction of change.

F. Effect of Initial Stretch on the Active Responses

1. Norepinephrine-induced Isometric Responses

a) Pattern of Response

Stimulation of the adventitia-intact segments with 5×10^{-7} g/ml norepinephrine gave maximum active pressures of about 36 mm Hg at an average passive pressure of about 150 mm Hg. Although the passive pressure value is still in the physiological range, the ratio of the active to passive pressure is quite low being equal to only 0.24. Often, the magnitude of the "activity" Q_{O_2} paralleled that of the active pressure, but the two maxima also often occurred at slightly different values of passive pressure. Generally, the "activity" Q_{O_2} maximum followed that of the active pressure, indicating that the efficiency of the contractile process was greater at the lower passive pressure values. The relationship deviated from a proportional one in one other important respect also, in that the "activity" Q_{O_2} decreased beyond its maximum at a slower rate than did the active pressure so that the efficiency of the contractile process remained less than its optimal value.

Essentially the same results were obtained with adventitia-free

segments. However, the active pressure and "activity" Q_{O_2} values of the adventitia-free segments reached their maxima at lower passive pressures than when adventitia-intact segments were used. Once again, it may be that the adventitial layer does absorb some of the fairly low pressure applied across the segment wall, necessitating higher passive pressures in order that the smooth muscle cells themselves will be affected. The actual values of the maximum active pressure ranged from 32.8 to 61.2 mm Hg and as would be expected, were equivalent to those obtained with the adventitia-intact segments. The maximum values of the "activity" Q_{O_2} in the two cases as a percentage of the "basal" Q_{O_2} were also not significantly different.

Since the maximum mechanical response was thought to represent a good estimate of the amount and contractility of the vascular smooth muscle present in each segment, it was decided to reduce the variation between responses from different arterial segments arising from these differences in their vascular muscle by normalizing each of the active tension and corresponding "activity" Q_{O_2} values of each segment relative to the corresponding maximum value obtained for that segment. In order to give more realistic curves, each of the two sets of data was then multiplied by the average maximum for that group. The responses obtained at resting length or zero mm Hg passive pressure, although used by some other workers as the normalization or reference point, were found to exhibit just as much variation as the maximum responses. The responses obtained at this unphysiological condition were also very small in magnitude.

Normalization relative to maximum responses still yielded considerable scatter at any one passive tension, but certain tendencies still

stood out. It appeared in these experiments that the responsiveness of a segment to a standard stimulus increased to a certain point with increased passive tension or stretch of the vascular smooth muscle cells. This result was also obtained in a less quantitative fashion in the preliminary group of experiments that was reported.

All of the adventitia-free segments used in the Warburg method attained maximum responses at passive tensions ranging from 17 to 45 g/cm. The average maximum active tension equalled 16.3 g/cm while the average maximum "activity" Q_{O_2} equalled 126% (of the average "basal" Q_{O_2}, approximately). In the majority of the segments the maximum "activity" Q_{O_2} occurred at a greater passive tension than the maximum active tension. With greater passive tensions the arterial segment apparently became progressively less responsive to the stimulus and the active tension and "activity" Q_{O_2} values steadily decreased, the former values decreasing more rapidly than the latter with the passive tension. The efficiency of the muscle at this stage was, therefore, comparatively low.

The behaviour of the "activity" Q_{O_2} and active tension or stress with increasing values of passive tension or stress was remarkably consistent even in the preliminary series of experiments where, though the contracting segments performed some isotonic work and also developed some isometric tension, the correlation between the increment in the Q_{O_2} and the degree of contraction was quite good. Only the ascending portion of both active response curves was obtained in this preliminary series which indicates that the maximum degree of passive stretch was still relatively low. Later series consistently showed that the "activity" Q_{O_2} began to decrease at fairly high values of passive tension or stress. This was observed both with the norepinephrine-induced and electrically-induced

responses of the arterial segment.

Our data generally shows that the rate of oxygen uptake of the contracted arterial segment varies with the stretch of the muscle in very much the same quantitative manner as the active mechanical response. Thus the correlation between the two types of response is reasonably good over a wide range of muscle lengths. This proportionality is demonstrated by the significant correlation between the two parameters obtained with both experimental procedures. In the Warburg arrangement the correlation line equation of $y = 6.8x + 16.9$, $(r = 0.89)$, shows that the muscle consumed per g/cm of maintained active tension an average amount of oxygen equivalent to 6.8% of its "basal" Q_{O_2} or about 0.038 µl/mg/hour. The corresponding values in the polarographic arrangement were a correlation line equation of $y = 0.30x + 0.21$, $(r = 0.93)$, and an "activity" Q_{O_2} of $0.30\%/g/cm^2$ or about 0.0016 µl/mg/hour/g/cm^2. Assuming a wall thickness of 0.015 cm for the arterial segment converts the last value to 0.053 µl/mg/hour per g/cm which is about 30% larger than the corresponding Warburg value.

Unpublished work has shown that the proportionality between the "activity" Q_{O_2} and active stress was not unique to those responses which were influenced by stretch alone but was also retained by those active responses which were obtained by stimulation of the muscle in bathing solutions containing various levels of sodium and potassium ions. The same situation was found to occur with changes in pH except that at the higher pH values (7.8) the ratio "activity" Q_{O_2}/active stress dropped. This may imply a higher metabolic efficiency at these pH values. Different concentrations of epinephrine and norepinephrine had equal effects on both the mechanical and oxidative responses of the arterial segment, i.e.,

their ratio remained constant. While the ratio was unchanged between 32-37°C, it decreased for lower temperatures and increased at the higher. Presumably temperature depresses the metabolic efficiency at the higher and elevates it at the lower temperatures.

The high correlation between the "activity" Q_{O_2} and active tension or stress is somewhat unexpected, because as Lundholm and Mohme-Lundholm (1962a) have demonstrated, the oxygen uptake alone of arterial muscle is not a reliable criterion or indicator of its energy metabolism. They found that adrenaline stimulated the glycolysis and that glycolysis inhibiting substances and other metabolic inhibitors such as dinitrophenol blocked the contractility in vascular muscle even under aerobic conditions and in the presence of glucose to such an extent that it was estimated that up to 50% of the total energy required for these contractions could have been obtained from glycolytic reactions. They also mention that the synthesis of high-energy phosphate compounds via respiratory metabolism probably bears a poor stoichiometric relationship to the oxygen consumption, since appreciable variations in the phosphorylation/oxidation ratio may occur; nonetheless, they found the relationship between the synthesis of these phosphate compounds and the lactic acid production from anaerobic glycolysis to be fairly close. Determination of the energy metabolism from the lactic acid production, however, also has its drawbacks such as the necessity of anaerobic conditions and the relatively long time periods between the discontinuous measurements.

b) Comparison of Warburg and Polarographic Data

The maximum active responses that were observed with stimulation by 5×10^{-7} and 5×10^{-8} g/ml norepinephrine in the Warburg and polarographic experiments, respectively, and the corresponding ranges of

TABLE 20

The Maximum Active Responses of Arterial Segments to Stimulation by Norepinephrine

Average Maximum Values				Passive Stress Range		Activity Q_{O_2} / Active Tension Average Values (%/g/cm)	
Activity Q_{O_2}		Active Tension					
Polarog.	Warburg	Polarog.	Warburg	Polarog.	Warburg	Polarog.	Warburg
115 %	126 %	391 g/cm^2 (12 g/cm)	16 g/cm	500-900 g/cm^2 (15-27 g/cm)	17-44 g/cm	10.2	6.8

passive tension values are shown in Table 20. The agreement between these values as well as between the average ratio of the "activity" Q_{O_2} to its active tension in the two sets of experiments is seen to be quite good. In both cases the efficiency of the muscle in maintaining an active tension decreases beyond the point of maximum active response since a greater "activity" Q_{O_2} was found to be necessary to maintain a given active stress or tension.

The above results are in relatively good agreement considering the differences between the two techniques. The method of loading the segments was quite different, in one case the segment being stretched longitudinally by 40% and developing its active tension in a direction tangential to its long axis, while the other technique involved no longitudinal stretching of the segment and the active stress was again developed at right angles to the length of the segment and the segment was not kept cylindrical.

The more physiological method of loading the segment in the Warburg technique may be the reason for the greater metabolic efficiency of the contracted segments when loaded in this manner as compared to the polarographic method. This was the case even though in the Warburg set-up 10% of the segment length was outside the sutures and therefore was not stretched and did not contract.

Regarding the scatter in the polarographic data, the spread of passive stress values or their equivalent passive tension values over which the maximum active responses are observed is somewhat less than the corresponding spread of passive tension values in the Warburg experiments. On the other hand, the scatter in each of the active responses at any given value of passive stress is comparable to that found at the corresponding passive tension in the Warburg data. Since any difference in the

cross-sectional area of the arterial segment affects the stress per muscle cell and the number of muscle cells under stress in an inversely proportional manner, taking into account the cross-sectional area rather than just the length of the segment may have little effect in reducing the scatter.

c) Comparison of our Data with that of other Reports

That the mechanical responses of other types of muscle are similarly dependent on the degree of initial stretch has been known since the work of Evans and Hill (1914) on frog sartorius muscle. Qualitatively similar results have also been obtained by Starling (1918) on heart muscle, Winton (1926) on dog's retractor penis smooth muscle, Brocklehurst (1926) on cat's ileum smooth muscle, Bulbring (1953) on guinea pig intestinal muscle, Abbott and Lowy (1958a) on molluscan smooth muscle and by Lee (1960) on isolated cat papillary muscle.

A similar proportional relationship between the activity oxygen consumption and the corresponding active tension values obtained at different degrees of muscle stretch has to some extent been observed by some other workers, including Bulbring (1953) with guinea pig intestinal smooth muscle, Fischer (1931) and Gemmill (1936) with frog skeletal muscle and Lee (1960) with cat papillary muscle. On the other hand, Whalen (1960, 1961) obtained data with both skeletal and cardiac muscle which indicated that although the active mechanical responses of these muscles increase to a maximum value and then fall off as the muscle is stretched, the "activity" Q_{O_2} and total Q_{O_2} values both keep increasing.

The proportionality between the tension developed and the energy liberated by isometrically contracting muscle has also been demonstrated for other muscles but where the heat production has been employed as the

criterion of the energy production. Good correlation between these two parameters has been demonstrated by Evans and Hill (1914) for heart muscle, Hill (1928) and Hartree (1931) for frog skeletal muscle and by Bozler (1930, 1936a, 1936b) for snail smooth muscle. The latter worker also found that the maintenance of tension by snail smooth muscle required a proportionate and continuous expenditure of heat or energy.

Chemical studies have revealed a similar correspondence between the active tension developed and the energy liberated by contracting muscle. This has been shown by Lundsgaard (1930) and Carlson and Siger (1960) who both measured the breakdown of creatine phosphate by active skeletal muscle and by Beviz, Lundholm et al. (1965) who measured the summed hydrolysis of ATP and CrP and the tension developed during the adrenaline-induced contraction of isolated bovine mesenteric artery. In all of these cases, however, the total energy produced during either the development of the tension only or during a muscle twitch or short tetanus was employed to calculate the value of the proportionality constant. The equivalent constant from our data includes a time factor since it represents the average or steady state rate of oxygen uptake during the maintenance rather than the development of tension so that any comparison with the above values would be meaningless. However, a quantitative comparison of our data will be made later with the results of a few studies in which the energy liberated during the maintenance of constant tension by electrically stimulated skeletal muscle has been measured.

Measurements of the rate of oxygen uptake during the development of tension could be made with electrical stimulation of the arterial segment. This phase of the contraction was quite short in duration and the oxygen uptake readings increased in an exponential manner from their initial

to their final steady state values during a one to three minute period. Since in no instance was there any evidence of an overshoot during this continuous increase in the rate of oxygen uptake it would appear from our data that the average rate of respiration during the development is actually less than the rate during the maintenance of tension. Since some evidence does exist which indicates the opposite to be the case it is probable that the time delay in our system was too large to permit the detection of any temporary rapid change in the respiratory rate.

A comparison of the energy demand of arterial muscle during the phases of tension development and tension maintenance has been made by Lundholm and Mohme-Lundholm (1965). They found the rate of lactic acid production under anaerobic conditions to be elevated by only 60% during the phase of constant maximum tension of an adrenaline-induced contraction of isolated bovine mesenteric artery. This change was quite moderate relative to the 300% to 500% elevation in the metabolism noted during the phase of tension development. The reduction in the content of high-energy phosphates of arterial muscle has been found by Beviz, Lundholm et al. (1965) to be significantly greater during the development than during the maintenance of active tension. Bozler (1930) has reached a similar result by measuring the rate of heat production of snail smooth muscle during the two phases of isometric contraction. The mechanisms influencing the energy production during the two phases of isometric contraction are not altogether different as shown by the work of Fenn and Latchford (1934) on frog skeletal muscle. They found the energy for the maintenance of active tension by the electrically stimulated muscle to vary with muscle length in exactly the same way as the energy for the development of tension.

The comparatively low energy requirements of arterial muscle during the phase of tension maintenance has led Lundholm and Mohme-Lundholm (1965) to postulate the existence of a passive "tonic" mechanism in arterial muscle which was independent of the "active state" and its commensurate stimulation of the metabolism during the phase of tension development and was primarily operative during the phase of maintenance of constant tension following the initial phase. They did conclude that this mechanism still demanded some energy. Somewhat similar ideas have been advanced by Laszt (1960) from studying the properties of the potassium-induced contraction of arterial muscle but he proposed that the "tonic" mechanism required no energy in performing its task of maintaining constant tension so that it is, in effect, equivalent to a "catch" mechanism. Although Johnson (1962) has presented considerable evidence which favours the presence of such a "catch" mechanism in tonic contractions of molluscan smooth muscle, several workers, including Bayliss (1928), Bozler (1930) and Abbott and Lowy (1958b) have considered a tetanic mechanism to be adequate for invertebrate smooth muscle to remain contracted for long periods without fatigue. Our results on the norepinephrine-induced contractions of arterial muscle have consistently showed a significant value for the maintenance energy which suggests that a tetanic mechanism could be responsible for the maintenance of constant tension in these contractions.

From a practical point of view, the energy of maintenance of a given tension or the maintenance efficiency of arterial muscle may be of more interest than that associated with the development of tension since in vivo one of the major functions of the arterial muscle is to maintain an active tone of the blood vessel wall for extended periods of time and

it appears that the energy demand of the muscle is even then significantly elevated for as long as the higher tone is maintained.

2. Electrically-induced Isometric Responses

a) Pattern of Response

As with the norepinephrine stimulated muscle, the electrically stimulated muscle yielded active mechanical and respiratory responses which increased in magnitude to a maximum value and then decreased as the passive tension or stress on the muscle was increased. This general behaviour was noticed both in the Warburg and polarographic experiments.

No attempts were made to keep the current density constant during any one experiment or the same for all segments, but the stimulating voltage was kept constant for each technique. It was intended to minimize the differences in responsiveness of the different segments to the stimulating voltage by normalizing all of the active responses of a given segment relative to its maximum active response, i.e., by employing the same procedure that was used for treating the data from the norepinephrine experiments.

By treating the data in this way no significant qualitative difference between the two sets of electrically-induced active responses was observed. The general behaviour of the active responses was an increase to a maximum value at a physiological value of passive tension.

Despite the shift which occurs with stretch in the peak value of the active mechanical and respiratory response, as in the norepinephrine-induced contractions the correlation between the two electrically induced active responses is in each case quite high and significantly different from zero. The correlation line equation of the Warburg data of $y = 5.5x + 5.0$, $(r = 0.86)$, indicates that the contracted muscle

consumed an amount of oxygen equal in magnitude to 5.5% of its "basal" Q_{O_2} in order to maintain an active tension of one g/cm or an average of 0.031 µl/mg/hour per g/cm. The corresponding polarographic data yielded the equation $y = 0.14x + 2.4$, ($r = 0.94$), with an "activity" Q_{O_2} of 0.14% per g/cm^2 or an average value of about 0.00075 µl/mg/hour per g/cm^2. This latter value is equivalent to 0.025 µl/mg/hour per g/cm which is only 20% less than the above Warburg value. Some of the possible reasons for this difference in the metabolic efficiency between the two methods have been discussed.

Once again, unpublished work has shown that the proportionality between the "activity" Q_{O_2} and active stress was not unique to those responses which were influenced by stretch alone but was also retained by those active responses which were obtained by stimulation of the muscle in bathing solutions containing high and low levels of sodium, potassium and hydrogen ions. With the exception of the high pH media, the ratio of the "activity" respiration to its corresponding active stress was constant and not significantly different from that in the normal media. Changes in temperature above 37°C increased the ratio while temperatures below 37°C reduced its value, presumably by influencing the metabolic efficiency of the electrically-induced contractions.

b) Hysteresis Effect

A hysteresis of the mechanical responses of electrically stimulated arterial muscle similar to that which we have observed has also been demonstrated by Sparks and Bohr (1962). In our experiments the hysteresis effect was found to be more pronounced with the active mechanical responses than with the "activity" respiration of the arterial segment. This relative decrease in responsiveness experienced with decreas-

ing load may have been the result of some irreversible stretch suffered by the muscle during the period of progressively increasing load.

Whether or not irreversible stretch is the cause of the hysteresis could have been tested by repeating the loading-unloading sequence a few more times and observing the change in the hysteresis of the active responses. Since the hysteresis of the passive tension-length curve of arterial muscle is known to progressively decrease with repeated loading and unloading, a comparison between the hysteresis of the passive and active responses might resolve this problem. Such a comparative study requires, however, a considerable amount of time.

The stretching of the segment during the stripping off of the adventitial coat and during the mounting procedure at the beginning of the experiment probably helped to minimize any changes in irreversible stretch during the experiment and therefore perhaps helped to minimize the hysteresis also and may thus have reduced the effect which these changes exert on the isometric contraction. Thus the scatter in the overall data may have been reduced by the initial stretching.

That the active responses of the arterial segment also experience a peak value with decreasing values of passive tension argues against the occurrence of any significant damage of the segment at the highest values of passive tension. The efficiency of the contractions during this unloading sequence was also reduced only slightly. The "basal" Q_{O_2} of the segment at the end of this long experiment was elevated above its initial value by the usual small amount which is also indicative of slight, if any, damage.

c) Comparison of Warburg and Polarographic Data

The maximum active responses in the polarographic experiments

were found to occur in the passive stress range extending from 300 to 650 g/cm^2 or from about 9 to 18 g/cm of passive tension. In the Warburg experiments, the range was similar, extending from 8 to 36 g/cm. The actual values of the average maximum active responses were 74% for the "activity" Q_{O_2} and 530 g/cm^2 (or about 15 g/cm) for the active stress (or tension) in the polarographic experiments, while in the Warburg experiments the corresponding values were 104% and 15.1 g/cm. Thus, the maximum active tension values are in close agreement, but the corresponding "activity" Q_{O_2} values are significantly greater in the Warburg experiments.

The similar data obtained by the Warburg and polarographic techniques is somewhat surprising in view of the obvious differences in the shape of the mechanical response curves with the two types of stimulation as well as the many differences between the two techniques themselves. Most of the important differences have already been mentioned in Results.

Besides differences in the method of loading the segment, the different strengths of stimulating voltage and differences in the physical arrangement of the stimulating electrodes which exist between the Warburg and polarographic techniques as well as the different method of measuring the rate of oxygen uptake in the two cases, all of which might affect the final result in a more quantitative manner, the behaviour of the stimulated muscle with increasing stretch could also have been influenced in a more qualitative fashion by changes in the actual current density which might occur with stretch. However, as mentioned before, the current density through our arterial segments actually decreases with increasing stretch of the segment so that the initial increase in the active responses that was observed with increasing passive stress or tension could in neither case be attributed to an increase in the current density

through the segment.

The smaller value for the ratio of the two maximum active responses and hence perhaps the greater metabolic efficiency of the segments in the polarographic set-up may have been due to the contraction occurring much more uniformly with time throughout the length and width of the segment in this set-up. If the speed of conduction of an action potential is as slow in our arterial muscle as Roddie (1962) has shown for turtle arteries and if the electrical resistance of arterial muscle cells is greater at right angles to their long axis than what it is parallel to it as Nagai and Prosser (1963) have demonstrated for intestinal smooth muscle cells, then it is possible that the greater distance between the stimulating electrodes in the Warburg arrangement and the slower conduction of the impulse between them could lead to a greater asynchrony in the response of these cells. Because of this asynchrony it is possible that some of the tension developed at any one time could be absorbed by the stretching of those muscle cells which have not yet begun contracting themselves. This then could lead to an underestimate of the active tension value but it does not follow that the "activity" Q_{O_2} would be decreased by a similar amount so that the metabolic efficiency could as a result be depressed. The long duration of the stimulation period (30 seconds) and of the period of increasing tension, might have helped to reduce this effect but if it is at all significant it would certainly be less in the polarographic arrangement where the two electrodes were generally closer together and extended along and made contact with the entire length of the segment.

Comparing these two sets of data further reveals that these Warburg experiments were the only instance in which the active tension

generally reached its maximum value at a higher value of passive tension than did the maximum "activity" Q_{O_2}. In the other Warburg experiments and in all of the polarographic experiments, the opposite was observed. Hence, with this one exception our results are in disagreement with those of Evans and Hill (1914), Fenn and Latchford (1934) and Lee (1960) all of whom found the maximal energy production of skeletal and cardiac muscle to occur at a lower passive tension than that at which the maximal contractile tension was obtained.

d) Comparison with other Reports

With respect to the effect of stretch on the mechanical responses of electrically stimulated arterial muscle our observations agree with those made by Sparks and Bohr (1962) on isolated strips of mesenteric artery, Winton (1926) on isolated dog's retractor penis smooth muscle, Fischer (1931) and Whalen, Collins and Berry (1962) on frog skeletal muscle, Lee (1960) and Whalen (1960) on isolated cardiac muscle strips of cat and rat, respectively. Direct electrical stimulation of the muscle was employed in each case to give mechanical responses which increased to a maximum value as the muscle was stretched and which declined with further stretch.

Both Fischer (1931) and Lee (1960) also found that the total as well as the activity oxygen uptake of their electrically stimulated muscles behaved in a manner similar to that of the mechanical responses at various degrees of muscle stretch so that a proportional relationship between the mechanical and respiratory responses, in effect, did exist. Lee (1960) found the ratio of the peak active stress to the passive stress producing it to be equal to about 0.5, and the corresponding value of the activity oxygen consumption to be equal to about 100% of the

resting value. Even though his values were determined for isometric twitches of cardiac muscle where the phase of tension maintenance is non-existent they are, nevertheless, somewhat the same as ours, both with respect to the relative value of the maximum mechanical response of the muscle and the change in its rate of respiration.

At variance with the tendencies outlined above are the metabolic studies of Whalen (1960, 1961) who found, once again, by direct electrical stimulation of strips of skeletal and cardiac muscle that it is the total length or total tension of the muscle rather than its active tension which best correlates with its oxygen uptake.

The average value for the ratio of the active mechanical and respiratory responses, obtained by direct electrical stimulation of the arterial segment in the polarographic set-up, of 0.00075 µl/mg/hour per g/cm^2 was used to give an approximate mean value of 0.00025 µl oxygen/g/sec per cm. Norepinephrine stimulation, in comparison, yields an average value of 0.00057 µl/g/sec per cm. This data can be compared in a quantitative fashion with the results of those few workers who have measured the absolute value of the steady heat production during prolonged isometric contractions of electrically stimulated frog sartorius muscles.

Thus, the data of Hartree and Hill (1921) has been recalculated to yield a value of 0.0044 µl of oxygen consumed per second per centimetre length of muscle per gram weight of tension that was maintained. This value is a minimum figure and therefore corresponds to the maximum efficiency of the frog sartorius muscles which were stimulated to contract at 20°C. In converting calories of heat to its equivalent volume of oxygen it was assumed that pure carbohydrate oxidation was taking

place. The length of the muscle must be included in the result, otherwise the result has no absolute meaning, since by increasing the length of the muscle - but not the cross-section - the value for the energy production can be increased without increasing the value of the tension maintained. A change in cross-section however affects both parameters equally in a maximal contraction of skeletal muscle, so that it was not considered. The above value can be reduced to equal simply 0.94 per second. By treating the data of Fenn and Latchford (1934) in a similar manner, an almost identical minimum value of 0.004 µl oxygen/g/sec per cm is obtained for the isometrically-contracted sartorius muscle at room temperature.

In poor agreement with the above values is the minimum figure of 0.1 µl oxygen/g/sec per cm calculated from the results of Abbott (1951) for the frog sartorius muscle at 0°C which compares very poorly with the minimum value of 0.0008 µl/g/sec per cm calculated from the data provided by Hartree and Hill (1921) at the same temperature of 0°C.

Considering that the maintenance heat rate increases greatly with temperature, the ratio of the active responses for arterial muscle at 37°C is very small. The value for frog skeletal muscle at 37°C can be calculated directly from the data of Hartree and Hill (1921) and equals 0.017 µl/g/sec per cm or is about 68 times greater than the arterial muscle value at the same temperature. No data exist for other smooth or skeletal muscles with which we can compare our results.

Hartree and Hill (1921) have suggested, as have many other workers since, that the high efficiency of smooth muscle in maintaining a constant tension is probably due to the slowness of its muscular response. On this basis, however, the electrically-induced response of

FIGURE 29

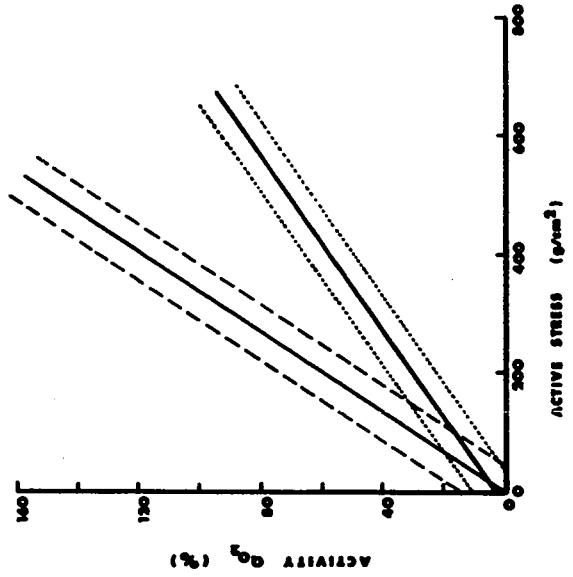
The variation of the "activity" Q_{O_2} of adventitia-free arterial segments with their active tension or stress when stimulated electrically and with norepinephrine.

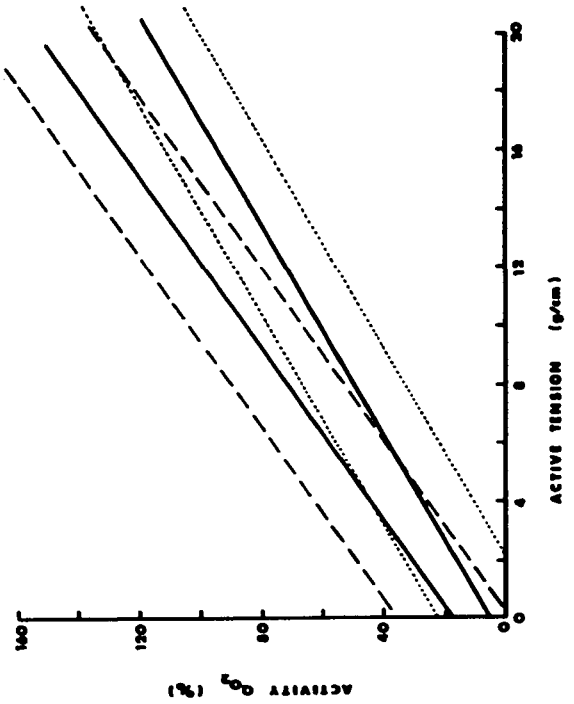
(a) Left

Method A - The upper solid line represents the regression line of the norepinephrine data and the lower solid line the regression line of the electrical data obtained by using the Warburg technique. The dashed lines drawn parallel to each of the regression lines represent plus and minus one standard error of the estimate. The two curves are not significantly different from one another.

(b) Right

Method B - The upper solid line represents the regression line of the norepinephrine data and the lower solid line the regression line of the electrical data obtained by using the polarographic technique. The dashed lines drawn on either side of each regression line and parallel to it represent plus and minus one standard error of the estimate in each case. The two regression lines appear to be significantly different within one and possibly two standard errors of the estimate.


ACTIVITY Q_{O_2} (%)
140
120
80
40
0
200
400
600
800
ACTIVE STRESS (g/mm²)


ACTIVITY Q_{O_2} (%)
140
120
80
40
0
4
8
12
16
20
ACTIVE TENSION (g/mm)

arterial muscle would be expected to have a lower rather than a higher efficiency relative to the norepinephrine-induced contraction. Since the tension-production energy consumption has been shown to be of similar magnitude for arterial and frog skeletal muscle by Lundholm and Mohme-Lundholm (1965) whereas the tension-maintenance energy consumption appears to be appreciably lower for vascular and snail smooth muscle than for frog skeletal muscle, the above workers postulated the existence of a passive "tonic" mechanism in arterial muscle to explain its low maintenance energy, while Bozler (1930) found a tetanic mechanism to be adequate to explain the low maintenance energy of his snail smooth muscle. Whether this maintenance mechanism be the same or essentially different from the mechanism responsible for the development of tension, it clearly requires a significant amount of oxidative energy for its operation.

3. Comparison of the Norepinephrine and Electrically-induced Isometric Responses

a) Differences between the Responses

The most important difference between the two types of response revealed by this study is the higher oxidative efficiency of the electrically-induced responses relative to those produced by norepinephrine stimulation. This difference was statistically significant in the polarographic experiments. Figure 29 shows the dependence of "activity" Q_{O_2} on active stress or tension for both the Warburg and polarographic methods, with norepinephrine and electrical stimulation. For the polarographic data the curves depicting the responses obtained with drug and electrical stimulation are significantly different within one standard error of the estimate at all except the lowest values of active stress.

It also appeared that the electrically-induced responses of arterial muscle were more responsive to the influence of stretch, especially to small degrees of stretch, than the norepinephrine-induced responses, since significantly lower values of passive stress were needed to attain maximum active responses with the direct electrical stimulus. Winton (1926) has observed this same difference between the active mechanical responses obtained with direct electrical stimulation and epinephrine stimulation of isolated dog's retractor penis smooth muscle.

b) Possible Causes of Differences

Several possibilities can be ruled out as being the cause of the different oxidative efficiencies that were observed with the norepine - phrine and electrically-induced contractions. There was no evidence of actual damage or "burning" of the segment where it came in contact with the electrodes. Also arguing against any irreversible chemical changes in or damage to the muscle by the electrical stimulus, is the fact that the responses could be faithfully reproduced a number of times with little change in magnitude when the passive stretch was kept constant.

It may be that the electrical stimulus exerts its contractile action partly through a harmless physical-chemical process requiring little or no energy. This may be the case with the fast component of the contraction but not with the slow component, since Furchgott (1952) and Gillis and Yates (1960) have shown that the slow component is due to the release of locally stored norepinephrine from the vascular wall. Even so, the muscle relaxes spontaneously at a very high initial rate indicating that there is little in the nature of a tonic contraction or contracture in either the fast or slow component. Such consistent and spontaneous reversibility renders the suggestion of a pure physical-chemical

process doubtful.

Also, two arteries which showed a drastic decrease in respiratory activity with time, were stimulated when their basal metabolism was equal to only about 10% to 15% of their values at the beginning of the experimental run. In both of these cases, electrical stimulation produced only a small active pressure response, about 1/10 the value normally expected with that passive pressure while the oxygen consumption, as low as it was, still increased noticeably. It appears that a steady supply of oxygen or of energy in some form is necessary for the electrically stimulated muscle to produce even a slight contraction. An inability to supply this energy, as indicated by the low demand for oxygen by both the relaxed and contracted muscle, severely limited the magnitude of the mechanical response.

A possible reason for the significant difference between the values for the oxidative efficiency that were obtained with the two different types of stimulation may lie in the different time course of their contractile responses. The "activity" Q_{O_2} of the norepinephrine-induced contraction was measured while the muscle was maintaining a constant value of active tension, while the "activity" Q_{O_2} of the electrically-induced contraction due to the very short duration of the latter, had to be measured during an interval that included a slight increase in the active tension and a slight relaxation of the muscle, as well as the one to two minute period of actual maximum response.

The oxygen uptake measurements with the Warburg set-up had to be taken over at least a four minute period in order that detectable differences could be accurately recorded during the contraction. The average value of the contractile response during this four minute interval

was often only about 3% to 7% less than the maximum value, and it was this average value that was considered in the results. This accords with the fact that the active responses in the Warburg method do not show a significantly different ratio for the two methods of stimulation.

In the polarographic experiments, even though the mechanical response remained maximal for only about two minutes, continuous recording of the oxygen uptake of the electrically-induced contractions indicated that oxygen was consumed at a surprisingly steady rate during five minutes of the contraction. While it is very probable that the delay of the oxygen recording system (time constant of about one minute) contributes to the constancy of some of the rates that were recorded it may equally well be that the steady rate of oxygen uptake that was measured during the short period of maximum contraction was not significantly influenced by the processes immediately preceding and following it since, in most respects, the time course of the rate of respiration could hardly be separated from the time course of the mechanical events of the muscle.

The degree of activation and the number of muscle cells activated at any one time may have been different for the two types of stimulation and may have influenced the time pattern of the contraction, but the net effect or the maximum active tension or stress developed in the two cases were almost always quite comparable. The net mechanical result was all that was recorded so that any asynchrony in the contractions of the individual muscle cells would go undetected. This effect would be expected to be more of a problem with electrical than drug stimulation and would tend, if anything, to reduce the metabolic efficiency of the electrically-induced contraction rather than increase it as is the case. Thus this explanation is hardly tenable.

On the assumption that electrical stimulation in the polarographic method really is different from drug stimulation, aside from the differences in the time pattern of the energy metabolism between the two types of stimulation due to evident differences in the time pattern of their contractions it could also be that the two types of stimuli affect the metabolism of the arterial muscle in a qualitatively different manner. Some of our experiments on arterial muscle which are not described here, have indicated that the electrically-induced response is less affected by anoxia and has a smaller tendency to repay an oxygen debt than the mechanically equivalent norepinephrine response. These results may have been due to a greater facility of the electrically stimulated muscle to draw an anaerobic mechanisms which supply energy, such as anaerobic glycolysis. In general support of this latter possibility is the observation that under anaerobic conditions adrenaline apparently often displays an anti-glycolytic effect and, also, the finding of Prasad (1935) that direct electrical stimulation of gut muscle increases its rate of glycolysis by 12%.

A possible clue to the higher metabolic efficiency of the electrically-induced contractions may be provided by some of our experiments as well as the work of Keatinge (1964) which show that low temperatures have much less influence on the amplitude of the electrically-induced mechanical responses of arterial muscle than on the mechanical responses produced by norepinephrine stimulation. This suggests that the electrical stimulus acts through or on later stages of the contractile sequence than do catecholamines, and that the reactions which occur in the case of electrical stimulation are linked quite intimately with the contractile mechanism. These reactions are probably less affected by immediate large

changes in the energy production, perhaps because the utilization of the muscle's energy stores is once again greatly facilitated as it is in the case of potassium stimulation. These contractions were physiological and not due to denaturation.

Variation in pH affects the two types of contraction markedly but differentially. Some of our studies in this respect have shown that the oxidative efficiency of the contracted arterial muscle increased with increasing pH with both types of stimulation and that the increase in efficiency was greater for the electrically-induced responses. Although the pH of the bathing solution would be expected to, and did, drop during both the drug and electrical stimulation of the arterial muscle, if a temporary local increase in pH does occur within the stimulated muscle due to CrP breakdown, for example, then the higher metabolic efficiency with the electrical stimulus could be explained on this basis. This symptom of high pH might indicate that there are some differences in the enzyme systems involved in supplying the necessary energy for the two types of active responses or that the metabolic pathways themselves are different or are present in different proportions in the two cases.

It has been shown by Lundholm and Mohme-Lundholm (1963b) that adrenaline can stimulate the metabolism of arterial smooth muscle, without any effect on the contractile process, presumably by its action on a separate receptor system. Noradrenaline may exert a similar but weaker effect on arterial muscle but whether the electrical stimulus can also activate the mechanical and metabolic processes independently is not known. It probably can to some extent since it has been demonstrated that the slow component of the electrically-induced response is due to the release of noradrenaline from the vascular wall. Essentially any

differences between the drug and electrical stimulus must, therefore, be due to the fast component of the electrically-induced response.

Using isotonic loading of their arterial muscle Lundholm and Mohme-Lundholm (1963a) also demonstrated that direct electrical stimulation but not stimulation by either adrenaline or noradrenaline stimulated the glycogenolysis of the muscle. This effect could result in an increased oxidative efficiency with electrical stimulation since glycogen is more efficient per mole of lactic acid produced than is glucose as substrate. In view of this difference, it is not altogether inconceivable that the electrical stimulus may have a different effect on the aerobic glycolysis mechanisms of arterial muscle than that of the norepinephrine stimulus. It has been shown that aerobic glycolysis can account for a large fraction of the energy demanded by resting and contracted arterial muscle but no data exists as to the energy demanded via glycolytic sources by electrically-induced contractions of this muscle. Since the oxygen demand of these contractions is relatively low it could be that glycolytic mechanisms are called upon to supply a greater proportion of the total energy required by these contractions compared to that supplied to the norepinephrine-induced contractions. If this is the case, the efficiency of the electrically-induced contraction, although it consumes less oxygen and hence appears more efficient, would in fact be definitely lower than that of the norepinephrine contraction, since much more glucose would have to be metabolized to produce the necessary energy by this glycolytic pathway. Lundholm *et al.* (1960a) have stressed the possibility of shifts occurring in the value of the phosphorylation to oxidation ratio of arterial muscle; so, speculating further, it may be that an increase in this ratio with the electrical stimulus would enable the

contracted muscle to consume less oxygen and yet be supplied with the required amount of energy. This shift would, therefore, be reflected in an increased metabolic efficiency of the active muscle.

It has been shown that high concentrations of potassium can stimulate the contractile process of arterial muscle without affecting its energy metabolism. Lundholm and Mohme-Lundholm (1963b, 1965) and Laszt (1960) have obtained results which suggest that the primary cause of the potassium-induced contraction was not to be found in an increased supply of high-energy phosphate compounds but rather in the muscle's capacity to utilize its existing energy reserves. If the fast component of the electrically-induced response should act through or on the same site of action as does potassium, the low demand for oxygen despite the large mechanical responses and hence the high efficiency of the electrically-induced contractions would be explained. In this respect, it can be said that Headings, Bohr and Rondell (1960) have shown that direct electrical stimulation influences the electrolyte composition of arterial muscle whereas catecholamine stimulation does not.

Other studies on the internal ionic environment of arterial muscle and the effect of changes in its external ionic environment on the muscle's responses have revealed other differences with drug and electrical stimulation. Several workers, including Barr (1959, 1961) and Waugh (1962) have shown that changes in the membrane potential of vascular smooth muscle cells strongly affected the responses to electrical stimulation but not those produced by catecholamine stimulation. Barr (1961) has also observed that the mechanical responses of arterial muscle to epinephrine stimulation in calcium-free media disappear much more slowly than those induced by direct electrical stimuli. He suggested that a

difference in the relative dependence of the two stimuli on calcium influx versus release from intracellular stores may be responsible for the difference.

In addition, many studies, such as that by Bohr, Brodie and Cheu (1958) and some of our unpublished work have demonstrated that the catecholamine-induced responses of arterial muscle are potentiated with high concentrations of potassium in the external environment and inhibited with low concentrations of potassium. In contrast to this behaviour, the results of Leonard (1957) and Dickinson (1960) as well as experiments of our own have all shown the effect of abnormal potassium environments on the electrically-induced responses of arterial muscle to be the exact reverse of that normally obtained with catecholamine stimulation. Similar observations have also been made by Singh (1940) with molluscan smooth and frog stomach muscle and by Hajdu (1953) on isolated frog heart muscle.

Lundholm and Mohme-Lundholm (1962a) and Beviz, Lundholm et al. (1965) have shown that the content of pre-formed high-energy phosphate compounds in arterial muscle is small and that it changes significantly within a few minutes of stimulation. From this it is apparent that over a small period of time the oxygen consumption could not be expected to represent a dependable criterion of the total energy metabolism. The rate of splitting and consequent resynthesis of these phosphate compounds might have been different with the two types of stimulation so that the corresponding elevations in the rate of oxygen uptake associated with the resynthesis of these compounds would have experienced different time delays as well as different durations. If the time delay in the electrical situation was more than a few minutes and the additional oxygen was consumed slowly over a prolonged interval, it would not be included in nor

have much of an influence on the steady state values which we recorded. The same increase in oxygen uptake would not go undetected in the norepinephrine situation and would most probably be included in the prolonged steady state rates of oxygen uptake associated with these contractions.

No studies have been made in which the metabolic requirements of electrically stimulated and drug stimulated muscle were compared in a quantitative way when isometric loading of the muscle was employed. Some scattered information is available, however, which generally supports our own observations on arterial muscle. Bulbring (1953) has observed the increase in the oxygen usage of guinea pig intestinal muscle to be of the order of 300% to 400% of its initial rate with relatively high values of drug-induced active tension. This was found to be the case with contractions of variable duration so that the increased oxygen uptake could apply equally well to the maintenance as to the development phases of the active tension. In comparison, if such a comparison has any real meaning, Rao and Singh (1940) and Weeks and Chenoweth (1952) noted that direct electrical stimulation of isolated frog stomach and rat diaphragm muscle caused an increase in the oxygen uptake of the isometrically contracted muscles of only 100% and 63% of their initial resting values, respectively. In contrast to the above, changes in respiration of several-fold for electrically stimulated frog and mammalian skeletal muscle have been reported by Fenn (1927), Meyerhof and Shulz (1927), Martin, Field and Hall (1932) and Quesnel and Kramer (1939). It is difficult to compare the above data with our own since not only were different muscle preparations employed but the amount of tension developed was probably different in each case due to differences in the applied passive load.

Fewer of the above objections are found in the data gathered from a series of studies on isolated cardiac muscle performed by Lee (1953) and Whalen (1957). Their data shows that the increase in respiration relative to the maximum tension developed by the addition of noradrenaline and adrenaline, respectively, to be significantly greater than that due to direct electrical stimulation of the cardiac muscle. The efficiency of the adrenaline-induced contractions was relatively small.

4. Norepinephrine-induced Isotonic Responses

a) Pattern of Response

In our experiments the influence of the applied load on the work performed by arterial muscle which was stimulated to contract with norepinephrine was such that the work increased to a maximum value as the load was increased and then decreased with further loading. This same relationship has been observed by Wallace and Speden (1959) on arterial muscle from sheep and also, in a more qualitative manner, by Bevan (1960) on rabbit aorta strips stimulated with epinephrine. The same effect has been shown to be true for isolated uterine smooth muscle by Csapo and Goodall (1954) and for intact nictitating membrane smooth muscle by Hampel (1933).

The pattern of response of the activity respiration with applied load was similar to that of the work performed by the arterial muscle. The correlation between the norepinephrine-induced mechanical and respiratory responses, although not quite as good as in the isometric experiments, was still significantly different from zero. The correlation line equation of $y = 21.1x + 38.8$, ($r = 0.57$), indicates that the contracted muscle consumed an amount of oxygen equivalent to 21.1% of its "basal" Q_{O_2} in order to perform one g-cm of work or an average of 0.11 µl/mg/hour/

g-cm. By assuming pure carbohydrate oxidation to be taking place which the RQ values tend to suggest to be the case, and other typical experimental values for our segments, the above value can be used to give an average mechanical efficiency for the arterial muscle of about 1.7%. This value which appears to be quite low would have been even smaller if the corrected "activity" Q_{O_2} values had been employed. Even as it is, the high value of the positive y-intercept of this data is the only one which is significantly different from zero within one standard error of the estimate.

This correspondence between the "activity" Q_{O_2} and the total external work performed by the segment when both are plotted against the passive load is somewhat unexpected since aside from implying that the main factor governing the steady state energy production of the active muscle is the total external work performed by it, it also implies that the activity of the respiratory mechanisms of arterial muscle even at the very beginning of each isotonic contraction is determined by the total amount of work that is to be performed during a given contraction and provides the energy needed or replaces that utilized from the energy stores at a predetermined steady rate. At least the equipment which we used could not detect any such changes in the rate of oxygen uptake during any given contraction even when lighter loads were used and the rate of doing work changed considerably over a short period of time.

Only the steady state oxygen consumption of the segment while it was actively shortening was considered in the calculation of the "activity" Q_{O_2}. Inclusion of the oxygen uptake values when the muscle was maximally shortened and not working would have resulted in a decrease in all of the "activity" Q_{O_2} values listed and a poorer correspondence be-

tween them and the work performed may have resulted.

b) Correction of the "Activity" Respiration

The oxygen consumption of isotonically-loaded uterine and intestinal smooth muscles after the addition of contracting agents was studied by Evans (1923, 1926) and Bulbring (1953), respectively. When using light loads they both observed a net decrease in the oxygen uptake of the contracted muscle compared to when it was at rest, and Bulbring attributed this to the large decrease in length and hence of the surface energy of the muscle which overshadowed the actual increase in the oxygen uptake which they observed during the initial contraction phase when the muscle was performing most of its work. In other words this result could be explained by a decrease in the "tension" Q_{O_2} (or length) which was greater in magnitude than the increase in the "activity" Q_{O_2} of the contracting muscle and could happen even more easily if the "activity" Q_{O_2} is measured during the final "isometric" state of the muscle which has a lower oxygen uptake than the isotonic or shortening state. Also, if the preparation consists of smooth muscle cells only which have a low Young's modulus of elasticity, then the decrease in the "tension" Q_{O_2} upon shortening would be greater due to the greater relative amount of shortening now possible with a given passive load. Combining these two effects, a large decrease in the "tension" Q_{O_2} with a small "activity" Q_{O_2} value, could lead to a net decrease in the oxygen uptake even with larger applied loads.

It was with the thought that this might be happening in our experiments that some sample corrected "tension" Q_{O_2} (and corrected "activity" Q_{O_2}) curves were drawn. These corrections were maximum corrections in that when they were applied to the uncorrected "tension" Q_{O_2} values they presumably gave the "tension" Q_{O_2} values of the maximally shortened segment. The "activity" Q_{O_2} values were in turn given a maximum positive

correction. Yet, it is apparent that if the "tension" Q_{O_2} of the contracting segment were decreasing, it would decrease in proportion to the length change which had taken place up to that time. But the total rate of oxygen consumption by the contracting segment was observed to be quite steady which means that if the "tension" Q_{O_2} were progressively decreasing it must have been accompanied by a progressive increase in the "activity" Q_{O_2}. That these two progressive changes in the oxygen uptake should cancel one another out so completely at all the different rates and magnitudes of shortening is hard to visualize.

For this reason, the "tension" Q_{O_2} was called by that name rather than the "length" Q_{O_2}, for although Sparks and Bohr (1962) have provided data which suggests that changes in the length rather than in the passive tension are primarily responsible for changes in sensitivity of isometrically-loaded arterial muscle to direct electrical stimulation, it may be in our experiments that the passive stress on the contractile element is maintained despite the decrease in the length of the segment. This possibility has been suggested by Sparks (1964); alternatively it may be that the same "tension" Q_{O_2} value is somehow maintained even with smaller values of passive stress; or speculating still further, the "basal" Q_{O_2} may experience an increase compensating for the decrease in the "tension" Q_{O_2}. This latter possibility might involve something approaching the "delta" state of Ramsey and Street (1940) in which the basal state has been shown to undergo a qualitative change during the isotonic contraction of skeletal muscle.

Although Stannard (1939) has suggested that the resting and activity metabolism of skeletal muscle are separable functions, Feng (1932) and Whalen, Collins and Berry (1962) have proposed the idea of a common

pool of energy supplying the needs of resting, stretched and contracting muscle. If this is the case with arterial smooth muscle, it makes the possibility of these components of the metabolic pool exerting an influence upon one another much more probable.

Whatever the reasons may be for the constancy of the total rate of oxygen consumption, it was finally assumed that the "activity" Q_{O_2} did not change and the "activity" Q_{O_2} values that were used in all of the graphs, unless otherwise stated, were "uncorrected" values. The correction, even if it were applied, would probably tend to make the "activity" Q_{O_2} values overly large.

c) Comparison of our Data with other Reports

The energy requirements of arterial muscle during an isotonic contraction have not been looked into except for the recent studies of Lundholm and Mohme-Lundholm and co-workers. They found that the isotonic contraction of arterial muscle is usually an energy-requiring process, depending on the stimulus employed, but that the magnitude of the energy utilized is always quite small. Thus Lundholm and Mohme-Lundholm (1965) found no increase in the lactic acid production with potassium-induced contractions whereas with adrenaline-induced contractions the content of high-energy phosphate compounds has been demonstrated to decline in a study by Beviz and Mohme-Lundholm (1965). However, Lundholm and Mohme-Lundholm (1965) have also found that under anaerobic conditions, tone-increasing agents stimulated the lactic acid production of isotonically-loaded bovine mesenteric arteries only during the initial contraction phase - when the muscle was performing work - and that the increased tone was then maintained without any effect on the energy production. In our experiments, the oxygen uptake decreased noticeably during this latter

stage but was still significantly greater than its prestimulation value.

It has been demonstrated by Lundholm and Mohme-Lundholm (1962a) that various contracting drugs, including adrenaline, noradrenaline and histamine, as well as electrical stimulation, caused contractions of the arterial muscle in which the lactic acid production under anaerobic conditions showed both a quantitative and temporal correlation to the degree of shortening. In determining to what degree the lactic acid production depended on the work performed by the muscle, the authors studied the effect of histamine on only very lightly loaded and very heavily loaded preparations and found no demonstrable correlation between the work performed and the lactic acid production. They accounted for this result by computations which showed that of the increased energy production of the contracting muscle only 0.6% could be attributed to external work. Their results are not really conclusive since an intermediate load might have given peak active responses similar to those in our isotonic experiments. In comparison, the corresponding figure that was obtained in our experiments, which also represents the efficiency of the muscle, was found to be not much greater and equal to about 1.7%.

Some of the difference between their efficiency value and our own may of course be due to our using the respiration rather than the anaerobic lactic acid production as the indicator of the magnitude of the energy production. These workers have shown that over long periods the rate of lactic acid production under anaerobic conditions represents a reliable criterion of the total energy production of their arterial tissue while no such reliability can be assumed in our experiments, and the

respiratory mechanisms may be contributing only a fraction of the total energy demand. Since their technique required long intervals of at least fifteen minutes between readings, it is probable that their energy production values partially include some of that energy produced during the phase of the contraction where the muscle was no longer shortening or performing work. This would tend to somewhat depress their value for the isotonic efficiency.

As a result of this low efficiency value, Lundholm and Mohme-Lundholm (1962a) concluded that under anaerobic conditions the activation heat is probably much more responsible for the high energy metabolism associated with the contraction of arterial muscle than are the external work or the shortening and recovery heats. Because of the prolonged excitation that is required for the arterial muscle contraction to reach its maximum value this may also apply to our own muscle under aerobic conditions, as well as explain the higher energy demand by the norepinephrine-induced contractions which take a somewhat longer time to reach maximal shortening than do the electrically-induced contractions at the same passive load.

It has also been suggested by Lundholm and Mohme-Lundholm (1962a, 1965) that the small energy demand of the isotonic contraction of mesenteric arterial muscle could be increased by a factor of almost five times if the maximal contraction of the muscle from its fully relaxed state were to be considered rather than from its "basal" initial length where the muscle cells apparently still possess considerable inherent tone. If this is the case with our femoral arterial segments as well, it is surprising that much greater scatter in the data was not obtained due to inter-segmental differences in the "basal" tone which surely must have

existed.

Considering the data available on other types of muscle, our results are in qualitative agreement with the oxygen uptake measurements of Fischer (1931) and Baskin (1965) on isotonically contracting skeletal muscle in that they observed a strong correlation between the rate of oxygen consumption and the external work performed by the muscle. The studies of Mommaerts, Seraydarian and Marechal (1962), and of Carlson, Hardy and Wilkie (1963) have demonstrated the same effect with skeletal muscle by biochemical means and the latter group of workers have, in addition, simultaneously observed proportional changes between the heat liberated and the work performed by the muscle, thus confirming similar earlier observations. Many studies on heart muscle have shown that the oxygen uptake increased with an increase in the work of the heart, whether it was the pressure or the output which was varied. This has been shown by Patterson, Piper and Starling (1914), Evans and Matsouka (1915), Starling and Visscher (1927), Clark and White (1928) and Lorber (1953).

In disagreement with the general conclusions described above are the data obtained by Whalen (1960, 1961, 1962) and Whalen and Collins (1963). These studies showed that in spite of the variations in the work done by isolated rat heart muscle and frog skeletal muscle, the oxygen consumption remained relatively constant. Whalen's contention that it is the total length or total tension of the muscle which is the primary determinant of the additional oxygen uptake of the isotonically contracting muscle is similar to that drawn by Hill (1913a, 1913b, 1915) from his earlier heat liberation studies on skeletal muscle. More recently, the conclusions of Fales, Heisey and Zierler (1960), Katz and Feinberg (1958) and of Sarnoff, Braunwald, Welch, Case, Stainsby and Macruz (1958) on

intact skeletal and heart muscle also provide some support for Whalen's conclusions. On the other hand, studies very similar to the above have been performed by Ramirez (1953) and Monroe and French (1961) who obtained good correlation between the energy utilized and the work performed by the whole heart.

Many of the studies have employed isotonic twitches of the muscle so that the energy liberated, in some cases, included that due to work being done by the load on the muscle during the relaxation phase. This effect may be of qualitative significance since with isotonic contractions of skeletal muscle of a tetanic nature, which resemble our own slow isotonic contractions, the heat liberated has been consistently found to vary with the external work performed by the muscle as has been shown by Fenn (1923), Hartree and Hill (1928) and Fischer (1928, 1930).

5. Electrically-induced Isotonic Responses

a) Pattern of Response

The correlation between the "activity" Q_{O_2} and the external work performed during the electrically-induced isotonic contractions of our arterial muscle was quite high and significantly different from zero. The correlation line equation for this data of $y = 20.2x - 12.8$, ($r = 0.78$), shows that the contracted muscle consumed an amount of oxygen equivalent in magnitude to 20.2% of the "basal" Q_{O_2} in order to perform one g-cm of work or an average of 0.11 µl/mg/hour/g-cm. Using this figure and other typical experimental values, the mechanical efficiency of these contractions can be calculated to equal about 2.6% assuming that only carbohydrate is being metabolized. This was the only case in which the y-intercept of the data actually had a negative value, but as in most of the other cases, it was not significantly different from zero

within one standard error of the estimate.

In every case the steady rate of oxygen uptake of our arterial segments was correlated with the total (or maximum) amount of work performed by the segment during a given contraction. Due to the much faster rates of shortening of even heavily loaded skeletal and cardiac muscles, it may be impossible, at present, to determine their steady state rates of oxygen consumption while they are actually shortening. Most of the investigators have, in fact, measured the total oxygen uptake of an isotonic twitch, without resolving the oxygen uptake into components corresponding to the various phases of the mechanical response. With the electrically-induced contractions of arterial muscle, this resolution of the rates of oxygen uptake is much less clear-cut than with the norepinephrine-induced contractions where the "activity" Q_{O_2} definitely corresponds to the shortening phase of the isotonic contraction, and yet, as has been shown, a good approximation is also possible to the steady rate of oxygen uptake during the brief shortening phase of the electrically-induced isotonic contraction.

This measurement of the rate at which oxygen was consumed by the muscle only while it was actually shortening was made possible primarily by the relatively slow rate of contraction of the arterial muscle. Yet, with some segments, when lightly loaded and stimulated by electrical means, the shortening was completed within less than one minute. The rate of oxygen consumption that was obtained with these segments did not reach a steady rate during the brief period of contraction. For this reason, very light loads were not applied and with some segments measurements could not be accurately made even when the passive stress was of the order of 400 to 600 g/cm^2. These segments were discarded. Segments

were not stimulated when completely unloaded since the external work would not only have equalled zero but the steady rate of oxygen consumption during contraction probably could not have been measured anyway.

Although the results obtained with some of the lightly loaded segments would tend to disprove the existence of a steady state rate of energy production during the entire shortening phase, the measurements made on most segments did show such a steady state to exist during the entire duration of the shortening period. It is possible, once again, that a delay of the oxygen recording system could cause a steady rate to be recorded even though the rate might have been higher initially and lower later on. It is unlikely that the delay could have completely accounted for the steady rate since with heavily loaded segments the oxygen consumption rates that were recorded both when the rate of shortening of the segment was maintained at its initial value for three to five minutes and when the rate of shortening began to slowly decrease, were both quite steady and equal in magnitude.

A complicating factor in these experiments which might have introduced additional scatter into the data was that during the 30 second period of stimulation, the segment had already shortened somewhat, the actual extent depending on the applied load, the smaller the load the greater the speed and magnitude of the shortening. With shortening, however, the thickness as well as the length of the segment changes so that there is a progressive increase in the applied current density during the stimulation period. This increase is roughly proportional to the decrease in the length of the segment at any one time. So besides the different initial values of the current density which were applied to the segment when it was stretched by different loads, these initial

values themselves were changed during actual stimulation at any one value of passive load. Little could be done to change this, since the long period of stimulation was necessary in order to give mechanical responses of the necessary magnitude and duration.

It was assumed in this series of experiments that the "activity" Q_{O_2} did not change with the shortening of the segment even though it was possible that the "tension" Q_{O_2} of the segment did progressively change. The correction, of necessity, was only a very crude one and as in the norepinephrine experiments, even if it were applied, it would probably tend to make the "activity" Q_{O_2} values overly large. On the other hand, if the correction were applied it would probably eliminate the slight negative value which was obtained for the "activity" Q_{O_2} at low values of external work.

b) Comparison of our Data with other Reports

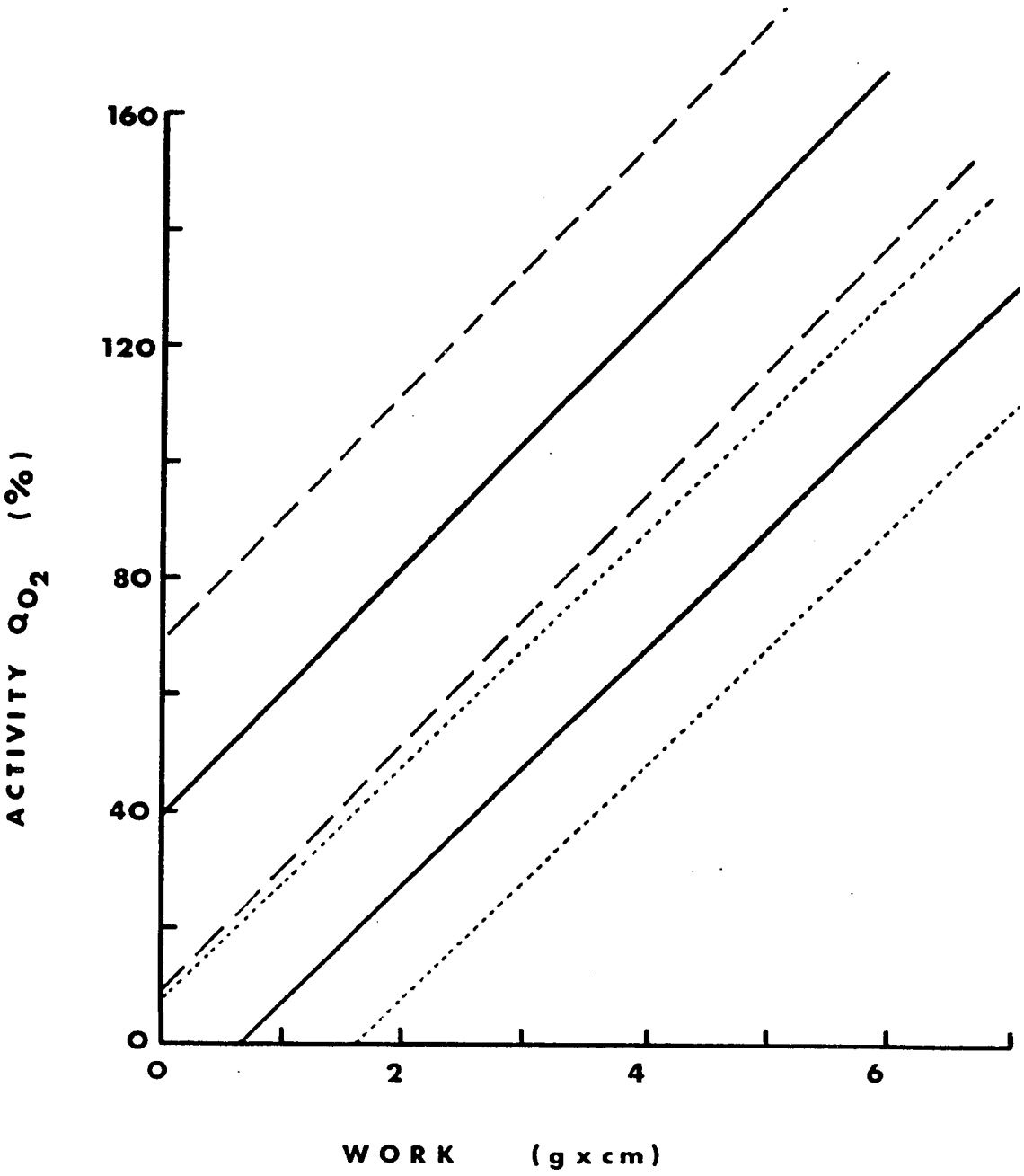
Few studies have been made on the energy metabolism of isotonically-loaded arterial muscle which has been stimulated electrically. The lactic acid produced under anaerobic conditions has been shown to bear both a quantitative and a temporal correlation to the degree of shortening of the electrically stimulated bovine mesenteric artery preparation in a study reported by Lundholm and Mohme-Lundholm (1962a). Electrical stimulation of the same isotonically-loaded preparation has also been shown to stimulate the glycogenolysis of the muscle under both aerobic and anaerobic conditions but no quantitative mechanical data are provided. This finding was reported by Lundholm and Mohme-Lundholm (1963a).

Many isotonic studies on electrically stimulated skeletal and cardiac muscle have already been mentioned in which the rate of oxygen consumption or heat production has been shown to behave in a manner paral-

FIGURE 30

Variation of the "activity" Q_{O_2} of adventitia-free arterial segments with the external work that they performed when stimulated electrically and with norepinephrine.

The "activity" Q_{O_2} values of each segment are expressed as a percentage of the initial "basal" Q_{O_2} for that segment and are not corrected for possible changes which might occur in the "tension" Q_{O_2} during the shortening process. The upper solid line represents the regression line of the norepinephrine data and the lower solid line the regression line of the electrical data. The dashed lines drawn on either side of each regression line and parallel to it represent plus and minus one standard error of the estimate. The only possible significant difference between these two regression lines may be in the value of their y-intercepts.


160
120
80
40
0
0
2
4
6
ACTIVITY Q_{O_2} (%)
WORK (g x cm)

leling the active mechanical responses of the muscle. These same studies, both those employing tetanic contractions of skeletal muscle and isotonic twitches have found that the work performed by the muscle varied with the passive load in a manner which was qualitatively the same as the behaviour of our arterial muscle.

6. Comparison of Norepinephrine and Electrically-induced Isotonic Responses

a) Differences between the Responses

Some significant quantitative differences were present in the results of the isotonic experiments where norepinephrine and electrical stimulation were used to cause the muscle to contract. The average value of the maximum work with the norepinephrine-induced contractions was significantly less than the average maximum value for the electrically-induced contractions while the corresponding average value for the maximum "activity" Q_{O_2} was actually significantly greater than the electrical value within one standard error of the mean. The two sets of active responses obtained with electrical stimulation were also displaced relative to those produced by norepinephrine stimulation so that the former maxima occurred at passive stress values about 200 g/cm^2 lower than the latter maxima. Otherwise, the shape and relative positions of the work and "activity" Q_{O_2} curves in the two situations were very similar.

The average oxidative efficiencies for the two types of contraction were almost identical in value. However, as shown in Figure 30 the two y-intercepts, or the activity respiration for very low work values, are just significantly different within one standard error of the estimate and some of the reasons which have been mentioned for the apparently high efficiency of the electrically-induced isometric contraction might thus

apply to the electrically-induced isotonic contraction at small values of passive load.

b) Possible Causes of Differences

One difference in the nature of the two types of induced response lies primarily in the fast component of the electrically-induced response since it has been shown by Gillis and Yates (1960) that the slow component is due to the release of norepinephrine from the vascular wall by the electrical stimulus. Although we have performed no experiments to investigate the effect of abnormal ionic environments on the isotonic responses of arterial muscle, Bohr (1964) has shown that calcium ion inhibits the fast component while potentiating the slow component and Brodie, Bohr and Smit (1959) have found the two components of the isotonic response to be affected differently by the amount of sodium and potassium in the bath, all of which indicates that separate mechanisms may be acting to produce the two components. Since the fast component appears to be the dominant one in the electrically-induced contraction, as it is associated with the greater amount of the shortening which takes place during contraction, while the two components in the norepinephrine-induced contraction appear to be of equal importance, it follows that basic differences between the two components could be, in part, responsible for the differences observed between these two types of contraction. Thus, aside from influencing the two components in a different manner, the amount of sodium and potassium in the medium has also been shown to influence the characteristics of the two types of contraction in a different manner as well. Waugh (1962) has found that the responses of arterial muscle to adrenergic neurohormones were not impaired while those to electrical stimulation were impaired by potassium depolarization. How these differences in the process

of excitation by the two types of stimulation affect the muscle cell respiration is not known. It may be that the fast component of the electrical response requires comparatively little energy or oxygen to perform the major portion of the initial increase in tension or work.

The carbohydrate metabolism of mesenteric arterial muscle stimulated to contract with adrenaline and by electrical stimulation has been observed by Lundholm and Mohme-Lundholm (1963a) to be influenced differently in the two cases. Increased glycogen breakdown was associated with the electrically-induced isotonic contractions but not with the equivalent contractions caused by adrenaline under aerobic conditions. The effect of utilizing glycogen rather than glucose may reflect an increase in the metabolic efficiency of the electrically stimulated muscle since less oxygen may have to be taken up in the latter case.

In addition to these differences between the drug and electrically-induced isotonic responses it is quite possible that some of those differences discussed previously with respect to isometric loading of the arterial muscle could be applied here to help at least partially explain some of the differences which we have observed between the two types of isotonic contraction of arterial muscle.

7. Comparison of Isotonic and Isometric Responses

a) Differences between the Responses

The range of passive stress values at which the maximum "activity" Q_{O_2} values occurred in the norepinephrine-induced isotonic and isometric contraction equalled 850 to 1300 and 650 to 900 g/cm^2, respectively. It appears, therefore, that these two passive stress ranges overlap by very little. The average maximum "activity" Q_{O_2} value for the isotonic contraction equalled 129% ± 11% (SEM) and for the isometric contraction it was

not significantly less at 115% ± 8% (SEM). Generally the isometric "activity" Q_{O_2} values are greater than the isotonic "activity" Q_{O_2} values with passive stress values up to 500 g/cm^2 which could be due to the occurrence of the maximum isotonic "activity" Q_{O_2} at a greater passive stress, or again it may be that these small isotonic "activity" Q_{O_2} values do require some positive correction. Beyond their maximum values both sets of "activity" Q_{O_2} curves have small negative slopes which are not noticeably different. In both our isotonic and isometric experiments the maximum value of active stress or external work usually occurred at a lower value of passive stress than did the maximum "activity" Q_{O_2} value for that segment. Otherwise, the shape of the two sets of curves was quite similar except for the greater amount of scatter usually encountered with the isotonic data.

The above differences are a little more striking when the electrically-induced isometric responses are compared with the electrically-induced isotonic responses. The isotonic values of the maximum "activity" Q_{O_2} occur at passive stress values ranging from 750 to 1050 g/cm^2 compared to the isometric range of 350 to 650 g/cm^2. These two passive stress ranges do not overlap and indicate that the optimal initial length of these isometric contractions is perhaps significantly less than that for the preloaded isotonic contractions. No isotonic experiments were performed when the segment was after-loaded. The average maximum "activity" Q_{O_2} value for the isotonic experiments of 96% ± 10% (SEM) was significantly greater than the corresponding isometric value of 74% ± 6% (SEM). Also, the isometric "activity" Q_{O_2} values are greater than the isotonic values at any value of passive stress up to about 500 g/cm^2. Beyond their optimal values of passive stress, both sets of "activity" Q_{O_2} curves decrease

equally slowly. In comparison, both the active stress and external work curves were relatively symmetrical with respect to their maximum values. These maxima usually preceded their corresponding "activity" Q_{O_2} maxima.

It is not surprising that the maximum "activity" Q_{O_2} values of the isotonic contractions are greater than those maxima associated with the isometric contractions since the latter were determined during the phase of tension maintenance which presumably requires less energy than the phase of tension development. As will be mentioned later, many workers have found the maximum isotonic "activity" Q_{O_2} value of skeletal and cardiac muscles to be even greater than the maximum isometric value associated with tension development rather than tension maintenance by these muscles.

b) Comparison of our Results with other Reports

Concerning the energy requirements of isometric and isotonic contractions of mesenteric arterial smooth muscle, Lundholm and Mohme-Lundholm (1965) found that although the isotonic contraction was an energy requiring process, except for the case of potassium ion stimulation, the energy demand as measured by the lactic acid production under anaerobic conditions was appreciably greater for the isometric than for the isotonic contraction, both during the phase of increasing tension and during the maintenance of constant tension. In addition, they observed that after the tension of the isotonically-loaded muscle had reached a fairly constant level, its metabolism returned to resting levels. We did not observe such a large decrease in the "activity" metabolism of our segments during this stage.

In order to explain the large disparity, in terms of energy metabolism, between the two types of contraction the above authors suggested

that a maximal isotonic contraction from the fully relaxed state of the muscle would give, theoretically at least, a metabolic increase somewhat exceeding that for isometric contraction. As in a previous study by Lundholm and Mohme-Lundholm (1962a), the isotonically-loaded muscle was usually only lightly loaded (with passive stress values equalling about 15 to 70 g/cm^2 being used) in order to easily observe the large changes in length upon stimulation (of about 20% of initial length) so that the isotonic responses, like the isometric responses, but probably much more so, were contracting against loads which were far removed from their optimal values.

An interesting qualitative effect of the different types of loading on the activity metabolism has been demonstrated by Lundholm and Mohme-Lundholm (1963b, 1965) by stimulating bovine mesenteric arteries, under anaerobic conditions, with potassium ions. With isotonic loading of the muscle strip, the potassium ions selectively stimulated the contractile process since the lactic acid production actually diminished during the contraction phase itself and afterward returned to control levels. On substrate-depleted and dinitrophenol-treated arterial muscle, potassium ions had a more pronounced stimulatory effect than did other tone-promoting drugs or glucose. These results suggested that the primary cause of the arterial muscle contraction was not to be found in an increased supply of high-energy phosphate compounds but rather in the muscle's capacity to utilize its existing energy reserves. If this is the case, it applies primarily to isotonically-loaded arterial muscle for these workers also found that under isometric conditions, the potassium ions elevated the lactic acid production both during the actual rise of tension and when the tension had reached maximal level. In contrast to

this we have found that the energy metabolism associated with the usual drug and electrical stimuli is not influenced nearly so much by the type of loading used and that it is generally greater rather than less with isotonic loading of the arterial muscle.

The effect of isometric and isotonic loading on the maximum energy liberated by skeletal and cardiac muscles has been looked into by a large number of workers. Although most of the early work on skeletal muscle by Hill (1913a, 1913b, 1915) revealed that an isometric contraction liberated, in general, more energy in the form of heat than an isotonic one, since the work of Fenn (1923) most workers have found the exact opposite. Fenn (1923) also found that the extra energy liberated in the isotonic contraction varied quantitatively with the work performed by the muscle. This is in agreement with our own results as well as with the observations made by Abbott and Lowy (1958b) on molluscan smooth muscle, Clark and White (1928) and Lorber (1953) on heart muscle, Fischer (1928, 1930) and Hartree and Hill (1928) on tetanic contractions of skeletal muscle and by Hill (1930) on simple isotonic twitches of skeletal muscle.

By measuring the oxygen uptake rather than the heat liberated by frog sartorius muscles, Fischer (1931) and Baskin (1965) found the maximum isotonic Q_{O_2} to be significantly greater than the maximum isometric Q_{O_2} at moderately heavy loads. These findings are in line with our data while on the other hand, the work of Bulbring (1953) on intestinal smooth muscle generally indicated that the rate of oxygen uptake was greater under isometric than under isotonic conditions. This may have been due to measurements being made primarily during the constant tension phase of the drug-induced isotonic contraction when work was no longer being performed by the muscle.

Although the earlier chemical methods gave contradictory results, recent studies tend to confirm the above observations. Thus, Mommaerts, Seraydarian and Marechal (1962), Carlson and Siger (1957) and Carlson, Hardy and Wilkie (1963) have recently found the extent of splitting of phosphocreatine to be directly related to the work done by frog skeletal muscle and to be greater with the isotonic contractions compared with the isometric contractions only when moderately heavy loads were used.

At variance with the general conclusions described above and hence with our own are the results reported by Whalen (1960, 1962), who observed that the oxygen consumption during the isotonic contractions of both rat myocardium and frog skeletal muscle did not differ significantly at any point from the oxygen consumption of their isometric contractions at the same initial length. Also, in spite of the variations in the work done, the oxygen consumption remained relatively constant. Whalen's data do gain some support from the work of Sarnoff et al. (1958) on the intact dog heart and that of Fales et al. (1960) on dog skeletal muscle.

Nevertheless, it appears that our results do agree qualitatively with the findings of many of those studies in which the energy demand of the isotonically-loaded muscles was primarily measured during the phase of shortening rather than the phase of tension maintenance, i.e., when the muscle was definitely performing work and when the effect of a wide range of applied loads on the two types of contraction was also investigated.

As has been mentioned before, the oxidative efficiency of arterial muscle in maintaining an active tension is a few orders of magnitude greater than that calculated for frog skeletal muscle maintaining an active tension. In contrast to this difference, the metabolic efficiencies

of both the arterial and skeletal muscles are approximately equal when the phase of tension development is considered. This similarity has been pointed out by Lundholm and Mohme-Lundholm (1965). From the data that is available on skeletal muscle it is seen that its average metabolic efficiency during isometric twitches is much greater than its value during the maintenance of prolonged tension while it follows that with arterial muscle the increase in efficiency with a phasic compared to a tonic contraction is perhaps non-existent or even negative in value. Considering isotonic loading of our arterial muscle, electrically-induced contractions yielded maximum oxidative efficiency values of less than 3% while the large amount of data available for the corresponding efficiency of skeletal and cardiac muscles gives minimal values of the order of 5%, maximal values of about 25%, with average values running between 10%-15%. All of these values are greater than the maximum oxidative efficiency of our isotonically-loaded arterial muscle. Some of the difference between our efficiency value for arterial muscle and that reported for skeletal and cardiac muscles may be due to our muscle being preloaded (or free-loaded) at all load levels while most of the other workers used after-loaded muscles especially at the higher loads.

8. Young's Modulus of Arterial Tissue

Curves shown in previous figures enable us to derive Young's modulus of elasticity for the adventitia-free arterial segment. The average Young's modulus was found to be quite high and equal to 1000 $g/cm^2/100\%$ elongation. A possible explanation of the great disparity between this value for the modulus of arterial muscle and the much lower values found for other smooth muscles by Bozler (1941) and Krafka (1939) may be due to the presence of elastin which elevates the modulus by a considerable

factor even when present in small amounts in the arterial wall. Hinke and Wilson (1962b) calculated for the entire wall of the rat tail artery which was estimated to contain only 5% to 8% elastin, a modulus which was about six times greater than that for the arterial muscle alone. Our value is in line with that determined by Krafka (1939) for the media alone of a dog's femoral artery and indicates that the proportion of elastic tissue in the media of this artery must be quite high. This has actually been shown to be the case by Harkness, Harkness and McDonald (1957) and Fischer and Llaurado (1966).

9. Possible Mechanisms for the Observed Phenomena

Some of the possible underlying causes of the observed mechanical and metabolic behaviour of arterial muscle are now discussed.

It is possible that the observed increase in force of the isometric contraction with increasing length could in part be due to the re-alignment of obliquely lying muscle fibres which could lead to a more favourable situation for exerting force. If the fibres are oblique then the isometric force developed by the segment in the polarographic method should be greater than in the Warburg method since the segment is stretched longitudinally in the latter case. But the forces developed in the two cases were about equal so that the obliqueness of the fibres is not evident and so is probably not involved in increasing the force of contraction with increasing passive stress. In addition, Strong (1938) has observed the distribution of muscle fibers in the media of a variety of distributing arteries to be a close helical arrangement and in some cases to be almost circular in nature so that the re-alignment with increasing stretch should be relatively small in extent.

Mechanical responses of arterial muscle qualitatively similar to

those which we observed have been reported by a number of workers. In an attempt to determine whether the length or the passive tension was more important in influencing the active response of stretched arterial muscle, Sparks and Bohr (1962) studied the response of the quickly stretched strip at different passive tensions but at constant length and obtained results which suggested that the length was the more important factor. However, Bulbring (1955) has reported that tension may be the determining factor in guinea pig taenia coli since it was related to the excitability and membrane potential in a more predictable way than the length when the strip was stretched or released.

There does exist some evidence which forms a basis for questioning the state of the membrane as a determinant of the contractility-stretch relationship. Thus, Burnstock and Prosser (1960) and Barr (1959) reported an absence of action potentials of vascular smooth muscle in a contracted state. Instead, the primary site for the action of stretch which in turn influences the contractility could be the contractile protein rather than the membrane. In support of this fact is that in spite of the great differences in membrane functions, length or tension is a determinant of contractility of all types of muscle. More specific evidence that length or tension directly affects the contractile protein is that the contractility of glycerol-extracted cardiac muscle and arterial muscle is dependent on length or tension as demonstrated by Benson, Hallaway and Turbak (1958) and Bohr, Filo and Guthe (1962), respectively.

A potential cause of the increase in the force of contraction experienced by arterial muscle with increasing stretch may be the result of the greater concentration of high-energy phosphate compounds which the elevated oxidative metabolism of the stretched muscle makes available to

the contractile protein of arterial muscle and which is utilized during contraction. In support of this theory, Beviz, Lundholm et al. (1965) have shown that the content of creatine phosphate and adenosine triphosphate in arterial muscle is significantly reduced during the development and maintenance of tension by this muscle. Since the "tension" Q_{O_2} does not decrease significantly at the higher values of passive tension but the active tension and "activity" Q_{O_2} certainly do, some factors other than the concentration of high-energy phosphates must be responsible for the decrease in the force of contraction at the higher values of passive tension.

G. The Respiratory Quotient of Arterial Muscle

The average "basal" respiratory quotient of all the segments on which measurements were taken in the polarograph series equals 0.95 ± 0.02 (SEM). The average "tension" RQ equals 0.93 ± 0.01 (SEM) while the various average "activity" RQ values have already been listed.

In the majority of cases, the variation in each of the "basal", "tension" and "activity" RQ values was very slight during the duration of any one experiment. Although these values are not as close to unity as the preliminary Warburg experiments indicated for the "basal" RQ, they are in good agreement with the values of Kirk et al. (1954) who obtained average values of 0.91 and 0.99 for the "basal" RQ of human and dog aortic samples, respectively. Their RQ values, unlike our own, were found to decrease slowly with time. Determination of the RQ of the aortic tissue of newborn and adult rabbits by Costa, Weber and Antonini (1950) showed values between 0.80 and 1.00 which, along with the average value of 0.8 mentioned by Furchgott (1955), are somewhat less than our own

average "basal" RQ value.

There appeared to be no significant difference between the "activity" RQ values of the norepinephrine and electrically-induced isometric contractions while the norepinephrine-induced isotonic contraction yielded an average "activity" RQ of 1.01 ± 0.01 (SEM) which was significantly greater than the electrical value of 0.93 ± 0.02 (SEM). This latter value is quite close in magnitude to the isometric "activity" RQ values. Since all of the RQ values were still quite high, for the most part it can be concluded that neither the nature of the stimulus nor the type of loading which we used had any consistent significant effect on the nature of the substrates that were metabolized by the arterial muscle.

It can be estimated, on the basis of the total oxygen consumed by our arterial segments during a typical experiment, that the total amount of glucose metabolized is equivalent to only about 1% of the segment weight. Thus it does not follow that the glucose stored in the arterial wall is depleted during these long experiments. Nevertheless, our high RQ values are consistent with nearly pure carbohydrate oxidation taking place whether the carbohydrate used be that stored in the wall or supplied externally. However, no measurements of the RQ were made in these series with plasma as the bathing medium. Also, it has been reported by Briggs, Chernick and Chaikoff (1949) and Furchgott (1955) that vascular smooth muscle can metabolize most of the intermediates of the glycolytic and Krebs cycles as well as some fatty acids, so that an RQ of about 0.95 for either resting or active arterial muscle may not necessarily be the value in vivo where a greater variety of substrates is available.

VIII. GENERAL COMMENTS

A. Applicability of our Data to the In Vivo Situation

It is difficult to extrapolate our results to the situation *in vivo*, especially to smaller vessels such as arterioles which are the primary determinants of vascular resistance. Since the dependence of the force of the isometric and isotonic contractions on the initial length or tension is seen to be a fundamental property *in vitro* of arterial muscle, and of many other contractile tissues, it is likely that arterial and possibly arteriolar muscle show the same dependence *in vivo*. If this is actually the case, then the length or passive tension of the muscle in the wall of these blood vessels will play a part in the mechanisms controlling blood pressure.

Regarding the energetics of vascular smooth muscle it can be said that in vessels of the size we used, a prolonged contraction will be accompanied by an increase in the energy turnover of the muscle. Of course, this does not eliminate the possibility that other relations may obtain in arterioles, such as a "tonic" or "catch" mechanism.

It may very well be that the responsiveness of the isolated blood vessels which we used to a given drug or electrical stimulus is much greater than what it is *in vivo* as has been shown to be the case with other denervated smooth muscle tissues. The effect of changes in the passive stress on the active responses of the isolated arterial segment may also be an exaggeration relative to its effect *in vivo*. All that

can be said in this connection is that the drug concentrations which we have used of 5×10^{-7} and 5×10^{-8} g/ml norepinephrine gave strong but submaximal responses. The peak responses were obtained with a norepinephrine concentration of about 10^{-5} g/ml. Although it is not known for certain to what concentration of norepinephrine arterial muscle is subjected when the hormone is liberated at the nerve endings, Vick, Ederstrom and Vergeer (1956) have considered 10^{-7} g/ml of epinephrine to be a physiological stimulus. Since we have found little difference in the response of the vessel wall to this hormone and to norepinephrine it appears probable that the drug stimulus which we used affected the isolated blood vessel to the same extent as *in vivo*.

B. The Possible Role of the Myogenic Reflex in Autoregulation of Blood Flow

Since initial stretch does itself generally increase the oxygen consumption of the resting arterial wall, some support is given by these results to the suggestion that either the stretch of vascular smooth muscle itself or the elevation in the metabolism accompanying it is capable of acting as a stimulus to contraction. Although our results do not significantly favour one factor over the other, if the increase in passive tension rather than in the length is the dominant factor influencing the muscle metabolism and contractility, the chances of the myogenic theory for the autoregulation of blood flow applying become still greater.

Also, stretching the arterial muscle or increasing its passive tension, greatly increases its response, both in the degree of its isometric or isotonic contraction and in oxygen consumption, to the stimulus of pressor drugs and to direct electrical stimulation. Since in normal

function there is always some vasoconstrictor arterial tone, stretching the wall by transmural arterial pressure will, therefore, further increase the active tone of its arterial muscle with subsequent decrease in the vessel diameter and decrease of blood flow. The maximum value of the active tension or stress that we observed in our experiments was about equal in magnitude to the optimal passive tension or stress value of the arterial muscle upon stimulation. Both these passive and active tension or stress values were in the physiological range when either norepinephrine or direct electrical stimulation was employed.

On the other hand, in completely relaxed vascular smooth muscle, as in dilated arteries, it is much less probable that a rise in blood pressure would produce an equally significant increase in tone and consequent constriction. On this basis, the role of the "myogenic reflex" may be limited to introducing only smaller changes in the tone of the arterial muscle, though, of course, its quantitative effect on the tone of smaller vessels such as arterioles might be greater.

Our data consistently showed that the active tension developed by arterial muscle depended on the length or passive tension of the muscle fibres and that the value of the active tension was often comparable in magnitude to the value of the passive tension. In addition, Leonard and Sarnoff (1957) have observed a marked decrease in the extensibility of strips isolated from dog peripheral veins when the strips were experiencing a strong, sustained drug-induced contraction of their vascular smooth muscle. These results suggest that contracted vascular smooth muscle is capable of contributing significantly to the total elastic tension of the blood vessel wall. This property of the vessel wall makes it possible for the myogenic theory of Bayliss (1902) to be operative in blood ves-

sels. In contrast to these observations are those reported by Landgren (1952) and Alexander (1954) who found the distensibility of blood vessels to increase when the arterial muscle in them was contracted which suggests that the vascular muscle itself possesses no significant elastic properties. These differences might be due to the use of isolated segments of very elastic arteries and vascular beds by the latter two workers as compared to perhaps more muscular isolated preparations by the other workers.

C. Metabolic Efficiency of Arterial Muscle

The general observation that the responses of arterial muscle to such dissimilar stimuli as norepinephrine and electrical current are similarly influenced by stretch of the muscle strongly suggests that the mechanism of the influence is a basic component of the contractile machinery of arterial muscle shared by the responses to perhaps all active agents. In this connection it is interesting to note that the slopes of the regression lines which were drawn for the polarographic data and which relate the variation of the "activity" Q_{O_2} with the active stress of the muscle can be reduced, eventually, to units of sec^{-1} or of rate and as such may simply represent the rate of a primary mechanical reaction which is utilizing the energy derived through the oxidative metabolism or it may, in turn, represent the rate of a basic biochemical reaction which is limiting the rate of the oxidative metabolism itself in the contracted arterial muscle such as the rate of breakdown of ATP by ATPase. The constancy of the velocity term for a given type of stimulus is somewhat surprising since respiration is not the only pathway supplying energy and, yet, may be made possible due to the very low energy stores which are available for contractile activity in arterial muscle.

Throughout this study it has been difficult to avoid applying the results obtained on other types of muscle to our own results on arterial muscle in view of what little is known about the properties of arterial smooth muscle. We are aware that such speculation may lead to some false interpretation but since these various muscles have been shown to share some similar properties it was felt that some generalizations in their behaviour were permitted.

IX. SUGGESTIONS FOR FUTURE RESEARCH

The conclusions of this study would be much more meaningful if they could be applied to arteriolar smooth muscle as well. A study of this nature on arteriolar muscle would probably involve the use of a system similar to our polarographic set-up but due to the smaller mass of muscle involved the sensitivity of the system would have to be increased considerably above its present value in order to retain the same degree of accuracy that is presently available. Spontaneous rhythmic contractions of this single-unit muscle may also make the data more difficult to interpret.

It was apparent in this study that factors which may affect the excitability of arterial muscle cells such as chemical agents, electrical stimulation, changes in the sodium, potassium and hydrogen ion levels and the degree of passive stretch, also significantly influence either directly or indirectly the contractility and consequent metabolic activity of the contracted muscle cells. In order to get a clearer separation of the membrane phenomena from those stages of the contractile process which are linked more intimately with the tension production itself, it might prove most profitable to repeat the measurements of the mechanical and metabolic activity on a preparation of contractile protein extracted from arterial and arteriolar smooth muscle.

The effect of indirect rather than of direct electrical stimulation on the active responses of arterial muscle could be determined.

This mode of stimulation is, of course, more physiological and still would permit the continuous recording of all phases of the contractile and metabolic responses, without the necessity of interruption as is the case with drug stimulation. Additional light might also be thrown on the mechanism whereby direct electrical stimulation influences arterial muscle so differently from catecholamine stimulation. A preparation of the rabbit pulmonary artery with its sympathetic nerve attached has been described by Bevan (1962) but unfortunately a large proportion of the smooth muscle cells are arranged longitudinally in this artery and the modulus of elasticity of the tissue is also very low.

The question as to whether the electrically-induced responses are metabolically as efficient as they appear to be could be dealt with by measuring the total heat production of the electrically-induced and drug-induced responses. The dependence of the electrical contraction on any non-oxidative aspect of the total muscle metabolism could be evaluated from its contribution to the total heat production. Although the work would be more biochemical in nature, the rate of utilization of glucose, the rate of lactic acid production or the rate of splitting of ATP and CrP during the two types of contraction would also help to resolve this issue. An investigation of the effect of low temperature on the above parameters during an electrically-induced contraction might disclose the source of energy, if any, of the normal electrically-induced mechanical responses of arterial muscle at these low temperatures which we noticed and as has been reported by Keatinge (1964). The drug-induced mechanical responses were strongly inhibited or eliminated by these same low temperatures. The use of multigrid electrodes in these studies would perhaps ensure a greater synchrony of response of the muscle cells

in the preparation.

It would also be of value to know the variation of the "activity" Q_{O_2} of arterial muscle with its rate of developing tension since this would permit quantitative comparison of the metabolic efficiency of arterial muscle with that of many other muscles where the time factor has been eliminated from the calculation. Some technical problems associated with these measurements include the necessity of a faster responding system to detect the changes in oxygen uptake as well as a mechanical recording system with minimum compliance. The rate of internal shortening of the muscle fibers themselves would have to be estimated in order to permit conclusions to be drawn regarding the responses during a pure isometric contraction.

There are several reports in the literature stating that arterial muscle is capable of metabolizing and synthesizing various lipids. The use of lipids rather than of carbohydrates might be preferred by the arterial muscle under some experimental conditions and this may result in an increase in its metabolic efficiency. The use of plasma as bathing medium and the determination of the RQ would reveal when lipid was being used and whether any shifts in the choice of substrate were occurring under these more physiological environmental conditions. The continuous presence, if possible, of 5% CO_2 in the bathing solution would also render experimental conditions closer to the in vivo framework. Along these same lines, the perfusion of bathing fluid through (and around) the arterial segment and the recording of changes in flow at constant pressure would permit a more meaningful evaluation of the degree of contractile and metabolic activity of the contracted arterial muscle present in the segment.

A clearer picture of the dependence of contracted arterial muscle on its oxidative metabolism might be obtained by a more thorough study of its oxygen debt. The investigation of the effect of different durations of the period of anoxia may yield significantly different values of the oxygen debt depending on the arterial muscle's content and rate of utilization of its preformed high energy stores.

Finally, in order to draw more specific conclusions regarding the mechanical characteristics of both relaxed and active vascular smooth muscle cells themselves, the net effect of the intimal endothelium, the elastin fibres and of the elastic membranes, all of which are present in the adventitia-free preparation of the blood vessel wall, could be quantitatively evaluated. In addition, more light might be thrown on the oxidative metabolism of arterial muscle itself under various conditions if the contribution of the metabolically active intima to the total respiration of the adventitia-free segment were known.

X. CONCLUSIONS

1. A preparation, the isolated segment of dog femoral artery both with and without adventitia, was found to give consistent responses over many hours, during which period contraction was not influenced by previous stimulations and contractions.
2. The "basal" respiratory rate of isolated segments of dog femoral artery which have been stripped of their adventitial layer was measured by means of a Warburg manometric technique and also by means of a polarographic technique. The former method yielded an average "basal" Q_{O_2} of 0.56 µl/mg wet weight/hour ± 0.02 (SEM) and the latter method a value of 0.53 µl/mg wet weight/hour ± 0.02 (SEM). These values are somewhat less than the value reported in the preliminary series of experiments. This may be due to the fact that the latter segments were usually stretched circumferentially. In general, the "basal" respiratory rate of arterial muscle was found to be in the upper range of values so far reported for other mammalian smooth muscles.
3. When the wall of the arterial segment was progressively stretched circumferentially, the extra oxygen consumed or the "tension" Q_{O_2} increased to reach a maximum value almost equal in magnitude to the "basal" Q_{O_2}, at which it either levelled off or decreased slightly with further stretch.
4. Stimulation of the arterial segment with adrenaline, noradrenaline

or by means of direct electrical stimulation yielded both isometric and isotonic mechanical responses which were highly dependent on the initial passive tension or stress of the segment. These mechanical responses consistently increased to a maximum value and then declined as the passive stretch was increased. The optimal value of passive stretch was in the physiological range.

5. The increase in the average or steady state rate of oxygen consumption of the contracted arterial segment was in each case proportional to the magnitude of the active stress developed or to the total external work performed by the segment. Thus, the "activity" Q_{O_2} of the contracted segment also increased to a maximum value and then decreased with increasing stretch but the decrease was not as steep as the decrease in the active mechanical response. Also, with few exceptions, the maximum values of the "activity" Q_{O_2} occurred at higher values of passive stretch than did the corresponding maximum mechanical responses. Despite the increase in the scatter of the results introduced by these two effects the correlation between the "activity" Q_{O_2} and the active stress or work was quite high.
6. The contraction induced by direct electrical stimulation utilized less oxygen than did the mechanically equivalent noradrenaline contraction when the polarographic but not when the Warburg technique was employed. Some possible reasons for the apparently greater metabolic efficiency of the electrically-induced isometric and isotonic contractions are discussed including the differential effect of temperature, pH, anoxia and of different concentrations of sodium and potassium on the electrical and drug-induced responses.
7. A comparison of the oxidative requirements of isometric and isotonic

contractions of arterial muscle shows that the isometric contractions consume more oxygen at small passive loads but less oxygen than the isotonic contraction at the optimal passive load. The load for the peak isotonic contraction was also generally greater than that for the peak isometric contraction.

8. Whether it was relaxed, stretched or contracting the RQ of the arterial muscle was 0.95 ± 0.03 (SEM). This is consistent with the metabolism of glucose, which was present in the bathing medium.
9. It appears that, apart from the nature of the stimulus employed, one of the major factors governing the rate of energy production of contracting arterial muscle with oxygen consumption as the criterion is the active tension developed and maintained by the muscle, or the total external work performed by the muscle.

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APPENDIX

A. Composition of Physiological Salt Solution

The salt solution we used was a modified Ringer solution which contained a phosphate buffer. Two stock solutions were prepared and designated A and B, with compositions respectively in millimoles/liter:

A	NaCl	145.3
	KCl	5.6
	Na_2HPO_4	4.2
	H_3PO_4	0.2-0.6
	$MgSO_4$	1.2
	Dicalcium edetate (Versenate)	0.027
B	Calcium chloride	2.16

These two stock solutions were kept at 4°C until required. The buffered physiological salt solution was then made by mixing eight parts of A with one part of B. Sufficient phosphoric acid was used in A to produce a pH of 7.4 when the solutions were mixed as specified. No trouble was encountered from precipitation of calcium since the two solutions were kept separate until required. The stock solution kept well and maintained the same pH for several weeks. The edetate was included to chelate trace amounts of heavy metals which would otherwise catalyze the auto-oxidation of catecholamines which were used to increase the tone of the arterial muscle. D-glucose was added to the mixed solution just before use to give a final concentration of 200 mg%.

B. Technical Considerations

1. Method A

(a) Manufacture of the Rubber Segments

The process simply involved the dipping of glass tubing (3 mm outside diameter) into a latex solution (Lewiscraft Rubbertex compound). The amount of ammonia which was added to this solution governed its viscosity and hence the amount of rubber solution which was deposited on the glass tubing per dip. Usually only one dipping was necessary. Upon the advice of a Goodyear engineer these rubber-coated tubes were allowed to dry in air for about 3 hours, then at 60°C for another 2 hours and then in air again for another 48 hours. After this time, with talcum powder smeared all over the rubber segment, the segment was rolled off of the glass tubing, then unrolled and cleaned up. To insure adequate drying of the internal surface of the rubber segment, the latter was allowed to dry in air for another two days before being used. This procedure resulted in minimum seepage of fluid through the thin rubber walls probably by eliminating the small water pockets present between the molecules of polymerized latex.

(b) Distensibility Measurements

The distensibility of the various components of the Warburg set-up was determined with the aid of a very simple device which consisted of a pressure transducer connected to a mercury manometer by means of which the desired pressure was also applied across the wall of the tubing to be investigated. This tubing was placed inside a flask filled with water with a calibrated capillary tube projecting horizontally through the stopper enclosing the flask. The change in the volume of the tubing which occurred with an increase in its transmural pressure was measured

as the displacement of the water along the capillary tube. The ratio of the final volume change to the corresponding pressure change gave the distensibility.

It was found that the distensibility of the polyethylene tubing with its connections equalled 0.013 mm^3/mm Hg, while that of the average rubber segment was 38.8 mm^3/mm Hg and that of the average adventitia-free arterial segment 1.92 mm^3/mm Hg. According to the manufacturer the distensibility or volume displacement of the Statham pressure transducer which was employed equals 0.00083 mm^3/mm Hg and is apparently constant up to 250 mm Hg pressure. Except for the transducer and rubber segment values, the other values are expressed relative to an initial transmural pressure of 50 mm Hg. Due to its very low distensibility, the rubber segment value was determined at an initial pressure of 0 mm Hg.

It appears that the rubber tubing was much too distensible to interfere with the stiffer arterial segment, whereas the connecting tubing and the pressure transducer were relatively indistensible and hence, absorbed comparatively little of the active pressure developed by the stimulated arterial segment. When the arterial segment develops an active pressure of 40 mm Hg, its volume decrease can be estimated to be less than 0.2%, so that the contraction was essentially isometric in nature.

(c) Limitations of the Equipment

The physical arrangement of the apparatus used in the Warburg experiments appears to be very poor in several ways. One of its primary drawbacks would seem to be the presence of oxygenated modified Ringer solution within the rubber and arterial segments which could act as a source or sink for oxygen gas present in the bathing medium. Depending

upon the concentration gradient of the oxygen across the wall of the two segments, oxygen could be diffusing one way at one stage of the experiment and then, if the bathing solution were replaced with fresh solution or if the transmural pressure were increased, thus reducing the thickness of the segments as well as adding more fresh fluid to the enclosed solution, the rate or direction of its diffusion could be changed. Any oxygen lost from the bathing solution to this enclosed solution would be erroneously interpreted as an increase in the arterial muscle Q_{O_2}, while any gain of oxygen by the muscle or bathing solution from this trapped fluid would give an error of opposite sign.

The only way in which the effect of this variable factor could be measured was to compare the results of the "basal" Q_{O_2}, "tension" Q_{O_2} and "activity" Q_{O_2} values determined with this set-up with those determined by using the polarographic technique. As it turned out, no consistently significant differences were observed between the various parameters that were compared. This was to be somewhat expected as the maximum ratio of the enclosed fluid volume to the bathing solution volume and hence, of their contents of oxygen as well, was only about 0.06.

In addition, paired measurements were later made of the "basal", "tension" and "activity" Q_{O_2} values of an adventitia-intact arterial segment when the enclosed fluid volume was saturated with oxygen and the same measurements were then repeated when the trapped fluid was saturated with air instead of oxygen. Despite the very high initial concentration gradient for oxygen in the latter case compared to the former case, there was no significant difference between the corresponding Q_{O_2} values. A second segment which also had a wall of ordinary thickness was stripped of its adventitia and the procedure repeated with similar

results. The thin homemade rubber tubing was used with both arterial segments.

The distensibility of the various components of the system has been given earlier. Due to the relatively high distensibility of the rubber tubing and the high degree of stiffness of the connecting tubing and pressure transducer compared to that of the arterial segment, it is probable that the passive and active transmural pressure values were not noticeably distorted because of the distensibility of the various components of the system.

In addition, the few control experiments which were performed to determine the magnitude of the oxygen consumption by or its diffusion through the rubber segment and polyethylene tubing, yielded results not significantly different from those of another control flask which contained neither the rubber nor polyethylene tubing. In any event, during the actual experiments involving arterial segments, the thermobarometer flask was identical to the one containing the arterial segment but did not contain the latter, so that the thermobarometer correction included the loss or gain of oxygen by all means other than the consumption by the arterial segment itself.

The leakage of fluid from within the stretched rubber and arterial segments at experimental values of transmural pressure was not significant enough to warrant correction, especially over the small interval of time that any one passive or active pressure was maintained. As a result, the oxygen consumption readings were hardly influenced by the very small leakage of fluid which sometimes occurred.

2. Method B

(a) Calibration of the Oxygen Electrode

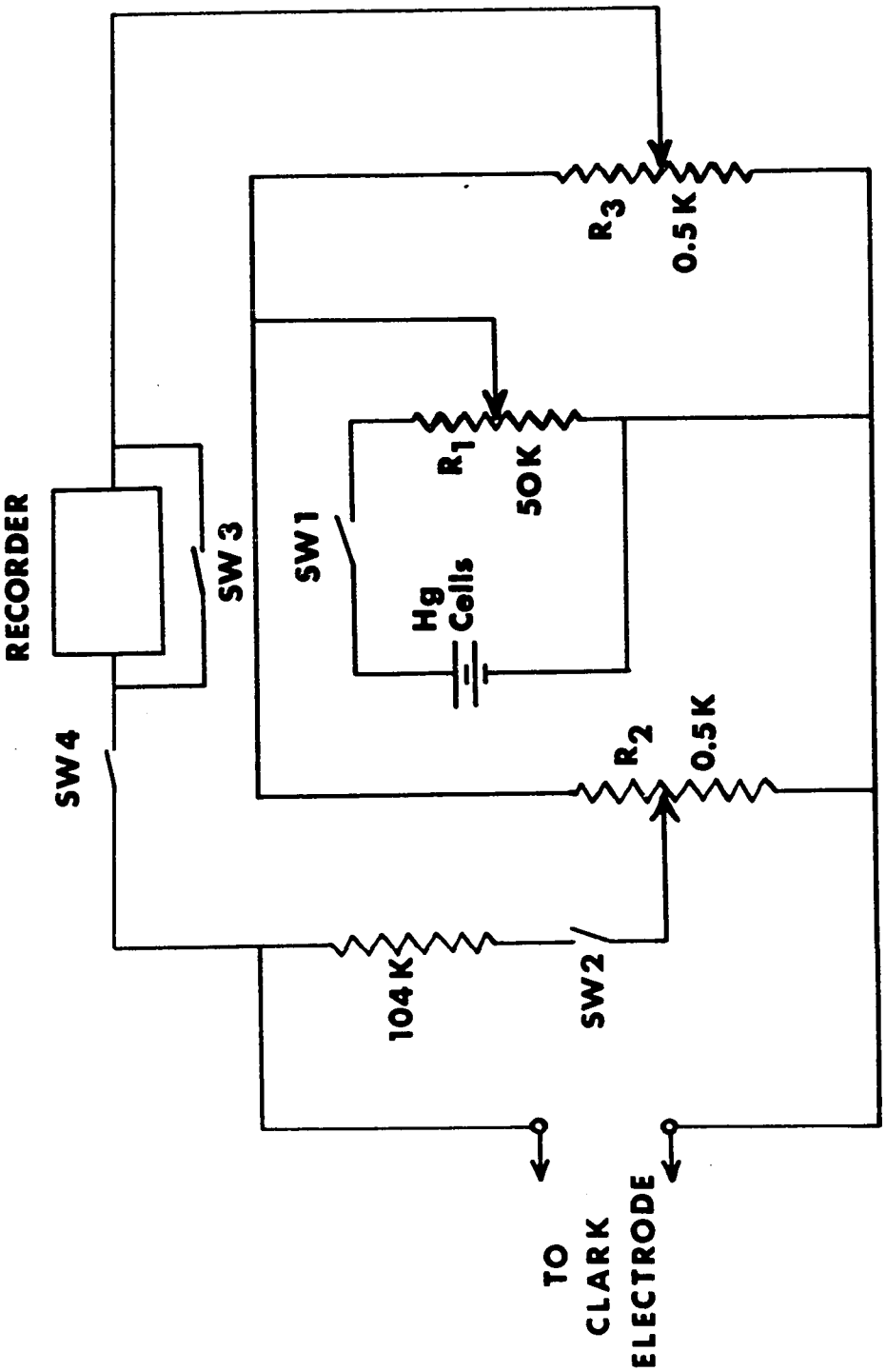
The electrode was calibrated by immersing its tip into a solution

of buffered modified Ringer-glucose at 37°C while the solution was saturated with one of the following preheated gas mixtures: 0% oxygen + 100% nitrogen; 4.5% oxygen + 95.5% nitrogen; 10.3% oxygen + 89.7% nitrogen; 20.6% oxygen + 79.4% nitrogen and 100% oxygen. The Fry gas analyzer described by Fry (1949) was used to determine the average amount of oxygen gas present in the various commercial gas mixtures. These gases were each bubbled through the solution at a vigorous rate with the electrode placed just a little to one side of the main stream of bubbles so that it measured the oxygen concentration in the fluid rather than that in the gas bubbles and yet the agitation of the fluid in the vicinity of the electrode was sufficient to give a steady reading. If the solution is not mixed adequately at the electrode tip, due to the consumption of oxygen by the electrode, the oxygen concentration near the tip decreases progressively with time and the readings decrease also. A very linear relationship (within ± 1% of full scale) was observed between the electrode current and the concentration of oxygen in the solution, with the average slope of the curve equalling 0.048 microamperes per 1% change in the oxygen concentration.

Two more additional commercial gas mixtures containing concentrations of oxygen which were more in our experimental range, were bubbled through the modified Ringer solution in the same manner as the above mixtures. They were also analyzed with the Fry gas analyzer to determine the average concentration of oxygen that was present. Both of these mixtures, consisting separately of 95.4% oxygen + 4.6% nitrogen, and 97.7% oxygen + 2.3% nitrogen, gave output current values which fitted quite closely on the above line. This showed that the 100% oxygen gas used before, upon vigorous bubbling through the modified Ringer solution, yielded an average

FIGURE 31

Schematic diagram of the electrical circuit used with the Clark oxygen electrode.


RECORDER
SW 4
SW 3
SW 1
Hg Cells
R_1 50K
R_3 0.5 K
R_2 0.5K
104 K
SW 2
TO CLARK ELECTRODE

concentration of oxygen in the solution equal to 99.4% ± 0.5%. That such a high percentage of the oxygen should dissolve in the solution and be "seen" by the electrode was necessary since this was the concentration that was consistently used at the beginning of each experimental run. It was also necessary that the linearity of the electrode output should be maintained at these high concentrations since it was later decided to let the oxygen concentration in the cuvette solution decrease by only 2% from its initial value, i.e., from about 99.4% to 97.4% oxygen.

Therefore, the steady state changes in the oxygen concentration occurring in the cuvette solution, due to consumption of oxygen by the arterial segment, could be calculated from the corresponding changes of the electrode output current (or voltage). With a fresh, fully oxygenated solution in the cuvette, after allowing a few minutes for equilibration of the temperature and of the oxygen throughout the cuvette solution as well as of the electrode itself, the electrode output current was completely suppressed (by adjusting the bucking resistor R_3 in Figure 31) so that the recorder recorded a net voltage of zero across the load resistor. As the arterial segment consumed oxygen, the output current decreased below its previous value so that the voltage across the load resistor decreased also.

Since the maximum change in the oxygen concentration that was to be allowed was -2%, when this was the case, the output current had changed by 0.096 μamp. To record this current change on the 10 millivolt full scale Varian recorder, the load resistor was made equal to 104,000 ohms. By putting in a load resistor of this value, the 2% change in the oxygen concentration became equivalent to a recorded voltage change of 10.0 millivolts. It is these voltage changes which were actually re-

corded and converted to the corresponding changes in the oxygen concentration. The polarity of the recorder input was set so that as oxygen in the cuvette solution was consumed, the recorder input voltage increased.

The cuvette solution was withdrawn and replaced with freshly oxygenated and preheated modified Ringer solution as soon as the voltage approached full scale. Whenever this procedure was performed, the recorder input was short-circuited and simultaneously another switch (SW4) opened, so as not to affect the electrode load or its polarizing voltage. Once the temperature and gaseous equilibration of the new solution appeared complete, about 1 minute later, and with bubbles eliminated etc., switch SW4 was closed and switch SW3 opened. All this was done simply to prevent the recorder pen from swinging off the scale while the solution was being changed. During any one day, once it was set, the suppressor voltage rarely needed readjustment. Two mercury reference cells in series were used to supply a constant polarizing voltage to the electrode. Switches SW1 and SW2 were kept closed during the entire duration of each experiment.

Since the oxygen concentration in our experiments changed rather slowly with time, the delay of the electrode itself in registering these changes was probably negligible but, as shown previously, there was a net delay of about 2 to 3 minutes before a doubling of the rate of oxygen consumption was actually recorded as such. The rate of stirring of the solution within the cuvette was adjusted at a maximum possible value so as to minimize the time required for equilibration of the oxygen throughout the entire solution. The rate of oxygen consumption by the electrode itself was too small (at the very most 1 μl O_2/hour or about 2% of the

arterial consumption) to warrant correction. The stability of the electrode also presented no problem, the maximum drift of the baseline being equal to about ± 6% of full scale (or about ± 0.6 mv) per 30 minutes which was the longest time period over which any one measurement of the oxygen uptake was made.

(b) Determination of the Q_{O_2}

In order to translate a change in the oxygen concentration to an actual change in the oxygen content of the solution, i.e., to the actual number of microliters of oxygen consumed by the segment per unit time, the effective solubility of the oxygen in the buffered modified Ringer-glucose solution at 37°C must be known. This was determined experimentally by comparing the output current of the electrode or the oxygen concentrations of two separate solutions. One of these was distilled water saturated with 100% oxygen at 37°C, the other was the buffered modified Ringer-glucose solution saturated with the same gas at the same temperature. The rate of bubbling of the gas and the method of measurement of the average oxygen concentration in each case were identical. Since the output of the oxygen electrode was, on the average 7% less when immersed in the solution of buffered modified Ringer-glucose which was saturated with oxygen compared to its output when immersed in water saturated with the same gas with all the other conditions remaining the same, it can be assumed that the effective solubility of oxygen in our modified Ringer solution was 7% less. This assumption is not too far fetched in view of the fact that the solubility of oxygen in Ringer solution at 30°C as given by Dixon (1943) is only 0.5% less than its solubility in water at the same temperature as given by the International Critical Tables (1928). Since the solubility of oxygen in water at 37°C and at a partial pressure of

760 mm Hg equals 0.0239 cc (STP)/ml (International Critical Tables (1928)), the effective solubility in our modified Ringer solution at 37°C and 760 mm Hg pressure was considered to be 7% less or equal to 0.0222 cc (STP)/ml. Therefore, a change in the oxygen concentration of the modified Ringer solution of 1% at 37°C and 760 mm Hg pressure was equivalent to an average change in the amount of oxygen that was dissolved of 0.00022 cc (STP) per ml of solution.

Since the temperature of the cuvette solution was always maintained at 37°C, it was only necessary to multiply this figure by the following factors in order to obtain the Q_{O_2} values of the arterial segment in conventional units: the effective volume of the cuvette solution in ml, the factor of 1000 in order to give the amount of oxygen in microliters, the ratio of the atmospheric pressure in mm Hg at the time of the experiment over 760 mm Hg, the percentage change in oxygen concentration caused by the respiring segment per hour, and finally, by dividing the result by the wet weight of the segment in milligrams yields the Q_{O_2} value in units of microliters oxygen (at STP) consumed per milligram wet weight per hour. Most of the oxygen consumption data that was obtained was expressed as a percentage of the initial "basal" Q_{O_2} so that only the rate of respiration of the resting unstretched muscle had to be converted to the above units.

The effective volume of the cuvette solution was determined at the end of each experiment by slowly withdrawing all of the fluid from the cuvette by means of a 100 cc syringe. The cuvette contents were not disturbed. The volume of this fluid was measured and from it was subtracted the approximate volume of fluid present in the polyethylene tubing and in the venting valve which was closed during the actual experi-

ment. The oxygen dissolved in the fluid present in the tubing had no opportunity to equilibrate with the oxygen in the cuvette solution, and for this reason, this fluid volume was discounted. The effective volume of the cuvette solution that was obtained in this manner was found to range from 50.1 to 50.8 ml and depended, among other things, on the volume of the arterial segment and the depth to which the electrodes were submerged in the cuvette solution.

(c) Calibration of the CO_2 Electrode

The electrode was calibrated by measuring its output voltage while it was immersed in buffered modified Ringer-glucose solution at 37°C which was saturated, in turn, with the following gas mixtures: 1.1% CO_2 + 98.9% N_2; 2.6% CO_2 + 97.4% N_2; 4.8% CO_2 + 95.2% N_2. The Fry gas analyzer was used to determine the average amount of carbon dioxide gas present in the various commercial gas mixtures. These particular mixtures were used since the functional range of the carbon dioxide concentration in our experiments was from 0% to about 2%. It was found that the response of the electrode was quite variable at these low concentrations of carbon dioxide gas, the average readings at each concentration, when plotted against the concentration, displaying not a linear but more of an exponential relationship. This non-linearity of response of the electrode and its long delay in responding, although troublesome, were minor problems compared to the extremely erratic behaviour of the electrode which was observed during almost all of the experiments. It was finally used, not to continuously monitor changes in the carbon dioxide concentration, but to check the RQ of resting, stretched and contracted muscle under various conditions. This involved taking a few readings of the electrode output over a 5 to 10 minute interval whenever it appeared to be reasonably con-

sistent and when the CO_2 concentration was initially about 0.5% or more.

An additional check on the performance of both the oxygen and the carbon dioxide electrodes was made by observing the effect of the highest concentration of CO_2 to be encountered on the oxygen electrode response and of a small change in the oxygen concentration with the carbon dioxide concentration kept constant, on the CO_2 electrode response. It was found that for a constant oxygen concentration of about 97%, the presence of about 3% carbon dioxide gave readings of the oxygen electrode which were not significantly different from those observed when the carbon dioxide was replaced by nitrogen. Also, with a constant concentration of about 2% CO_2, when the remaining gas was changed from 98% oxygen to 95% oxygen + 3% nitrogen, no significant difference was observed in the readings of the CO_2 electrode. Even though the electrodes were not affected by these changes, it could not be assumed that the arterial tissue was unaffected, especially by the drop in pH accompanying the accumulation of carbon dioxide. For this reason, the changes in concentration were limited to a 2% drop in the oxygen and, as it turned out, about an equal rise in the carbon dioxide concentration.

(d) Determination of the Q_{CO_2}

To convert the changes in the CO_2 concentration to the actual volume of carbon dioxide that was being produced, the effective solubility of CO_2 in buffered modified Ringer-glucose solution at 37°C had to be determined. This was done experimentally by, once again, comparing the measured CO_2 concentrations, under identical conditions, in the modified Ringer solution with that in distilled water. Both of these solutions were gassed with a gas mixture containing 4.8% CO_2 + 95.2% N_2 and which was preheated at 37°C. It was found that the CO_2 electrode gave average

readings which were about 3% less with the modified Ringer solution than those obtained with the water. This difference is very similar to that given by Dixon (1943) who found the solubility of CO_2 at 38°C to be 2.4% less in Ringer solution compared to that in water.

This small difference raises the question of the importance of retention of the CO_2 by our solution on the actual output readings of the CO_2 electrode. The effect of this retention was checked by adding enough 3N HCl to the cuvette solution to lower its pH to 5.0 both when the CO_2 concentration was equal to its highest value of 2% and its lowest value of about 0.2%. Separate segments were used to produce these gas concentrations in each case. The electrode output was elevated by an equal and just noticeable amount in the two instances. Similar small changes were observed in testing two more segments in a similar manner. As a result, it was decided that in these experiments no correction was needed to compensate for the retention of CO_2 by the solution.

Therefore, it was assumed that the effective solubility of CO_2 in our modified Ringer solution was 3% less than that in water or equal to 0.55 cc (STP)/ml at 37°C and 760 mm Hg partial pressure since it was found that the solubility of CO_2 in water under these conditions equals 0.567 cc (STP)/ml (International Critical Tables (1928)). Therefore, it follows that a change in the CO_2 concentration of the modified Ringer solution of 1% at 37°C and 760 mm Hg pressure was equivalent to an average change in the amount of CO_2 that was dissolved of 0.0055 cc (STP) per ml of solution. Multiplying this figure by the same or equivalent factors used in the calculation of the Q_{O_2}, yielded the Q_{CO_2} of the arterial segment in the units of microliters of carbon dioxide produced (reduced to STP) per mg wet weight per hour.

(e) Loss of Oxygen from the System

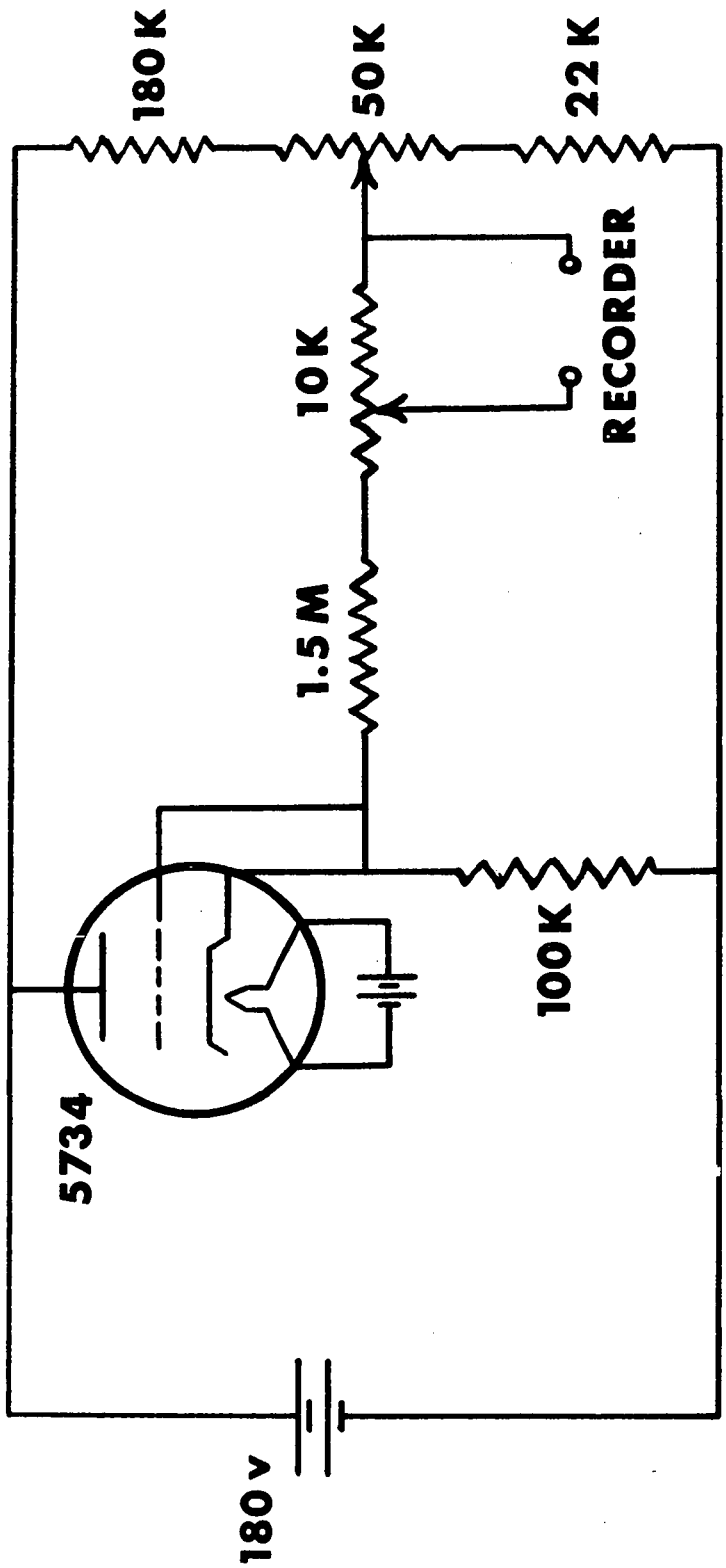
Upon several different occasions, while using this technique to measure the oxygen uptake of arterial muscle, control experiments were performed to determine the rate of loss of oxygen from the cuvette solution when no arterial segment was present. Otherwise, the physical set-up of the apparatus was unchanged. This leakage or loss of oxygen from the freshly oxygenated solution was usually equivalent to a decrease in the oxygen concentration of less than 0.1% over a period of one hour. This rate of loss is equivalent to less than 5% of the "basal" oxygen consumption of an average segment. No significant cumulative effect was noticeable due to this slow loss of oxygen since, during actual experiments, the bathing solution was replaced with fresh solution every 10 to 20 minutes anyway.

(f) Time Delay of the Recording Systems

The time delay of the equipment in registering a change in the oxygen concentration of the cuvette solution, from its initial value of 100% to its new steady value, was determined by injecting some modified Ringer solution saturated with nitrogen gas into the cuvette. This fluid was preheated to 37°C and its volume was varied from 0.5 ml to 2 ml so that the corresponding changes in the final oxygen concentration of the cuvette solution varied from -0.5% to -2%. The time taken for the oxygen electrode to register to within 95% of its eventual final output varied from 2 to 4 minutes from the time of injection of the nitrogen saturated solution. The longer delay times were generally associated with the larger changes in the oxygen concentration of the cuvette solution. The speed at which the solution was stirred was always set at the maximum value possible. Since the oxygen electrode itself has a very short delay

FIGURE 32

Schematic diagram of the circuit used with the mechano-electrical transducer (RCA No.5734) which was used for the measurement of the isometric tension of arterial muscle.


180 K
50 K
22 K
10 K
RECORDER
1.5 M
100 K
5734
180 v

time of 10 to 30 seconds in air, it is probable that the uniform distribution of the oxygen molecules throughout the cuvette solution requires much more time and is thus responsible for most of the delay that is observed. When the carbon dioxide concentration of the cuvette solution was increased by 1%, a delay of about 4 minutes was observed in the CO_2 electrode output, i.e., it reached its final new value 4 minutes after the change was made. The delay that was observed when the pH was changed from its normal value of 7.4 by +0.15 or -0.15 pH units in each case averaged about 2 minutes.

(g) Isometric Loading of the Arterial Segment

As shown in Figure 32, the transducer circuit was such that the recorder input voltage, with zero load on the lever, could be adjusted to equal zero by adjustment of the 50,000 ohm helipot. With the maximum experimental load on the lever, the 10,000 ohm helipot was adjusted to give a full scale deflection of 10 millivolts on a Varian recorder. Since the maximum load that could be applied to the bristle, without damaging the tube, was about 20 grams, a small stiff compression spring was fitted between the bristle and the plastic strip supporting the transducer. The effect of adding this spring was to increase the maximum safe load that could be applied to the bristle to well over 100 grams. Since a few of the loads that were applied to some segments were as high as 80 grams, the 10,000 ohm helipot was usually adjusted to make the recorder read full scale when a 100 gram load was attached to the bristle. Intermediate loads of 20, 40, 60 and 80 grams were also applied to check the linearity of the transducer output at the beginning and end of every experiment.

The wire or thread from the arterial segment was passed through the openings in the wooden and plastic strips supporting the transducer

and pulled up through the centre of the spring. It was attached to the lever at the same point from which the calibrating weights were previously suspended. The spring had to be compressed and tilted out of the way to permit the wire or thread to be tied on the lever, but it was returned to the same initial position after each such manoeuver. A large plywood strip that supported the entire transducer arrangement was then elevated until a barely perceptible tension was exerted on the segment, and the strip was then locked into position. By rotating the small brass screw shown in Figure 3, the plastic strip supporting the transducer was elevated, until no slack remained in the wire or thread and yet there was still a little slack in the arterial segment which was noticed by observing the side view of the segment. The recorder reading at this point represented the effective weight of the wire or thread together with the supporting rod and the attached arterial segment. This weight was quite small and of the order of 0.2 to 0.3 grams. The same screw was then slowly rotated to increase the effective load on the arterial segment to the desired value.

Since no stainless steel wire was available at the beginning of these experiments, silk suture thread (size 00) was used instead to connect the arterial segment to the transducer. Once the steel wire was obtained and straightened out, it was used in place of the thread. The relative amounts of stretch of the wire and thread were compared by securing one end of the 3-foot length of wire or thread rigidly, while the other end was attached to the transducer lever. The transducer supports were then elevated to give an initial load of 100 grams on the thread or wire. The changes in the load with time were recorded. With the thread, it was found that within about 3 minutes, the load dropped by about 2 to

5 grams, while with the steel wire, a drop of 0.5 to 1 grams was observed over the same period of time. In both cases, after the initial decrease, the load remained essentially unchanged. Since the time duration of the yield or stress relaxation of the loaded thread or wire is less than that required for the arterial tissue, it did not interfere with the constancy of the load once the arterial tissue had completed its own stress relaxation. The actual value of the final load on the arterial segment, therefore, needed no correction. However, the "active" force developed by the stimulated segment was sometimes as great as 40 grams and was attained within 2 to 3 minutes after stimulation. It is possible, by using the thread to support the segment, that the "active" force recorded in such cases could be an underestimate of the true value by as much as 2 grams. With the steel wire the maximum under-estimate would be somewhat less, i.e., about 0.4 grams.

It is realized that other components in the actual experimental set-up could be yielding as well when a load is applied or developed. Among these are the arterial support with its retaining screws, the two stainless steel rods, and the self-tightening knot in the wire or thread. Two heavy pieces of brass were placed on the cuvette cover plate to keep the whole cuvette itself from floating up. At the upper end of the set-up, the various transducer supports, the transducer lever and spring were the other possible sources of "give" in the system. The stainless steel, plastic and plywood supports were made as husky as space would permit to reduce the amount of yield in these components. The actual deflection of the transducer lever, with the compression spring in place, was 0.013 mm/ 50 g load. So it is probable that the approximate total external shortening of the arterial segment when maximally contracted with this isometric

arrangement would be of the order of 0.005 mm or about 0.1% of its initial vertical length.

C. Reasons for Discontinuing some Experiments

There were several reasons why segments failed to last the duration of the experiment. Among these, the most common one was the inability of increased stretch, even at the very beginning of the experiment, to have hardly any effect on the magnitude of the mechanical responses. Otherwise, the "basal" Q_{O_2} of these segments was in the normal range and normal active responses were obtained by stimulation at zero load. The cause of this behaviour, besides some inherent defect in the segment, was probably that the segment was damaged by overstretching especially while it was being stripped of its adventitial coat. While care was taken not to stretch the arterial tissue excessively, at times the adventitial tissue was attached more tenaciously than usual and it was difficult to avoid considerable longitudinal stretch of the segment. The hysteresis effect shown previously indicates that the responsiveness of the segment decreases significantly at a given passive tension when it has been previously stretched beyond this tension. So with rough handling of the adventitia-free segment it is possible that the increased responsiveness with passive stretch might be almost completely eliminated.

Another reason for discontinuing some experiments was the appearance of spasm of the arterial muscle. This was usually noticed after the first or second drug or electrical stimulus, when the segment refused to relax or relaxed very slowly. Vasomotion or spontaneous changes in tone of the arterial muscle were also sometimes noticed when the segment was passively stretched in order to determine its "tension" Q_{O_2}. These var-

iations in the tone of the muscle sometimes made both the "tension" Q_{O_2} and the subsequent active stress and "activity" Q_{O_2} values fluctuate to such an extent that their accuracy was seriously reduced. Most of these segments were discarded before a complete set of measurements could be made.

Occasionally, the "basal" oxygen consumption would greatly increase after a few hours and even after replacing the bathing solution with fresh solution would soon start increasing significantly once again. It is probable that, in these cases, bacteria present in the solution or more probably within the arterial wall or very closely attached to it and which were multiplying at an ever increasing rate were responsible for the elevated oxygen uptake, since the addition of some penicillin or streptomycin temporarily reduced the "basal" Q_{O_2} almost to its original level.

Finally, several segments showed a considerable decrease in their final "basal" Q_{O_2} values as compared with their initial values rather than the usual slight increase. The responsiveness of these segments to stimulation usually became progressively smaller with time as well.

The results of those experiments that were prematurely discontinued for any one of the above reasons were not analyzed. Only the results from those segments that gave consistent responses throughout the experiment both while active and while relaxed were analyzed and used in total. About two-thirds of the total number of segments used fell into this latter category.