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THE EFFECT OF HIGH HELIUM PRESSURE ON THE INHERENT TONE OF GUINEA PIG TRACHEAL SMOOTH MUSCLE

By

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ABSTRACT

All submariners undergo ascent training from a maximum of 100 feet of seawater (fsw). Since the training tank opened in 1954 there have been a number of serious incidents, including loss of consciousness, paralysis and death which have been attributed to pulmonary air embolism. Work on isolated tissues in the 1930's suggested that voluntary muscle underwent a spontaneous contracture as a result of compression. It was proposed that rapid compression and/or decompression could result in bronchoconstriction, leading to airway closure and air trapping. Recently, the limit to which man can dive safely has been extended by the use of saturation diving. Some divers have reported sensations of breathlessness during exercise while at depth under saturation conditions. Thus, bronchoconstriction may also occur under static, high pressure conditions.

The guinea pig tracheal chain has been used as a model for human airway smooth muscle, since both have inherent tone in vitro. This preparation was studied at atmospheric pressure to establish its characteristic features with respect to nerve population, receptor population and the mechanism by which tone is initiated and maintained. Particular attention was paid to the response of the tissue to hypoxia.

Some preliminary hyperbaric experiments revealed the importance of controlling other variables known to affect tracheal smooth muscle function such as P_{O_2}, P_{CO_2} and pH. Helium gas was shown to be non-toxic at 1 bar and was used as compression gas in the majority of experiments.

Tracheal smooth muscle responded to compression with a relaxation. Neither receptor blocking drugs, nor the neurotoxin Tetrodotoxin (TTX) had any effect on this response. A 3-fold increase in calcium concentration of the bath fluid reduced but did not abolish the response to compression. Quinidine and ouabain, two agents known to cause intracellular Ca^{2+} to rise, were also tested for their effect on the response to compression. Both quinidine and ouabain caused inherent tone to rise. However, neither drug significantly reduced the response to compression.

The effect of pressure on the response to field stimulation was also investigated. When expressed as a percentage of the tone at the time of stimulation, the response was not significantly reduced, even at 67 bar. This can be contrasted with the effect of hypoxia on the response to field stimulation where, following a similar decrease in tone, the response was significantly reduced.

The effect of pressure is discussed with reference to the results of the experiments performed at atmospheric pressure.

Tracheal smooth muscle does not contract in response to compression and the hypothesis is refuted. The possible implications of the results of this study for submarine escape training and saturation diving are discussed.

"It cannot be too strongly emphasised that there is no reasonable prospect of saving an acceptable proportion of the complement of a sunken submarine unless the methods adopted are founded upon physiological principles."

J.B.S. Haldane

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I would like to thank the Curator and staff of the Submarine Museum HMS Dolphin who opened their archives to me while I was researching the history of submarine escape. I am also pleased to acknowledge technical assistance from Mr. P. Sanderson who visited AMTE(PL) when a sandwich student at the University of Bath and many hours of helpful discussion with Mr. J. Florio of AMTE(PL). I am grateful to Mrs. B. Atherton who has typed this thesis with cheerful competence.

Finally, I am indebted to Alastair, who has kept my chin up!

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SYMBOLS AND ABBREVIATIONS

Pressure. In this thesis the commonest unit of pressure used is the bar. One bar is equivalent to the pressure exerted by 10 msw at a relative density of 1.01972. It is also equivalent to 1 ata, 14.696 psi and 1.01325×10^5 $N.m^{-2}$. Where quoting the pressures reported by other authors the units have been converted to bar, or ata, unless it is not clear whether the authors are referring to absolute pressure, or gauge pressure. It is hoped that the use of the bar, rather than the equivalent SI unit will help to remind the reader that the aim of the study is to further our understanding of man in the underwater environment.

Ach	acetyl choline
ANS	autonomic nervous system
Ar	argon
ata	atmospheres absolute (roughly equivalent to 1 bar).
atm	atmosphere
ATP	adenosine triphosphate
bar	unit of pressure. 1 bar is equivalent to 10 msw. The total pressure at 10 msw = 2 bar or 2 ata.
BIBS	built in breathing system
^{o}C	degrees centigrade
Ca^{2+}	free (ionised) calcium
$CaCl_2$	calcium chloride
CA	catecholamine
CNS	Central nervous system
CO_2	carbon dioxide
DSEA	Davis submerged escape apparatus
ED50	dose expected to initiate 50% of the maximal response.
f	fugacity
FEV_1	forced expired volume in one second
FVC	forced vital capacity
f/s	feet per second or feet of sea water per second with respect to decompression.
fsw	feet of sea water

g	grams
GH	growth hormone
HIS	Hood inflation system
He	helium
HPNS	High pressure nervous syndrome
Hz	Herz, cycles per second
K	dissociation constant
K^+	potassium ion
KOH	potassium hydroxide
La	lanthanum
M	molar
MacI, MacII	the pressure chambers used in this study
mepc	miniature end plate current
Mg	magnesium
m/s	metres per second or metres of seawater per second when referring to decompression.
msw	metres of seawater
N_2	nitrogen
Na_2O_2	sodium perioxide
NSAID	Non steroidal anti inflammatory drugs
O_2	oxygen
OMEC	One man escape chamber
p	probability limits
P	partial pressure
P_c	critical pressure
PG	prostaglandin
pH	-log of the H^+ concentration
PO_4^{2-}	phosophate ion
PIT	pyridyl isatogen tosylate
psi	pounds per square inch. 14.696 psi = 1 ata = 1 bar ≡ 10 msw.
psig	pounds per square inch gauge, i.e. the absolute pressure.
r	correlation coefficient
$\%R_{/5}$	the percentage of precompression tone recovered 5 minutes after stimulation.
s	solubility
SD	standard deviation of the mean
SE	standard error of the mean

SEIE	Submerged escape immersion equipment
SETRC	Submarine Escape Training Review Committee
SETT	Submarine escape training tank
SR	sarcoplasmic reticulum
T	inherent tone
T_c	critical temperature
T_i	initial tone
T_t	transition temperature
TTX	tetrodotoxin
V	volts
v/V	volume by volume
w/V	weight by volume
<	less than
>	greater than
α	Bunsens coefficient of solubility
π	reduced pressure
Θ	reduced temperature
Δ	change
m	milli (10^{-3})
μ	micro (10^{-6})
n	nano (10^{-9})
$[\ ]_{o,i}$	concentration of bracketed ion or atom, subscript o = outside subscript i = inside

LIST OF DRUGS USED

Drug	Supplier
Acetylcholine chloride (Ach)	Sigma Chemical Company
Adenosine (Mg salt)	- Ditto -
Adenosine triphosphate (ATP) (Na salt)	- Ditto -
Adrenaline hydrogentartrate	- Ditto -
Aminophylline	- Ditto -
Atropine sulphate	- Ditto -
Indomethacin	- Ditto -
Propranolol hydrochloride	ICI
Quinidine sulphate	British Drug Houses
Tetrodotoxin (TTX)	Sigma Chemical Company
Verapamil	- Ditto -

All ingredients for Krebs' solution were obtained from British Drug Houses (Analar Grade).

1. INTRODUCTION

DIVING AND SUBMARINE ESCAPE:

AN HISTORICAL REVIEW

Man has explored the underwater world for a variety of purposes: military, commercial and adventurous. As this exploration has extended to greater depths, a variety of problems have been encountered which the physiologist has been called upon to explain and to overcome, so that exploration can continue.

The first part of this thesis consists of a review of the historic developments in diving, which have lead in turn to interesting physiological observations.

Although advances in engineering have allowed submariners to explore and exploit the sea, while themselves living in a normobaric environment, the occasional need to escape from a sunken submarine means that the submariner may be subjected to the same difficulties as the diver. However, the submariner may have to escape from a submarine without the luxuries usually available to the diver, such as extensive decompression tables and a support team on the surface. Thus, the history of submarine escape is full of references to the physiological effects of rapid changes in pressure, gas tension and temperature. Submarine escape and the measures taken to train for such events are reviewed here also.

Man has penetrated the underwater environment for military and commercial purposes for thousands of years. Some of the earliest references to commercial diving must be the wall paintings found in the region of the Nile. These show duck hunters equipped with spears and papyrus reeds for breathing underwater. There are references to pearl inlay dating from 4000 BC and pearl shell at Thebes in 3200 BC. The emperor of China is known to have received gifts of pearls in 2250 BC.

Pearl diving would at this time have been performed without assistance from breathing apparatus. There is evidence from the third century BC in Rhodes that a diver's union enforced a sliding scale for treasure trove according to the depth from which it was recovered, so the difficulties of breath-hold diving were undoubtedly understood. At about the same time Herodotus gives an account of Scyllus who dived to cut the anchoring cables of Xerxe's fleet during a fierce storm.

Breath-hold diving of this kind has its own dangers including drowning and shallow water blackout, due to oxygen (O_2) lack and the accumulation of carbon dioxide (CO_2). Hyperventilation to wash out

CO_2 (the principal trigger to breath-taking) can improve the endurance, as no doubt, can practise. It has been claimed that depths of 101 m can be reached by this method (see Triton, 1978). Dangers not associated with breath-hold diving include decompression sickness. Although 30 ft (10 m) is an easily obtainable depth, and a lung full of air contains sufficient nitrogen (N_2) to be associated with decompression sickness, the duration of the dive is insufficient to allow for a critical amount of N_2 to be absorbed. Similarly, oxygen (O_2) toxicity is not a problem of breath-hold diving.

There are also ancient references to the use of apparatus to assist breathing during diving. For example, Pliny claimed that a breathing tube from mouth to surface was employed by divers during the Persian wars. Lorini, in 1609, described a similar device shown in Fig 1.1 which employed iron hoops to stiffen the tube, preventing its collapse under the pressure of the surrounding water. Such devices, however, cannot support breathing more than a few feet underwater, since the water pressure on the diver's chest exceeds that of the air pressure in his lungs. This results in great difficulty in breathing and in haemorrhages into the airways. Stigler (1911) found that when submerged to a depth of 1 metre (m) he could breathe atmospheric air by such a method but that he could dive for longer by breath-hold methods. After 18 seconds at 2 m he was forced to surface suffering from cardiovascular and respiratory distress.

Probably a more successful aid would be a simple rebreathing bag described by Mariano (1450) and da Vinci (1500). While this contained no means of removing CO_2, the additional volume would allow a greater depth to be achieved before the chest wall collapsed under the water pressure. Some dilution of the CO_2 accumulating in the lungs would be achieved and the relief of the strained ventilatory drive would also be beneficial.

For breathing apparatus to be successful it must supply air at the same pressure as the surrounding water. One method of providing this would be the diving bell described by Lord Bacon (1645) which was lowered full of air on to a tripod, allowing access for the diver, who returned to this sub-aquatic air supply for each breath. A similar device which included a platform for the diver

to work from was employed by Sinclair in 1665 to recover treasure from a wreck of a ship from the Spanish Armada. Using this type of apparatus the diver is restricted to the distance from the bell he can travel in one breath since he must return to the bell for each lung full of air.

Perhaps the diving apparatus of Borelli was designed with a view to giving the diver greater freedom on the sea bed (Fig 1.2). Despite the ingenuity of the device, which has provision for "regeneration of air by condensation in the metal pipe JKL", the designer has ignored the likelihood of the diver being forced up into the rigid metal helmet as he descends through the water. Again, the necessity for the pressure of the air breathed to equal the water pressure was ignored.

An adaptation of the diving bell can be seen in Fig 1.3. This allowed a diver working on the sea bed to breathe air from the bell via a leather tube. An attendant who remained inside kept the air pure by allowing fresh air to enter the bell from a barrel lowered just below its open mouth (see Hill, 1912). Five men could remain at 9-10 fathoms for 1 hour carrying out work for which the bell was designed.

Kleingert (1798) devised an ingenious, if cumbersome, diving machine (Fig 1.4), the piston compressed the large volume of air within the reservoir to the equivalent pressure of the surrounding water. The diving suit consisted of a metal cylinder covering the head and trunk, a leather jacket and trousers completing the outfit. Sufficient air for 2 hours work at the surface was thus continuously supplied to the diver. However, the diver's endurance whilst at depth would be considerably less than this.

The revolutionary diving suit of Siebe (1819) followed the development of an efficient air force-pump to supply air at high pressure to a diver working below. The original open suit allowed air to escape beneath the waist of the jacket. If the diver should fall, however, water would fill the helmet, thus drowning him. The close dress (1837) consisted of a suit of flexible material completely enclosing the diver. Air was vented via a relief valve in the copper helmet. The "standard dress" diving apparatus is based on the 1837 design and is still in use today (Fig 1.5).

The introduction of a demand valve which automatically supplied

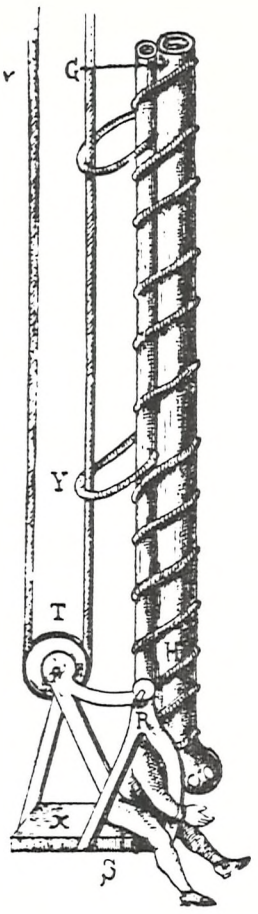
FIGURE 1.1 The diving apparatus described by Lorini (1609).

FIGURE 1.2 The diving apparatus of Borelli.

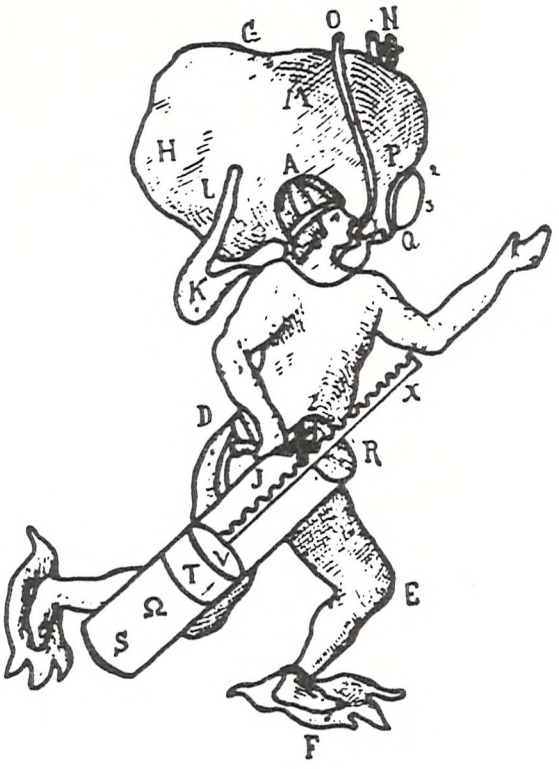
FIGURE 1.3 Spalding's diving bell.

FIGURE 1.4 Kleingert's diving machine (1798).

Reproduced from Caisson Sickness, Hill (1912).



1.1



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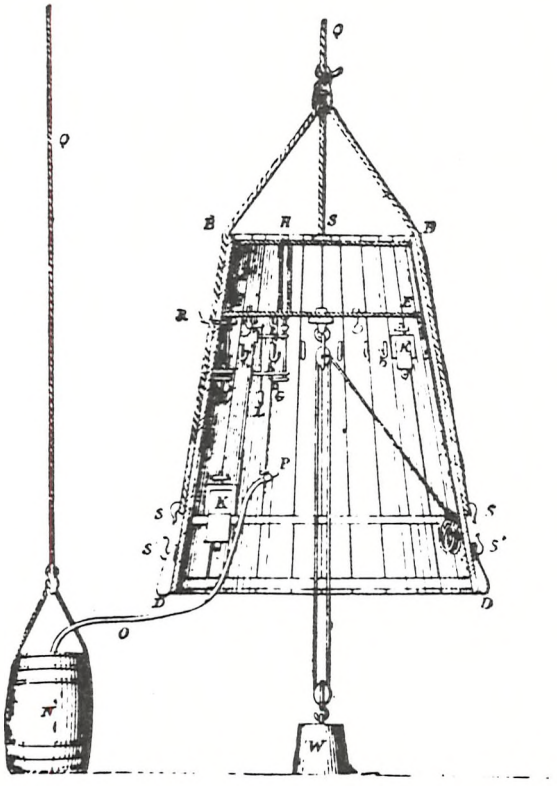

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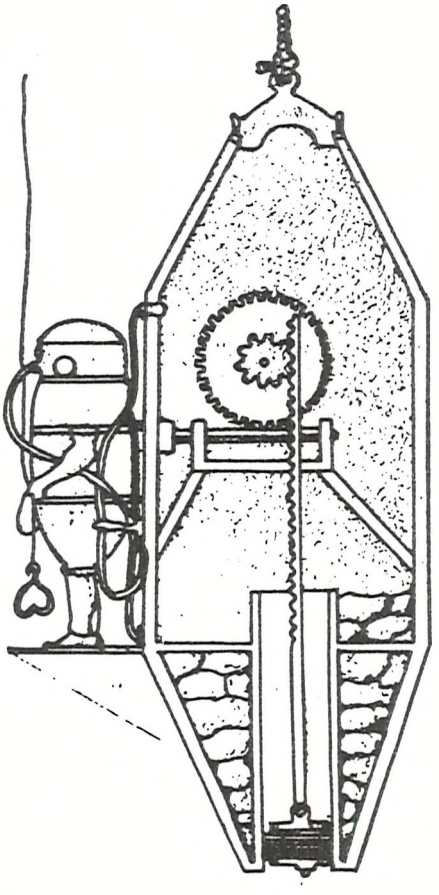


1.4

air at the pressure of the surrounding water (Rouquarayrol and Denayrouse, 1865) greatly benefitted the close dress diver. In order to protect him against the possibility of 'squeeze', air was formerly supplied to the diver at a pressure slightly greater than that of the surrounding water. This in turn made it necessary for the diver to be over ballasted to overcome his positive buoyancy. Each of these contingencies restricted the movements of the diver on the sea bed. The demand valve improved the mobility and independence of the diver and the optimism surrounding its use was reflected in Jules Verne's famous tale "10,000 Leagues Under the Sea", whose divers wore similar diving suits. However, it was not until 1942 that Cousteau improved the design of the demand valve and employed high pressure steel bottles to form the first independent diving apparatus.

Storage cylinders had been employed previously, but were of insufficient strength and limited endurance. For example, Fleuss in 1878 filled his copper cylinders to 30 ata with O_2. The inhaled O_2 was then exhaled into a rebreathing bag, the cylinder being used to top up metabolised O_2. This system requires the efficient removal of CO_2 which in this case was achieved by soaking tow in potassium hydroxide (KOH). This was placed in a separate canister through which the diver exhaled.

In 1952 Payne described an automatic constant mass reducing valve which rendered the O_2 rebreathing sets obsolete. Through these gradual changes in design it is possible to see how the modern diving suit has evolved.

As the diver gained more freedom and was able to dive deeper and stay down longer, the problems of N_2 narcosis, decompression sickness and increased breathing difficulties became apparent. The drunken behaviour of divers at depths greater than 150 ft on air is now recognised as being a result of N_2 dissolving in the tissues, particularly in the fatty membranes of excitable cells. At greater depths the so-called "raptures of the deep" can lead to unconsciousness and death.

If a diver remains at 100 fsw breathing air for more than 20 minutes, then he must ascend or decompress, slowly. This is to allow N_2 dissolved in the blood to be exhaled. Gases dissolve in fluids in proportion to the partial pressure of the gas, and the time to which the fluid is exposed to the gas. If the diver

FIGURE 1.5 The standard dress diver, based on the close dress of Siebe Gorman (1837). Reproduced with permission from the Photographic Department, HMS Vernon.

ascends without decompression stops, the non-metabolised or inert gases form bubbles in the blood. Since the N_2 will not be removed by the tissues the only cure is to recompress the diver, thus reducing the bubble size. This is followed by a very slow decompression. According to the position of the bubble different symptoms arise, varying from itching and joint pain to quadriplegia and death.

Some early distinguished physiologists: Paul Bert (1878) and Boycott, Damant and Haldane (1908) recognised the dangers of breathing air at high pressure and of rapid decompression. Bert was able to detect bubbles in the bloodstream of animals by means of a stethoscope. Haldane developed the first decompression tables.

Oxygen alone cannot be used as a breathing gas at high pressure since at pressures of 2-3 ata it causes convulsions, pulmonary oedema and alveolar damage and forms an obvious hazard to the diver.

In addition to the problems of N_2 narcosis and bends, or decompression sickness there is that of increased work of breathing due to the increased gas density. As the gas pressure is increased, so the effort required to ventilate the lungs reduces the diver's work capacity to zero.

In 1937 a dive to 420 fsw was achieved using a new breathing mixture containing helium (He) and oxygen. This took advantage of the poor narcotic properties of He and its low density (only $1/7$th that of N_2 at 1 ata) to improve considerably conditions for the diver. Since He is an inert gas with a lower solubility than N_2, different decompression tables are required for He diving.

In the early 1960's, the differing solubilities of N_2 and He were employed to speed up decompression procedures. During the ascent from 1000 fsw in a chamber dive and then in the open sea, Keller used air for the final part of the ascent. The rationale for this was that since N_2 was more soluble in blood than He, the N_2 would displace He but would not saturate the blood sufficiently to provoke narcosis or the bends.

Also in the 1960's the first operational saturation dives were carried out. Under saturation conditions the diver lives at high pressure breathing He-O_2 mixtures for several days or even weeks. Once equilibrium has been reached between the partial

pressure of the inert gas and the amount dissolved in the tissues, then the decompression profile is the same whatever the duration of the dive. Any dive to great depth is bound to require a lengthy decompression schedule, so it is more practical and economical for the diver to be saturated. The diver can then perform tasks requiring several days work without the hazards and inconvenience of repeated compression and decompression. Both of the above techniques are now employed successfully in commercial diving.

To date the deepest experimental He-O_2 saturation dive was achieved by a team from COMEX-Marseilles in 1974, when they reached a simulated depth of 2001 fsw. COMEX also hold the record for the deepest operational dive (1575 fsw).

With saturation diving a new physiological problem has been encountered: that of the High Pressure Nervous Syndrome (HPNS), reviewed by Bennett 1971). Although first described in man, HPNS has also been shown to occur in a variety of vertebrate and invertebrate species (Brauer, 1977). In man HPNS describes a collection of symptoms including nausea, vomitting, vertigo, tremors etc, while in animals very high pressures result in convulsions and death. HPNS appears to be due to the effect of pressure per se since it occurs in animals breathing O_2 dissolved in liquids (Kylstra, 1960). Furthermore, it is thought to be due to an action on the central nervous system (CNS) since following section of the spinal cord tremors and convulsions are observed from the head to the point of section, but not beyond.

In man the symptoms appear at about 180 msw (19 bar) and can be severe on reaching 220-240 msw. The severity of the symptoms appears to be related to the compression rate, but even very slow compression does not abolish the syndrome (see AMTE(PL) report no. R80 402).

The most recent development in diving techniques has been the introduction of trimix diving. In 1980, at Duke University, three men were compressed to the simulated depth of 477 m (46.7 bar) At the maximum depth the compression gas comprised 0.5 bar O_2, 43.9 bar He and 2.3 bar N_2. This combination of gases was chosen since it was proposed that the narcotic properties of N_2 would ameliorate the symptoms of HPNS.

The same technique was used in November 1980, when two British divers, both scientists at AMTE(PL), were compressed to the world record depth of 67 bar in "trimix". The compression gas contained 10% N_2 throughout the dive.

The relative success of these experimental dives has lead to the hope that trimix diving will extend the depth at which man can live and perform useful work.

Despite the great achievements of civilian and service divers, the depths presently attainable even under experimental conditions would allow only one tenth of the ocean floor to be explored. This puts six tenths of the surface of the earth beyond the reach of even 20th century man.

One solution to this problem was recognised long before HPNS was encountered, ie the enclosure of the diver or divers within a rigid container, so that he is not exposed to the water pressure. Kessler (1616) described a barrel-like apparatus made of leather and hoops. This was fitted with a window, and weights for sinking it beneath the water. However, it is not clear if this was intended to be an armoured bell, or if it was somewhere open to the water.

Both P. Marsenius (1638) and Robert Boyle (1647) described a submarine boat which was to be rowed underwater. A demonstration of this boat carrying 12 oarsmen plus passengers was conducted on the Thames. This boat employed an innovation still in use in modern submarines and described by Robert Boyle: "Debrell conceived that it is not the whole body of air, but a certain spirituous part of it, that fits for respiration, so that besides the mechanical contrivances of his boat he had a chemical liquor, the fumes of which when the vessel containing it was unstopped, would speedily restore to the air fouled by respiration, such a portion of vital parts as would make it again fit for that office." This outstanding liquid was probably caustic soda or potash which would absorb CO_2 from the atmosphere.

The first armoured diving suit to be fully described was that of John Lethbridge in 1715 (Fig 1.6). It consisted of a watertight barrel with a window and two holes through which his arms could project. Leather cuffs were secured round his arms to prevent water entering when he was lowered beneath the waves. He wrote:

FIGURE 1.6 The armoured diving suit of Lethbridge (1715). Reproduced from Caisson Sickness, Hill (1912).

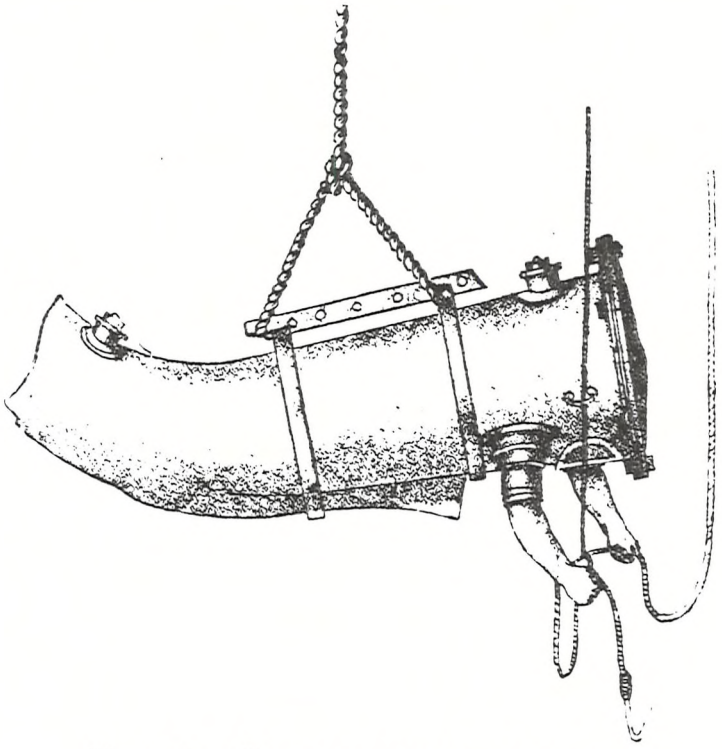


FIG. 8.—DIVING APPARATUS OF LETHBRIDGE.

"I can move about 12 square feet at the bottom where I have staid many times thirty to forty minutes. I have been 10 fathoms deep many times and I have been 12 fathoms but with great difficulty." Lethbridge's machine has recently been reconstructed in a Clydeside shipyard and tested at Comex Marseilles. It seems that although his claims were a little extravagent, some useful work could be performed before the exclusion of blood from the arms, and the coldness of the water made lifting impossible.

The first true submarine boats were tested underwater in the late 18th century. Bushnell provided his boat with chambers which could be filled or emptied of water making the boat rise or sink. This vessel was used unsuccessfully against HMS Eagle during the American War of Independence. Fulton demonstrated a submarine boat in the Seine in 1800. At least one of the submarines of this period had a commercial interest: that of Pazerne in 1844. Certain chambers in which men worked were filled with compressed air, while others could be filled or emptied of water to raise or lower it to the sea bed, where excavation work was carried out. The boat could be moved along the sea floor using poles and was the forerunner of the modern caisson.

In 1851, the first recorded submarine escape was attempted. "Sea-diver" was a single compartment sheet iron vessel, propelled by two hand wheels. In the previous year she had been used to break the blockade of Kiel Harbour by the Danish Fleet when it seems that the mere appearance of the submarine was sufficient to disperse the enemy. By 1851 the Danes had returned and made a second attempt. On this occasion the device which controlled the rate of descent broke and the submarine sank in 60 fsw. Ba er persuaded his crew of two to flood the submarine allowing the pressures to equalise. As equilibrium was reached the hatch opened and the three men were propelled towards the surface by a great rush of expanding air. This escape illustrates well the first essential procedure of submarine escape, ie in order to open the hatches the submarine pressure must equal the surrounding water pressure. Thus, during the flooding of the vessel the unfortunate submariner is exposed to many of the hazards normally associated with diving - N_2 narcosis, O_2 toxicity, cold water immersion and decompression sickness. Furthermore, since the

eventual ascent rate is so fast, there is the additional risk of ruptured lung due to the expansion of gas in the airways. This risk is, of course, accentuated if the escapee should attempt to hold his breath. The history of submarine escape is concerned with the development of apparatus and escape techniques to reduce each of these risks.

It was not until the early 1900's that submarines were taken at all seriously. Previously many senior officers of the Royal Navy were of the opinion that it was contrary to the rules of war to approach and attack an enemy whilst unseen.

At this time it was considered that the only way to rescue men from a sunken submarine was to salvage the whole vessel. The loss of the British A-1 and French "Farfardet" with all hands in 1901 and 1904 respectively, clearly demonstrated the difficulties of this approach. In both cases salvage was begun, but had to be abandoned due to bad weather. In the case of the A-1 it was five weeks before the submarine could be lifted from her resting place off Spithead and brought home to Gosport.

Following these two disasters submarine officers in the US Navy initiated some experimental escapes from submarines. In 1909 the commanding officer of US Porpoise (Ensign Whiting) left a submerged submarine via the torpedo tube, which had to be first flooded to equalise the pressures. On several subsequent occasions the torpedo tubes were used to leave and enter submerged submarines. The disadvantages of torpedo tube escapes as a practical means of evacuating a sunken sumbarine are many. It may not be possible to empty the torpedoes loaded in the tubes and the bow of the vessel may well be buried in the mud as in the case of HMS Thetis (see p. 26). Finally, the last man of the crew would have to sacrifice his life for those of his companions, even if they were prepared to face the further ordeal of being shut up in the narrow, dark tube.

R H Davis, working for the renowned firm of Siebe, Gorman and Co, was working with Fleuss who had designed the closed circuit O_2 breathing system for divers (see p. 9). They hoped to adapt the Fleuss, diving suit, making it small enough to be stowed compactly in a submarine, and for a man wearing the suit to exit a submarine via the conning tower hatch or torpedo tube. This

Fleuss-Davis self-contained diving apparatus had already proved its value in 1878 in the Seaham colliery disaster. By 1903, this suit, although considerably smaller, was still too large to be officially adopted for use in British submarines. The principal obstacle was the size of the O_2 bottle, which in any case could only be filled to 400 psi.

Georges Joubert of France proposed an interesting modification. Sodium peroxide (Na_2O_2) was used as a CO_2 absorbent. In so doing it released O_2, thus avoiding the need for O_2 cylinders. However, the evolution of O_2 was slow, the suit itself was very bulky and Na_2O_2 is inflammable when in contact with water. However, the Hall-Rees apparatus as it was known (see Fig 1.7) was officially adopted, but when the C11 sank with these suits on board no attempt was made to use the apparatus.

Dräger of Lübeck, now a well known maker of diving equipment manufactured a small "Fleuss-type bathing rescuer" which was adopted by the German Navy in 1912. No details of escapes using this suit survive and it appears that the majority of escapes from submarines during the period 1900-1917 were made without the assistance of breathing apparatus.

The policy of the US and British Navies regarding submarine accidents continued to be that of salvage, despite mounting evidence which showed that unless the submarine was sunk close to shore, quickly found and good weather held the men inside would succumb to CO_2 poisoning in less than 48 hours. Indeed, the real danger of CO_2 accumulation was not recognised until after the Thetis disaster in 1939 (see p 26).

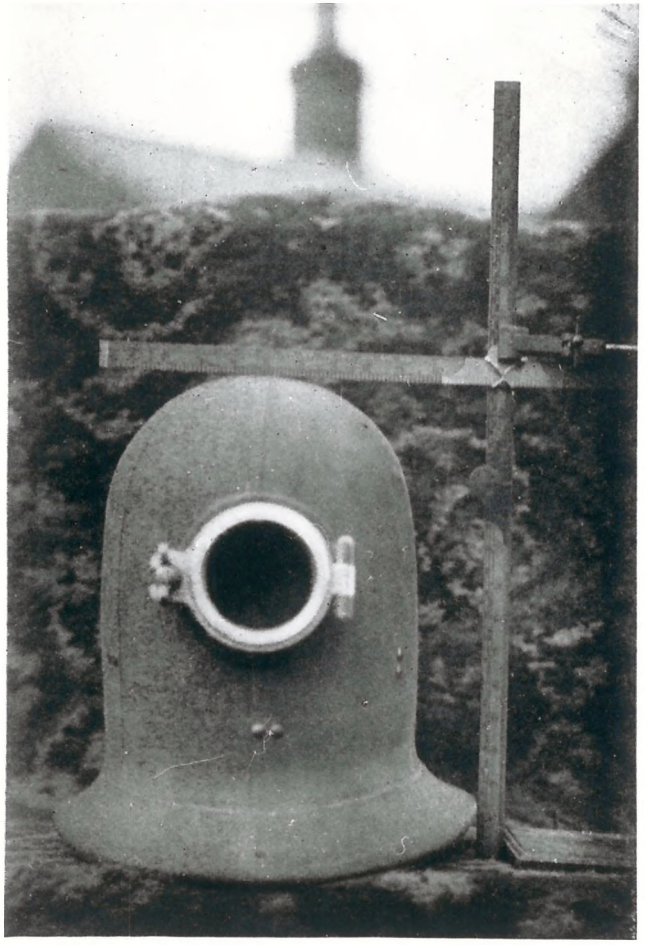
In all the history of submarine accidents there has been only one successful rescue where men have been brought to the surface without alteration of pressure. This occurred in 1939 when the US submarine Squalus was sunk in 40 fathoms twelve hours out of Portsmouth, New Hampshire. The McCann Rescue Bell (see Fig 1.8) designed for just such an emergency was nearby and within 26 hours the support ship was moored over the submarine and the bell ready to make its first rescue bid. By pumping out water from between the bell and the submarine hatch into the ballast tanks, bell and submarine pressures were equalised and the men could enter the bell. In order to regain positive buoyancy water was blown out of the

FIGURE 1.7 The Hall Rees Apparatus.

a. The slogan "one cubic foot per man" was applied to this apparatus.

b. A submariner "wading ashore after a submarine accident".

Reproduced from Submarines of the World's Navies, C. Domville - Fife (1910).



"One cubic foot per man"—showing the small space required in a Submarine for each dress.

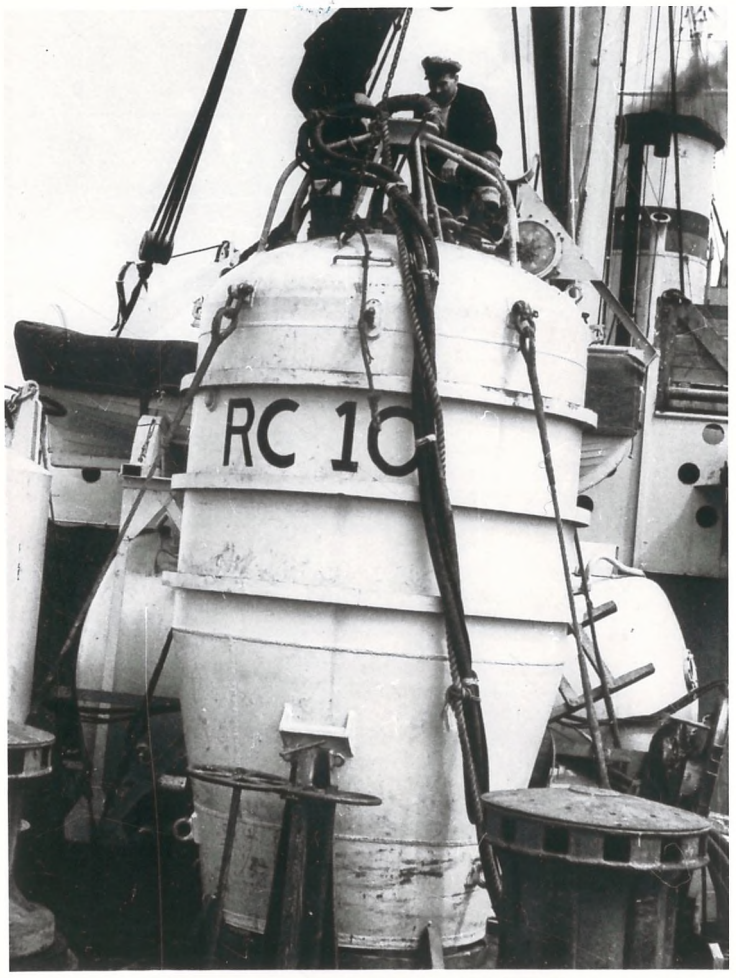
Wading ashore after a Submarine Accident. This dress can also be used for diving purposes.

By permission.

FIGURE 1.8 The McCann Rescue Bell after the rescue of 23 men from Squalus (1939).

FIGURE 1.9 The Momsen Lung.

Both photographs are reproduced from Subsunk, W.O. Shelford, 1960, by kind permission of Mrs. J. Shelford.



"THE GREAT RESCUE BELL ON HER FANTAIL"

Record House, Glasgow

[*See p.* 74.]

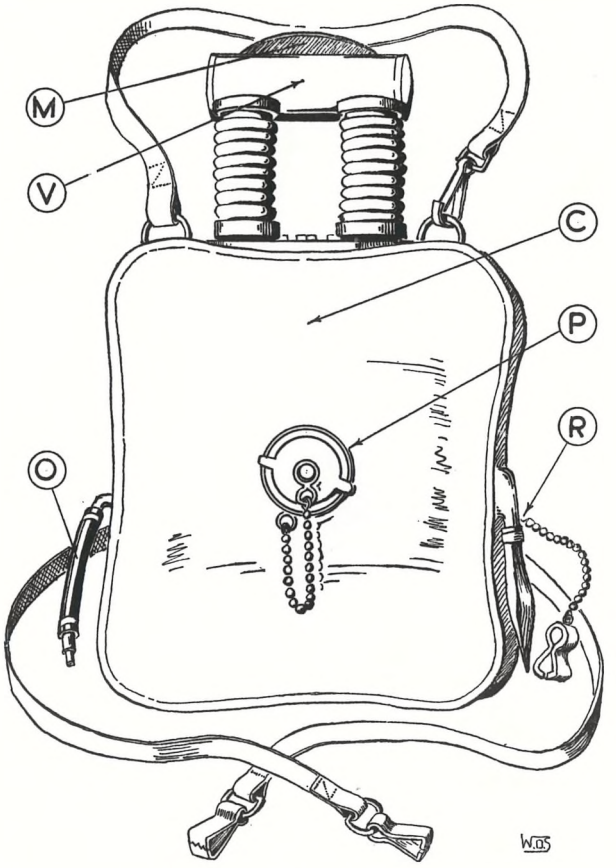


Fig. 4. The "Momsen" Lung (American)

(M) Mouthpiece
(V) Valve-box
(O) Oxygen connexion from cylinder on manifold at escape position
(C) Canister inside bag
(P) Plug for connecting extra canister for protection against smoke and carbon monoxide
(R) Relief valve and clip for blanking off

ballast tanks by compressed air and the ascent to the surface was slowed by a braking cable. Twenty three men were rescued from Squalus. This is still the most successful submarine accident survival story.

At that time the crew of Squalus was also equipped with individual escape sets known as the Momsen Lung, to be used only as a last resort. This apparatus was first described in 1929 following a number of unsuccessful submarine salvage attempts. The Momsen Lung (Fig 1.9) was not a self-contained breathing set but was essentially a rebreathing bag which could be recharged from O_2 bottles fitted around the escape hatches. Exhaled CO_2 was absorbed as the bag was filled. The 'lung' also provided buoyancy once the submariner reached the surface.

Also in 1929, the Royal Navy adopted the DSEA (Davis submerged escape apparatus) (Fig 1.10). This suit employed a small high pressure O_2 cylinder from which to refill the rebreathing bag. The principle behind both the Momsen Lung and the DSEA was identical; expanding gas in the lung was exhaled into the canvas bag during the ascent. The bag itself was fitted with a pressure relief valve. Submariners in the Royal Navy were trained to use the DSEA sets in a tank of water 10 feet deep.

The first escapes using the DSEA were from HMS Poseidon which sank off Wei Hai Wei, North China in 1931 in 130 fsw. Eight men were trapped behind a water tight bulkhead in the torpedo compartment. Six men escaped using DSEA although one died after reaching the surface. Although it was hailed as a successful escape attempt several disadvantages of the compartment escape using DSEA were apparent from this incident.

Firstly, it took 3 hours to flood the compartment, during which time the survivors were subjected to increasing pressure of air, up to 65 psi; secondly, each of the survivors suffered from decompression sickness, two of whom received permanent injuries; thirdly, following the escape each man developed aseptic, or avascular, necrosis of the long bones. This is a crippling disease in which there is disintegration of the articulating surface of the hip, shoulder and/or knee joints.

A further disadvantage of these sets, which became apparent in subsequent escape attempts, was the temptation to relieve the effects

FIGURE 1.10 The Davis Submerged Escape Apparatus (DSEA) demonstrated by Warrant Officer Lacey (1929).
Reproduced by kind permission of the Submarine Museum, HMS Dolphin.

Drill for Use of Davis Submerged Escape Apparatus

A. For use underwater:

The apparatus in the "Ready" position means:

1. Apparatus slung round the neck, and strapped round waist.
2. Goggles on.
   Note They should be dipped in water before being put on.
3. Paper clip off.
4. Mouthpiece elastic round neck.
   When water reaches nut on CO_2 absorbent canister, and not before or oxygen will be wasted:

1. In mouthpiece.
2. Charge bag from manifold, if possible, otherwise from small flask.
   NB Care should be taken not to charge bag more than about two-thirds full as, if it is charged too full, the pressure inside the bag, when the depth is decreased, may not have time to escape quickly enough through the exhaust valve and breathing will become difficult.
3. Exhale through the nose and on nose clip.
4. Open mouthpiece valve and carry on breathing through the mouth.
   NB Care must be taken to ensure that oxygen does not escape through the nose, due to a badly fitting clip nor through the mouth, due to not making a good seal with the lips round the stem of the mouthpiece.

Continued ...../over

Drill for Use of Davis Submerged Escape Apparatus continued ....

B. As a lifebelt:

On arrival on the surface:

1. On paper clip.
2. Blow bag up to full extent either from the oxygen flask or by shutting the mouthpiece valve, removing the nose clip, inhaling through the nose, open the mouthpiece valve blow into the bag, then shut mouthpiece valve.
3. Close the mouthpiece valve; out mouthpiece.
   NB When floating lie as still as possible with the head held well back. The bag can be reinflated, if required, either from the oxygen flask, if not already expended, or by blowing into it.

C. For use out of water:

The apparatus to be in the "Ready" position as in A. except that the paper clip must be on the outlet valve.

The drill is then the same as in A. above.

Note The bag must be recharged, either from a manifold, from the small oxygen flask, or as a last resort by breaking the oxylets, whenever it is felt to be getting soft or breathing becomes laboured.

of O_2 lack and CO_2 accumulation during flooding up by breathing O_2 from the DSEA. Not only does this use up valuable O_2 before the escape attempt, but also it could cause retching and convulsions due to O_2 poisoning. However, it was recognised that O_2 breathing immediately before escaping could wash out some dissolved N_2, thus reducing the likelihood of decompression sickness. A further problem associated with the DSEA was the complicated procedure for its use (see p.22). Of the 6 Poseidon survivors only one definitely followed the correct routine.

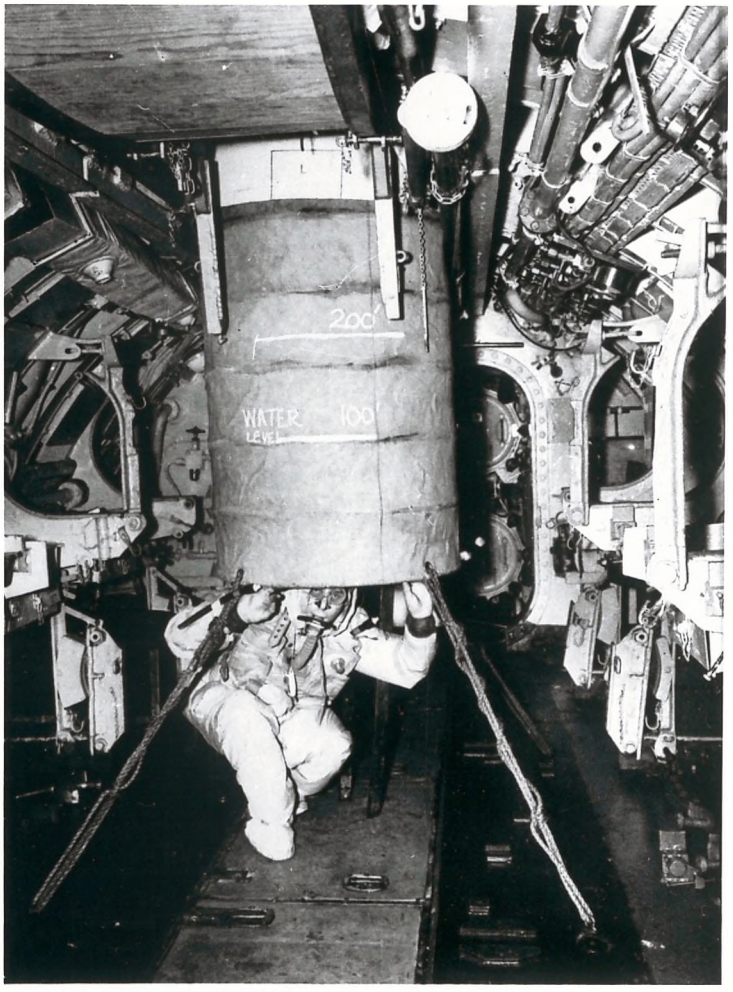
Following the sinking of HMS Poseidon the Royal Navy fitted escape hatches to every submarine and included fast flood valves to speed up the compression. Furthermore, fabric or 'twill' trunks were fitted beneath each escape hatch which could be pulled down in event of an emergency. This was to provide an air lock within the compartment which would otherwise be lost every time the hatch opened (Fig 1.11).

In addition to these positive measures, fittings which would enable air to be piped to a sunken submarine from the surface were blanked off. The purpose of this was to discourage the crew of a submarine from waiting for rescue or salvage until such a time as the air became too foul for them to think or act sensibly and effect independent escapes.

The twill trunk was replaced in the late 1930's by the Davis Escape Chamber which could be flooded more rapidly than the whole compartment. This method had the advantage of allowing the men to escape two by two, leaving the others in the dry and comparative safety of an intact compartment. The escape chamber could then be drained for the next pair to escape.

Many other techniques were patented at about this time, including the McCann Rescue Bell. Some, such as the Gerolami-Arata Olivati system employed a one-man escape pod which could be released from the hull of the submarine, and winched back down to the wreck for the next man. Few of these inventions, however, were practical since they either required the submarine to sink in a horizontal position, or required compressed air, or power, which was unlikely to be available to a crippled submarine. However, in the eyes of any submariner, the worst fault of all was for the escape equipment to occupy too much space. Even the compact

FIGURE 1.11 The Twill Trunk Apparatus. This may be used in combination with life-jacket assisted escape from a depth of 150 fsw or less.



A Rating demonstrating the Use of the "Twill Trunk" as fitted in the Torpedo Compartment of a British Submarine

DSEA sets were often regarded as unnescessary bulk.

Only 8 days after the successful rescue of 23 men from USS Squalus, the British submarine Thetis sank during its initial trials in Liverpool Bay. Of the 103 crew and civilian engineers aboard, there were only 4 survivors. The bow of the vessel was stuck in the mud at 160 fsw while the stern was visible above water. Whilst waiting until ships could be heard overhead (almost 18 hours) the CO_2 content of their inspired air must have been damaging, affecting both their reasoning and their capacity for useful work. In a last minute attempt to leave the submarine they tried to release 4 men from the 2 man Davis escape chamber. When the chamber was drained down for the next attempt the 4 men were still inside, 3 of whom were dead. This so discouraged the remaining men that only 2 more sought to escape. Nevertheless, it is significant that of the 4 men who followed the correct procedure, all 4 lived totell the tale.

Two principal recommendations were made following the Thetis disaster. Firstly, submarine escape training should have been carried out at more than the 15 fsw then practised. Secondly, the Davis escape chamber was abandoned and the twill trunk reintroduced.

Thus, with the DSEA and the twill trunk the Royal Navy went to war for the second time in one century. Submarine escape research continued throughout the war.

In 1940 the second progress report of Admiral Sir Dunbar-Nasmith's Physiological Sub-Committee for Saving Life from Sunken Submarines summarised the principal physiological problems of submarine escape:

1. CO_2
2. O_2
3. N_2
4. Cold
5. Compression
6. Decompression

1. $\underline{CO_2}$

After experiments on themselves, J B S Haldane and E M Case discovered that a concentration of 4% CO_2 in the atmosphere could

be tolerated at 300 fsw for a very short time. 5% CO_2 at the surface certainly resulted in poor success in arithmetic tests. Therefore, Haldane et al recommended that before compression or flooding up began the atmospheric CO_2 should be reduced to 0.5%. For this purpose a number of absorbents were tested but not introduced due to their bulk, caustic nature or inflammability.

2. O_2

This report considered that O_2 lack was more likely to be a problem than O_2 toxicity which was poorly understood at that time.

3. N_2

The problems of decompression sickness were already understood. The problem of N_2 narcosis became more apparent when the Committee members underwent 300 fsw experimental air dives. They discussed the Nuffield Escape Apparatus which employed He:O_2 mixtures and recommended its use. This has never been followed up.

4. Cold

The Committee tested an "anti-cold" suit which protected the wearer from cold immersion at 55.5°F for at least 40 minutes.

5. Compression

The Committee recommended rapid compression in individual or 2 man escape chambers, plus additional training in the clearing of ears. They also suggested the use of Benzedrine inhaler "to improve the opening of the Eustachian tube".

6. Decompression

The maximum safe ascent rate to avoid ruptured lung was considered to be 50 ft per minute. As a result of this the DSEA was fitted with a drogue skirt to slow the ascent through the water.

In addition to these recommendations the Committee stressed the need for escape training and drill in an effort to reduce the strangeness of escape routine and the likelihood of a mistake in the use of the DSEA.

Thus, by 1940 the physiological hazards of submarine escape were largely understood. These were almost all reiterated in 1946 by the Ruck-Keene Committee. However, few of their recommendations were implemented until the 1950's following the

loss of the Truculent (see p. 29).

There is no doubt that many daring and successful escapes were attempted from the sunken submarines of all Navies during World War II. However, many of the men who reached the surface probably died of hypothermia, or drowning before surface ships could rescue them. The escape of 14 men from HMS Umpire in 1941 reflects the success with which the DSEA was used. Although rammed and sunk by a British armed trawler in the North Sea, several nearby ships gave assistance to the survivors. Three other men who escaped with no sets were swept further out to sea and lost, thus demonstrating the advantage of a buoyant life jacket once on the surface.

New escape techniques based on the recommendations of the 1939 and 1946 committees and on the work of the newly founded Royal Naval Physiological Laboratory were gradually introduced after the second world war.

It was first suggested in 1946 that mixtures of N_2 and O_2 be available in the submarine for breathing during emergencies. By reducing the P_{N_2} and raising the P_{O_2} the likelihood of decompression sickness occurring from a given depth would be reduced. Until the P_{O_2} reached 2-3 ata the hazards of O_2 toxicity would not be encountered. This was not tried out until 1954 when the Built In Breathing System or BIBS was introduced. Men could breathe 60:40 N_2:O_2 gas mixture from a mouthpiece connected to a gas line close to the escape hatch. This mixture has since been replaced by air as a cheap appropriate N_2/O_2 mixture, always available to the submarine.

Also in the 1950's individual breathing sets were finally replaced by life jackets. This was partly due to the success of the BIBS and to the evidence that just as many men reached the surface with DSEA as without during emergency escapes. This brought about a big change in escape training techniques.

It was felt that the only way to avoid deaths due to ruptured lungs was to train the submariners to exhale the air as it expanded in their lungs.

In 1946 it was again recommended that trainees should learn to do this from depths of 100 fsw. It was not until 1954, however, that the 100 foot escape training tower was finally opened at

HMS Dolphin.

An experimental one-man escape chamber (OMEC) was introduced in 1946 and dropped in favour of the twill trunk/compartment escape in 1960, although it was recognised that this method was only practical in water less than 150 ft deep. The OMEC proved to be inefficient and required considerable maintenance. This reduced the confidence of the submariner in escape technique.

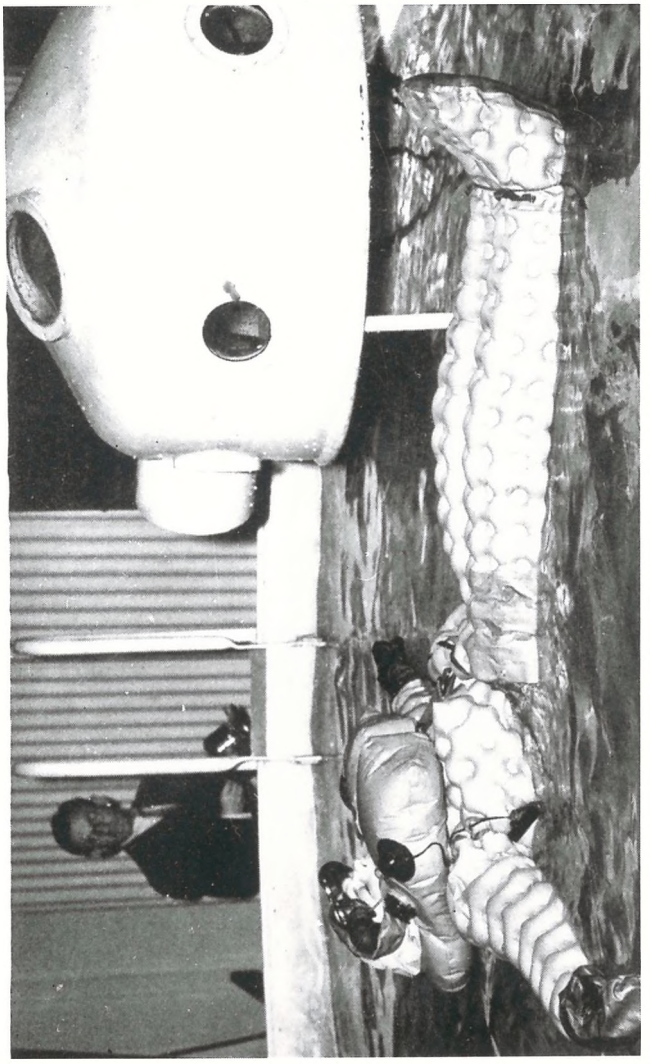
The problem of survival once on the surface was highlighted by the loss of the Truculent (rammed by a merchant ship in the Medway, 1950). Although 72 men successfully escaped from the stricken submarine using DSEA, only 15 men were finally picked up alive, the rest dying of exposure and drowning. Once more a recommendation of the 1939 and 1946 committees was not followed up until a disaster highlighted the problem. In December 1946 an immersion suit of rubberised cotton was tested and it was demonstrated that a man could survive with a reasonable degree of comfort in a water temperature near freezing for up to 4 hours. There is little doubt that had these been available to Truculent, more lives would have been saved.

The present day submarine escape policy and training programme (outlined below) closely follows the physiological principles and recommendations laid down 40 years ago. These principles were understood by Bauer in 1851, who realised that he must first equalise the external and internal pressures, and that rapidly expanding gas in his lungs could escape via his open mouth. Research efforts at HMS Dolphin and at RNPL (now AMTE(PL)) continue to improve the survival chances of a submarine crew.

Every modern submariner undergoes thorough training in escape techniques which are now designed to allow escape from anywhere on the continental shelf (300 fsw). During trials in 1960 several training officers and crew 'escaped' from HMS Osiris, underway at 600 fsw. The revised and improved one-man escape chamber or single escape tower was used perfectly by all men whether instructors or crew. Each used the Submarine Escape and Immersion Equipment (SEIE Mark VII), (Fig 1.12).

The submariner enters the SET wearing the SEIE, and inflates the transparent hood via a HIS (Hood Inflation System) valve connected to the BIBS in the hull of the submarine. The controls

FIGURE 1.12 Submarine Escape and Immersion Equipment (SEIE Mark VII).



A 'Survivor' floating on the Surface of the Hundred-foot Tower wearing an Immersion Suit

can be operated from inside the submarine or within the SET itself. Water enters the SET via a flood valve and compression begins when water reaches an overflow pipe which is then closed. Compressed air and seawater then enter the SET until pressures are equalised. The hatch opens automatically and the buoyancy of the hood takes the escapee to the surface. The pressure within the SET doubles every 4 seconds, i.e. 16 ata in 16 seconds. During this time he breathes air at automatically increasing pressure and can clear his ears. Since the total time at pressure is short the risk of decompression sickness is much reduced, and is negligible down to 600 fsw. During the ascent at 8-12 f/s he can breathe from the air in the inflated hood while he exhales actually more than he inhales (J. Florio, 1978). Expanding air escapes from the bottom of the hood. Once on the surface the double skin suit can be inflated with CO_2 from a small canister providing buoyancy and thermal insulation, even in the arctic, for several hours.

Each submariner is trained for this type of ascent from a mock SET in 92 fsw at HMS Dolphin. He also undergoes three buoyant (life jacket assisted) ascents, one from 30 fsw and two from 60 fsw without breathing apparatus. During these ascents he is taught to exhale steadily thus relieving the expanding gas in his lungs (Fig. 1.13a,b). During the training the simplicity and safety of the techniques are emphasised.

Throughout the history of both diving and submarine escape an increasing understanding of human physiology has enabled man to overcome many of the hazards encountered underwater. As man continues to explore the sea bed further problems may be solved by the same diligent attention to physiological detail.

FIGURE 1.13a Trainee Submariner completing a successful "escape" from 92 fsw wearing the SEIE Mark VII in the escape training tower at HMS Dolphin.

Reproduced by kind permission of the Submarine Museum, HMS Dolphin.

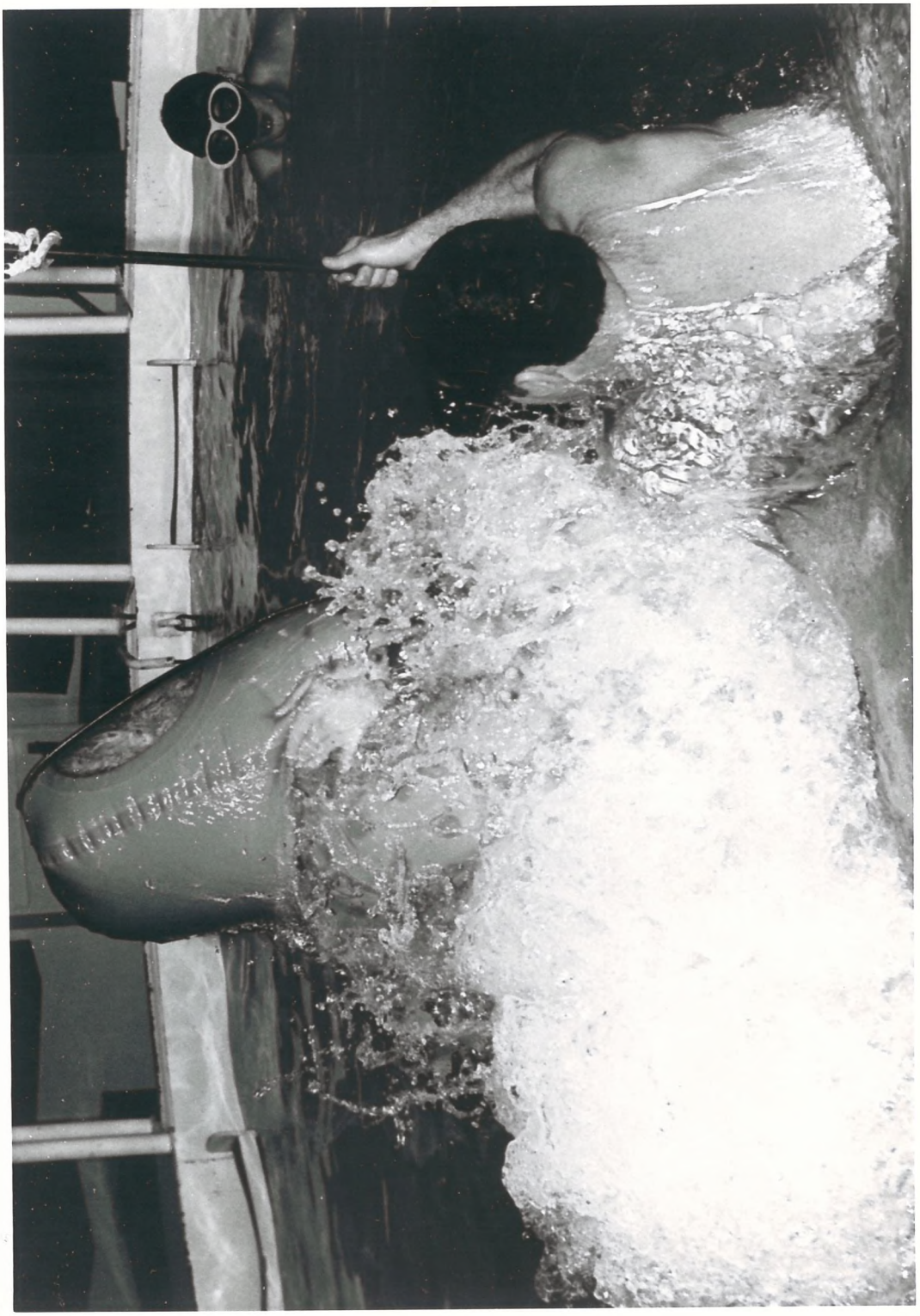


FIGURE 1.13b Trainee submariner undergoing buoyant ascent from 60 fsw. The submariner exhales continuously during the ascent.

Reproduced from "Subsunk", W.O. Shelford, 1960, by kind permission of Mrs. J. Shelford.

A Rating making a "Free Ascent" in the Hundred-foot Tower at H.M.S. "Dolphin"

GENERAL INTRODUCTION

Despite the advances which have been made both in apparatus and training techniques in submarine escape, there remains a finite risk associated with rapid compression and decompression. Submarine disasters will inevitably result in deaths, not all of them associated with the escape procedure. Thus, a true measure of the risk associated with present methods can only be obtained by studying the casualties which occur during training.

Since the opening of the 100 ft training tower at HMS Dolphin, only buoyant ascent and SEIE ascent have been taught and practised routinely. Between 1954 and 1977, 212,000 ascents from depths of 9 m (30 ft), 18 m (60 ft) and 31 m (94 ft) have been accomplished. During these ascents, 91 incidents requiring recompression have occurred, including 4 fatalities. Each of these incidents has been attributed, retrospectively, to arterial air embolism (Greene, 1978). It is believed that arterial air embolism is the result of air becoming trapped in the lung and expanding during decompression before entering the pulmonary circulation. Since in each reported case the instructors verified that the trainees exhaled continuously (buoyant ascent), or breathed normally (SEIE ascent), lung damage did not occur because of breath-holding during the simulated escapes. Indeed, in some cases lung damage could not be demonstrated by X-ray, or where fatalities occurred, by post-mortem examination.

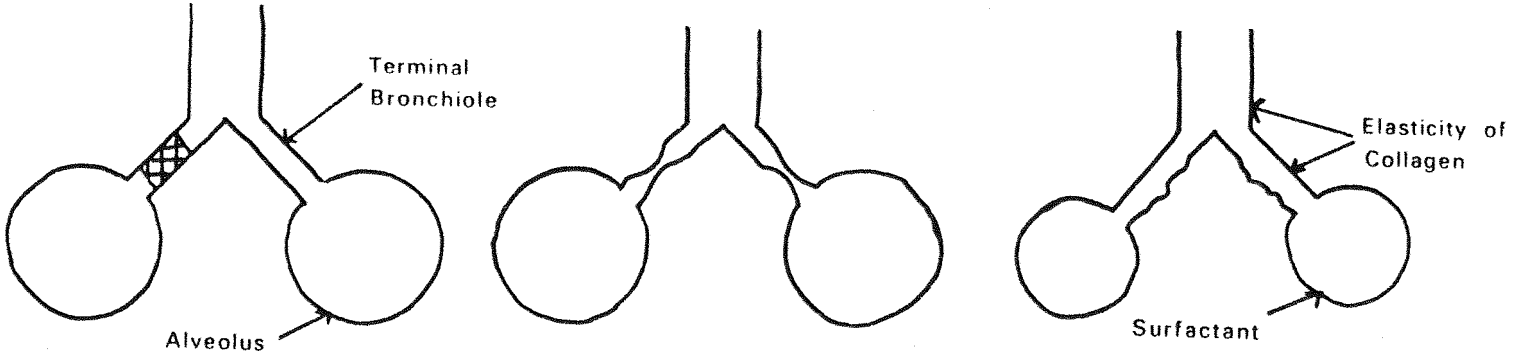
In 1974 the Submarine Escape Training Review Committee (SETRC) urged that greater research effort be made to investigate the possible causes of air trapping during submarine escape training.

Some possible causes of air trapping are outlined below and illustrated in Fig 2.1.

1. It is possible that certain individuals may be predisposed to pulmonary air embolism. Those trainees with a previous medical history of respiratory disease do not undergo training, neither do those who cannot exhale 75% of their Forced Vital Capacity in one second ($FEV_1/FVC < 75\%$). The maximum rate at which the trainee will be required to exhale is his vital capacity in 3 seconds (assuming an ascent rate of 3.3 m/s). Clearly, it is possible for any individual to comply with the test requirements, but still have one or more airways blocked by mucus plug or scar tissue, and for air to be trapped beyond this.

Fig. 1.14

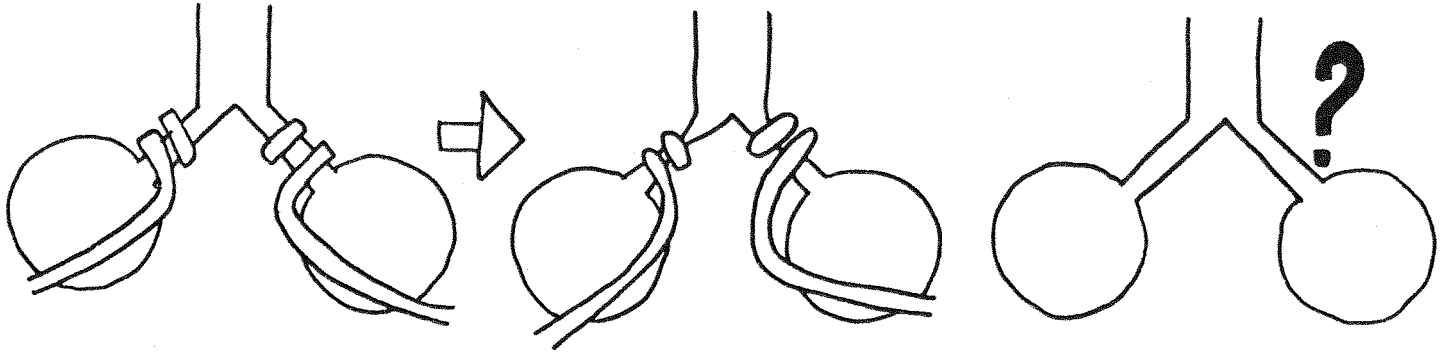
SOME MECHANISMS OF AIRWAY OBSTRUCTION RELEVANT TO SUBMARINE ESCAPE



1. MUCUS PLUG
(PREDISPOSITION)

2. PRESENCE OF SPASMOGENS
VAGAL ? (FEAR?)

3. SOME ASPECTS OF OXYGEN TOXICITY
(ONLY WHEN SATURATED)



4. INCREASED FILLING OF SMALLEST BLOOD VESSELS
DUE TO IMMERSION

5. DIRECT EFFECT OF PRESSURE
(a) ON MUSCLE (c) ON RECEPTOR
(b) ON A.N.S. INTERACTIONS?

2. It has been suggested that the more frightened trainees are more prone to injury as a result of submarine escape training. Fear may result in both vagal and sympathetic stimulation. Vagal stimulation would result in bronchoconstriction, while the result of sympathetic stimulation would depend on the relative proportions of α and β adrenoceptors present in the airways. It has been suggested that when present in the airways α adrenoceptors mediate bronchoconstriction. Thus the net effect of sympathetic stimulation will be determined by the variation in receptor population to a given airway.

3. High partial pressures of oxygen (P_{O_2}) can lead to oedema of the bronchioles, thus increasing airway resistance. High P_{O_2} can also lead to a reduction in the elasticity of the parenchyma, thus reducing the effort independent elastic recoil of the lung. Fortunately, due to the rapid compression techniques currently employed, oxygen toxicity is not a problem in modern submarine escape. It has been proposed that O_2 may be employed as a breathing gas for escapes from depths greater than 400 fsw, but this is yet to be tried operationally.

4. Many investigations into the effect of rapid decompression have used goats and human subjects in dry decompression chambers. These studies ignore the fact that submarine escape is performed with the subject completely immersed in water. Thus two important physiological mechanisms, both tending to cause air trapping, have been neglected.

i. Immersion of the neck and face leads to vagal stimulation, and slowing of the heart has been recorded as a result of this so-called "diving reflex". The effect of sudden immersion on airway resistance in man has not been studied.

ii. When a person is immersed upright in water, the normal pooling of blood in the abdomen and lower limbs is opposed. Since the water pressure on the legs is greater than that on the chest, blood tends to pool in the upper abdomen and thorax. As a result of this, small airways collapse and air trapping can be demonstrated (Dahlback, 1978).

5. It is possible that compression, maintained high pressure or rapid decompression could exert direct effects on the smooth muscle

of the respiratory tract, or its innervation, leading to contracture and air trapping which would increase the risk of arterial air embolism during submarine escape. In addition, a sustained contracture of airway smooth muscle could have important consequences for the diver. Increased airway resistance would add to the work of breathing and lead to a reduction in effective gas exchange during saturation diving.

Effect of high pressure on isolated tissues

The effect of pressure on isolated tissues has been studied sporadically since the experiments of Regnard in 1891. Several investigations have revealed that isolated skeletal muscle and some smooth muscle preparations undergo a spontaneous contracture when exposed to high pressure. These experiments have been reviewed by Cattell (1936), MacDonald (1975) and MacDonald and Wann (1979). The major findings relevant to this study are described below.

i. Cardiac Muscle.

In 1891 Regnard described the effect of hydrostatic pressure on isolated heart muscle from the frog. The cardiac fibres became swollen and ruptured when bathed in distilled water and compressed to 400-500 bar. Hill (1912) reported that compression to 300 bar for 2 hours had no effect on the beating frog heart (in vivo). In 1932 Cattell and Edwards reported that high pressure increased the force of contraction of spontaneously beating frog heart. In 1930 the same workers had reported the positive inotropic effect of low temperature (5-20^{o}C) on cardiac muscle. The authors suggested that high pressure and low temperature exerted these effects via restriction of molecular movement.

It was shown (Edwards and Cattell, 1930) that the contraction time of turtle cardiac muscle was prolonged at 1,000 psig (68 bar) and that tension increased by 42%. At even greater pressures (4,000 psig, 272 bar) contraction height was increased by "several hundred percent". Edwards and Draper (1932) reported that isolated frog heart preparations responded to high pressure with an initial tachycardia, followed by a bradycardia. Ornhagen (1977) demonstrated bradycardia in liquid breathing mice at high hydrostatic pressure. Subsequently, Ornhagen and Hogan (1977) showed that when exposed to 150 bar, the beating frequency of

isolated sinus node preparations was reduced by an average of 43%. Ornhagen did not observe the tachycardia reported in earlier studies because of the rigid control of temperature in his experiments; whereas workers in the 1930's took no account of the adiabatic temperature changes. Sadly, this criticism applies to much of the earlier work.

ii. Skeletal Muscle.

In 1914 Ebbecke published a major study of the effects of high hydrostatic pressure on frog voluntary muscle. He showed that pressures of 300-400 bar enhanced both twitch tension, and tetanic tension initiated by electrical stimulation. Ebbecke also revealed that a pressure step of 300-400 bar alone could cause contracture. These contractures could be demonstrated in the presence of d-tubocurarine, which indicated that pressure was acting directly on the muscle. Furthermore, the contracture was not accompanied by a change in "action current" as monitored by a string galvonometer.

In 1928 Edwards and Cattell investigated the effect of pressures up to 1,000 psig (68 bar) on frog gastrocnemius muscle stimulated to contract tetanically. The contraction immediately following the pressure step was always augmented. The heat evolved by the muscle during contraction was increased proportionately. This indicated that there had been an increase in chemical energy conversion, but no change in the efficiency of the contractile process.

Cattell and Edwards (1932) using the same tissue, reported an increase in twitch tension of 20-40% following compression to 4,000 psig (272) bar).

Nagao (1955) (cited by Hayashi, 1961) demonstrated that frog muscles placed in K^+ ion replete Ringer's solution, in isotonic sucrose, isotonic urea or even distilled water will respond to high pressure with a contraction, even when electrically inexcitable. Another Japanese author (Murakami, 1958) observed that when glycerinated muscle fibres were compressed, no spontaneous contracture could be recorded. This indicates that aspects of the excitation-contraction coupling mechanism of skeletal muscle could be responsible for pressure-induced contracture.

iii. Smooth Muscle.

In contrast to the results obtained with voluntary muscle,

early work on smooth muscle at high pressure revealed a variety of effects. Brown and Edwards (1932) reported the effect of high hydrostatic pressure (72,000 psig, 1326 bar) on the turtle retractor penis muscle. A slowly developing, but prolonged contracture was observed which declined gradually if pressure was maintained. Sudden decompression resulted in an early increase in tension followed by a return to zero. However, Edwards (1935) demonstrated a decrease in tension in electrically stimulated pyloric smooth muscle and in spontaneously active duodenal muscle strips (Cattell and Edwards, 1936) at pressures of 82-89 bar.

A review of work largely by Japanese authors (Kanae Hayashi, 1961) states that smooth muscle contracts in response to pressures between 100 and 2,000 bar (Miki, 1960 and Miki and Yasuda, date not given). However, Miki himself summarises his data thus "when the pressure of about 300-500 kg/cm^2 (approximately 300-500 bar) is applied to the muscle strip, it lengthens quickly at first and then conversely shortens gradually after a certain limit of extension, and again the strip contracts rapidly but temporarily and then relaxes slowly. These lengthening and subsequent shortenings of the strip under high hydrostatic pressure of 300-500 kg/cm^2 seem to be of myogenic nature and not neurogenic nature." This work was performed using the isolated longitudinal muscle strip of the frog intestine.

A subsequent investigation by the same author revealed that the spontaneous activity of frog intestinal smooth muscle was depressed by adrenaline and that this depression was reversed by high hydrostatic pressure.

Akers and Carlsen (1976) have investigated the interaction of adrenaline and also of acetylcholine (ACh) on mammalian duodenal smooth muscle. Their studies revealed that the time to reach the maximum response, or the maximum response latency to ACh was increased at pressures as low as 30 bar, while the magnitude of the response was increased. This work suggests that agonist-receptor interactions could be altered at pressures encountered in modern saturation diving.

At a meeting of the Society for Experimental Biology, Ornhagen and Sigurdson (1978) reported the effect of 25-100 bar on spontaneously contracting rat portal vein strip. At each

pressure the frequency and force of contraction were reduced with respect to one bar, the maximum reduction in frequency (48%) being seen at 100 bar. They observed no effect due to rate of pressure change but reported an increased frequency of contraction during compression.

The most recent study of a smooth muscle preparation at high pressure was published in 1979 (Little and Paton). Guinea pig ileum was subjected to pressures of up to 136 bar and the superfusate collected for bioassay of Ach. Ach release was significantly increased both by He pressure and by a variety of anaesthetic agents. The activity of the muscle was not recorded during these experiments so it is not possible to compare this result with those of earlier workers with similar tissues.

Previous studies on the effect of high pressure on isolated tissues have produced a wide variety of results which are difficult to interpret. Often very high pressures were used, far in excess of any yet experienced by man, but to which some deep sea creatures have adapted. In many of the experiments reviewed by Cattell and McKeen (1936) there was no control of temperature, so that sudden compression and decompression resulted in heating and cooling. Furthermore, the P_{O_2} and pH of the environment were not monitored.

Aims of Study

The aims of this study are fourfold: firstly, to investigate the effect of rapid compression and decompression and maintained high pressure on the inherent tone of guinea pig airway smooth muscle; secondly, to ensure as far as possible that the effects are the function of a single variable, pressure; thirdly, from a knowledge of the pharmacology of tracheal smooth muscle at atmospheric pressure, to determine the mechanism of the response recorded, and finally to relate the effect of pressure on the isolated model tissue to the pathophysiology of submarine escape and saturation diving.

The experiments described in this thesis have been conducted on two conventional models of the human respiratory tract: guinea pig tracheal chain and lung strip. The effect of high pressure on smooth muscle from the respiratory tract has never been investigated previously.

2. MATERIALS AND METHODS

A. General Methods

(i) Tracheal chain

Guinea pigs of either sex (weight range 400-1000 g) were used. The animals were stunned by a blow to the head and exsanguinated via the brachial artery. The trachea, from the larynx to the carina was removed and placed in cool Krebs-Henseleit solution (see Appendix A). Two tracheal chains were prepared from each trachea in a manner similar to that described by Foster (1963).

The trachea was cut into 10 segments of 2-3 cartilage rings. Each segment was cut through the cartilage, opposite the trachealis muscle, to form a strip. Alternate strips were joined together with cotton thread. Thus, contraction and relaxation of the muscle which modified the diameter of the trachea in vivo, resulted in shortening or lengthening of the chain in vitro. The preparation is illustrated in Fig 2.1.

Since two chains are prepared from each trachea, the method is known as the matched, or paired, tracheal chain. During the majority of experiments reported here, matched tracheal chains were used simultaneously to obtain both experimental and control data from the same animal.

The tracheal chain preparation has been used both isotonically and isometrically. Spontaneous tone is a characteristic feature of guinea pig tracheal smooth muscle and it can be observed in either recording mode. Contraction is shown by an upward deflection in every record included in this report.

(ii) Lung strip preparation

In order to compare the effect of pressure on smooth muscle from upper and lower portions of the respiratory tract, guinea-pig lung strips were prepared.

Lung strips measuring 2-4 mm in width were trimmed from the edge of the lower lobes of both lungs, after the method of Lulitch, Mitchell and Sparrow (1976). Two pieces of lung parenchyma could be obtained from each strip (see Fig 2.2).

(iii) Isotonic recordings

Paired tracheal chains of 5 segments each were mounted on matched isotonic transducers (Harvard smooth muscle transducer) under identical loads of 750 mg. The load applied to the lung strip

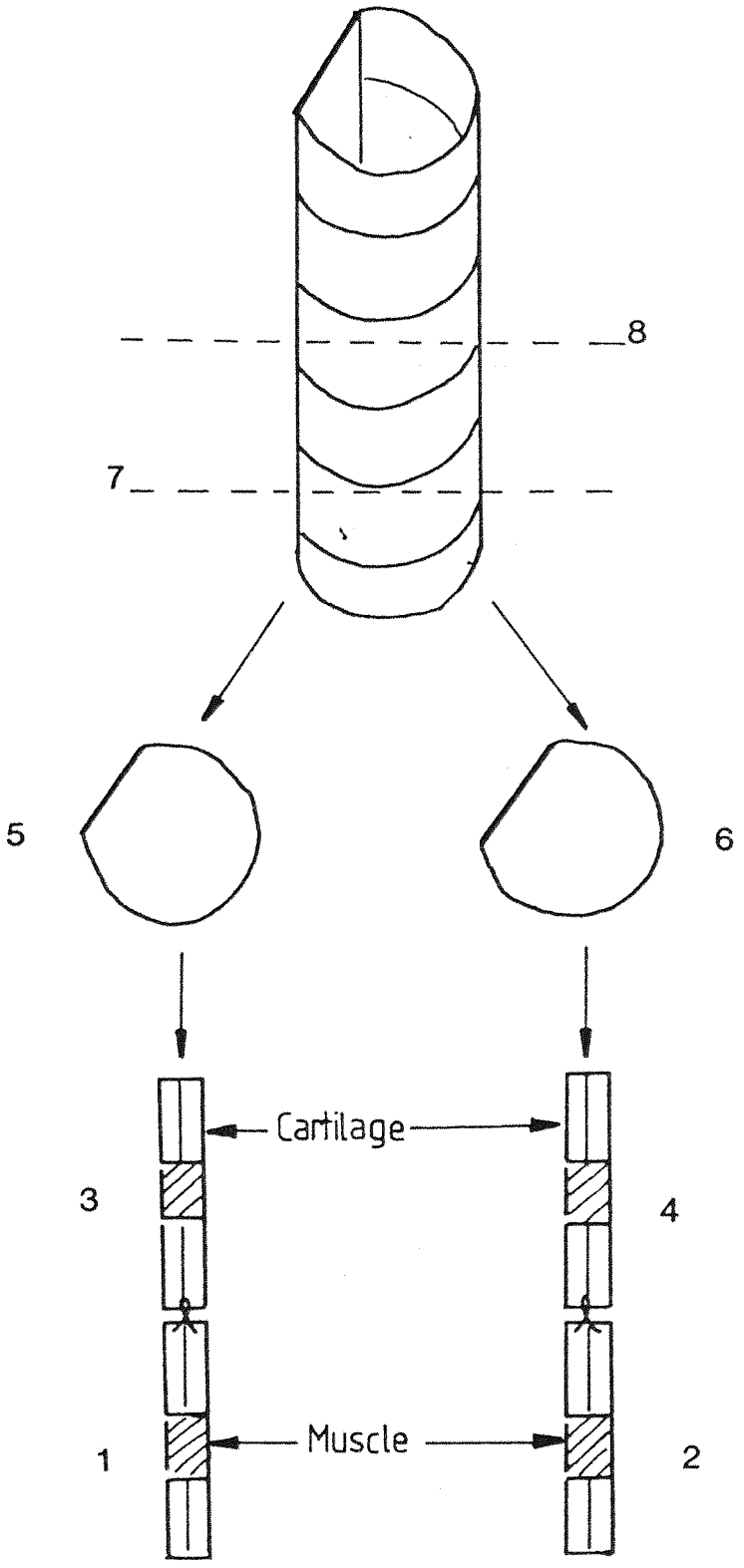
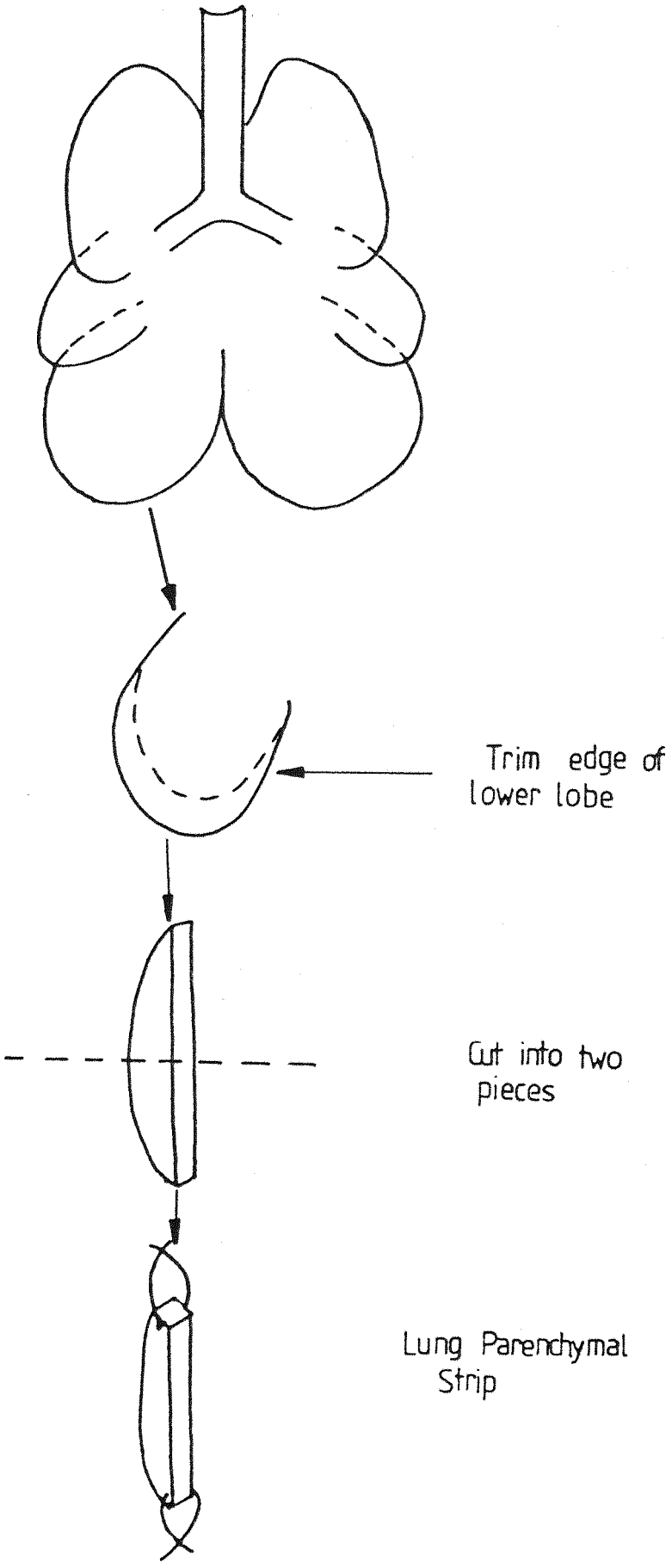


FIGURE 2.1 The paired tracheal chain preparation. The trachea is divided into 10 segments. Alternate pieces are joined together, forming 2 chains, which are matched. Experimental and control data are thus obtained from the same animal.

FIGURE 2.2 The lung strip preparation. Two lung strips are prepared from the edge of the lower lobe of the guinea pig lung. The preparations were suspended from the transducer and attached to the electrode holder by thread which was knotted gently, but securely close to the tissue.



preparations was 500 mg. All hyperbaric recordings were made using this device (see Appendix D). When it was available, the Harvard transducer was also used for normobaric experiments. However, frequent failure of the transducer, and delay in obtaining parts made use of an alternative transducer necessary.

(iv) Isometric recordings

Matched chains, each prepared from 3 segments, were used for the majority of normobaric investigations. Chains comprising segments 1, 5, 9 and 2, 6, 10 (see Fig 2.1) were mounted on Grass FTO3C isometric transducers under identical loads of 1.25 g.

The output of both transducers was displayed on a Grass pen recorder.

(v) Experimental methods

(a) Bath conditions.

All preparations were suspended vertically in 10, 15 or 30 ml of Krebs' solution at 37°C and attached to a perspex electrode holder by cotton thread. At ambient pressure the tissue was maintained at pH 7.35-7.40 by bubbling the fluid with 5% CO_2:95% O_2 (0.05 bar CO_2: 0.95 bar O_2).

Since the recording method and bath volume varied from one experimental series to another, the conditions of each experiment are given in detail alongside each set of data.

(b) Electric field stimulation.

Two parallel platinum wires each 2 mm in diameter were inserted through a perspex holder alongside the tracheal chain and used for field stimulation of the intramural nerves (see Fig 2.3). Grass stimulators were used to supply pulses of 1ms width at a nominal 100V (voltage supplied to the tissue 2V ± 10%) and variable frequency. The duration of each pulse train was 15 seconds.

(c) Agonist dose-response curves.

Tracheal smooth muscle responds slowly to a variety of spasmogens and relaxants and recovery from the effects of these agents is also slow. This makes it laborious to obtain data using a conventional dose-wash-dose cycle. Furthermore, deterioration of the preparation can render the results inaccurate. Instead, cumulative dose-response curves can be constructed for drugs which do not undergo significant breakdown, or uptake by the tissue and for which tachphylaxis does not occur to a significant extent (see Van Rossum, 1963). This method is quicker than

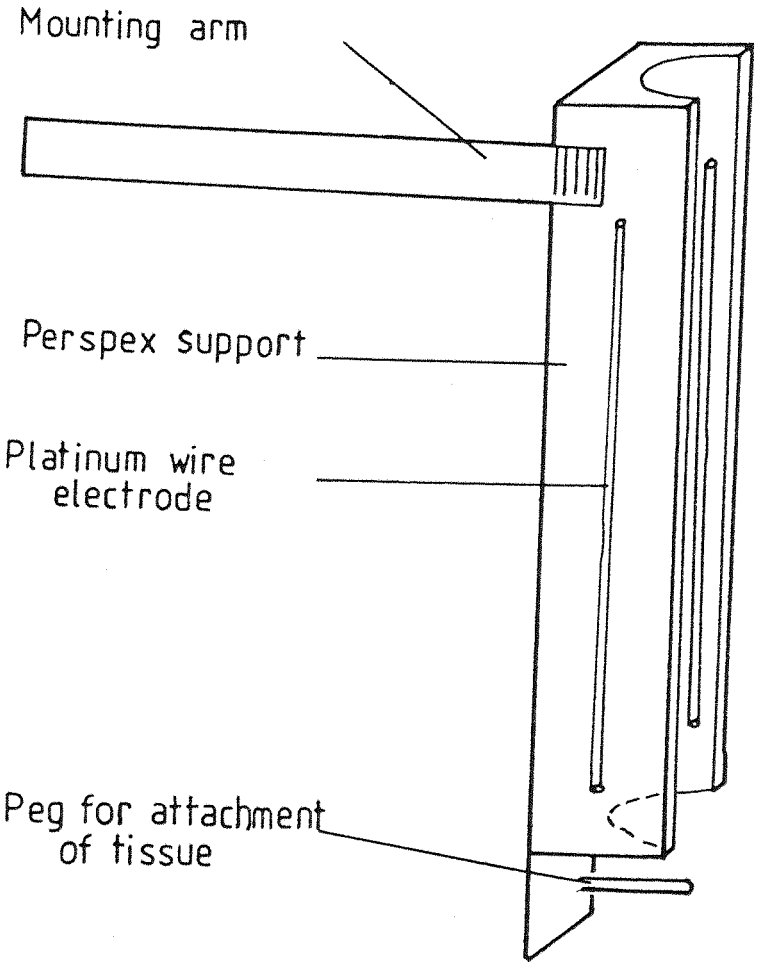


FIGURE 2.3 The perspex electrode and tissue holder used with both tracheal chain and lung strip preparations.

the conventional method and is, therefore, less subject to inaccuracy due to deterioration of the preparation. The response of the tissue to adrenaline, ATP and adenosine were evaluated in this way.

Despite the advantages offered by the cumulative dose method, it was unsatisfactory for use with the agonist acetyl choline (Ach). Repeated addition of the latter resulted in an overall reduction in tone, after the drug had been washed out, while the response to Ach was enhanced (Table 3.20a,)*. Because of this, three cumulative dose response tests could not be obtained. Pilot experiments revealed that a bath concentration of 6 µg/ml Ach Resulted in approximately 50% of the maximal response. This was known as the ED.50. The ED.50 method was used to investigate the response of tracheal chain to Ach under normoxic and hypoxic conditions (section B(xii)).

(d) Ca^{2+} content of the bathing fluid.

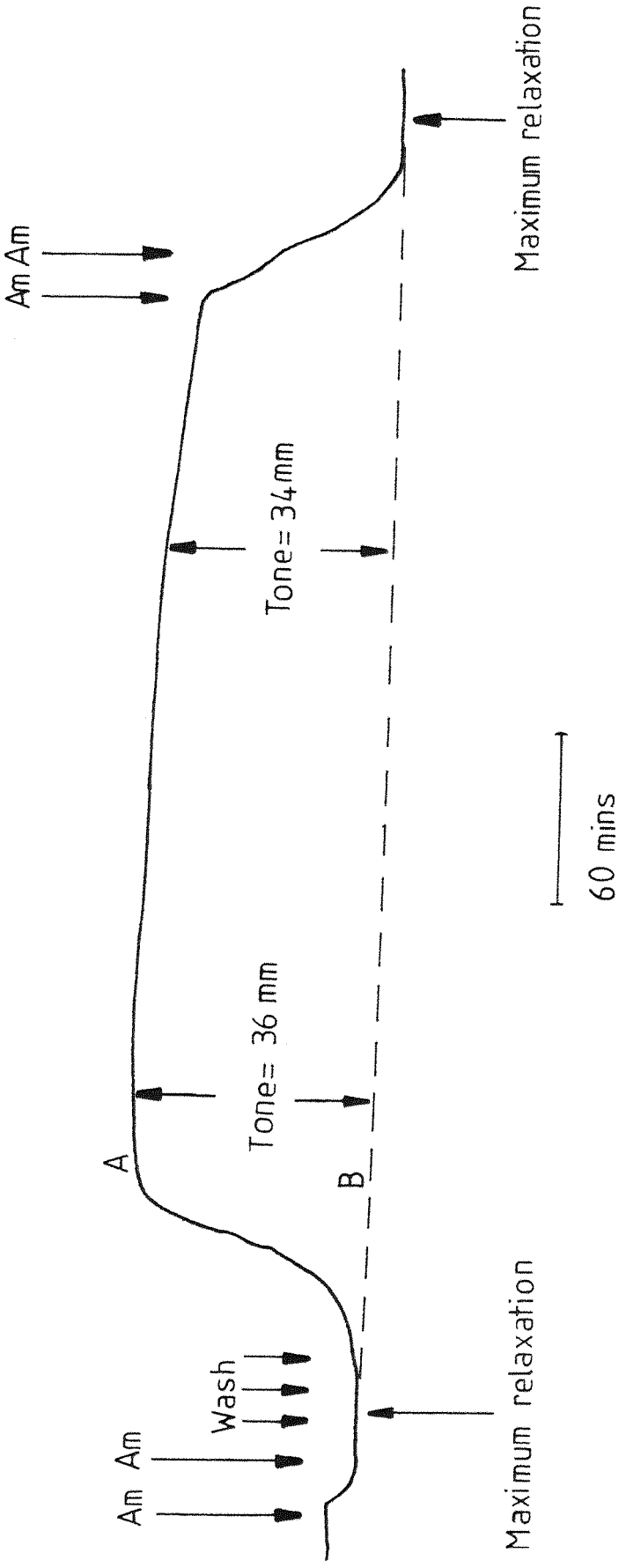
During some normobaric and hyperbaric experiments tracheal chains were bathed in Krebs' solution containing low (less than 2.4 mM) or high (greater than 2.4 mM) Ca^{2+}. These solutions were prepared in the following manner: Krebs' solution containing no Ca^{2+} was bubbled vigorously with 5% CO_2:95% O_2 for 10 minutes. Stock $CaCl_2$ solution (10 M) was added slowly to this solution. The vessel was then stoppered and warmed slowly to 37°C in a water bath. Solutions were discarded if a precipitate formed.

Solutions containing a maximum of six times normal [Ca^{2+}] (14.4 mM) were prepared. Free [Ca^{2+}] was not assayed in these solutions. Since Ca^{2+} interacts with PO_4^{2-} in Krebs' solution, the true [Ca^{2+}] was probably less than the nominal values recorded in this thesis.

(Even in solutions where no visible precipitate formed, the Ca^{2+}:PO_4^{2-} interaction could be demonstrated by measuring the pH of the solution. The pH of the modified Krebs' solution varied from 7.8 (0.13 mM [Ca^{2+}]) to 7.2 (14.4 mM [Ca^{2+}]). The effect of pH on tracheal smooth muscle tone is outlined in Appendix C, where it can be seen that within the range 7.3-8.5 tracheal tone is unaltered).

*The mechanism of this effect is unclear. However, it is unlikely to be due to classical desensitisation as described by Rang and Ritter (1970) for the following reasons: (1) Inherent tone is not altered by cholinergic antagonists; (2) The response to exogenous Ach was enhanced by pre-exposure, rather than reduced.

FIGURE 2.4 This figure shows a hypothetical trace which illustrates the method used in this study to assess inherent tone. Line A is the maintained contracture recorded during the experiment. Line B joins the two points of maximal relaxation, recorded at the beginning and end of the experiment in response to supramaximal concentration of aminophylline. The value of inherent tone at any time during the experiment is the difference between lines A and B.


Am Am
Maximum relaxation
Tone= 34 mm
Tone= 36 mm
A
B
60 mins
Am Am
Wash
Maximum relaxation

(vi) Methods of analysis of results

A survey of the literature on tracheal smooth muscle revealed a variety of methods of analysis. Some authors have recorded the absolute size of a given response, regardless of the inherent tone of the tissue. However, this method does not take account of any changes in tone, which can occur spontaneously or as a result of the experiment. In other reports the responses have been measured as a percentage of the maximum possible response. Other authors record the response as a percentage of inherent tone at the beginning of the experiment and, finally, others record the response as a percentage of the tone immediately prior to the addition of the agonist.

During the course of this study it became clear that the size of the response to an agonist is related in some way to the inherent tone of the preparation. Some methods of analysis tend to obscure this relationship and can lead to incorrect conclusions. The methods used in this thesis, and the reasons for their use are outlined below and shown diagrammatically in Figs. 2.4, 2.5 and 2.6.

(a) Measurement of inherent tone.

Tracheal smooth muscle from the guinea pig develops a spontaneous contracture which can be maintained for several hours. This contracture is known as inherent tone (but has been referred to by some authors as "tone").

Inherent tone was assessed as described below and shown in Fig. 2.4.

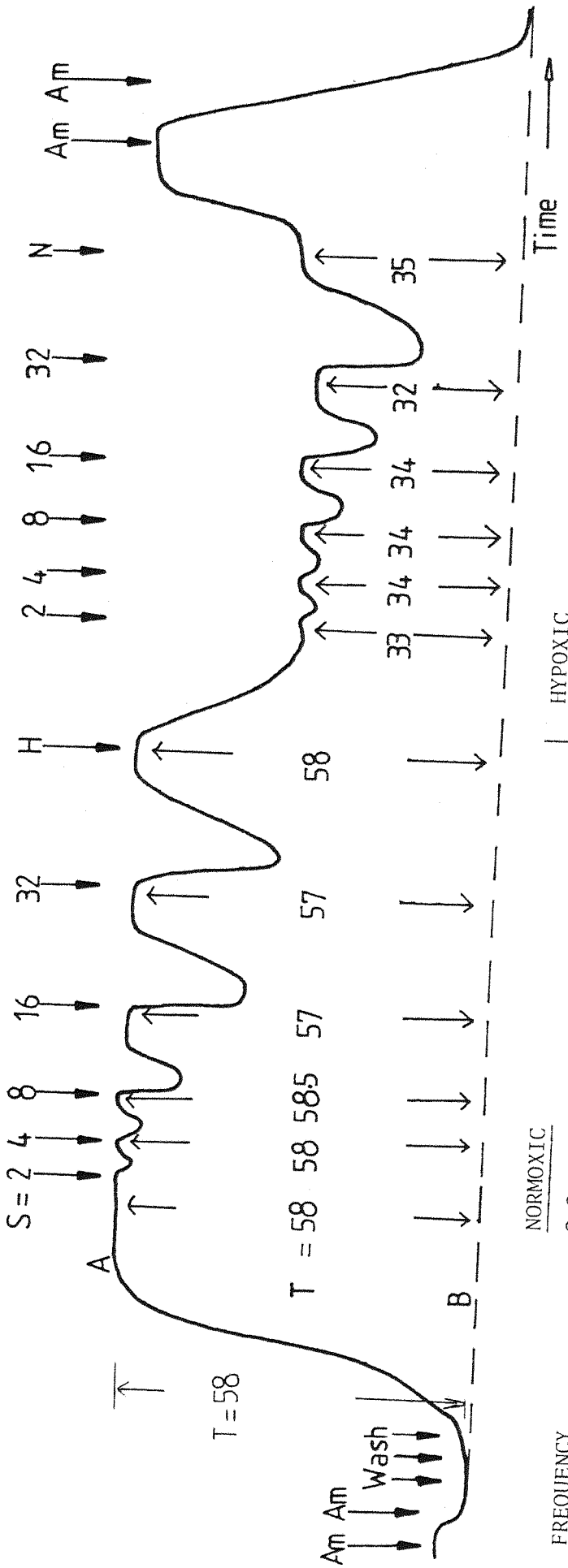
At the beginning of each experiment two doses of aminophylline (final concentration 4.4 mM, pH 8.2) were added to the bathing fluid to obtain a maximal relaxation (see Foster, 1963). Following thorough washing, the tissue contracted spontaneously and reached a stable value after 30-60 minutes. A maximal relaxation was also obtained at the end of the experiment. A line was drawn on the recording chart between the two points of maximal relaxation, in order to define the maximal relaxation throughout the experiment (see Fig. 2.4). The value of the inherent tone at any time during the experiment is the difference between the maintained contracture (line A on Fig. 2.4) which is recorded, and the maximum relaxation (line B on Fig. 2.4) which is extrapolated.

This method was first adopted by Foster (1963) and is particularly useful for experiments which take several hours to complete.

FIGURE 2.5 This figure illustrates how small changes in tone (as during the first frequency response curve) are compensated for if the responses are expressed as a percentage of the inherent tone recorded immediately prior to stimulation. This method has been recommended by Foster (1963).

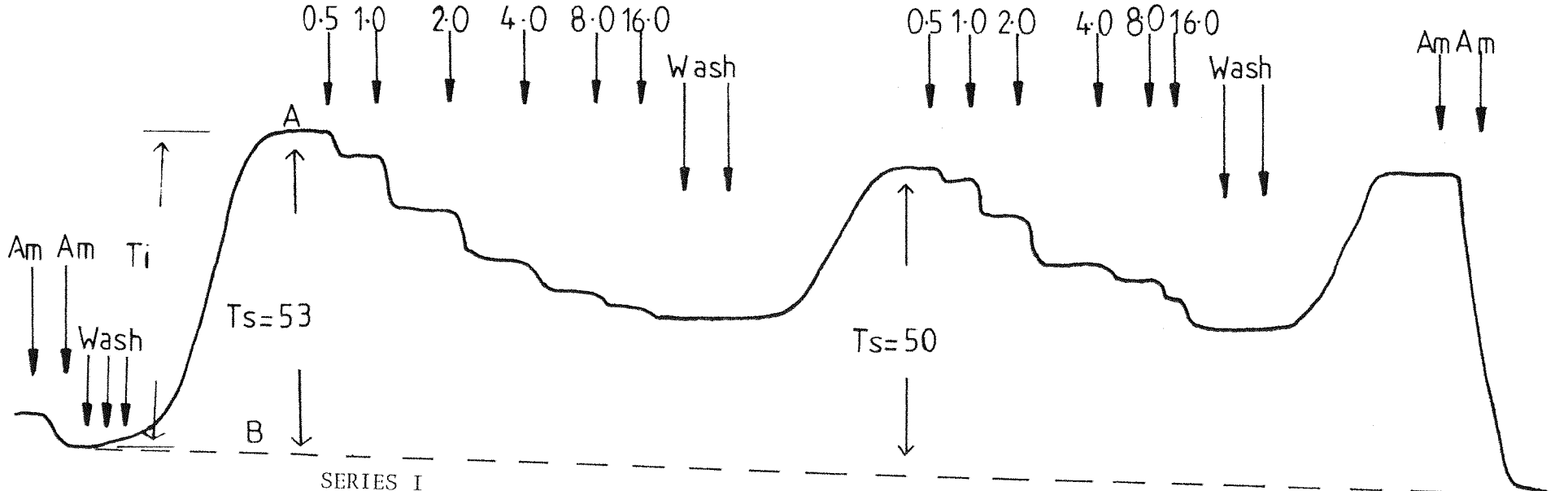
However, this method can be misleading if large changes in tone are recorded. It can be seen that although the absolute size of the response is slightly reduced during the second frequency-response curve, when the result is expressed as a percentage of the inherent tone (T), the response appears to be enhanced.

The same data may also be expressed as a percentage of initial tone (T_i). This simply summarises the data but allows the results to be combined with those from other experiments for statistical analysis.



NORMOXIC					HYPOXIC				
FREQUENCY	2.0	4.0	8.0	16.0	32.0	2.0	4.0	8.0	16.0	32.0
Response (mm)	2.0	4.0	11.0	18.0	23.0	2.0	3.0	7.0	12.0	16.0
Response (%T)	3.4	6.8	18.8	31.5	40.3	6.1	8.6	20.5	35.3	50.0
Response ($\%T_i$)	3.4	6.8	18.9	31.0	39.6	3.4	5.2	12.1	20.7	27.6
$T(\%T_i)$	100.0	100.0	100.8	98.3	98.3	56.8	59.5	58.6	58.6	55.2

FIGURE 2.6 During dose-response curves the relaxation response can be expressed as a percentage of the starting tone, T_S, ie the tone recorded immediately before the addition of the first dose of the agonist. This method is useful when small changes in tone occur. However, when large changes in inherent tone are recorded the data may be expressed as a percentage of initial tone (T_i).



	SERIES I						SERIES II					
Dose	0.5	1.0	2.0	4.0	8.0	16.0	0.5	1.0	2.0	4.0	8.0	16.0
Response size (mm)	4.0	13.0	21.0	26.0	29.0	30.0	2.0	7.5	15.0	18.0	21.0	26.0
Response (%T_S)	7.5	24.5	39.6	49.0	54.7	56.6	4.0	15.0	30.0	36.0	42.0	52.0
Response (%T_i)	7.5	24.5	39.6	49.0	54.7	56.6	3.7	14.1	28.3	33.9	37.7	49.0
Tone (%T_i)	100.0	100.0	100.0	100.0	100.0	100.0	94 3	94.3	94.3	94.3	94.3	94.3

The value of inherent tone is reported in grams (isometric recording) or as mm deflection of the pen (isotonic recordings).

(b) Analysis of field stimulation experiments.

Because of the large differences between preparations obtained from different animals, combination of the raw data for statistical analysis do not demonstrate the trends observed in many experiments. Other authors have overcome this problem by expressing the results as a percentage of another parameter, usually a measure of inherent tone (see Fig. 2.4). This tends to reduce the variation between preparations. Two such methods were used here to assess the field stimulation data.

α Firstly, each response was expressed as a percentage of the tone recorded just prior to stimulation. Often there were small changes in inherent tone during the course of frequency response curve and this method compensated for these changes.

β Secondly, in some experiments the addition of a drug or the introduction of a new experimental variable was followed by a significant alteration in tone. Results of this kind were obtained during hypoxia, and hyperbaric studies, and a diagram of such a result is shown in Fig. 2.5. In this hypothetical experiment the responses to field stimulation during period one increased with increasing frequency. The tone was relatively constant throughout the period. Following hypoxia (H) there was a fall in tone and a second frequency response curve was constructed (period two). The sizes of the responses were smaller than in period one, but when expressed as a percentage of the tone recorded at the same time, the responses appear larger. This is because the tone is 50% less than during the first recording period.

In the type of experiment described above the reader could arrive at the conclusion that hypoxia enhanced the relaxation response to field stimulation, when the opposite may be closer to the truth. This misconception arises because the method of analysis does not give sufficient information about the inherent tone.

Another method of presenting the same data is shown in Fig. 2.6. Both tone and response can be reported as a percentage of the inherent tone recorded before any experimental procedures had begun, ie when the inherent tone had been stable for 10 minutes.

This is known as the "initial tone". Both tone and response can be plotted on the same graph, so that all the information is immediately available.

The method described above simply summarises and condenses the information on the recording chart. Both methods described above allow the results to be combined for statistical analysis.

(c) Results of cumulative dose-response tests.

Two methods of analysis proved useful for the understanding of data obtained from cumulative dose-response curves.

α Firstly, inherent tone was assessed prior to the addition of the first dose of the agonist: this was the "starting tone" (T_s). The response to the total concentration of drug present at any time was measured as a percentage of the starting tone.

Where successive cumulative dose-response curves were constructed on the same preparation, starting tone was assessed at the beginning of each dose-response cycle. Thus starting tone is not the same as initial tone, except during the first dose-response test. This method is illustrated in Fig. 2.6 and can be compared with (b_α) used for the field stimulation experiments.

β As in (b_β) both tone and responses can be recorded as a percentage of initial tone. This method is also recorded in Fig. 2.6.

(vii) Statistical methods

Student's t-test has been used throughout this study to determine the significance of a result. All data was screened primarily using non parametric methods (e.g. Wilcoxon Ranked Pairs Test) and then re-tested using the Students' T-test. Paired tests were used when considering data from preparations obtained from the same animal. Unpaired tests were used when considering data from different animals. A result was found to be significant if $p < 0.05$. Regression analysis was used to test the relationship between two parameters, and to obtain a best-fit straight line (section B(xii)).

B. Normobaric Experiments

(i) The effect of $[Ca^{2+}]_o$ on tracheal smooth muscle tone

(a) Seven single tracheal chains were prepared and bathed in 15 mls of normal Krebs' solution ($[Ca^{2+}]_o$ was 2.4 mM). The results were recorded on the Harvard smooth muscle transducer. When inherent tone was established the $[Ca^{2+}]_o$ was halved by replacing the bathing fluid with Krebs' solution containing 1.2 mM Ca^{2+}. The bath volume was filled twice with this solution and inherent tone was recorded when it became steady. The above procedure was repeated with solutions containing 0.6, 0.3 and 0.15 mM Ca^{2+} respectively. The steady tone was recorded at each concentration.

(b) Five single tracheal chains were prepared and bathed in 30 mls of Krebs' solution. The results were recorded on the Harvard smooth muscle transducer. Once inherent tone was established, the preparation was bathed in Krebs' solution containing 7.2 mM Ca^{2+}. When inherent tone was steady, the value was recorded.

In six further experiments single tracheal chains were prepared and used as above. Inherent tone was established in normal Krebs' solution, which was then replaced with Krebs' solution containing 14.4 mM Ca^{2+}. When inherent tone was steady, the value was recorded as before.

(ii) The effect of hypoxia on tracheal smooth muscle tone

In pilot experiments hypoxia, ie the absence of bubbling gas, was shown to reduce the inherent tone and alter the response of the tissue to field stimulation and drugs. These effects were investigated more thoroughly by replacing the usual bubbling gas 5% CO_2:95% O_2, with 5% CO_2:95% N_2. Thus, the pH was maintained at 7.4, while hypoxia was achieved rapidly.

Seventeen pairs of tracheal chains were prepared in the usual manner and bathed in 15 mls of Krebs' solution. Grass FT03C transducers were used. One chain of each pair was maintained with 5% CO_2:95% N_2, while the other was bubbled with 5% CO_2:95% O_2 as a control. When the tone was steady, its value was recorded. Each of the tracheal chains was then used to record the effect of hypoxia on the response to field stimulation or to addition of drugs (sections B (x, xi and xii)). Following this the O_2 was restored and

inherent tone was allowed to recover.

(iii) The effect of substrate withdrawal on the response to hypoxia

In six experiments freshly excised trachea were placed directly into glucose-free Krebs' solution. Glucose was replaced by equimolar sorbitol in each of these experiments. Tracheal chains were prepared as before, bathed in 15 mls of glucose-free Krebs' solution and the results recorded on Grass FTO3C transducers.

When a steady tone had been established the preparations were bubbled tith 5% CO_2:95% N_2. The effect on tone was recorded. After 20 minutes 5% CO_2:95% O_2 was restored and the tone recorded for a further 30 minutes. The bathing fluid was then replaced by unmodified Krebs' solution and the tone was recorded for a further 20 minutes.

In control experiments, matched tracheal chains were bathed in glucose-free Krebs' solution and bubbled with 5% CO_2:95% O_2 throughout.

(iv) The effect of raised $[Ca^{2+}]_o$ on the response to hypoxia

Four tracheal chains were prepared in the usual way and allowed to take up tone. The preparations were then bubbled with 5% CO_2: 95% N_2 for 20 minutes and the response was recorded. The tone was allowed to recover in 5% CO_2:95% O_2. The bathing fluid was replaced by Krebs' solution containing 4.8 mM Ca^{2+}. The hypoxic exposure was then repeated. Once again tone was allowed to recover under normoxic conditions, following which the bathing fluid was replaced by Krebs' solution containing 9.6 mM Ca^{2+}. After tone had recovered the bathing fluid was replaced with Krebs' solution containing 14.4 mM Ca^{2+}.

Two tracheal chains were also subjected to four consecutive 20 minute periods of hypoxia. Between each hypoxic exposure the bathing fluid was replaced with unmodified Krebs' solution.

In the experiments described in sections (v-ix) drugs have been used, in an attempt to define the role of Ca and prostaglandins in the initiation and maintenance of inherent tone: the presence of cholinergic, adrenergic and purinergic systems in the tissue and their control of tracheal smooth muscle contractility.

The prime pharmacological action of the drugs used is described below:

Ouabain inhibits Na^+/K^+ ATPase, a ubiquitous enzyme present in plasma membranes. Ouabain also affects Ca transport in a variety of cells (Akera and Brody, 1977).

Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) which inhibits prostaglandin synthesis in a variety of tissues (Vaue, 1971).

Pyridyl isatogen tosylate (PIT) is a purinergic antagonist (Hopper, Spedding and Weetman, 1974).

Verapamil is a Ca channel blocker (Colatsky and Hogan, 1980).

Tetrodotoxin (TTX) is a sodium channel blocker which prevents the occurrence of action potentials in nerves (Kao, 1966).

Atropine is a muscarinic antagonist (Innes and Nickerson, 1975).

Reserpine depletes noradrenaline from sympathetic nerve terminals (Bein, 1956).

Propranolol is a β-adrenoceptor antagonist (Black, Duncan and Shanks, 1965).

Quinidine is an antimalarial drug with antiarrhythmic properties which may possess purinergic blocking action (Burnstock, 1972).

(v) The effect of drugs on tracheal smooth muscle tone

The drugs considered in this section include only those agents not usually described as spasmogens, autacoids or putative neurotransmitters.

(a) Ouabain.

Matched tracheal chains were prepared from six guinea pigs and bathed in 15 mls of Krebs' solution. The tone was recorded using Grass FT03C transducers. After tone had been established, atropine (1 µg/ml, 1.4 µM) was added to the bathing fluid of one of each pair of tracheal chains. Atropine (1 µg/ml) and propranolol (1 µg/ml, 3.8 µm) were added to the second chain of each pair. Subsequently, ouabain (11.0 µM) was added to both preparations and the responses were recorded for 25 minutes.

(b) Indomethacin.

Five pairs of tracheal chains were prepared and bathed in 15 mls Krebs' solution. Grass FT03C transducers were used to record the response to indomethacin.

Indomethacin (6 µg/ml, 2.8 µM) was added to one chain of each pair of tracheal chains after aminophylline (see p. 47) had been washed out of the bath, but before tone had begun to rise. The indomethacin was dissolved in ethanol; the solvent alone was 0.2% (v/V).

(c) Pyridyl Isatogen Tosylate (PIT).

Four pairs of tracheal chains were prepared and bathed in 30 mls of Krebs' solution. After tone had been established, PIT (50 µM) was applied to one chain of each pair. The effect on tracheal smooth muscle tone was recorded and compared with untreated paired chains.

(d) Verapamil (D600).

Four pairs of tracheal chains were prepared in the usual manner. The preparations were bathed in 15 mls of Krebs' solution and the results recorded using the Harvard smooth muscle transducer.

Verapamil was dissolved in methanol and applied to one of each pair of chains after aminophylline had been washed out, but before tone had begun to rise. The final bath concentration of verapamil was 5×10^{-5}M. Solvent alone was added to the matched control chains. The final bath concentration of methanol was 0.02% (v/V). The responses of both chains were recorded for 60 minutes.

Subsequently, 5×10^{-5}M verapamil was added to the second of each pair of chains. The effect of verpamil on established tracheal smooth muscle tone was recorded for a further 80 minutes.

(vi) Effect of drugs on the response to field stimulation

(a) Tetrodotoxin (TTX).

Five pairs of tracheal chains were prepared in the usual manner and bathed in 10 mls Krebs' solution. The results were recorded isotonically (Harvard smooth muscle transducer).

Both tracheal chains were stimulated at 16 Hz for 15 seconds. One of each pair was then treated with TTX (1 µg/ml, 3.1 µM). Both tracheal chains were stimulated again at 16 Hz for 15 seconds. The effect of TTX on tracheal smooth muscle inherent tone was noted.

(b) Atropine.

Five tracheal chains were prepared in the usual manner and bathed in 15 mls Krebs' solution. The results were recorded using the Grass FT03C transducer.

Each tracheal chain was stimulated at 4, 8, 16 and 32 Hz. After each stimulation the preparations were allowed to regain a steady base-line tone. This usually took 8-10 minutes. Each chain was then treated with atropine (1 µg/ml) and the frequency response tests were repeated.

(c) Reserpine pre-treatment.

Six female guinea pigs were injected with reserpine (2 mg/kg) on two consecutive days. Single tracheal chains were prepared from these animals two days after the initial injection. The chains were bathed in 15 mls unmodified Krebs' solution and stimulated at 4, 8, 16 and 32 Hz. The results were recorded isotonically using Harvard smooth muscle transducers.

(d) The effect of propranolol and quinidine on the relaxation response to field stimulation.

Five pairs of tracheal chains were prepared in the usual manner. and bathed in 15 mls Krebs' solution and the effect of field stimulation was recorded on Grass FT03C transducers.

Both tracheal chains were stimulated at 4, 8, 16 and 32 Hz. Then, to one chain of each pair, atropine (1 µg/ml) and propranolol (1 µg/ml) were added. After 10 minutes the frequency-response series was repeated on both treated and untreated chains. Subsequently, quinidine (0.05 mM) was added to the drug-treated chain. After 10 minutes the frequency-response series was repeated on both treated

and untreated preparations. The effects of the various drugs on the tone of the preparations was noted.

(e) Quinidine.

Frequency response curves were constructed on 4 pairs of tracheal chains as in (ivd). Quinidine (0.05 mM) was added to one chain of each pair. The effect of the drug on inherent tone was recorded. After 30 minutes the frequency response tests were repeated.

(vii) Effect of quinidine on the response to adrenaline

Four pairs of tracheal chains were prepared and bathed in 15mls Krebs' solution containing 1 g/l ascorbic acid (0.1% w/v). The results were recorded on Grass FTO3C transducers.

A cumulative dose-response curve for adrenaline was constructed over the range 1.0 - 8.0 mM (final bath concentration) on both chains of the pair. When the final dose had been washed out and inherent tone had recovered a stable value, atropine (1 µg/ml) and quinidine (0.05 mM) were added to one chain of each pair. The dose-response curve was then repeated on both control and experimental chains.

(viii) Effect of quinidine on the response to Adenosine Triphosphate (ATP)

Four pairs of tracheal chains were prepared as described in (vii) above. Cumulative dose-response curves to ATP were constructed over a range of 10 µM to 3mM. After wash-out and recovery, one chain of each pair was treated with atropine (1 µg/ml) and proporanolol (1 µg/ml). The dose-response tests were repeated on both treated and untreated chains. After wash-out and recovery atropine (1 µg/ml) propranolol (1 µg/ml) and quinidine (0.05 mM) were added to the bath of the chain previously treated with atropine and propranolol. Finally, the dose-response tests were conducted on both treated and untreated chains, as before.

(ix) Effect of quinidine on the response of tracheal chain to adenosine

Four pairs of tracheal chains were prepared as described in (vii) and (viii) above. A cumulative dose-response curve to adenosine was constructed, over the range of 0.2 µM to 1.5 mM.

Atropine (1 µg/ml) and propranolol (1 µg/ml) were then added to the bathing fluid of one chain of each pair and the dose response tests repeated on both chains. Atropine, propranolol and quinidine (0.05 mM) were present in the bath fluid of the same chain during the construction of a third cumulative dose-response curve.

(x) Effect of hypoxia on the response to field stimulation

Paired tracheal chains were prepared from six guinea pigs. The preparations were bathed in 15 mls Krebs' solution and Grass FT03C transducers were used to record the results. Three consecutive frequency-response curves were constructed on both control and experimental chains. During the second frequency-response series the experimental chain was made hypoxic by bubbling with 5% CO_2: 95% N_2. The bath fluid was bubbled with this mixture for 20 minutes before the start of the first stimulation, by which time a steady tone had been achieved. 5% CO_2:95% O_2 was restored to the experimental chain during the third and final frequency response test.

(xi) Effect of hypoxia on the response of tracheal chain to adrenaline

Three consecutive dose-response curves to adrenaline were recorded on six pairs of tracheal chains. One of each pair of tracheal chains was bubbled with 5% CO_2:95% N_2 for 20 minutes before and throughout the second of three dose cycles. The bubbling gas was unchanged for the control chain. The dose range required to relax the tracheal chains varied from (0.01 mM) to (1.0 mM) final bath concentration.

(xii) Effect of hypoxia on the response of tracheal chain to Ach

During preliminary experiments the concentration of Ach which produced 50% of the maximal response was determined. This concentration was 6 µg/ml Ach (33 µM) and was known as the ED50.

The ED50 was applied on three consecutive occasions to 6 pairs of tracheal chains. One chain was made hypoxic by bubbling with 95% N_2:5% CO_2 for 20 minutes before and throughout the second challenge with Ach. Normoxic conditions were restored before the third and final challenge. The bubbling gas was unchanged for the control chain.

C. Hyperbaric Materials and Methods

(i) High pressure chambers

All normobaric experiments were conducted in conventional glass organ baths, as described. Hyperbaric experiments were conducted in one of two hyperbaric chambers constructed by the late W. McNaughton in the Workshops at AMTE(PL). The basic design is shown in Fig 2.7. Both chambers consisted of a cylinder of steel pressure piping which rested on a recessed 'O' ring seal in the base plate. A plate of identical dimensions formed the lid of the chamber. The base and lid were held in place by 6 bolts (MacI) or 8 bolts (MacII). Both chambers had two gas entry ports and one vent or exhaust. One of the gas entry ports was of $\frac{1}{4}$" bore and was used to fill the chamber quickly with gas. This was therefore known as the compression line. The gas entered the chamber from beneath a baffle plate which protected the instruments from disturbance during compression. The second gas entry port was a steel tube of $\frac{1}{8}$" diameter which entered the chamber through the baffle plate and was fixed to the side of the organ bath. A polythene tube secured to the end of this gas line could be inserted into the organ bath. Gas which entered the chamber via this line was used to bubble the Krebs' solution within the organ bath. This was known as the bubbling line.

Each pressure chamber had an organ bath constructed of stainless steel which comprised a pressure tight water jacket into which a glass liner could be inserted. Water at 1 atmosphere and at 37^{o}C was circulated through this jacket and the water jacket of the conventional glass organ bath, in series with the hyperbaric chamber, by a Grant Instruments pump.

Both pressure chambers were supplied with six two-way conhex electrical connectors which were mounted in a perspex plate. Each connector was sealed with a tiny 'O' ring. The perspex plate was secured by a brass backing plate. An 'O' ring around the rim of the perspex plate formed a pressure seal. The first pressure chamber available for this study (MacI) is shown in Figure 2.8. The volume of the organ bath was 10 mls.

Although satisfactory this chamber had a number of disadvantages. In particular the overall size of the chamber

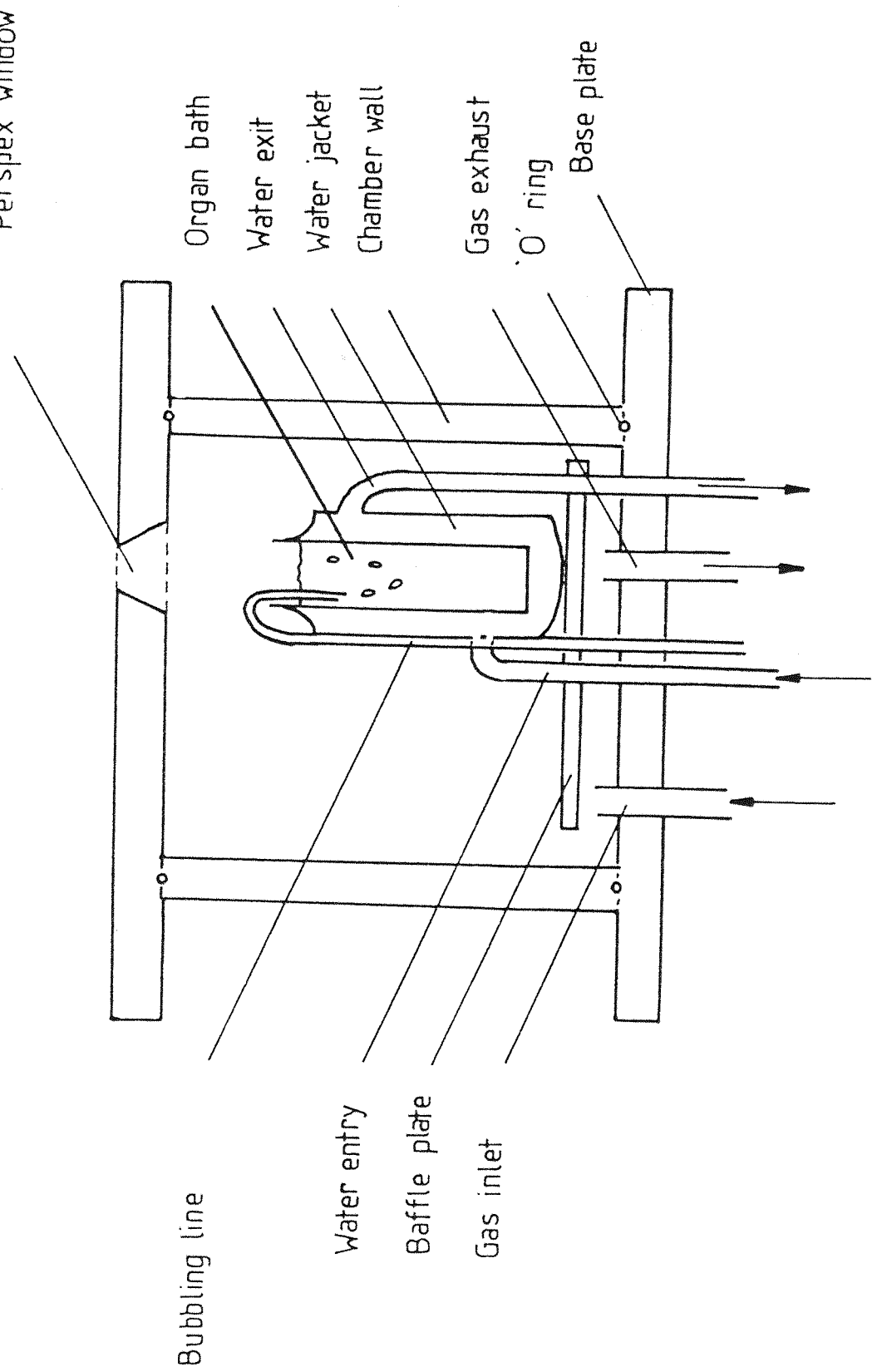


FIGURE 2.7 The hyperbaric chambers were constructed of stainless steel pressure piping which rested on a circular base plate. The organ bath was mounted on a baffle plate which protected the instruments from excessive disturbance during compression and decompression. The lid containing a perspex window was secured by six bolts.

FIGURE 2.8a The interior of MacI is shown in this figure, alongside the conventional organ bath. Harvard smooth muscle transducers were used to monitor the responses of control and experimental tissues simultaneously.

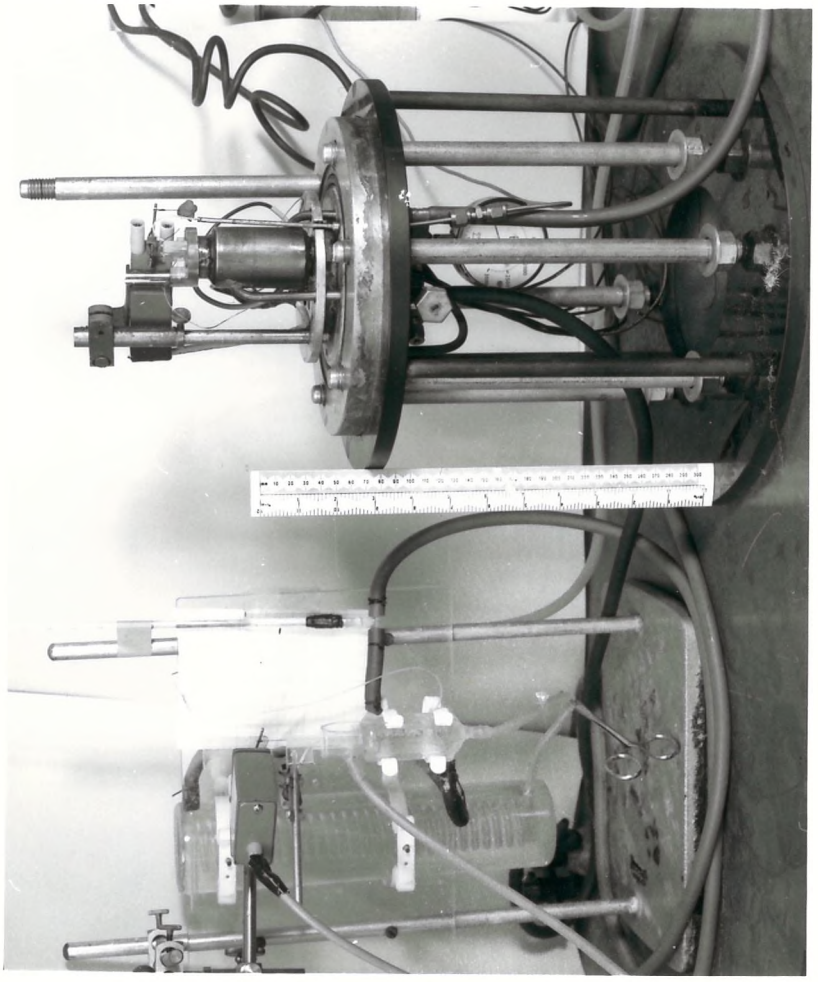


FIGURE 2.8b The interior of MacII was similar to that of MacI but was less cramped. The experimental preparations could be handled easily inside the constructed chamber.

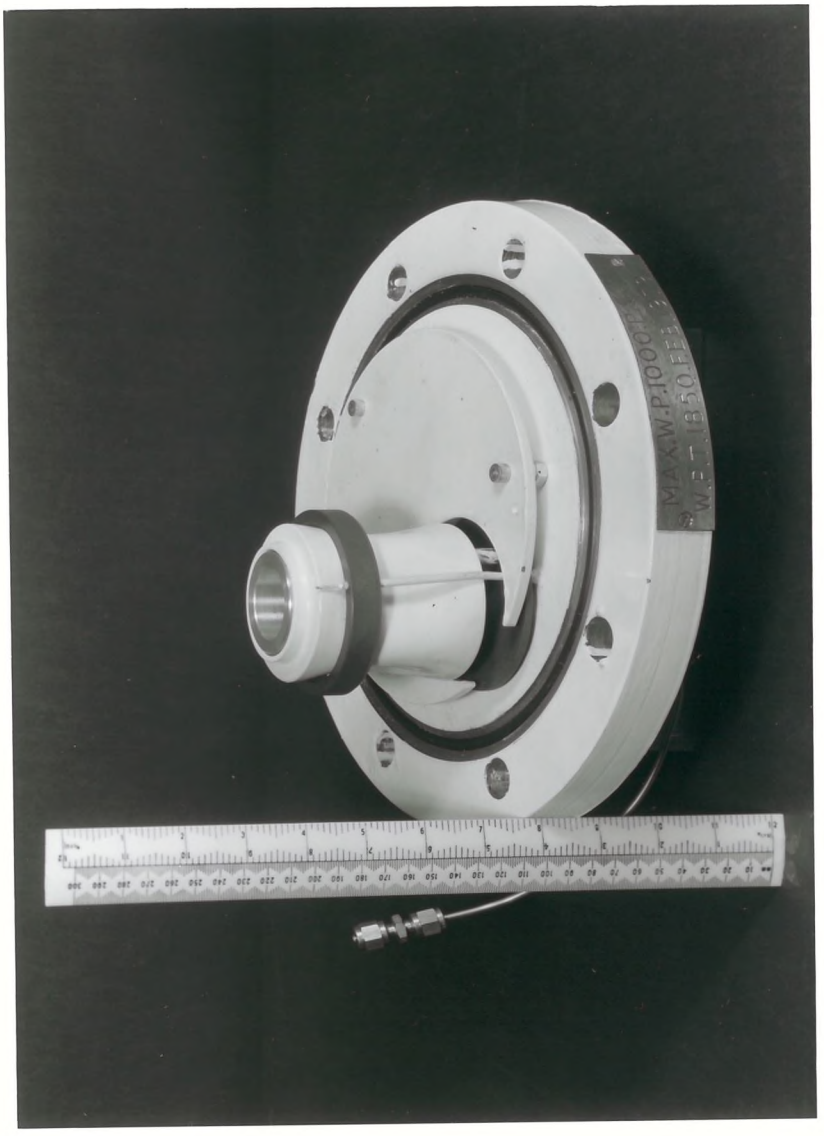

MAX W.P. 1000
WET 1850 FE

FIGURE 2.9 The exterior of MacII, showing the port via which pressure tight electrical contacts were made. The position of the port made the interior of the chamber more spacious, and made servicing and repairs of the connectors much easier.


A.X.W.P.1000 P.S.I.
T.1850 FEB.1972

was small, and while this prevented wastage of gas, there was hardly enough space for the organ bath and transducer. Once the preparation was set up in the organ bath, the chamber was constructed around the preparation, by lowering the cylinder over the apparatus onto the base plate. Any inaccuracy in placing the cylinder on the base could disturb the whole apparatus.

In order to make best use of the available space, the organ bath was mounted off-centre on the baffle plate. However, the only window in the centre of the lid was mounted in the centre of the lid. Thus it was not possible to observe the preparation, or the surface of the bathing fluid once the chamber was closed. This made it difficult to determine if compression had disturbed the lever arm of the transducer, or if gas was bubbling through the bathing fluid. Gauges on the outside of the chamber indicated that gas was entering the chamber via the bubbling line. However, on a few occasions the polythene tube was blown off the end of the bubbling line due to a sudden increase in the line pressure. This could only be determined after the chamber was re-opened.

A further disadvantage of this chamber was that the electrical connectors were mounted in the centre of the base plate. Although all the leads were insulated and protected by a plastic sheath, the metal connectors were often splashed with Krebs' solution leading to corrosion. The electrical connectors had to be replaced frequently. Furthermore, the chamber atmosphere was always humid and contained a high oxygen concentration. Consequently, every part of the chamber was affected by corrosion including those made of "stainless" steel.

A second hyperbaric chamber (MacII) was designed and constructed with the above problems in mind. MacII is shown in Figures 2.9 and 2.10. This chamber was constructed according to the same basic design as MacI. However, the overall size was larger. The organ bath was also of a larger total volume (50ml) for which aluminium spacers and appropriately sized glass liners were made (Fig 2.11). Thus bath volumes of 10, 30 and 50ml were available. The port via which the electrical connections were made was mounted 7 ins. up the side of the cylinder (see Fig 2.10 and 2.11). Thus the connectors were protected from corrosion

FIGURE 2.10 The base plate, cylinder and lid of MacII. The electrical connectors were mounted on the cylinder wall to protect them from splashing and corrosion.

FIGURE 2.11 A port was constructed to allow the electrical leads to enter the chamber through its wall. The components of the port are shown on the white background. The 1 cm port (extreme right) was screwed into the chamber wall. The electrical leads entered the chamber via this small opening. Six Conhex connectors were mounted on a perspex plate and a pressure seal was made between this and the steel port by a neoprene 'o' ring. The perspex plate was supported by a steel backing plate and secured with a threaded sleeve to the outside of the port (left).

The organ bath (shown lying on its side) could be varied in volume by inserting aluminium spacers and glass liners of different sizes. Total bath volumes of 10, 30 and 50 mls could be achieved.

by splashing. If necessary the connectors could be replaced by removing the port, instead of dismantling the whole chamber. This arrangement also allowed greater space within the chamber. It was possible to manipulate the preparation inside MacII without removing the cylinder, so that disturbance was less likely to occur while the chamber was being closed. A perspex window was placed off centre in the lid. This could be positioned over the organ bath, or some other part of the chamber if required, thus visibility was greatly improved.

Once MacII was in use, an unexpected advantage became apparent. In MacI the small volume of Krebs' solution was constantly bubbled with gas of known composition, calculated to maintain the tissue at 0.05 bar CO_2; 0.95 bar O_2; balance He, whatever the total pressure. Table 2.1 shows the gas mixtures used at each simulated depth.

When the tissue was bathed with larger volumes of Krebs' solution (as in MacII), it was demonstrated that short duration hyperbaric exposures could be achieved without constant bubbling of the solution with gas. In these experiments the chamber was flushed with 0.05 bar CO_2; 0.95 bar O_2 before compression. Pure He was then added via the compression line to increase the pressure.

The high pressure circuit is shown in Figure 2.12.

(ii) Special problems

The application of pressure to a preparation which is easily manipulated at atmospheric pressure, requires the solution of some problems peculiar to the demands of this type of experiment. Above all, it is essential to ensure that pressure is the only variable to affect the preparation. For example, temperature pO_2 and pCO_2 must all be rigidly controlled. Where pressure directly exerts unavoidable effects, e.g. by altering ionisation of components of the bathing fluid, these effects must be quantified and their relevance to the results must be assessed.

Pressure, and more importantly the bulk movement of gases during compression and decompression can affect measuring devices. Therefore the effect of pressure and pressure change on transducers, thermistors, and pH electrodes must be evaluated before they can be used to interpret a physiological response to pressure. Each

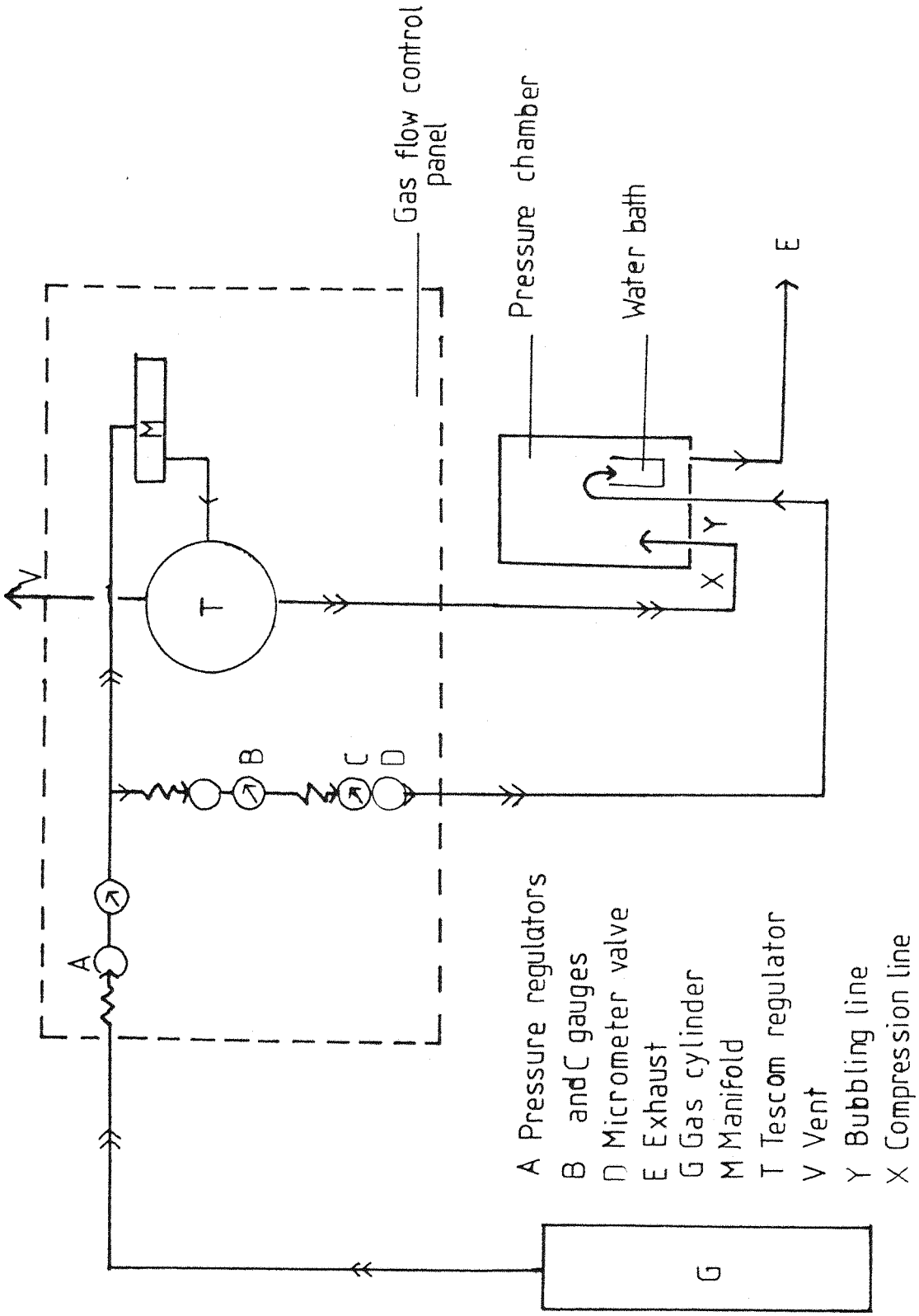
PARTIAL PRESSURE (bar)			TOTAL PRESSURE (bar)	%		DEPTH	
O_2	CO_2	He		O_2	CO_2	m	ft
0.95	0.05	Nil	1.0	95.00	5.00	Surface	
0.95	0.05	0.914	1.914	49.63	2.60	9.14	30) Submarine
0.95	0.05	1.829	2.829	33.58	1.76	18.29	60) escape
0.95	0.05	2.804	3.804	24.97	1.31	28.04	92) training depth
0.95	0.05	6.096	7.096	13.380	0.704	60.96	200)
0.95	0.05	12.192	13.192	7.201	0.379	121.92	400) Sea
0.95	0.05	18.288	19.286	4.925	0.259	182.88	600) escape
0.95	0.05	22.860	23.860	3.981	0.209	(228	750)* depth
0.95	0.05	30.0	31.0	3.06	0.16	300)	
0.95	0.05	42.0	43.0	2.209	0.118	420) Saturation diving	
0.95	0.05	66.0	67.0	1.418	0.074	660) depth	
0.95	0.05	100.0	101.0	0.94	0.04	(1000)	
				Balance He			

* Deepest possible attempt likely, 600-630 fsw deepest operational ascent.

TABLE 2.1 Composition of gases used for hyperbaric experiments in MacI.

FIGURE 2.12 The high pressure circuit.

Gas was supplied from a cylinder charged to a maximum of 2000 psi. The regulator (A) on the gas bottle was set to the desired chamber pressure plus 10 psi and the valve opened. Gas flowed as far as regulator B, and the Tescom regulator was set at the desired chamber pressure and gas then flowed into the closed chamber. When the set pressure was reached, regulator C was set at the chamber pressure plus 2 psi and micrometer Valve D was opened. Gas then flowed into the chamber and through the fluid in the organ bath. The excess gas was vented via the Tescom regulator to the atmosphere. The Tescom regulator could detect changes in pressure of less than 1% at pressures of up to 600 psig (43 bar).


Gas flow control panel
Pressure chamber
Water bath
E
M
T
V
A
B
C
D
X
Y
G
A Pressure regulators
B and C gauges
D Micrometer valve
E Exhaust
G Gas cylinder
M Manifold
T Tescom regulator
V Vent
Y Bubbling line
X Compression line

of these aspects of experimental design are outlined and discussed in detail in separate appendices at the end of this thesis. The results of these investigations revealed that:

(a) The temperature of the bathing fluid was constant during the compression, decompression and throughout the hyperbaric exposure at 37 $\pm$ 0.5°C (Appendix B).

(b) pO_2, pCO_2 were maintained as described on p. 69. The success of these procedures is examined fully in the General Discussion.

(c) The measurement of pH at pressure is unsatisfactory.

During a compression to 43 bar in MacI, using the appropriate gas mixture, a rise in pH from 7.4 to 7.8 was recorded. However, when the pH electrode was returned to 1 atmosphere it continued to give a high reading in solutions of known pH. This change was reversible only slowly. A rise in pH of the bathing fluid to 7.8 had no effect on tracheal smooth muscle at 1 atmosphere (Appendix C). Theoretical predictions suggest that pressure should cause the pH to fall, due to increased dissociation of ions. The effect of pressure on the pH of biological buffers and on the measurement of pH is discussed in Appendix C and in the General Discussion.

(d) Several isometric and isotonic muscle transducers were tested at high pressure and during changes in pressure. Of the commercially available devices, the Harvard smooth muscle transducer proved the most reproducible at pressure. When this transducer was tested, control responses were reliably reproduced at pressures up to 143 bar. However, small random base line shifts and changes in sensitivity were observed during compression and decompression (Appendix D). It was concluded that apparent tissue responses during compression and decompression might be of mechanical or electrical, rather than physiological origin.

(iii) Compression rate

All compressions were completed in 30 seconds, the maximum rate of compression being 134 bar/minute (final pressure 67 bar). Adopting this rapid compression rate ensured that gas movement had ceased before any tissue responses were observed. The compression rates used in this study are unrealistic for use with whole animals, even during simulated submarine escape. However, since

the transducer sensitivity changes during gas movement, tissue responses which occur during compression and decompression can not be reliably assessed. Thus the duration of the compression represents a compromise between the time interval from compression onset to the first response of the tissue, and the compression time experienced during submarine escape training.

It can be seen from the protocol (p. 73) that during compression the tissue receives no bubbling gas at all. This is because each mixture provides the correct pCO_2 only at the final depth. Bubbling with a gas suitable for the final total pressure during compression would result in rapid changes both in tissue pO_2 and in tissue pH (see Appendix C). Similarly a lack of O_2 and CO_2 for even a few minutes can result in a reduction in smooth muscle tone (section B ii). Consequently, any period without bubbling gas must be as short as possible. This was of particular importance using MacI, where the volume of the bathing fluid was limited to 10 mls.

(iv) Decompression rate

Decompression was carried out at approximately half the rate of compression, the maximum rate being 67 bar/minute. Again all bulk gas movement was complete before the tissue responded to the altered pressure. Thus the results obtained are not complicated by any change in the sensitivity of the transducer. During submarine escape training the ascent rate is 3-4 m/s. Thus it would take 50-70 seconds to ascend from 600 fsw (21 bar). The two lowest pressures investigated in this study (13.1 bar and 15 bar) are within the operational limits for submarine escape. Furthermore, the decompression rates used in this study correspond approximately to those experienced during submarine escape for these depths.

Because of the difficulties experienced with transducers of all types during compression and decompression, this study is limited to the effects of high, but static pressures on respiratory smooth muscle. Using the compression and decompression profiles described, which represent a considerable stress to the tissue, all gas movement is completed before tissue responses are recorded. These responses are, therefore, uncomplicated by sensitivity changes in the transducer due to the changing pressure.

PROTOCOL

The protocol adopted for the hyperbaric experiments was as follows:

1. Prepare and set up tracheal chains as described in Materials and Methods. Add aminophylline to achieve maximal relaxation. Wash three times. Leave for 30-60 minutes to take up tone.

2. Obtain control data at 1 bar (0.05 bar CO_2:0.95 bar O_2).

3. Construct pressure chamber, remove bubbling gas, close lid.

4. MacI. Compress to selected depth using appropriate gas mixture (see Table 2.1). When final pressure achieved, recommence bubbling with same gas mixture as used for compression. Allow excess gas to vent via Tescom regulator.

   MacII. Compress to selected depth using He alone. Do not bubble gas through the bath fluid. There will be no excess gas to bleed off via the regulator.

5. Obtain experimental data at high pressure.

6. MacI. Remove bubbling gas, decompress rapidly, remove lid and restore 0.05 bar CO_2:0.95 bar O_2.

   MacII. Decompress rapidly, remove lid and restore 0.05 bar CO_2: 0.95 bar O_2.

7. Repeat control data, if appropriate.

8. Add aminophylline to achieve maximal relaxation.

D. Hyperbaric Experimental Procedures

(i) Comparison of He and air as compression gases; comparison of the effects of bubbling with 5% CO_2:95% O_2, 100% O_2 or nil bubbling gas

(a) Six tracheal chains were prepared as described previously. One chain of each pair was set up in the organ bath of MacI (volume 10mls). The matched chain of each pair was also bathed in 10mls and maintained at ambient pressure with 5% CO_2:95% O_2 as a control.

Three of the preparations described above were compressed to 14.8 bar air; after a period of observation these were decompressed and the inherent tone allowed to recover a steady level. The same preparations were then recompressed to 14.8 bar, this time with He as the compression gas. After a second period of observation, they were decompressed, and the recovery of inherent tone was recorded. The remaining three tracheal chains were subjected to the compression to 14.8 bar, He, followed by air compression to 14.8 bar.

Throughout these recordings the bath fluid was bubbled with 5% CO_2:95% O_2. This resulted in excessively high, but unknown P_{O_2} and P_{CO_2} during the hyperbaric observation periods.

(b) Six tracheal chains were prepared as described in (a) above and subjected to identical compression procedures. Once inherent tone had been established using 5% CO_2:95% O_2, the bubbling gas was replaced with 100% O_2. Throughout the subsequent recording the bath fluid was bubbled with 100% O_2.

(c) Six tracheal chains were prepared as described in (a) above and subjected to identical compression procedures. Once inherent tone had been established with 5% CO_2:95% O_2, the bubbling gas was removed. After recording the inherent tone for 20 minutes at ambient pressure, the compression procedure was begun.

(d) Four tracheal chains were prepared as described above. One chain of each pair was compressed to 31 bar He, in the absence of any bubbling gas. The effect on inherent tone was recorded for 2 hours, observed for a further 60 minutes after decompression.

(e) The effect of He at atmospheric pressure on the inherent tone of tracheal smooth muscle was recorded in four experiments. Matched tracheal chains were prepared, one of which was set up in a

conventional organ bath. The surface of the bath fluid was therefore in contact with air throughout. The second chain of each pair was mounted as before in the organ bath of MacI. The lid of the chamber was closed but the exhaust vent was open. After steady tone had been recorded, the chamber was flushed gently with He. Both preparations were bubbled with 5% CO_2:95% O_2. Inherent tone was recorded for 160 minutes. 5% CO_2:95% O_2 was then withdrawn from both preparations, and the tone was recorded for a further 140 minutes.

In the remaining hyperbaric experiments the P_{O_2} and P_{CO_2} were either controlled by the use of special gas mixtures for compression and bubbling (MacI) or in MacII, a larger volume of bath fluid provided a sufficient reserve of O_2 and CO_2, so that inherent tone was unaffected by hypoxia during short experiments. The type of compression (He:O_2:CO_2) mixture or He alone will be indicated.

(ii) The effect of pressure on inherent tone

The effect of pressure on inherent tone of guinea pig tracheal smooth muscle was recorded in 56 separate experiments. A matched chain was maintained in air at atmospheric pressure in each case.

(iii) Effect of repeat compression

In 7 experiments tracheal chains were compressed twice to the same depth and for the same duration. Each tracheal chain was compressed to 43 bar using He alone. After decompression tone was allowed to regain a steady level, 30 minutes later the compression was repeated.

(iv) Effect of propranolol

Four preparations were compressed to 31 bar (He:O_2:CO_2) in MacI. After decompression the preparations were allowed to recover for 30 minutes. A dose of propranolol (10^{-6} g/ml) was added to the bath fluid and after 10 minutes the compression was repeated.

(v) Effect of atropine

Five preparations were compressed to 31 bar (He:O_2:CO_2 mixture) in the presence of atropine (10^{-6} g/ml). Four further preparations were treated with the same dose of atropine and compressed to 43 bar using the appropriate gas mixture. These experiments were conducted in MacI.

(vi) Effect of TTX

Five preparations were compressed with a $He:O_2:CO_2$ mixture to 43 bar in MacI. After decompression and a 30 minute recovery period, they were treated with TTX (10^{-6} g/ml). After a further 10 minutes, the preparations were recompressed to 43 bar. Matched tracheal chains were also treated with TTX.

(vii) Effect of $[Ca^{2+}]$ on the response to pressure

(a) Effect of reduced $[Ca^{2+}]$ on the response to 13.1 bar

Four single tracheal chains were prepared and one chain of each pair mounted in the organ bath of MacII, in unmodified Krebs' solution. Each chain was compressed to 13.1 bar (He alone). After 15 minutes the preparations were decompressed and inherent tone allowed to regain a steady level. The bath fluid was replaced twice with Krebs' solution containing 1.2 mMCa^{2+}. When inherent tone was steady, the preparations were recompressed to 13.1 bar for a further 15 minutes. After decompression and recovery the bath fluid was replaced three times with unmodified Krebs' solution. Then, the preparations were compressed to 13.1 bar for a third time.

(b) Effect of raised $[Ca^{2+}]$ on the response to 31 bar

Six tracheal chains were prepared as described above and compressed three times to 31 bar using He alone. During the second compression the preparations were bathed in Krebs' solution containing 7.2 mM Ca^{2+}.

(c) Effect of raised Ca^{2+} on the response to 43 bar

Six tracheal chains were prepared and used as in (b) and (c) above. Each chain was compressed three times to 43 bar, using He alone. During the second compression the chains were bathed in Krebs' solution containing 14.4 mM Ca^{2+}.

(viii) Effect of ouabain on the response to 43 bar

Five tracheal chains were compressed to 43 bar (He alone) in MacII. After 15 minutes the preparations were decompressed, and allowed to recover a level tone. 10^{-6} g/ml propranolol was added to the bath fluid, followed by ouabain (11 μM). When the inherent tone was again steady the preparations were recompressed to 43 bar.

(ix) Effect of quinidine on the response to 43 bar

Four pairs of tracheal chains were prepared and used as above. Each chain was compressed to 43 bar and after 15 minutes was decompressed. When inherent tone had recovered quinidine (50 µg/ml) was added to the bath fluid when tone was steady, the preparations were recompressed to 43 bar.

(x) Effect of pressure on the lung strip

Ten pieces of lung strip were prepared from 5 guinea pigs. Five strips were bathed in 30 mls Krebs' solution in MacII and allowed to take up tone. The remaining matched strips were bathed in 15 mls Krebs' solution in a conventional glass organ bath.

The experimental strips were compressed to 43 bar (He alone). The tone was recorded for 30 minutes before decompression.

(xi) Effect of compression to 43 bar argon (Ar)

Three tracheal chains were compressed to 43 bar (He alone in MacII. After decompression and recovery the preparations were recompressed to 43 bar with pure Ar. Three further preparations were recompressed to 43 bar Ar alone. After decompression and recovery the preparations were recompressed to 43 bar (He alone).

(xii) The effect of pressure on the response to field stimulation

The response to field stimulation was studied at three different pressures: 31, 43 and 67 bar. At 31 and 43 bar the response to field stimulation was also recorded in the presence of atropine (10^{-6} g/ml).

Preparations were set up as described in MacI and after tone was established a frequency response curve was constructed over the range 2-16 Hz. The preparations were compressed to 31, 43 or 67 bar using the appropriate $He:O_2:CO_2$ gas mixture. When tone was steady the frequency-response tests were repeated. After decompression and recovery, the frequency response tests were repeated for a third time. Paired control chains also underwent three consecutive frequency-response tests, each at ambient pressure.

3. RESULTS

3. Normobaric Results

In the following sections, all data are illustrated in the form of Figures. Some Tables are included where they are of particular relevance to the text. Tables followed by the Roman numeral I contain detailed information about each experiment and are to be found in Appendix I (pages 212-235).

(i) The effect of $[Ca^{2+}]_o$ on tracheal smooth muscle tone

(a) When tracheal chains which had been pre-equilibrated in normal Krebs' solution were bathed in Krebs' solutions containing less than normal concentrations of Ca^{2+}, inherent tone fell to a lower but steady value. The results are shown in Fig 3.1, Table 3.1. When $[Ca^{2+}]$ was halved to 1.2 mM some preparations showed a marked decrease in inherent tone, while other preparations responded with only a small loss of tone. The mean $\%T_i$ when $[Ca^{2+}]_o$ was 1.2 mM was 76.2 ± 3.4%. When $[Ca^{2+}]_o$ had been reduced to 0.3 mM inherent tone was almost abolished.

(b) Tracheal smooth muscle tone was not altered when chains previously equilibrated in 2.4 mM $[Ca^{2+}]_o$ were bathed in 7.2 mM Ca^{2+}. The mean percentage of initial tone ($\%T_i$) recorded was 104.1 ± 3.5%. It can be seen from Table 3.2(a) that one preparation responded with a net reduction in inherent tone.

When tracheal chains were bathed in 14.4 mM Ca^{2+} the $\%T_i$ recorded was 114.4 ± 5.5% (Table 3.2b). Once again, one preparation responded with a reduction in tone. These data are shown in Fig 3.2.

(ii) The effect of hypoxia on tracheal smooth muscle tone

In the majority of preparations inherent tone began to fall within two minutes of bubbling with 5% CO_2:95% N_2. However, some preparations showed a slight rise in tone which was then followed by a steady reduction in inherent tone. Fig 3.3 shows some typical examples of the two responses. Within 15 minutes of the onset of hypoxia a lower, steady level of tone was recorded in all preparations, which was relatively constant for periods of up to one hour even during drug application (Section B(x)) or repeated electrical stimulation (B(xi), (xii)). During hypoxia, the mean value of inherent tone fell from 1.55 ± 0.07 g to 0.84 ± 0.08 g (n = 17). The reduction in tone is statistically different, $p < 0.01$. The mean percentage of initial tone ($\%T_i$) remaining after hypoxia was 53.5 ± 3.7% (Table 3.3).

2.4mM		1.2mM		0.6mM		0.3mM		0.15mM	
Initial Tone (mm)	% Initial Tone	Tone (mm)	% Initial Tone	Tone (mm)	% Initial Tone	Tone (mm)	% Initial Tone	Tone (mm)	% Initial Tone
20.0	100.0	16.5	82.5	08.0	40.0	2.00	0.0	0.00	0.0
23.5	100.0	21.5	91.4	12.0	51.1	2.00	8.5	2.00	8.5
15.0	100.0	09.5	63.3	03.0	20.0	0.00	0.0	0.00	0.0
11.5	100.0	08.0	69.5	02.0	17.4	0.00	0.0	0.00	0.0
14.0	100.0	11.0	78.5	-	-	-	-	-	-
16.0	100.0	12.0	75.0	09.0	56.2	0.00	0.0	0.00	0.0
22.0	100.0	16.0	72.7	10.0	45.4	1.00	4.5	0.00	0.0
Mean	100.0		76.2		38.3		3.8		1.4
SD			09.1		16.2		4.5		3.4
SE			03.4		6.6		1.5		1.4

TABLE 3.1 Seven tracheal chains were prepared and inherent tone was established. The Krebs' solution containing 2.4 mM Ca^{2+} was replaced by a solution containing 1.2 mM Ca^{2+} and inherent tone was recorded. Subsequently the Ca^{2+} concentration of the bath fluid was halved 3 times until the Ca^{2+} concentration was 0.15 mM.

TABLE 3.2a Six tracheal chains were bathed in normal Krebs' solution and inherent tone was established in the usual way. The bath fluid was then replaced by Krebs' solution containing 7.2 mM Ca^{2+} and the inherent tone recorded when it became stable.

TABLE 3.2b Six tracheal chains were bathed in normal Krebs' solution and inherent tone was established in the usual way. The bath fluid was replaced by Krebs' solution containing 14.4 mM Ca^{2+} and the inherent tone recorded when it became stable.

$[Ca^{2+}]_o$ = 2.4mM Initial Tone (mm)	$[Ca^{2+}]_o$ = 7.2mM Tone (mm)	% Initial Tone
24.0	25.0	104.0
21.0	19.0	90.9
24.0	27.0	112.5
21.0	23.0	109.5
12.0	12.0	100.0
24.0	26.0	108.0
Mean		104.1
SD		7.8
SE		3.5

TABLE 3.2a Effect of raising $[Ca^{2+}]_o$ on established inherent tone of tracheal smooth muscle.

$[Ca^{2+}]_o$ = 2.4mM Initial Tone (mm)	$[Ca^{2+}]_o$ = 14.4mM Tone (mm)	% Initial Tone
15.5	20.0	129.0
26.5	30.0	113.2
21.0	27.0	128.6
29.0	27.0	93.1
21.0	24.0	114.3
24.0	26.0	108.3
Mean		114.4
SD		13.5
SE		5.5

TABLE 3.2b Effect of raising $[Ca^{2+}]_o$ on established inherent tone of tracheal smooth muscle.

FIGURE 3.1; 3.2 The data from Tables 3.1 and 3.2 are summarised in this figure. Inherent tone was established and the bathing fluid was then replaced with Krebs' solution containing different concentrations of Ca^{2+}. Six preparations were bathed in 7.2 mM Ca^{2+} and six further preparations in Kreb's solution containing 14.4 mM Ca^{2+} (3.2). Seven preparations were bathed in Krebs' solution containing progressively lower concentrations of Ca^{2+} (3.1).

Inherent tone was recorded on Harvard smooth muscle transducers and expressed as a percentage of the initial tone (% T_i).

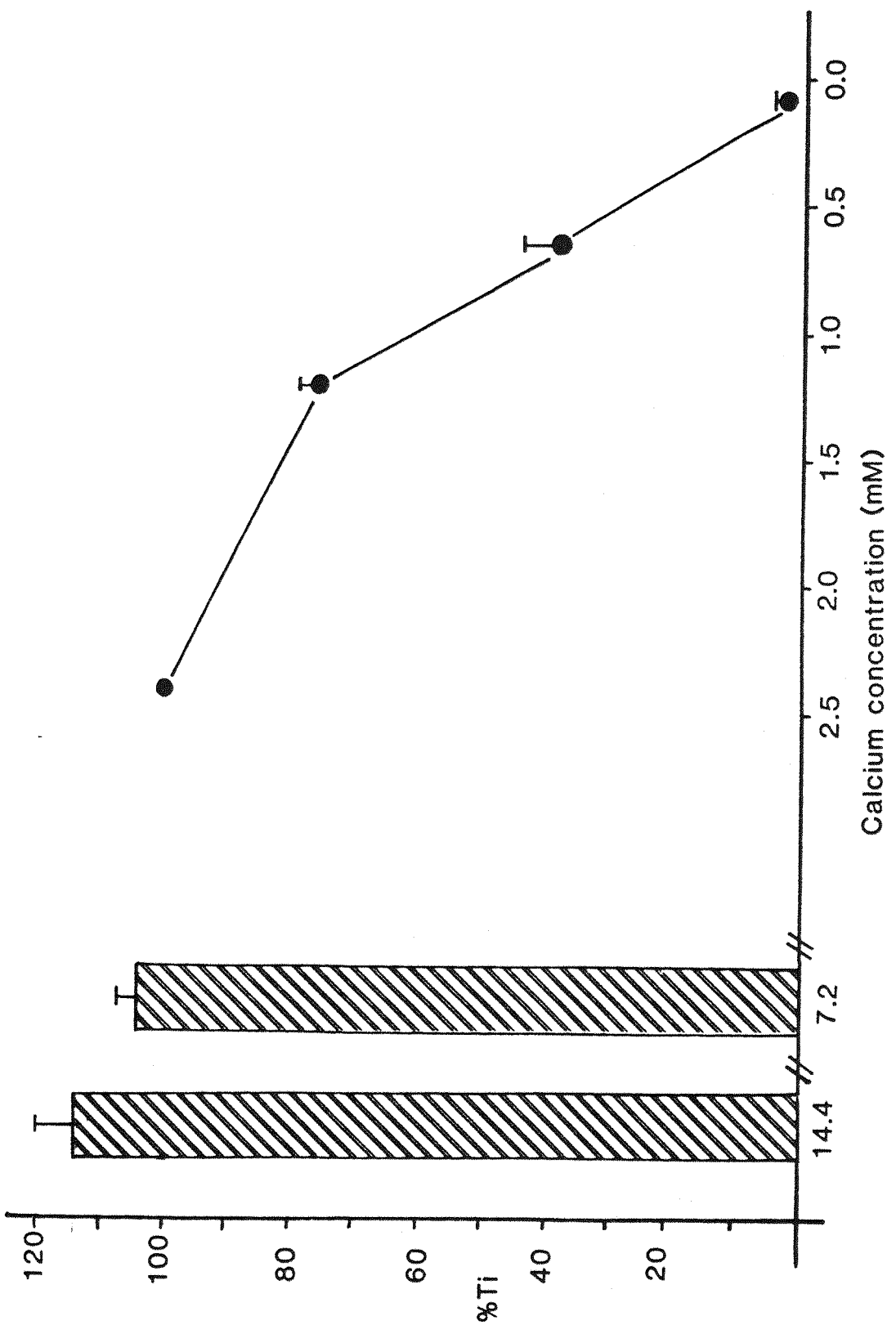

120
100
80
60
40
20
%Ti
14.4
7.2
2.5
2.0
1.5
1.0
0.5
0.0
Calcium concentration (mM)

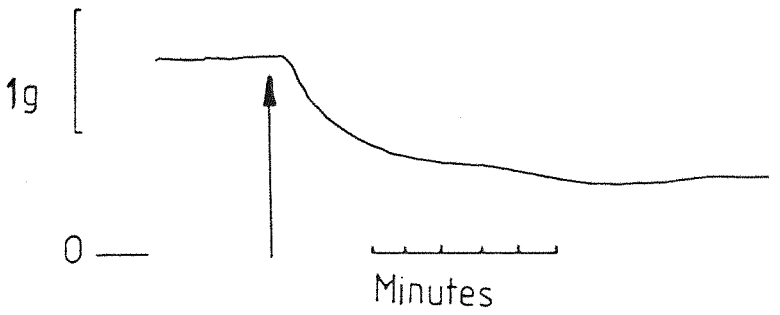
FIGURE 3.3 Tracheal smooth muscle responded in one of two ways to hypoxia:

A. Inherent tone began to fall within 2 minutes of bubbling with 5% CO_2 95% N_2 and reached a steady value within ~15 minutes.

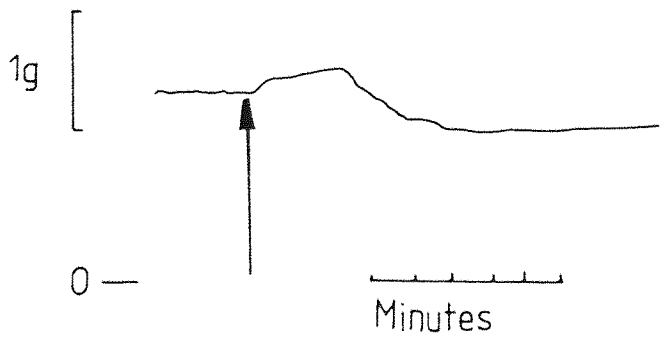
B. Inherent tone rose slightly following bubbling with 5% CO_2 95% N_2 and this contraction was maintained for 2-3 minutes. Within 5 minutes the preparations began to relax and a lower steady level of tone was reached within 15 minutes.

Inherent tone was recorded on Grass FTO3C transducers.

A



B



EXPT NO	T_i(g)	T_H(g)	$\%T_i$
1	1.5	1.1	77.4
2	1.5	0.9	59.1
3	1.4	0.8	57.1
4	2.0	1.0	50.0
5	1.6	0.8	50.0
6	1.8	1.0	55.5
7	1.3	0.5	36.8
8	1.3	0.2	15.7
9	1.8	1.5	81.5
10	1.1	0.5	47.1
11	1.5	0.9	59.1
12	1.4	0.7	47.6
13	1.1	0.5	47.1
14	1.6	0.7	45.8
15	1.7	1.2	69.2
16	1.8	0.8	44.4
17	2.1	1.4	65.6
Mean	1.5	0.8	53.5
SD	0.3	0.3	15.4
SE	0.1	0.1	3.7

TABLE 3.3 Effect of hypoxia on tone.

Seventeen preparations were exposed to hypoxia when the usual bubbling gas 5% CO_2:95% O_2 was replaced by 5% CO_2: 95% N_2. The value of inherent tone was noted prior to hypoxia (T_i) and after it reached a stable value (T_H). The percentage of initial tone remaining after hypoxia ($\%T_i$) is also recorded. These values are recorded in grams using Grass FT03C transducers.

(iii) Effect of substrate withdrawal on the response to hypoxia

When tracheal smooth muscle was bathed in sorbitol Krebs' solution, the value of inherent tone attained (2.0 ± 0.1 g) was not significantly different from that recorded in 50 similar chains bathed in Krebs' solution, when inherent tone was 1.79 ± 0.07 g. When exposed to hypoxia the inherent tone of the sorbitol-bathed chains fell from 2.0 ± 0.2 g to 0.04 ± 0.03 g, a significant decrease, $p < 0.001$. When normoxic conditions were restored tone rose again to 1.7 ± 0.1 g. However, this value was not maintained and tone fell to 1.02 ± 0.22 g within the next 45 minutes, $p < 0.005$. When sorbitol-Krebs' was replaced by glucose-Krebs' solution the mean value of inherent tone was raised significantly to 2.1 ± 0.2 g, $p < 0.005$.

Control chains were bathed in sorbitol Krebs' solution under normoxic conditions for 125 minutes during which time there was no significant reduction in inherent tone. However, when these chains were transferred to glucose Krebs' solution they showed a small but significant increase in tone from 1.8 ± 0.1 g to 2.2 ± 0.01 g, $p < 0.005$. The results of these experiments are summarised in Table 3.4 (a and b) (I), Fig. 3.4 (a and b).

(iv) The effect of raised $[Ca^{2+}]_o$ on the response to hypoxia

Table 3.5(I), Fig. 3.5 show that the presence of high $[Ca^{2+}]_o$ had no effect on the response tohypoxia over the range 2.4 to 9.6 mM. Both control and experimental chains responded with a relaxation which increased with each repeated hypoxic exposure and the differences between them were not significant. However, during the fourth exposure to hypoxia, the response of the control chains was noticeably less than any previous response, while the response in the presence of 14.4 mM $[Ca^{2+}]_o$ continued to be high.

FIGURE 3.4 The effect of substrate withdrawal on the response to hypoxia.

Paired tracheal chains were bathed on Krebs' solution containing sorbitol instead of glucose and allowed to take up tone in the usual way. The results were recorded on Grass FTO3C transducers.

After 60 minutes one chain of each pair was made hypoxic by bubbling with 5% CO_2:95% N_2. The responses are recorded in grams and as a percentage of initial tone, ie the inherent tone recorded after 60 minutes. When O_2 was restored, inherent tone was recorded for a further 65 minutes.

The bathing fluid was then replaced by Krebs' solution containing glucose and the inherent tone noted after 15 minutes when it had become steady.

S indicates the period during which the preparations were bathed in sorbitol Krebs' solution. G indicates that the tissues were bathed in glucose Krebs' solution. H indicates the hypoxic interval. Time in minutes is shown on the x axis. The inherent tone, expressed as a percentage of initial tone ($\%T_i$) is shown on the y axis.

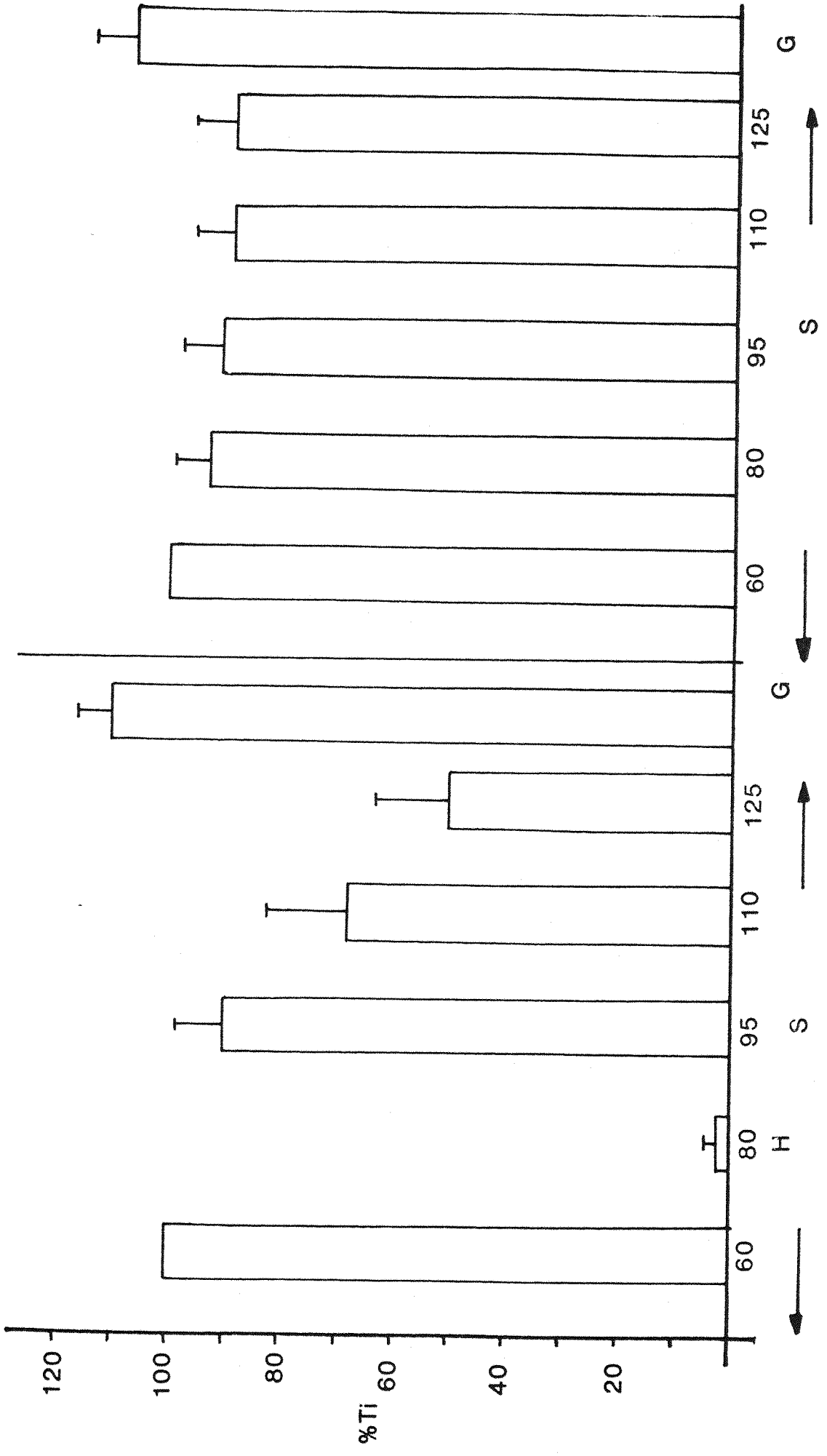
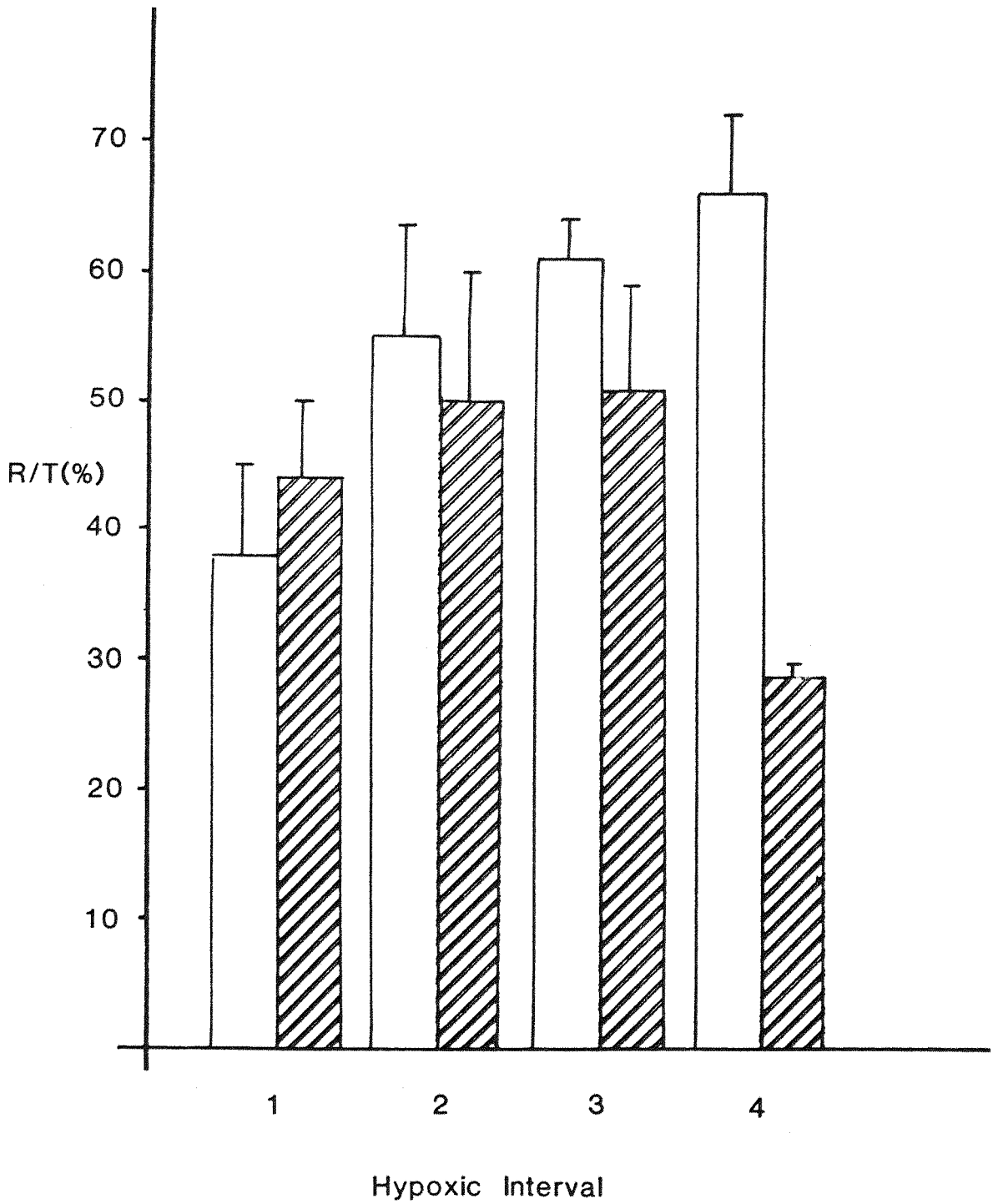

%Ti
120
100
80
60
40
20
60
80
95
110
125
H
S
G
60
80
95
110
125
G
S

FIGURE 3.5 The effect of raising the extracellular [Ca^{2+}] on the response to hypoxia is shown in Fig 3.5.

Tracheal chains were prepared and allowed to take up tone (T) in the usual way. 5% CO_2:95% O_2 was replaced by 5% CO_2:95% N_2. When tone was again steady the response (R) to hypoxia was noted and expressed as a percentage of tone recorded prior to hypoxia. This procedure was repeated after the tone had recovered and the bathing fluid replaced with Krebs' solution contining 4.8, 9.6 and finally 14.4 mM Ca^{2+}. In each case the tone and the response to hypoxia were noted and the response expressed as a percentage of the tone prior to hypoxia.

Two further tracheal chains were prepared and allowed to take up tone as above. Inherent tone was recorded on Harvard smooth muscle transducers. These chains were subjected to hypoxia on four consecutive occasions. After tone had recovered, the bathing fluid was replaced with Krebs' solution containing 2.4 mM Ca^{2+}.

The histograms (± SE) show the response to hypoxia, expressed as a percentage of the tone recorded just prior to hypoxia. The control data are shown in the hatched columns, the experimental data are shown in the white columns. The hypoxic intervals are numbered 1-4.


70
60
50
R/T(%)
40
30
20
10
1
2
3
4
Hypoxic Interval

(v) The effect of drugs on tracheal smooth muscle tone

(a) Effect of ouabain

In the presence of atropine and ouabain tracheal chains underwent a small contraction ($\%T_i$ = 118.1 ± 4.5%), Table 3.6 (a and b) (I), Fig.3.6). However, this contraction was not maintained and after 15 minutes a net relaxation had been recorded. The $\%T_i$ remaining was 66.1 ± 9.5%. When propranolol was included in the bathing fluid of the paired chains, the same concentration of atropine and ouabain resulted in a similar concentration ($\%T_i$ = 126.1 ± 7.4) which was maintained for at least 15 minutes, after which time the $\%T_i$ remaining in propranolol treated chains was significantly different from the untreated chains ($p < 0.005$).

(b) Indomethacin

Indomethacin in ethanol or ethanol alone was administered to paired tracheal chains before tone had begun to rise: both control and experimental chains showed similar increases in tone over the first 10 minutes (Fig.3.7, Table 3.7 (a and b) (I)). However, after 15 minutes the inherent tone of the indomethacine treated chain was significantly less than the untreated chain ($p < 0.01$) and as the experiment continued the treated chain steadily lost tone. After 60 minutes the inherent tone of the treated chains was 20.6 ± 4.9% of the untreated chains.

After 60 minutes the inherent tone of the control chains which had been exposed to ethanol (0.2% v/v) was 2.2 ± 0.2 g. This was not significantly different from that recorded in 50 similar chains where ethanol was not present.

(c) Pyridyl Isatogen Tosylate (PIT)

The effect of PIT on established inherent tone can be seen in Table 3.8(a)(b)(I); Fig.3.8. Initially, the tissue contracted and tone was increased from 16.5 ± 1.4 mm to 18.7 ± 1.8 mm (n = 4). This was a mean increase of 13.6%. However, the contraction was not maintained and 15 minutes after the addition of PIT, the tissue began to relax. After 60 minutes inherent tone was reduced to 2.5 ± 0.6 mm. This was 19.2 ± 4.9% of inherent tone recorded in the untreated chains.

FIGURE 3.6 The effect of ouabain on inherent tone of tracheal chain.

Tracheal chains were prepared and inherent tone recorded on Grass FTO3C transducers.
■ The response to ouabain (11 μM) in the presence of atropine (1 μg/ml) ± SE.
● The response to ouabain (11 μM) in the presence of atropine (1 μg/ml) and propranolol (1 μg/ml) ± SE.
The time in minutes is shown on the x axis and the response, expressed as a percentage of initial tone is shown on the y axis.

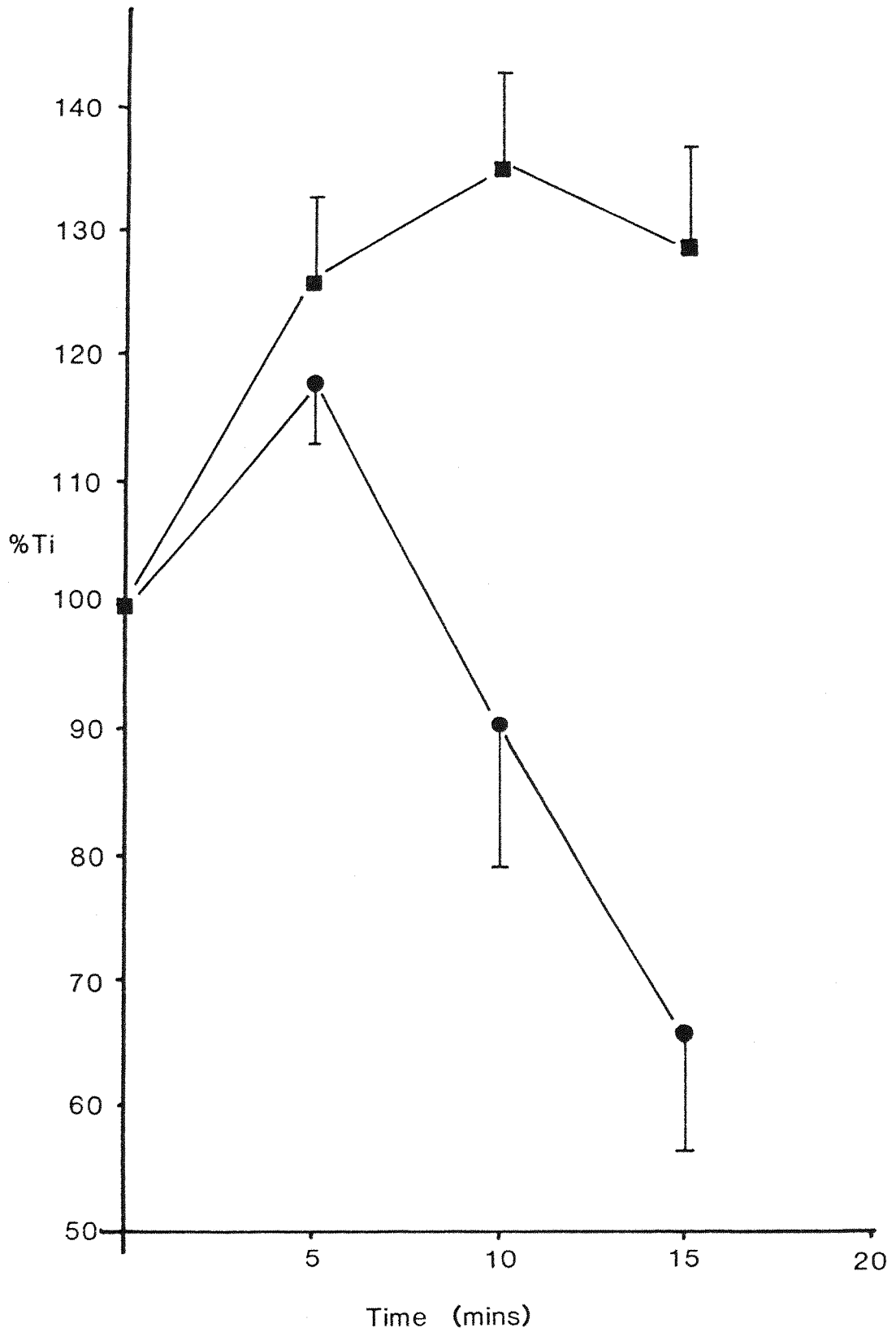

140
130
120
110
%Ti
100
90
80
70
60
50
5
10
15
20
Time (mins)

FIGURE 3.7 The effect of indomethacin (6 μg/ml) on the uptake of inherent tone in tracheal smooth muscle. The response of the tissue was recorded using Grass FT03C transducers.

■ Indomethacin (6 μg/ml) dissolved in ethanol (0.2% v/V) was added at time 0.
● Ethanol (0.2% v/V) was added at time 0.
Inherent tone is shown in grams on the y axis.
Time in minutes is shown on the x axis.

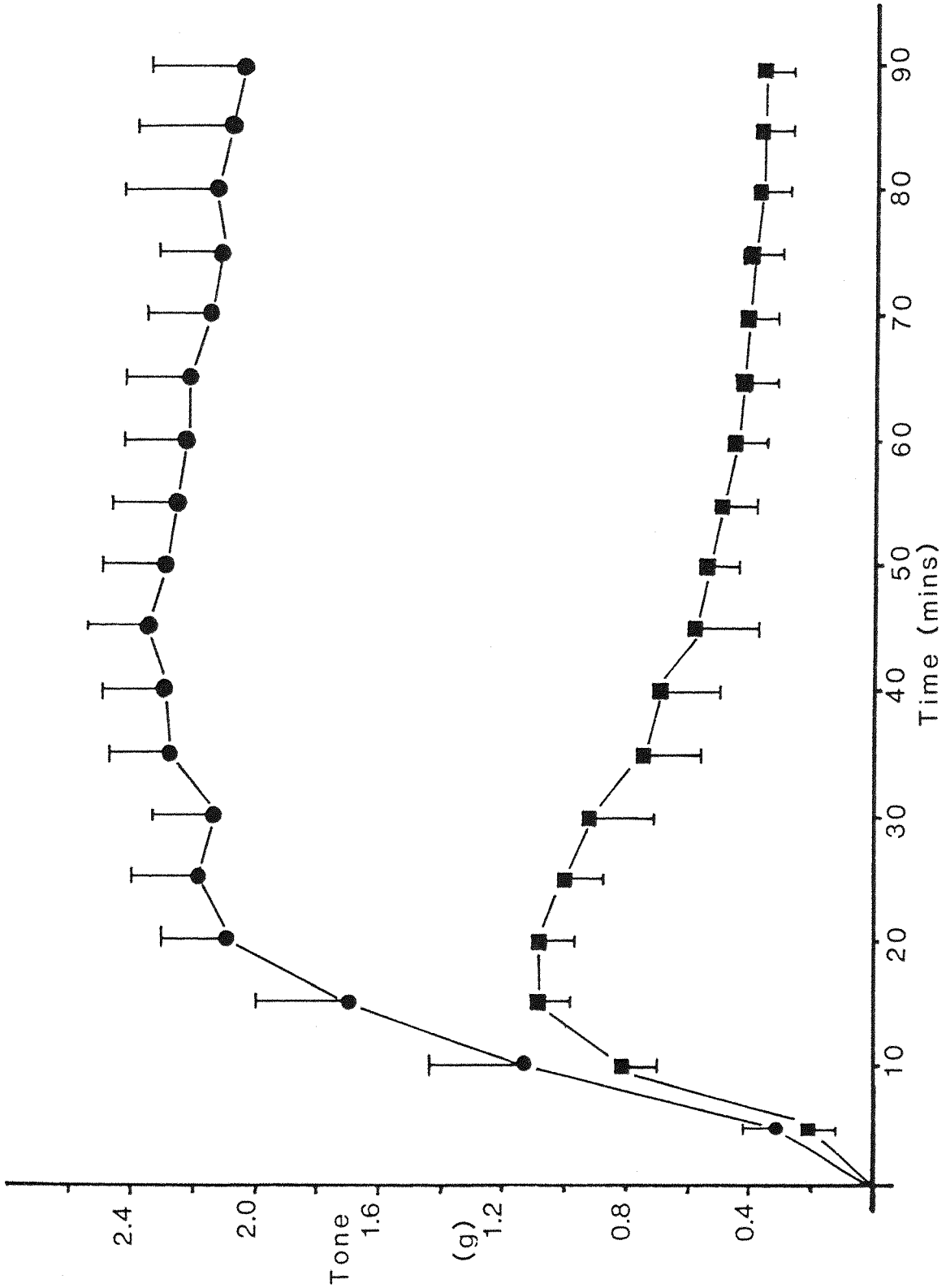
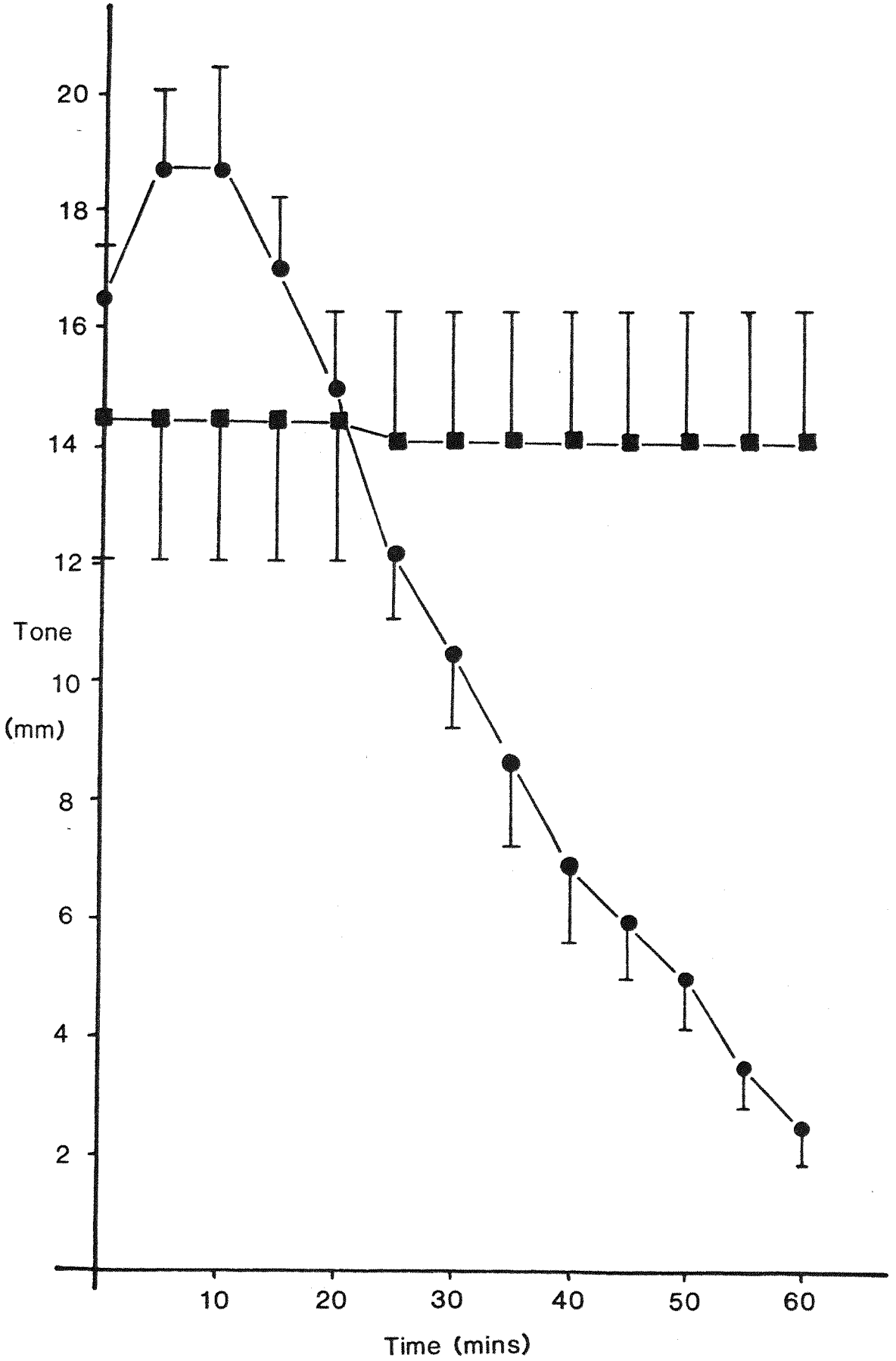

Tone
(g)
2.4
2.0
1.6
1.2
0.8
0.4
10
20
30
40
50
60
70
80
90
Time (mins)

FIGURE 3.8 The effect of PIT on established inherent tone of tracheal chain.

Paired tracheal chains were prepared in the usual way and allowed to take up tone, which was recorded on Harvard Smooth Muscle Transducers.

● The mean response in mm (± SE) to PIT (50 μM), added at time 0.
■ The mean inherent tone in mm (± SE) of the paired control chains, in the absence of any drug.
Tone in mm is given on the y axis.
Time in minutes is given on the x axis.


20
18
16
14
12
Tone
10
(mm)
8
6
4
2
10
20
30
40
50
60
Time (mins)

(d) Verapamil

Methanol or verapamil in methanol was added to pairs of tracheal chains before tone began to rise. Both chains took up tone in an apparently normal manner, Table 3.9(a)(b)(I), and both chains reached a maximum value of inherent tone after 25 minutes. However, 5 minutes later the verapamil treated chain had begun to relax. The relaxation which followed was slow and was not complete 100 minutes after the addition of verapamil.

When verapamil was added to the previously untreated chain, a slow relaxation was initiated which was not complete 60 minutes after the addition of verapamil (Fig.3.9).

(vi) Effect of drugs on the response to field stimulation

(a) Tetrodotoxin (TTX).

The response of tracheal smooth muscle to field stimulation was biphasic: an initial small contraction which was rapidly reversed to a relaxation. The recovery from relaxation was slow. The contraction phase was best recorded under isometric conditions, although it could be recorded at high frequency stimulation using the Harvard smooth muscle transducer.

TTX (1 µg/ml) completely abolished the response to field stimulation in all preparations tested. Inherent tone was unaffected by this concentration of the drug. A typical trace is shown in Fig.3.10.

(b) Atropine

Atropine (1 µg/ml) had no effect on tracheal smooth muscle tone, but completely abolished the contractile response to field stimulation at all frequencies tested (Table 3.10(a)(I) (Fig.3.11a). However, muscarinic blockade had no effect on the relaxation response to field stimulation (Fig 3.11b, Table 3.10(b)(I)).

(c) Reserpine pretreatment

The inherent tone of tracheal chains prepared from animals treated with reserpine was 26.2 ± 1.5 mm which was not significantly different from that recorded in 67 similar preparations. Reserpine pretreatment did not abolish the relaxation response to field stimulation (Table 3.11(I), Fig.3.12).

FIGURE 3.9 The effect of verapamil on the uptake of inherent tone, and on established inherent tone of tracheal smooth muscle.

Paired tracheal chains were prepared and the inherent tone recorded on Harvard Smooth Muscle Transducers.

● Verapamil (5×10^{-5} M) in methanol (0.1% v/V) was added at time 0.
■ Methanol (0.1% v/V) was added at time 0, verapamil 5×10^{-5} M in methanol (0.1% v/V) were added at time 40 minutes. Tone in mm is shown on the y axis. Time in mins is shown on the x axis.

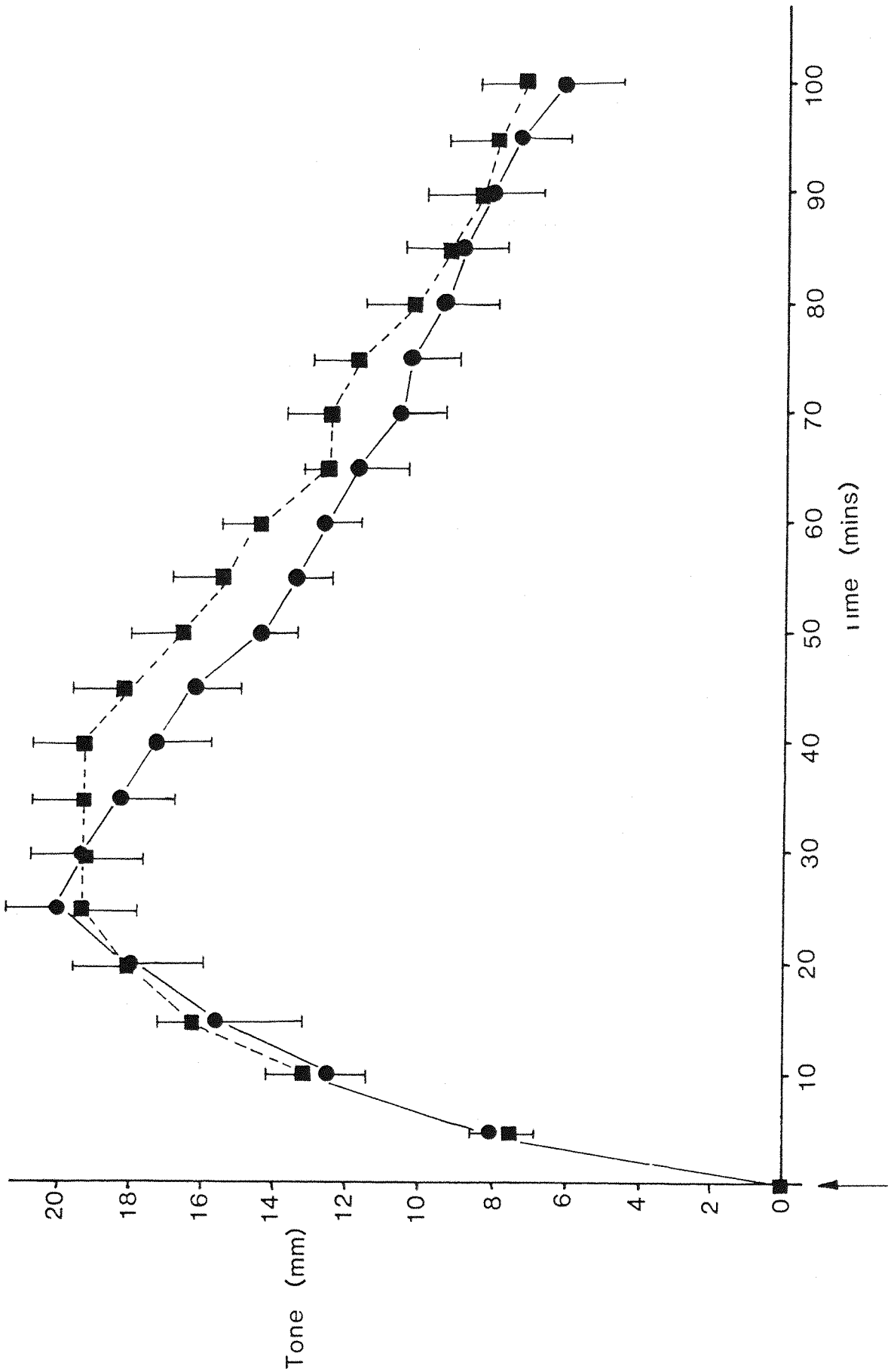

Tone (mm)
0
2
4
6
8
10
12
14
16
18
20
10
20
30
40
50
60
70
80
90
100
Time (mins)

FIGURE 3.10 The effect of TTX on the response of tracheal chain to field stimulation.

A. Figure 3.10A shows the typical response pattern of tracheal chain to field stimulation at increasing frequency. This trace was obtained using a Grass FTO3C transducer and is calibrated in grams. Zero tone is shown at the bottom left hand corner of the trace, and time in minutes in the bottom right hand corner. Contraction is shown in the upward direction and relaxation in the downward direction.

B. TTX (10^{-6} g/ml) abolished the response of tracheal chain to transmural stimulation. In this typical trace the tissue was stimulated at 32 Hz and following recovery, TTX was added to the bath fluid. Ten minutes later the stimulation was repeated, but no response was recorded.

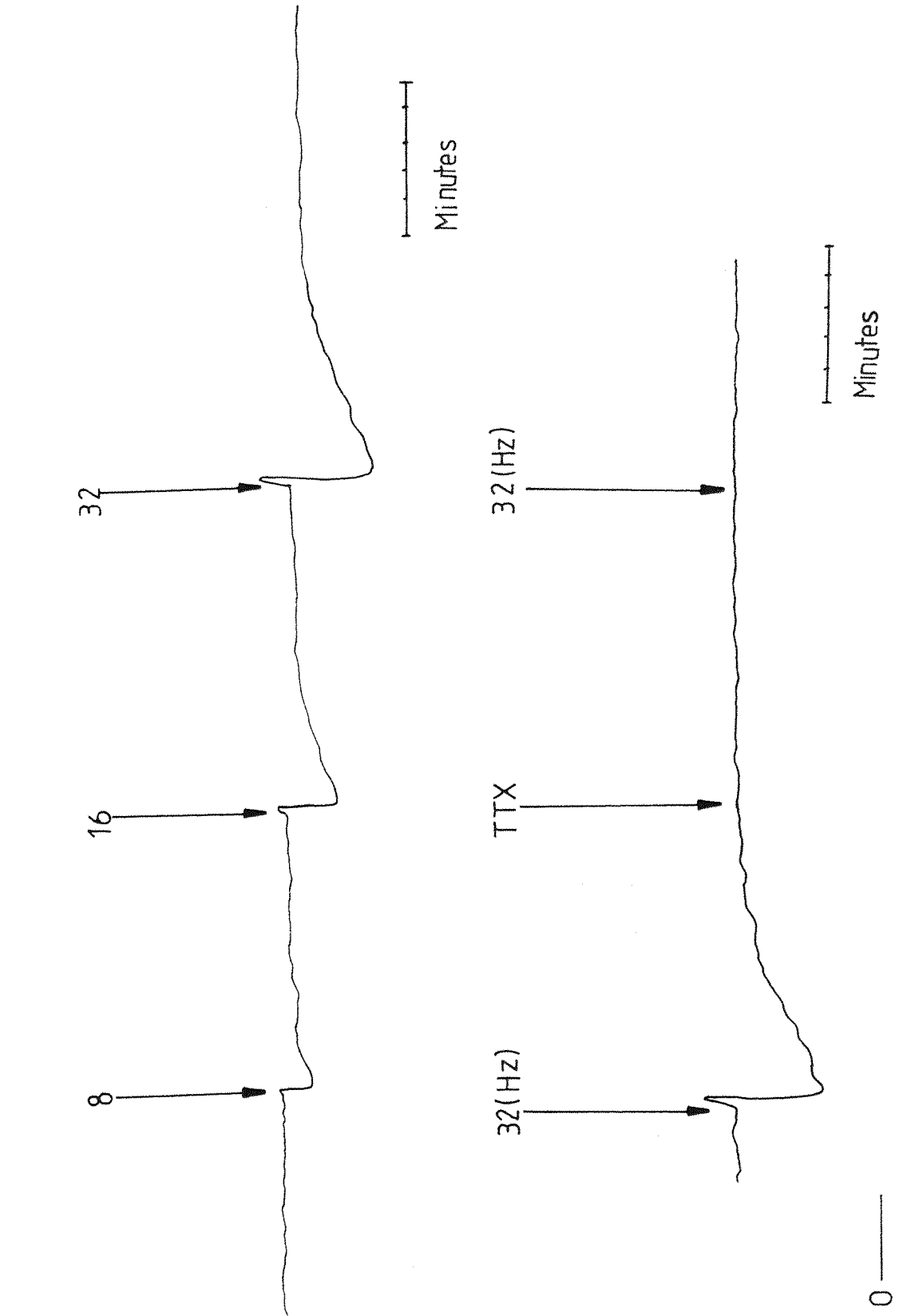

A
4(Hz)
8
16
32
1g
0
Minutes
B
32(Hz)
TTX
32(Hz)
1g
0
Minutes

FIGURE 3.11a The effect of atropine (10^{-6} g/ml) on the contractile response to field stimulation.

Five tracheal chains (unpaired) were prepared and allowed to take up tone in the usual way. Tone and the response to field stimulation were recorded on Harvard Smooth Muscle Transducer.

Contraction expressed as a percentage of the inherent tone prior to stimultion (C (%T)) is shown on the y axis.
Frequency (Hz) is shown on the x axis.
● The mean response ± SE, no drug.
■ The mean response in the presence of atropine 10^{-6} g/ml.

Data and conditions given in Table 3.10.

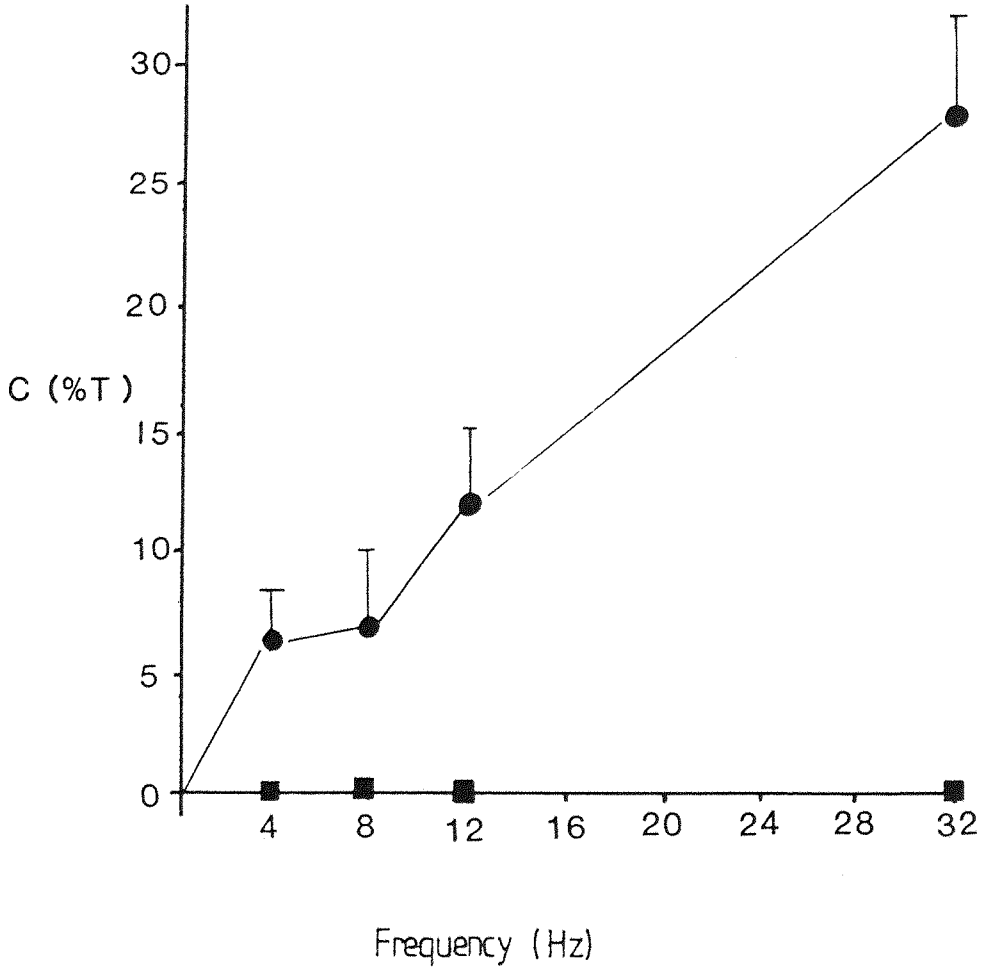

30
25
20
C (%T)
15
10
5
0
4
8
12
16
20
24
28
32
Frequency (Hz)

FIGURE 3.11b The effect of atropine (10^{-6} g/ml) on the relaxation response to transmural stimulation.

Relaxation expressed as a percentage of inherent tone prior to stimulation (R(%T) is shown on the y axis.
Frequency (Hz) is shown on the x axis.
● The mean response ± SE, no drug.
■ The mean response ± SE, atropine (10^{-6} g/ml) present throughout.

Data and conditions given in Fig. 3.11a.

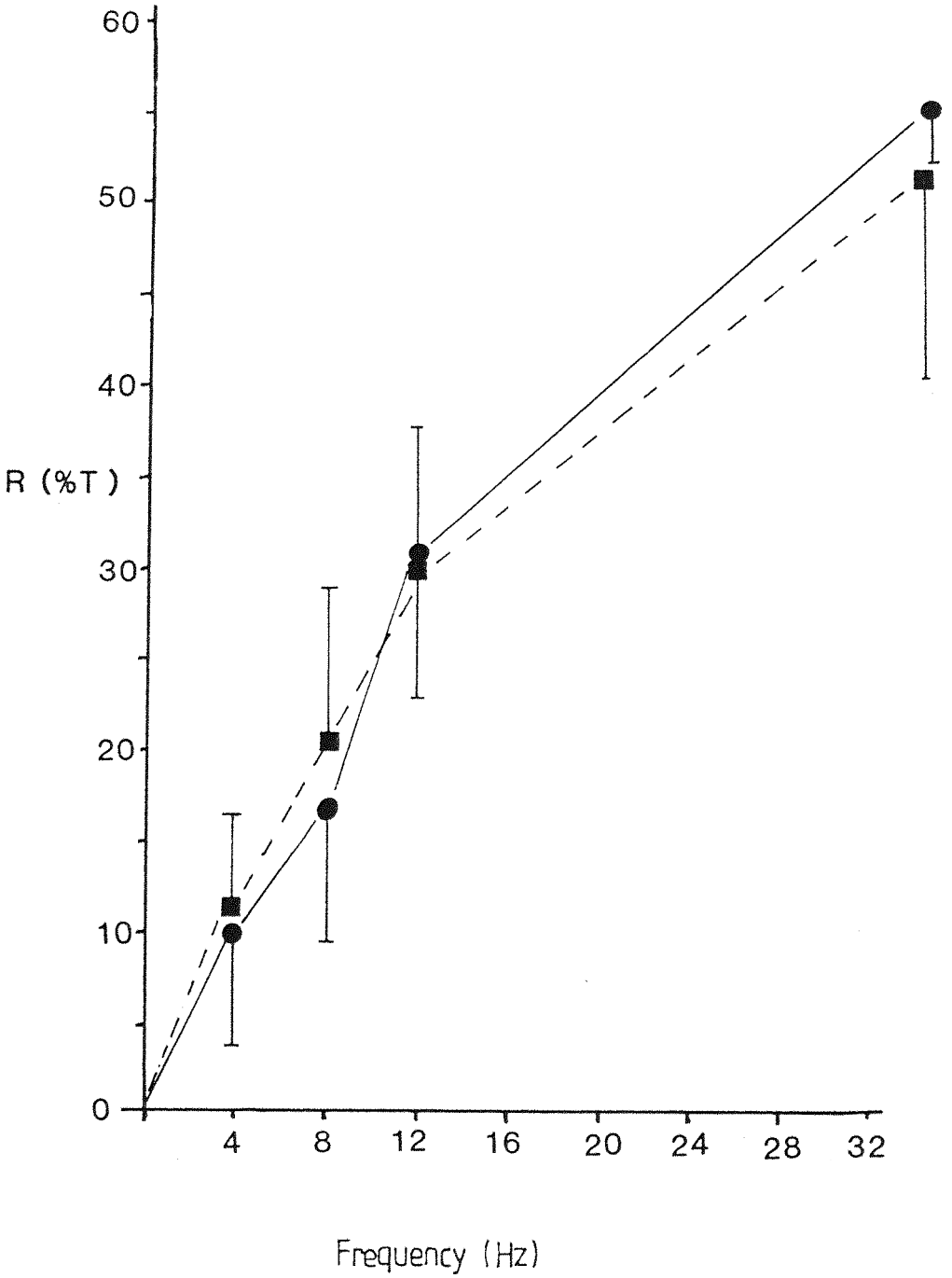

60
50
40
30
20
10
0
R (%T)
4
8
12
16
20
24
28
32
Frequency (Hz)

FIGURE 3.12 The effect of reserpine pretreatment on the relaxation response to field stimulation is illustrated in Figure 3.13.

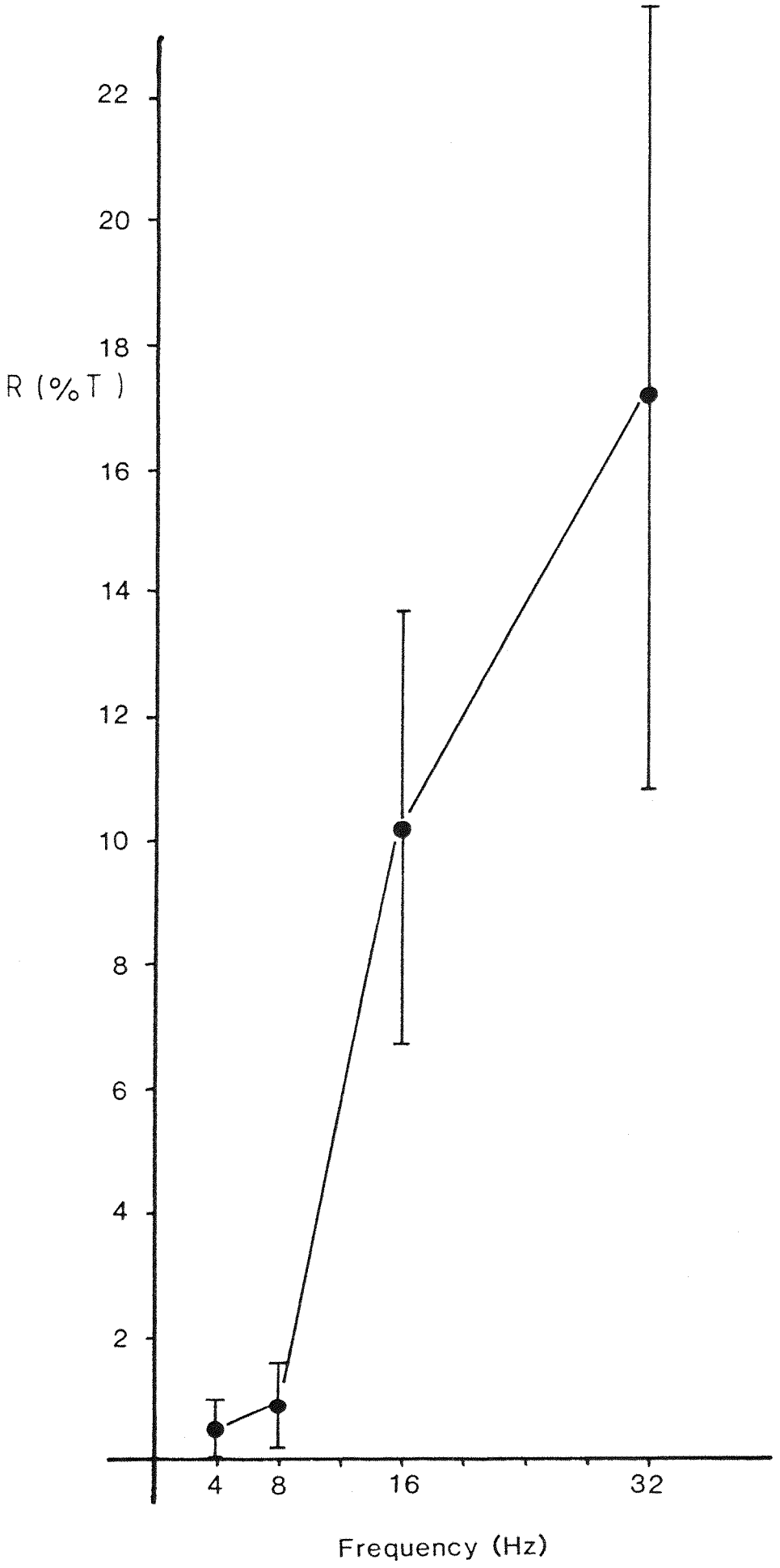
Tracheal chains were prepared from six animals which were pretreated with reserpine (1 mg/kg) on two consecutive days, prior to sacrifice.

Relaxation expressed as a percentage of inherent tone recorded just prior to stimulation R(%T) is shown on the y axis.

Frequency (Hz) is shown on the x axis.

The mean response ± SE.

Data and conditions given in Table 3.11.


22
20
18
R (%T)
16
14
12
10
8
6
4
2
4
8
16
32
Frequency (Hz)

(d) The effect of propranolol and quinidine.

In Fig.3.12 it can be seen that treatment with atropine and propranolol had no significant effect on the response to field stimulation at frequencies of 4 and 8 Hz. However, at 16 and 32 Hz the relaxation response was significantly reduced ($p < 0.01$) by their presence. Further drug treatment with quinidine completely abolished the relaxation response to field stimulation at all frequencies (Table 3.12).

(e) Quinidine.

Quinidine, at a final concentration of 0.05 mM completely abolished the contractile response to field stimulation (Table 3.13(a)(b) (I). In the same preparations (Tables 3.14(a)(b), the relaxation response to field stimulation was significantly reduced at all frequencies greater than 4 Hz, $p < 0.025$, Figs. 3.14 and 3.15.

(vii) Effect of quinidine on the response to adrenaline

The effect of quinidine on the cumulative response of tracheal smooth muscle to adrenaline is shown in Fig.3.16. Quinidine (0.05 mM) had no effect on the response of tracheal chain to adrenaline (Table 3.15(a)(b) (I).

(viii) Effect of quinidine on the response to ATP

ATP caused a dose-related relaxation of tracheal smooth muscle (Fig. 3.17). The response of tracheal chain to ATP was not affected by the presence of 0.05 mM quinidine (Tables 3.16(a) (b) (I)).

(ix) Effect of quinidine on the response to adenosine

A dose-related relaxation of tracheal chain was elicited with adenosine (Fig.3.18; Table 3.17(a) (I). The presence of quinidine (0.05 mM) had no effect on the cumulative dose-response curve to adenosine (Table 3.17(b)) (I).

FIGURE 3.13 The effect of atropine, propranolol and quinidine on the response to field stimulation.

Five tracheal chains were prepared in the usual way. The results were recorded on Harvard Smooth Muscle Transducers.

● The relaxation response (R(%T) in the absence of drugs.
■ The relaxation response in the presence of atropine (10^{-6} g/ml) and propranolol (10^{-6} g/ml).
◖ The relaxation response in the presence of atropine (10^{-6} g/ml), propranolol (10^{-6} g/ml) and quinidine (0.05 mM).
Relaxation expressed as a percentage of inherent tone prior to stimulation R(%T) is shown on the y axis.
Frequency of stimulation (Hz) is shown on the x axis.

Data and conditions are given in Table 3.12.

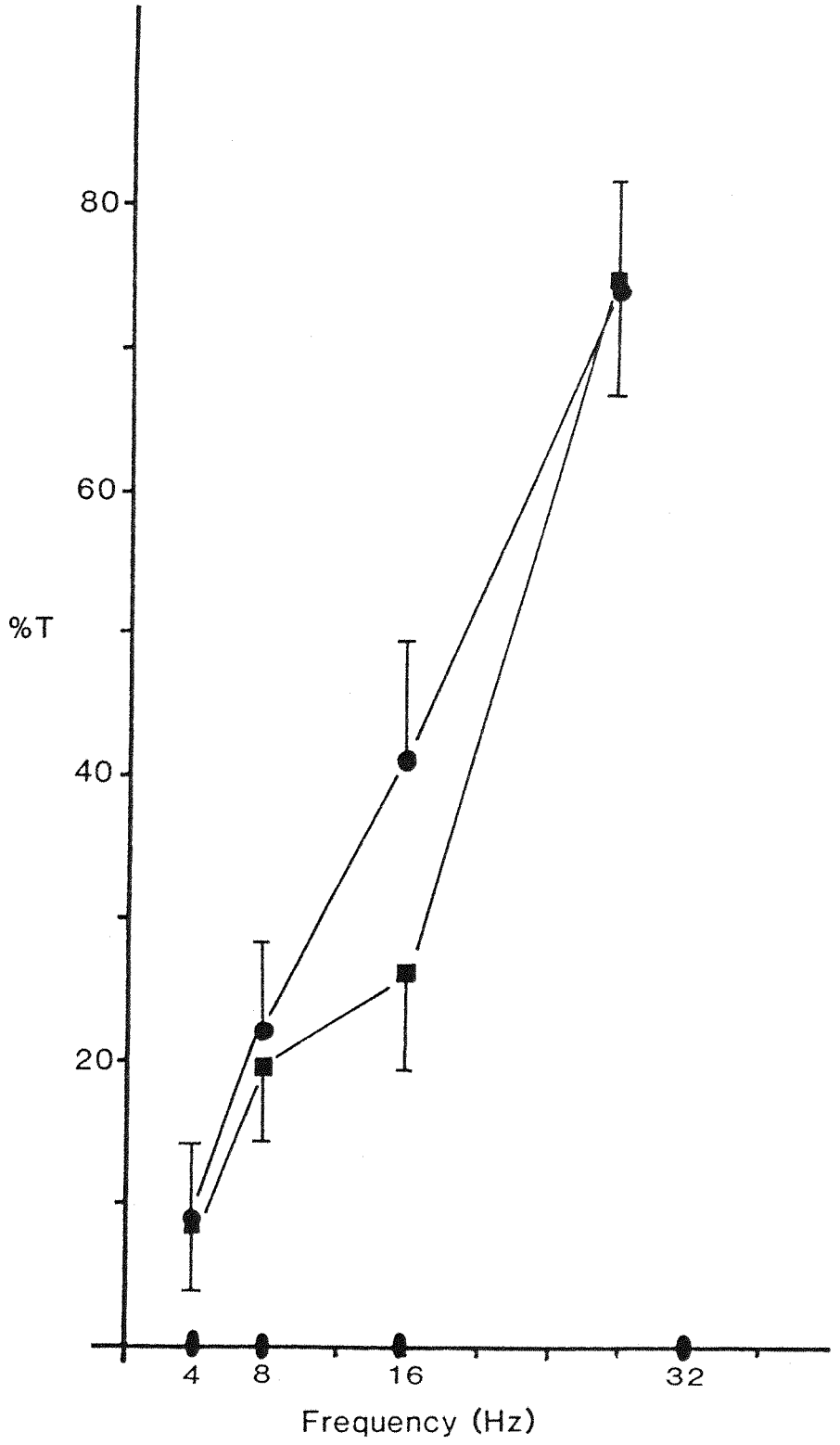

80
60
%T
40
20
4
8
16
32
Frequency (Hz)

FIGURE 3.14 The effect of quinidine on the contractile response to field stimulation.

Four paired tracheal chains were prepared in the usual way. Inherent tone and response to field stimulation were recorded on Grass FTO3C transducers.

● The mean response ± SE in the absence of drug.
■ The mean response in the presence of quinidine (50 μM).
Contraction (C) expressed as a percentage of initial tone ($\%T_i$) is shown on the y axis.
Frequency in Hz is shown on the x axis.

FIGURE 3.15 The effect of quinidine on the relaxation response to field stimulation (see above).

● The mean response ± SE, in the absence of drug.
■ The mean response ± SE in the presence of quinidine (50 μM).
The relaxation response (R), expressed as a percentage of initial tone ($\%T_i$) is shown on the y axis.

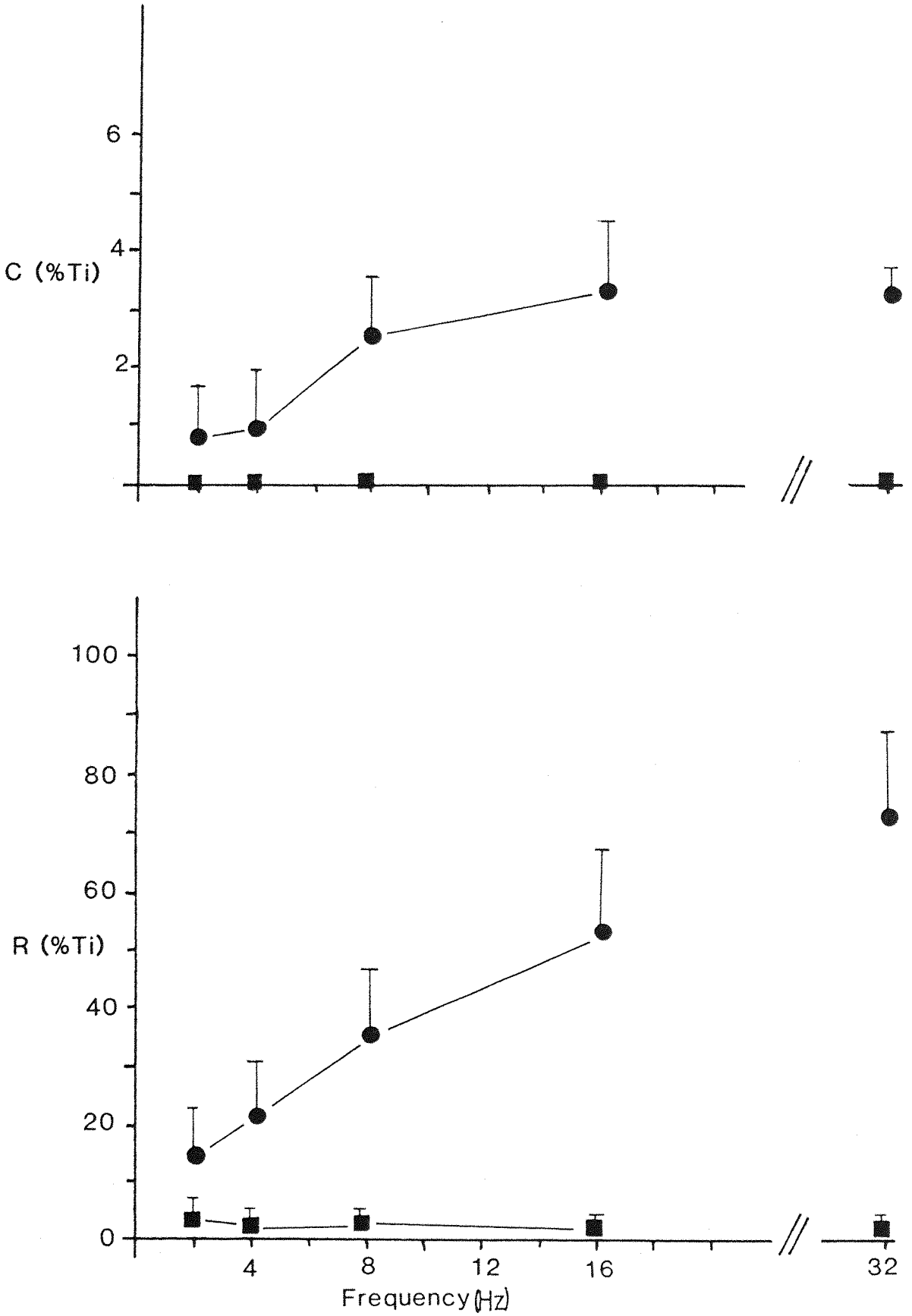

C (%Ti)
6
4
2
R (%Ti)
100
80
60
40
20
0
4
8
12
16
32
Frequency (Hz)

FIGURE 3.16 The effect of quinidine on the response of tracheal chain to adrenaline.

A dose response curve to adrenaline was obtained over the range 1.0-8.0 mM on four pairs of tracheal chains. The inherent tone at the start of each dose cycle (T_S) is recorded in grams, as is the relaxation response (R). R is also given as a percentage of T_S ($\%T_S$).

The second chain of each pair was subjected to two consecutive dose cycles of adrenaline, in the absence of any other drug.

● Dose Cycle A
■ Dose Cycle B

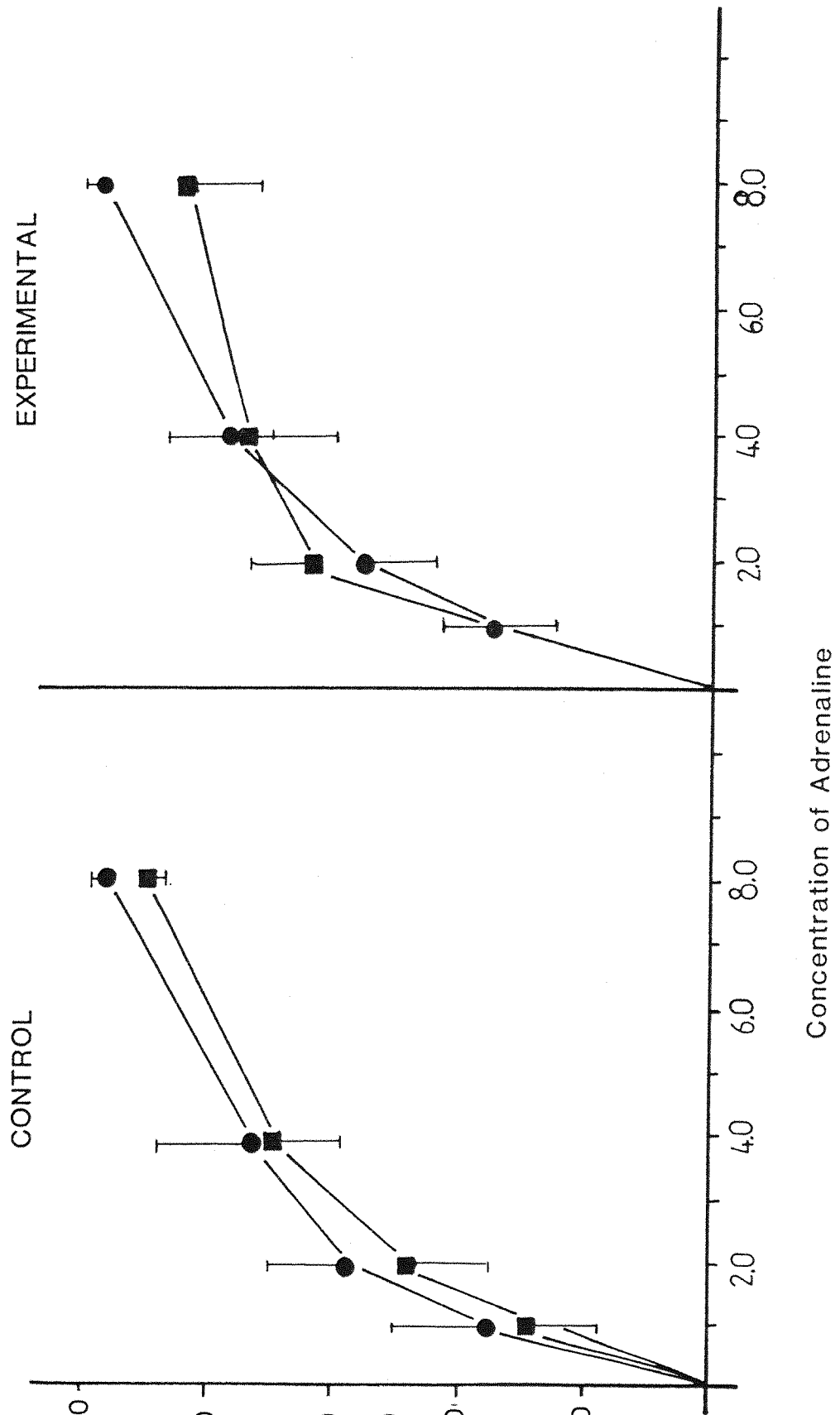

CONTROL
EXPERIMENTAL
R (%Ti)
100
80
60
40
20
2.0
4.0
6.0
8.0
Concentration of Adrenaline

FIGURE 3.17 The effect of quinidine on the response of tracheal chain to ATP.

A dose response curve to ATP was obtained over the range 0.01-3.0 mM on five pairs of tracheal chains. The inherent tone at the start of each dose cycle (T_S) is recorded in grans, along with the relaxation response (R). R is also given as a percentage of T_S ($\%T_S$).

The second chain of each pair was subjected to two consecutive dose cycles of ATP, in the absence of any other drug.

● Dose Cycle A
■ Dose Cycle B

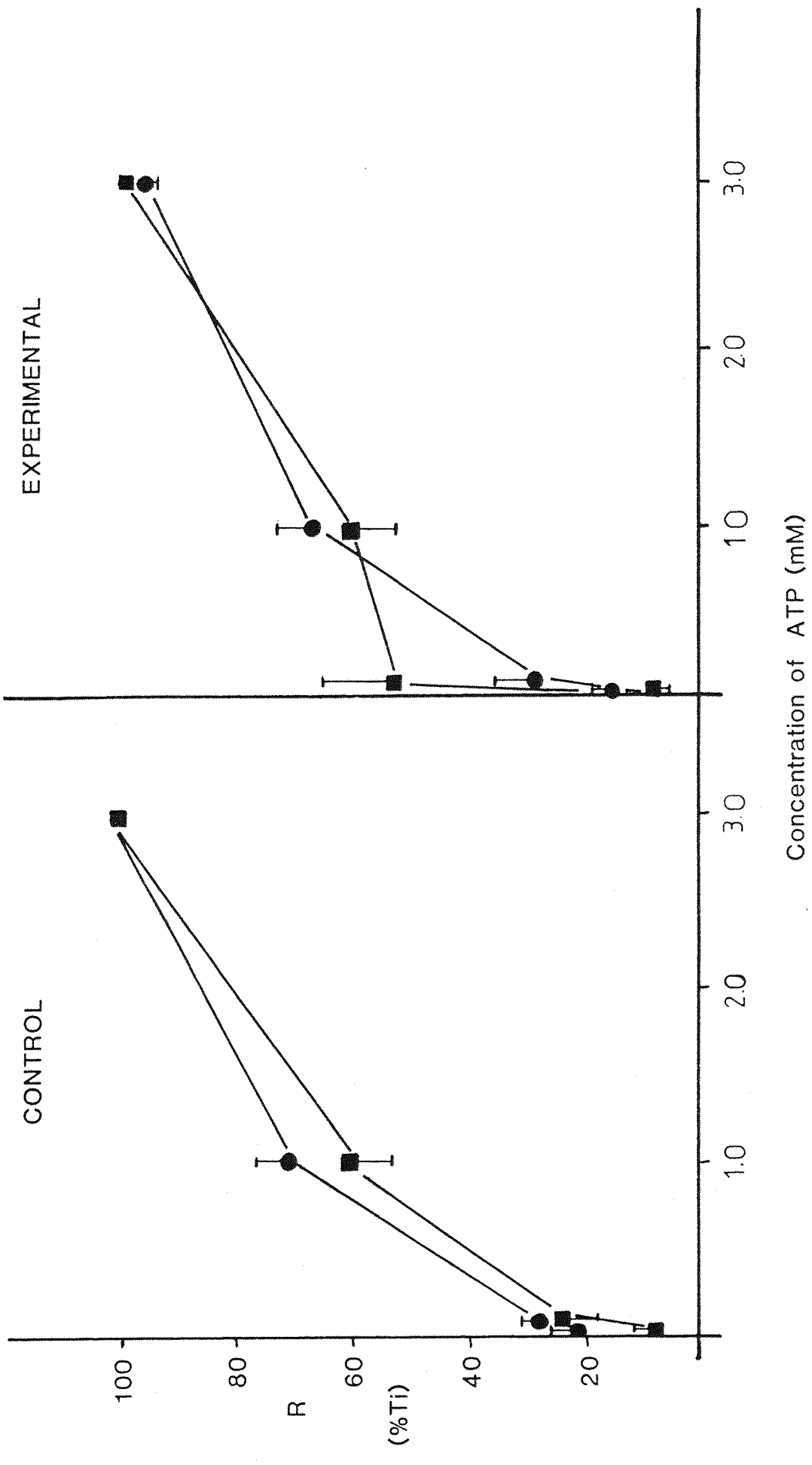
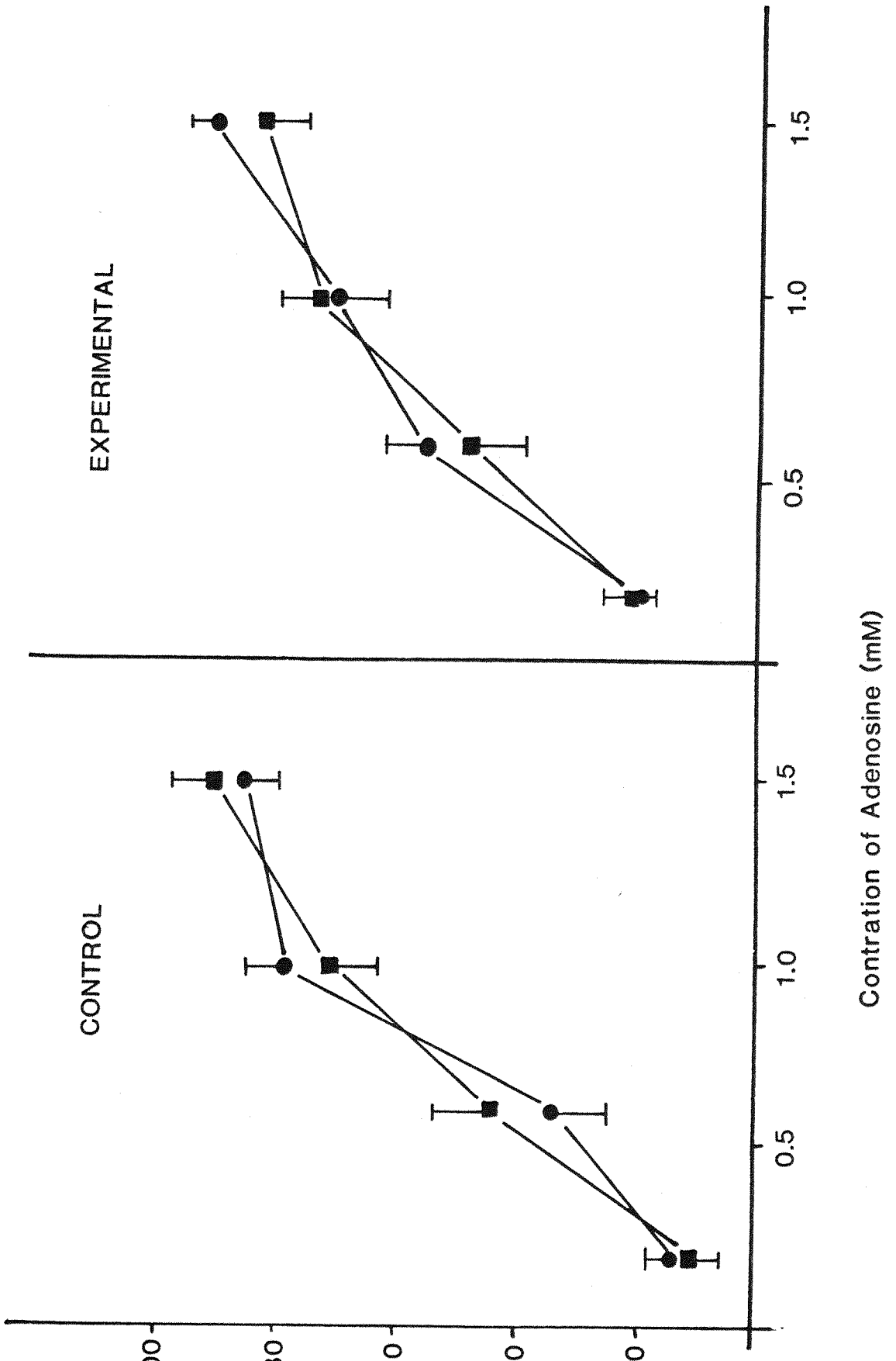

CONTROL
EXPERIMENTAL
R (%Ti)
100
80
60
40
20
1.0
2.0
3.0
1.0
2.0
3.0
Concentration of ATP (mM)

FIGURE 3.18 The effect of quinidine on the response of tracheal chain to adenosine.

A dose response curve to adenosine was obtained over the range 0.2-1.5 mM on four pairs of tracheal chains. The inherent tone at the start of each dose cycle (T_S) is recorded in grams, along with the relaxation response (R). R is also given as a percentage of T_S ($\%T_S$).

The second chain of each pair was subjected to two consecutive dose cycles of adenosine in the absence of any other drug.

● Dose Cycle A
■ Dose Cycle B


CONTROL
EXPERIMENTAL
R
(%Ti)
100
80
60
40
20
0.5
1.0
1.5
0.5
1.0
1.5
Contration of Adenosine (mM)

(x) Effect of hypoxia on the response to field stimulation

As seen previously, hypoxia resulted in a reversible reduction of inherent tone B(ii). The relaxation response to field stimulation was expressed as a percentage of tone recorded immediately prior to stimulation and is shown in Tables 3.18(a)(b) (I).

The mean response to field stimulation was significantly reduced ($p < 0.01$) at each frequency with respect to the same chain under normoxic conditions. After O_2 had been restored, inherent tone recovered and the relaxation response was not significantly different from that recorded prior to hypoxia.

In parallel control experiments there was no significant difference between three consecutive frequency-response curves (Fig. 3.19).

(xi) Effect of hypoxia on the response to adrenaline

Adrenaline caused a dose-related relaxation of tracheal smooth muscle. Although the threshold concentration of adrenaline required varied from one preparation to another, it remained constant for each individual pair of tracheal chains (see Tables 3.19(a) and 3.19(b) (I). This made the results difficult to combine. The results of one typical experiment are shown in Figs.3.20 (a)(b). Hypoxia had no significant effect on the response to adrenaline. After O_2 had been restored the response to adrenaline was similarly unaltered. There was no significant difference in the responses obtained in three consecutive dose-response curves to adrenaline on normoxic paired chains, carried out simultaneously.

(xii) Effect of hypoxia on the response to Ach

Tracheal smooth muscle undergoes a dose-related contraction in response to Ach. The response to Ach is enhanced by hypoxia (Table 3.20(a); Fig.3.20(a)). However, it can be seen from the control data (Table 3.20(b); Fig.3.20(b)) that, following each successive addition of Ach the inherent tone is reduced and that the response to Ach is also enhanced in these preparations. In Tables 3.20(a) and (b) the response to Ach is expressed as a percentage of the tone recorded immediately prior to addition of the agonist. In Table 3.21(a)(b) the same data are presented so that both tone and the response to Ach are expressed as a percentage of T_i, the initial tone. These data are shown in Fig.3.21 where tone as $\%T_i$ is plotted against response as $\%T_i$. Linear regression analysis reveals that there is a significant correlation ($r = -0.7$; $p < 0.0005$; $n = 36$), so that as tone was reduced, the response to Ach was enhanced.

FIGURE 3.19 The effect of hypoxia on the frequency response curve of tracheal chain. Paired tracheal chains were stimulated at 2, 4, 8, 16 and 32 Hz and the results were recorded using Grass FTO3C transducers.

A. ● The mean relaxation response ± SE, control chains, normoxic conditions.
■ The mean relaxation response ± SE, experimental chains, normoxic conditions.

B. ● The mean relaxation response ± SE, control chains, normoxic conditions.
■ The mean relaxation response ± SE, experimental chains, hypoxic conditions.

C. ● The mean relaxation response ± SE, control chains, normoxic conditions.
■ The mean relaxation response ± SE, experimental chains, normoxic conditions.

The response, expressed as a percentage of inherent tone recorded just prior to the stimulus is shown on the y axis.
The frequency of stimulation (Hz) is shown on the x axis.

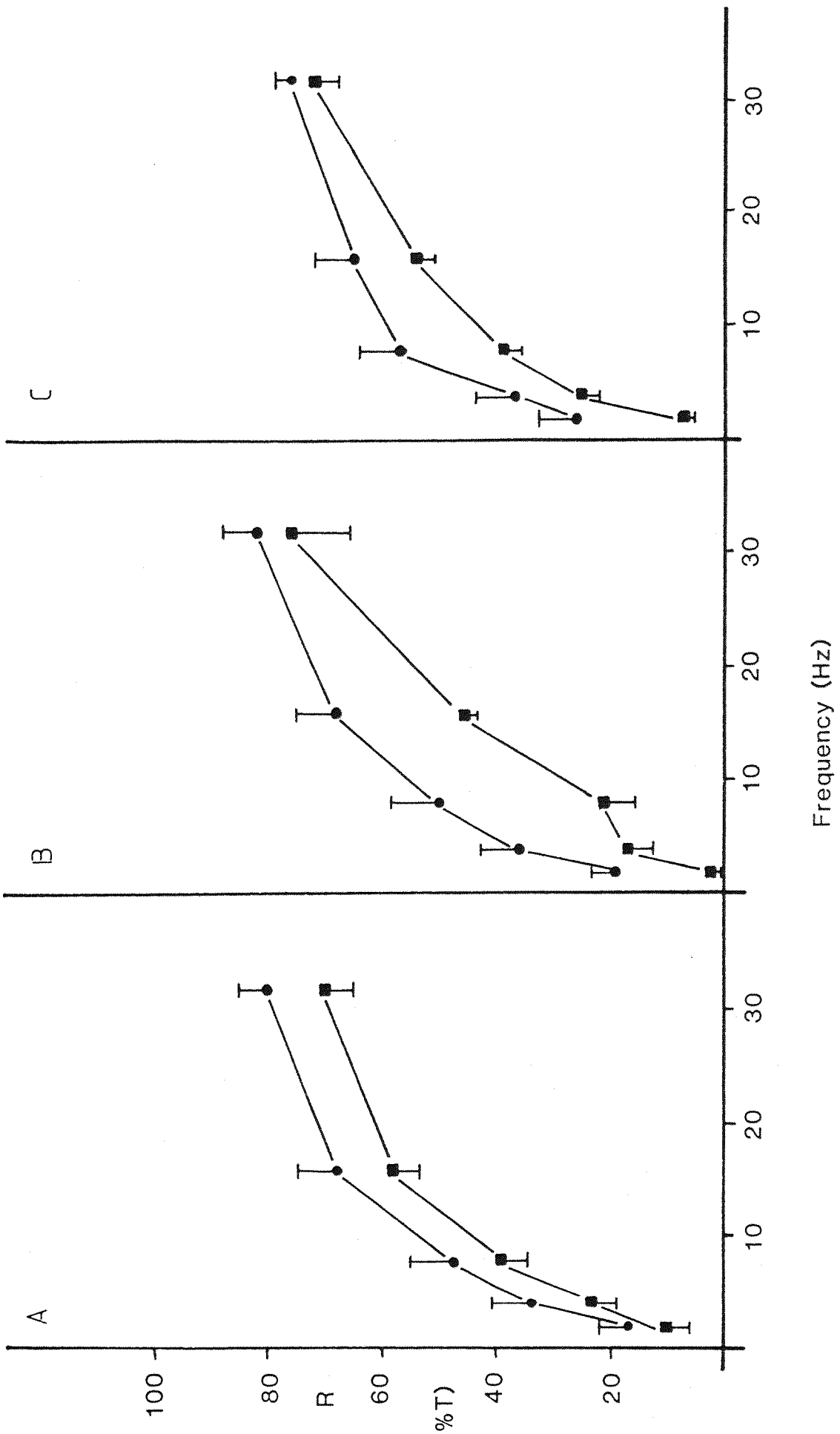

A
B
C
100
80
60
40
20
R
(%T)
10
20
30
Frequency (Hz)

FIGURE 3.20a The effect of hypoxia on the response to adrenaline.

Paired tracheal chains were prepared in the usual way and the response to adrenaline was recorded using Grass FT03C transducers. A typical result is shown in Fig. 3.20a and b. The results of five similar experiments are recorded in Table 3.19a,b (I).

● Response to adrenaline expressed as a percentage of tone recorded prior to the addition of adrenaline, Expt No. 6, period A, normoxic conditions.
■ Response to adrenaline, Expt No. 6, period B, hypoxic conditions.
○ Response to adrenaline, Expt No. 6, period C, normoxic conditions.

The response, expressed as a percentage of inherent tone prior to the first dose of adrenaline in each dose cycle is shown on the y axis.
The concentration of adrenaline (in µg/ml) is shown on the x axis.

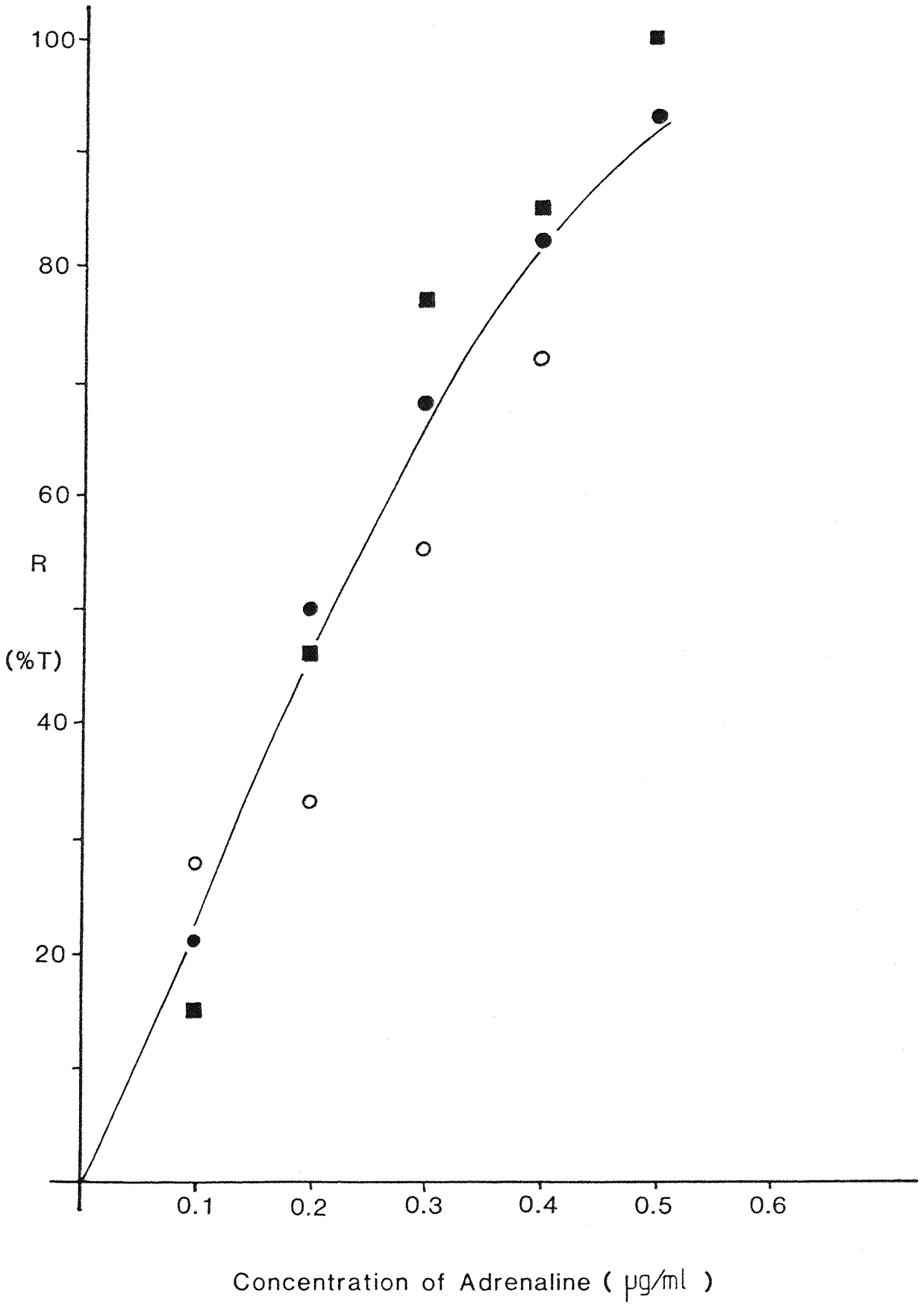

100
80
60
R
(%T)
40
20
0.1
0.2
0.3
0.4
0.5
0.6
Concentration of Adrenaline (μg/ml)

FIGURE 3.20b The effect of hypoxia on the response to adrenaline (normoxic controls).

Three consecutive dose response curves were recorded from Expt No. 6 under normoxic conditions (periods A-C).

● 1st dose response curve, period A.
■ 2nd dose response curve, period B.
○ 3rd dose response curve, period C.

The response, expressed as a percentage of inherent tone prior to the first dose of adrenaline in each dose cycle is shown on the y axis.
The concentration of adrenaline (in μg/ml) is shown on the x axis.

Data and conditions are given in Fig.3.20a.

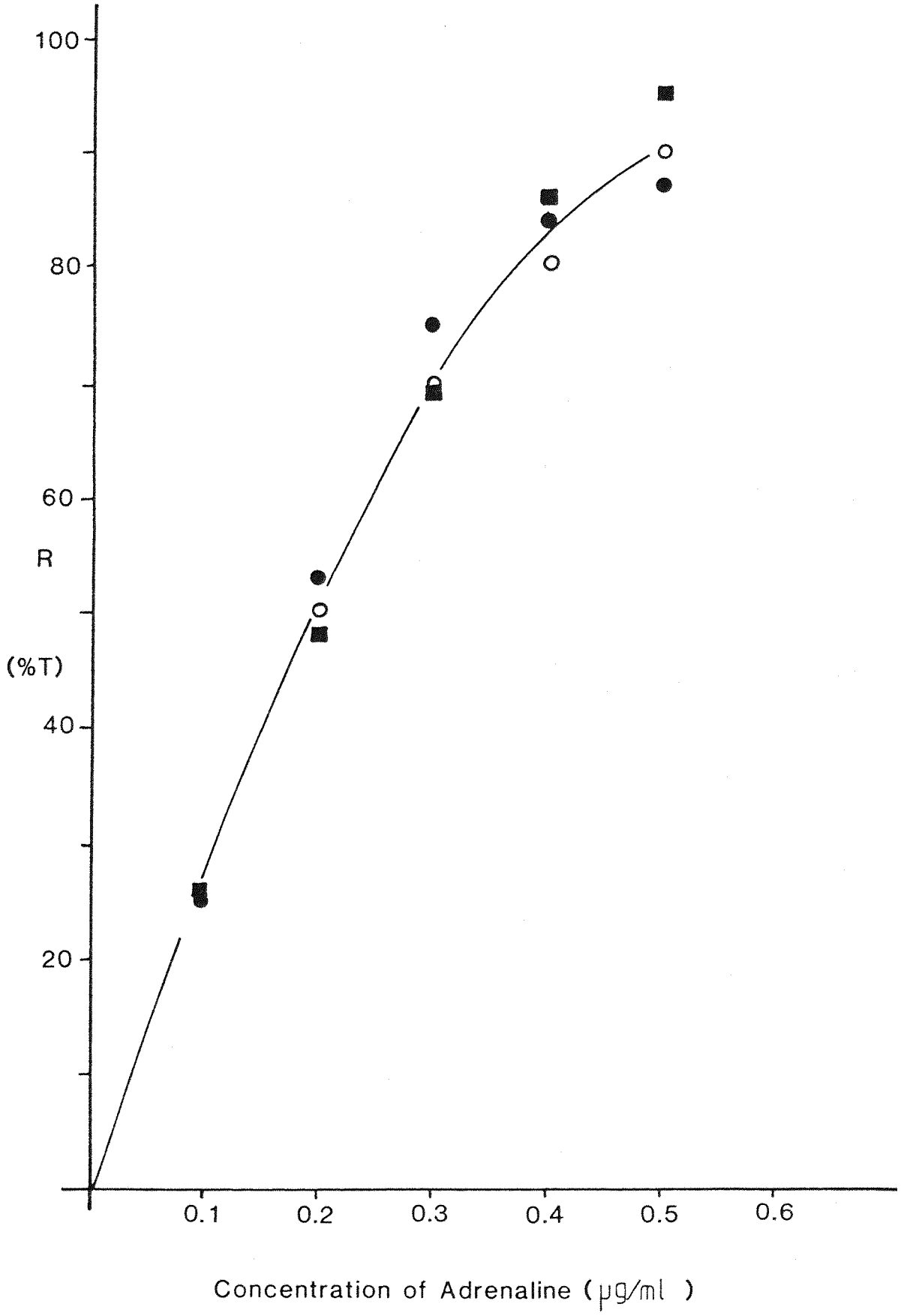

100
80
60
R
(%T)
40
20
0.1
0.2
0.3
0.4
0.5
0.6
Concentration of Adrenaline (μg/ml)

TABLE 3.20a Paired tracheal chains were prepared and set up in the usual way. The inherent tone (T) and the response to Ach (R) (6 μg/ml) were recorded using Grass FTO3C transducers. The response, expressed as a percentage of inherent tone (%T) is shown. In addition, the inherent tone and the response to Ach have been added together and are shown as T+R in the final column of the table. During periods A and C the tissues were maintained under normoxic conditions, however, during period B the conditions were made hypoxic by replacing 5% CO_2:95% O_2 with 5% CO_2:95% N_2.

EXPT. NO.	Tone (g)	Response (g)	%T	T+R (g)
A 1	2.1	1.2	56.2	3.3
2	2.2	1.3	60.6	3.4
3	1.9	0.8	44.8	2.7
4	1.9	0.9	48.2	2.8
5	1.2	0.5	44.4	1.7
6	1.2	0.5	44.4	1.7
Mean			49.7	2.6
SD			6.9	0.7
SE			2.8	0.3
B 1	0.7	2.6	371.4	3.3
2	0.6	2.8	466.6	3.4
3	0.8	2.5	316.0	3.3
4	0.8	2.3	291.0	3.3
5	0.5	0.8	150.0	1.3
6	0.3	0.6	200.0	0.9
Mean			299.2	2.6
SD			114.6	1.1
SE			46.79	0.5
C 1	1.2	2.1	172.0	3.3
2	1.1	2.5	217.6	3.6
3	1.4	1.1	80.9	2.5
4	1.4	1.6	114.3	3.0
5	0.7	0.6	90.9	1.3
6	0.6	0.8	130.0	1.4
Mean			134.3	2.5
SD			51.9	0.9
SE			21.2	0.4

TABLE 3.20a Effect of hypoxia on the response of tracheal chain to Ach.
B = Hypoxia.

EXPT. NO.	Tone (g)	Response (g)	%T	T+R (g)
1	2.3	1.0	44.0	3.3
2	2.5	1.3	50.0	3.8
3	2.3	0.8	34.0	3.1
4	2.2	0.9	39.3	3.1
5	1.0	0.5	53.3	1.5
6	1.1	0.5	43.7	1.6
Mean			44.0	2.7
SD			6.9	0.9
SE			2.8	0.4
1	2.5	1.4	56.7	3.9
2	2.1	1.8	87.5	3.9
3	1.9	1.1	55.2	3.0
4	1.6	1.3	83.3	2.9
5	1.0	0.5	53.3	1.5
6	0.8	0.6	83.3	1.4
Mean			69.8	2.7
SD			16.3	1.1
SE			6.7	0.5
1	1.8	2.1	110.7	3.9
2	1.5	2.0	136.4	3.5
3	1.5	1.5	100.0	3.0
4	1.5	1.4	91.3	2.9
5	0.7	0.7	100.0	1.4
6	0.7	0.7	100.0	1.4
Mean			106.4	2.7
SD			15.9	1.0
SE			6.5	0.4

TABLE 3.20b Effect of hypoxia on the response of tracheal chain to Ach: control chains.

TABLE 3.20b Paired control chains were set up as in Table 3.20b and subjected to three consecutive challenges with Ach (6 µg/ml). Inherent tone, the response to Ach and their sum are shown in Table 3.20b. As before R is expressed as a percentage of the inherent tone recorded prior to each dose of Ach.

EXPT. NO.	T_i (g)	$\%T_i$	R (g)	$\%T_i$	T (g)	$\%T_i$	R (g)	$\%T_i$	T (g)	$\%T_i$	R (g)	$\%T_i$
1	2.3	100.0	1.0	43.4	2.5	10.0	1.4	60.8	1.8	78.3	2.1	91.3
2	2.5	100.0	1.3	52.0	2.1	80.0	1.8	72.0	1.5	60.0	2.0	80.0
A 3	2.3	100.0	0.8	34.1	1.9	82.6	1.1	47.8	1.5	65.2	1.5	65.2
4	2.2	100.0	0.9	40.9	1.6	72.7	1.3	59.1	1.5	68.2	1.4	63.6
5	1.0	100.0	0.5	50.0	1.0	100.0	0.5	50.0	0.7	70.0	0.7	70.0
6	1.1	100.0	0.5	45.4	0.8	72.7	0.6	54.5	0.7	63.6	0.7	63.6
Mean				44.3				57.4				72.2
SD				6.5				8.7				11.2
SE				2.6				3.6				4.6
1	2.1	100.0	1.2	56.8	0.7	33.3	2.6	123.8	1.2	57.1	2.1	100.0
2	2.2	100.0	1.3	59.1	0.6	27.3	2.8	127.3	1.1	50.0	2.5	113.6
3	1.9	100.0	0.8	42.1	0.8	42.1	2.5	131.5	1.4	73.7	1.1	57.9
4	1.9	100.0	0.9	47.4	0.8	42.1	2.3	121.0	1.4	73.7	1.6	84.2
5	1.2	100.0	0.5	41.6	0.5	41.6	0.5	66.6	0.7	58.3	0.6	50.0
6	1.2	100.0	0.5	41.6	0.3	25.0	0.6	50.0	0.6	50.0	0.8	66.6
Mean				48.1				103.4				78.7
SD				7.9				35.5				24.9
SE				3.2				14.4				10.1

TABLE 3.21

The data from Tables 3.20a and 3.20b are presented in a different form in Table 3.21.

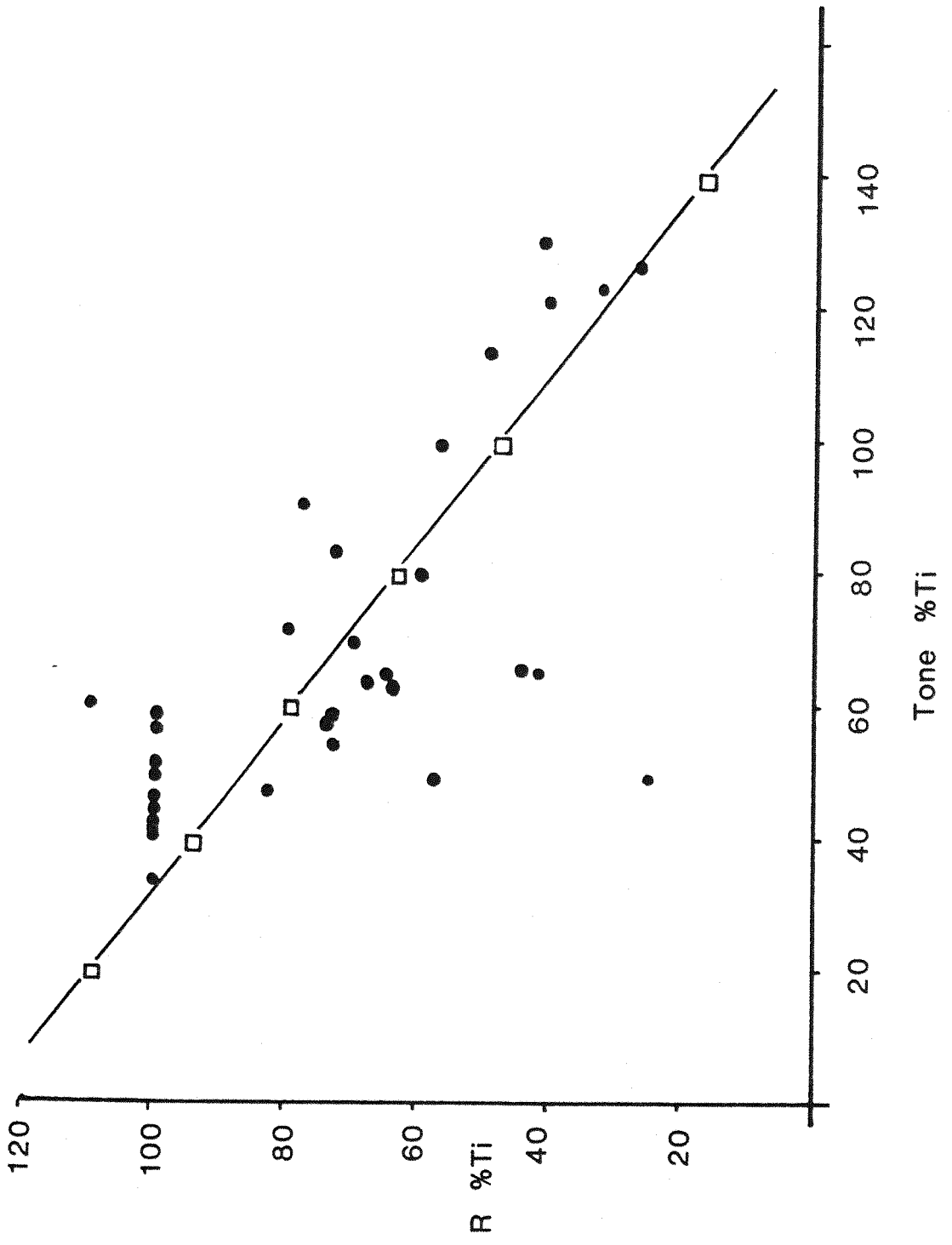
Both inherent tone (T) and the response to Ach (R) are expressed as a percentage of the initial tone T_i. This strategy summarises the data in a form which enables all the experimental results to be treated in a simple statistical manner.

FIGURE 3.21 The influence of inherent tone on the response to Ach.

The inherent tone (T) and the response (R) to a standard dose of Ach (6 μg/ml) were expressed as a percentage of the initial tone in Table 3.23 and these data are plotted in Figure 3.22.

● The data points, both normoxic and hypoxic.
□ Points calculated from linear regression analysis of the data to show the "best fit" line through the data. The value of r is -0.7 and is highly significant ($p < 0.0005$).
$R(\%T_i)$ is shown on the y axis.
Tone $(\%T_i)$ is shown on the x axis.

Conditions given in Tables 3.20a and 3.20b.


120
100
80
60
40
20
R %Ti
20
40
60
80
100
120
140
Tone %Ti

D. Hyperbaric Results

In the following sections the majority of the data is presented in the form of Tables and may be found with the bulk of the text. Figures and Tables followed by the Roman numeral II may be found in Appendix II (pages 236-250).

(i) Comparison of He and air as compression gases; comparison of the effects of bubbling with 5% CO_2:95% O_2, 100% O_2 or nil bubbling gas

The results of sections a-d are summarised in Figs. 3.22, 3.23.

(a) Compression of tracheal chains to 14.8 bar.

He in combination with abnormally high (but unknown) P_{O_2} and P_{CO_2} resulted in complete loss of tone by 45 minutes. When air was the compression gas, complete loss of tone occurred in 55-60 minutes. After decompression 60.0 ± 7.8% of the precompression tone was recovered when He was the compression gas. However, only 38 ± 4.0% of the precompression tone was recovered following air compression.

(b) Substitution of pure O_2 for 5% CO_2:95% O_2 slowed, but did not prevent the loss of tone recorded. When the chains were exposed to the same total pressure as in (a) above with He as the compression gas, tone was completely abolished after 160 minutes. Only 46.0 ± 6.2% of the precompression tone was recovered following decompression.

A similar result was obtained when air was used as a compression gas; inherent tone was completely absent after 120 minutes, but 55.0 ± 5.7% of the precompression tone was recovered after decompression.

(c,d) Both He and air were used to compress tracheal chains which were not bubbled with any gas. At both 14.8 bar and 31 bar He, the loss of tone was similar to that observed at atmospheric pressure (Figs. 3.22, 3.24). However, tone was sustained when air was used as the compression gas. Following decompression (and in the continued absence of bubbling gas), inherent tone began to fall.

(e) He gas at atmospheric pressure had no significant effect on the inherent tone of tracheal smooth muscle. Furthermore, He had no effect on the loss of tone recorded when the tissue was made hypoxic and hypocapnic by the removal of 5% CO_2:95% O_2.

A typical result is shown in Fig. 3.25.

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FIGURE 3.22 The effect of compression to 14.8 bar (helium) on inherent tone of guinea pig trachealis muscle in the presence of

- ● Nil bubbling gas.
- ■ O_2 as bubbling gas.
- ▲ 95% O_2:5% CO_2 as bubbling gas.

Each point is the mean of 3 experiments.

C = The time at which compression was completed.
D = The time at which decompression was completed.

The inherent tone was expressed as a percentage of the maximum tone recorded during the entire experiment as is shown on the y axis. The tone was recorded continuously but is shown only at 5 minute intervals which can be seen on the x axis.

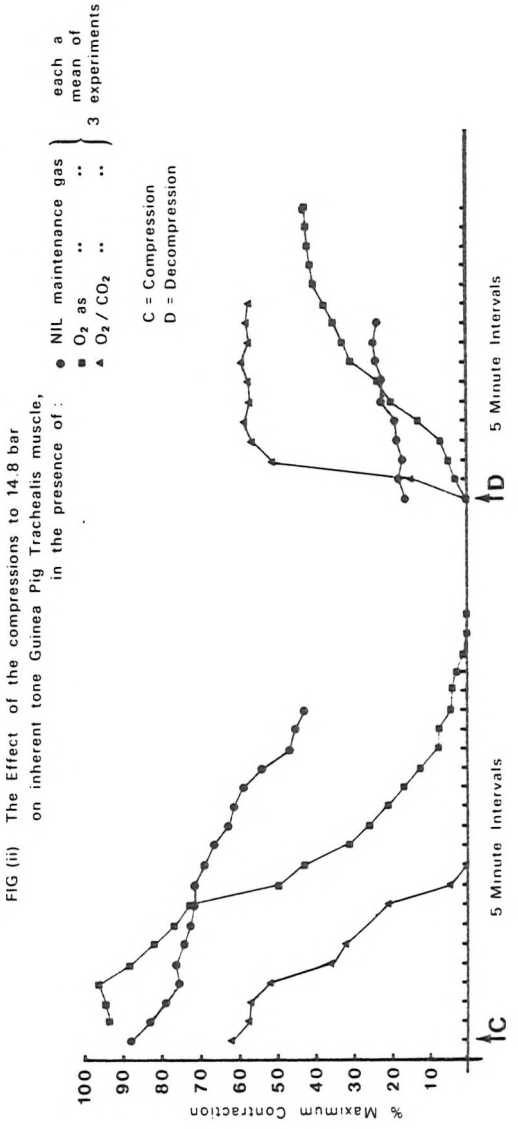


FIG (ii) The Effect of the compressions to 14.8 bar
on inherent tone Guinea Pig Trachealis muscle,
in the presence of :
● NIL maintenance gas
■ O_2 as ..
▲ O_2 / CO_2 ..
each a mean of 3 experiments
C = Compression
D = Decompression
% Maximum Contraction
100
90
80
70
60
50
40
30
20
10
C
D
5 Minute Intervals
5 Minute Intervals

FIGURE 3.23 The effect of compression to 14.8 bar (air) on inherent tone of guinea pig trachealis muscle in the presence of

- ● Nil bubbling gas.
- ■ O_2 as bubbling gas.
- ▲ 95% O_2:5% CO_2 as bubbling gas.

Each point is the mean of three experiments.

C = The time at which compression was completed.
D = The time at which decompression was completed.

Inherent tone, expressed as a percentage of the maximum recorded during the experiment is shown on the y axis and time in 5 minute intervals is shown on the x axis.

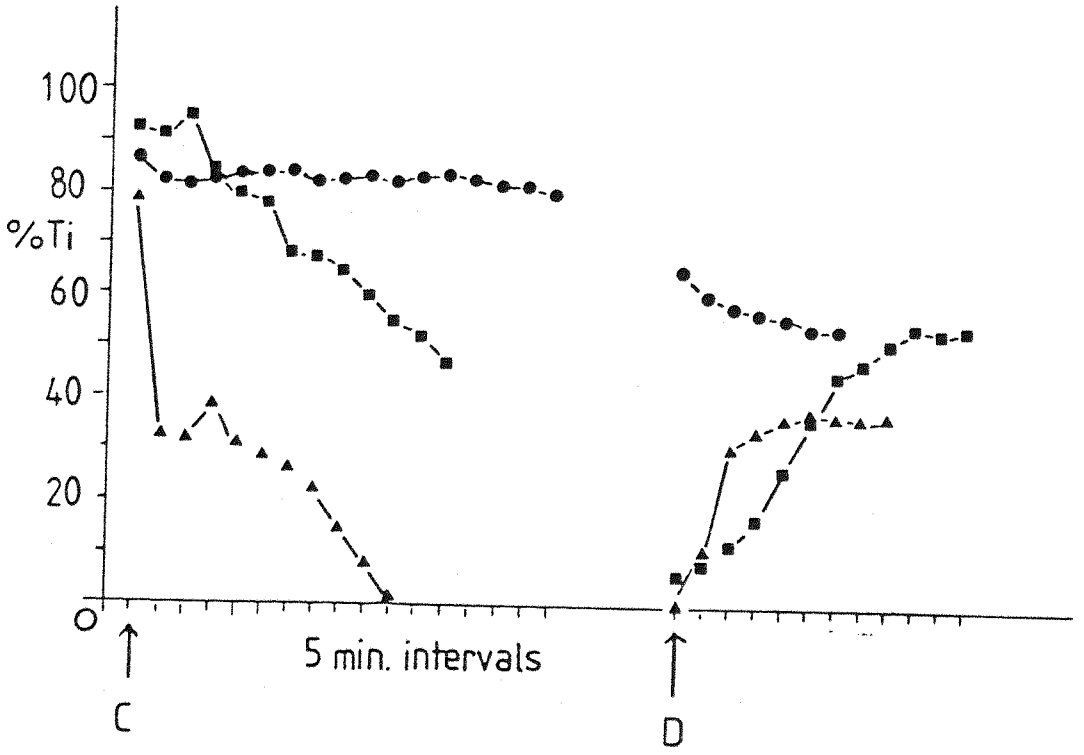


FIGURE 3.24 Four pairs of tracheal chains were prepared in the usual way. One chain of each pair was maintained in 1 bar air throughout. ●

The second chain of each pair was mounted in MacI (bath volume 10 mls).

- O A The chain was maintained in 1 bar air, with 5% CO_2:95% O_2 as bubbling gas.
- O B The bubbling gas was withdrawn.
- O C The air was replaced by the gas.
- O D The preparation was compressed to 31 bar He (nil bubbling gas).
- O E The preparation was decompressed to 1 bar He (nil bubbling gas).

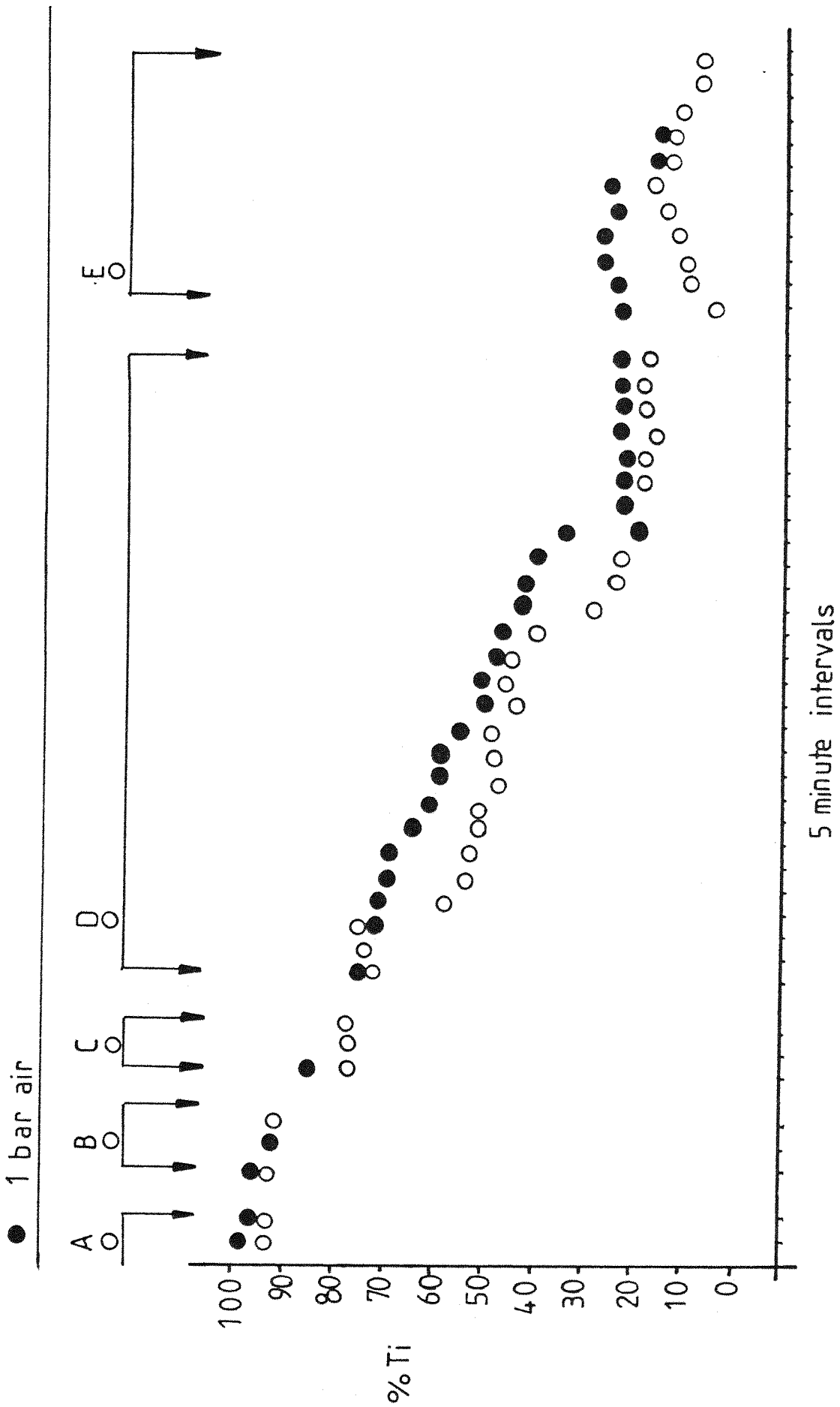

1 bar air
A
B
C
D
E
100
90
80
70
60
50
40
30
20
10
0
% Ti
5 minute intervals

FIGURE 3.25 The effect of He on the inherent tone of guinea pig tracheal smooth muscle.

Paired tracheal chains were placed in 30 ml organ baths bathed in Krebs' solution and bubbled with 95% O_2:5% CO_2. Inherent tone was recorded on Harvard smooth muscle transducers. When inherent tone had been established, chain 1 (O) was enclosed in the hyperbaric chamber which was flushed continuously with He gas at 17 psi (101% of atmospheric pressure). Inherent tone was recorded for a further 3 hours. At y the bubbling gas was withdrawn from both preparations and inherent tone recorded for a further 140 minutes. Zero tone was determined by the addition of aminophylline at the beginning and end of the experiment (A).

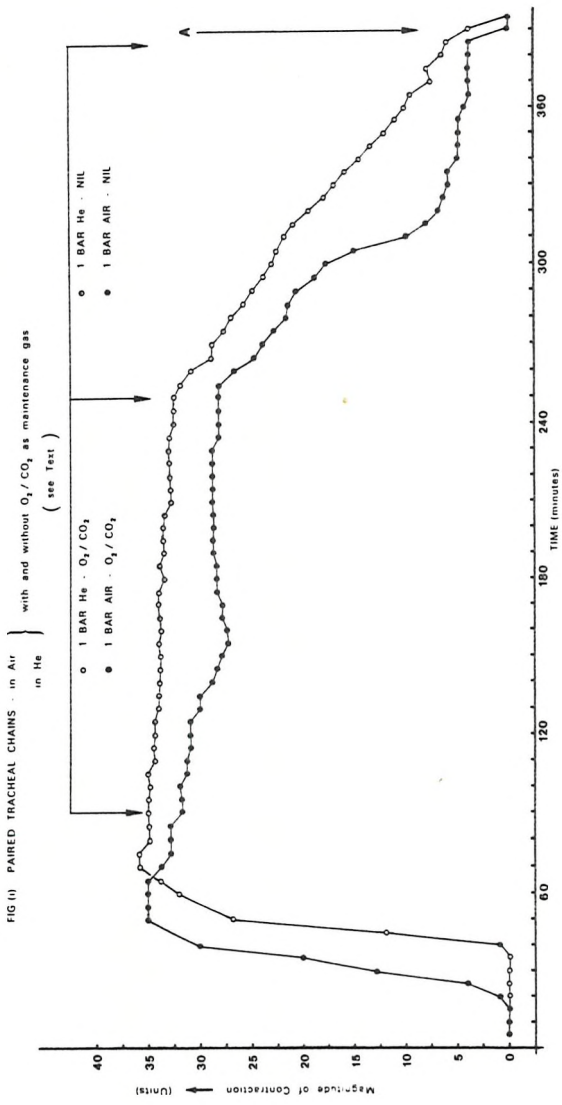


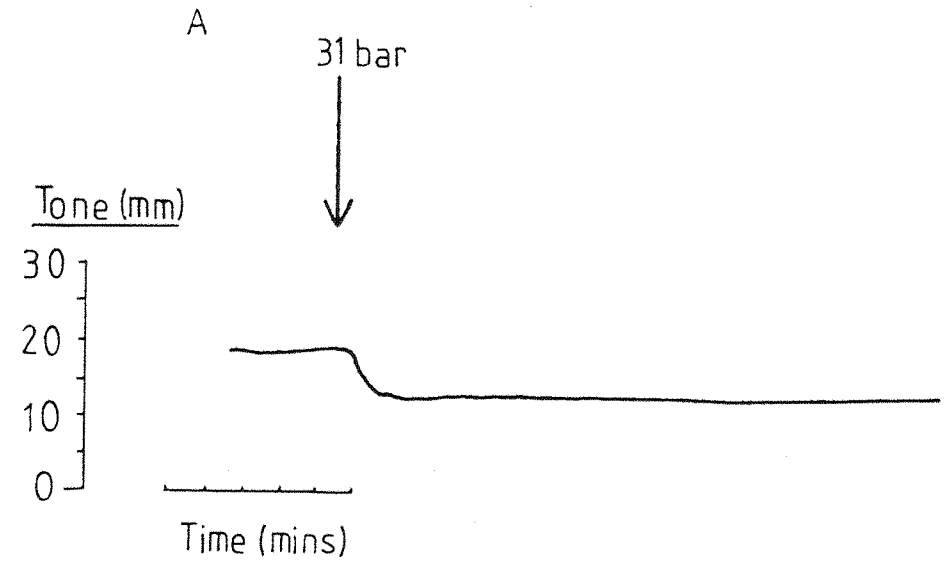
FIG (i) PAIRED TRACHEAL CHAINS - in Air } with and without O_2 / CO_2 as maintenance gas
in He } (see Text)
o 1 BAR He - O_2 / CO_2
• 1 BAR AIR - O_2 / CO_2
o 1 BAR He - NIL
• 1 BAR AIR - NIL
A
Magnitude of Contraction ← (Units)
0
5
10
15
20
25
30
35
40
60
120
180
240
300
360
TIME (minutes)

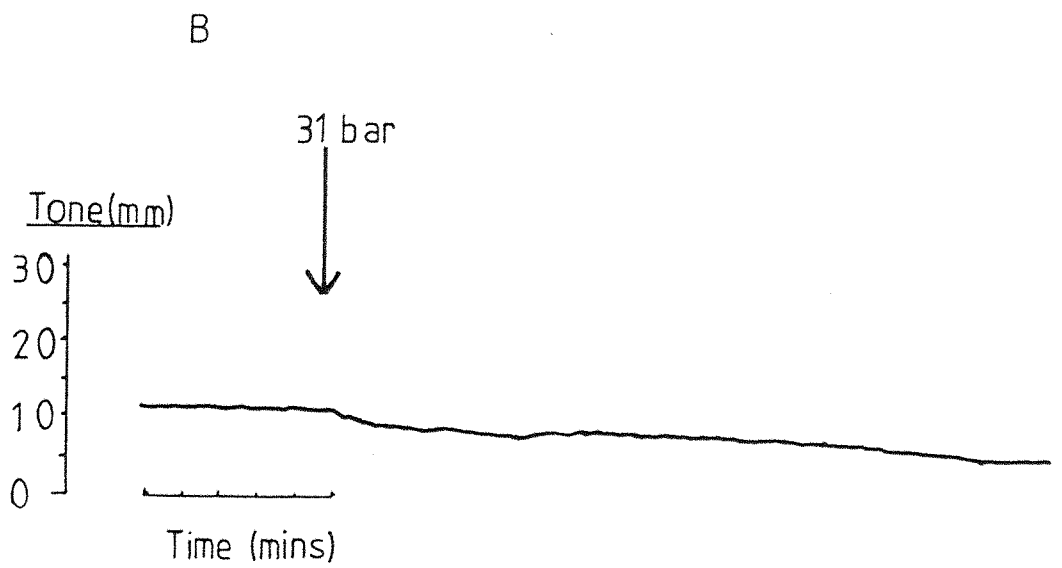
FIGURE 3.26 Typical responses to compression to 31 bar.

A. The most frequently observed response was an abrupt decrease in inherent tone, sustained until decompression was initiated.

B. Slower relaxations were also observed, relaxation was complete within 5 minutes.

The way in which inherent tone fell, either slowly or abruptly, was not determined by the final pressure or by the type of compression.


A
31 bar
Tone (mm)
30
20
10
0
Time (mins)


B
31 bar
Tone(mm)
30
20
10
0
Time (mins)

(ii) Effect of pressure on inherent tone

When tracheal chains were compressed to 13.1, 31, 43 or 67 bar, compression was usually followed by an abrupt relaxation of the tissue which was complete in 10-20 seconds. The new level of tone was maintained until decompression. However, on a few occasions, a slower relaxation was observed, which took as long as 5 minutes to complete. Examples of both types of response are seen in Fig. 3.26. The way in which tone fell, either slowly or abruptly, was not influenced by either the final pressure or by the type of compression. On no no occasion was compression followed by an increase in tone.

The effects of pressure on 47 different tracheal chains is shown in Table 3.22 and in Fig. 3.27. The data has been collated from experiments in sections (iii)-(xii). All compressions were carried out in the absence of drugs, and at normal $[Ca^{+}]_{o}$ (2.5 mM). The compression gas is indicated in the Table. There were several important features of the response:

a. The mean response was greatest at 43 bar.

b. The method of compression, whether He alone or $He:O_2:CO_2$ mixture had no significant effect on the size of the response.

c. The size of the response was not related to the precompression tone. This is reflected in the variability of the response when the latter was expressed as a percentage of tone, and in Fig. 3.28 where response has been plotted against precompression tone.

TABLE 3.22 The effect of pressure on inherent tone of guinea pig trachealis muscle.

Forty-seven individual tracheal chains were compresssed to 13.1-67 bar using $He:O_2:CO_2$ mixtures (a) or He alone (b). No drugs were present. The inherent tone prior to compression (in mm) and the decrease is recorded 10 minutes after compression. This decrease is also expressed as a percentage of the pre-decompression tone.

Harvard smooth muscle transducers were used to record the results in all hyperbaric experiments.

Pressure (bar)	Tone before compression (mm)	Decrease (mm)	% Decrease
31.0	12.0	4.0	33.0
	31.0	3.0	9.0
	40.0	2.0	5.0
	15.0	0.0	0.0
Mean			11.7
SD			14.6
SE			7.3
43.0	14.0	5.0	35.0
	23.0	5.0	21.7
	16.0	5.0	31.2
	16.0	8.0	50.0
	25.0	6.0	24.0
Mean			32.5
SD			11.2
SE			5.0
43.0	22.5	5.5	24.4
	28.5	1.5	5.2
	18.0	5.0	27.7
	21.0	3.0	14.2
	17.0	1.0	5.8
Mean			15.5
SD			10.3
SE			4.6
67.0	11.5	4.5	39.1
	18.0	4.0	22.2
	17.0	0.0	0.0
	25.0	3.0	12.0
Mean			24.4
SD			13.6
SE			6.8

TABLE 3.22a

Pressure (bar)	Tone before compression (mm)	Decrease (mm)	% Decrease
	23.0	4.0	17.0
	19.0	5.0	26.0
13.1	25.0	1.0	4.0
	16.0	2.5	18.0
Mean			15.8
SD			9.0
SE			4.5
	29.0	5.0	17.2
	26.0	3.0	11.5
31.0	15.0	7.0	46.0
	7.0	2.0	28.5
Mean			26.0
SD			15.0
SE			7.5
	24.0	1.0	4.2
	28.0	3.0	10.7
43.0	28.0	6.0	21.4
	21.0	6.0	28.5
	24.5	3.0	12.2
Mean			16.2
SD			10.8
SE			4.8
	21.0	3.0	14.2
	12.0	4.0	33.3
	26.0	2.0	7.6
43.0	24.0	7.0	29.1
	12.0	3.0	25.0
	15.0	7.0	46.0
	19.0	5.0	26.0
Mean			26.0
SD			12.6
SE			4.7
	10.0	7.0	70.0
	23.0	8.0	34.7
43.0	30.0	3.5	11.6
	12.0	2.0	16.6
	19.0	2.0	10.5
Mean			28.7
SD			25.0
SE			11.2
	15.0	2.0	13.3
	28.0	4.0	14.3
43.0	9.0	3.0	33.3
	14.0	4.0	28.5
Mean			22.3
SD			9.9
SE			4.9

TABLE 3.22b

FIGURE 3.27 The effect of pressure on inherent tone of trachealis muscle.

The results shown in Table 3.24 are plotted in Figure 3.27.

The decrease in inherent tone which followed compression is expressed as a percentage of precompression tone and is shown on the y axis. Pressure (in bar) is shown on the x axis.

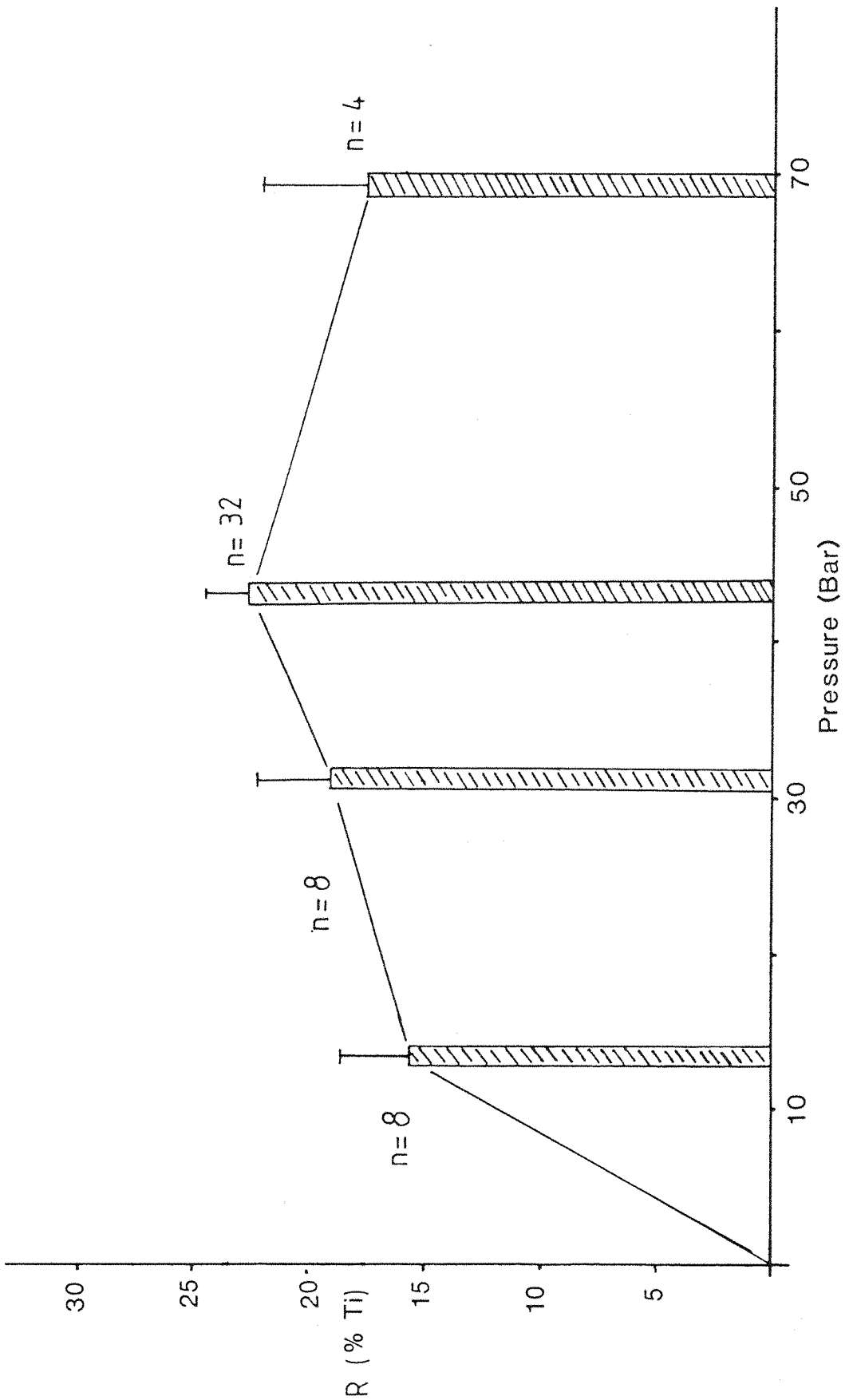
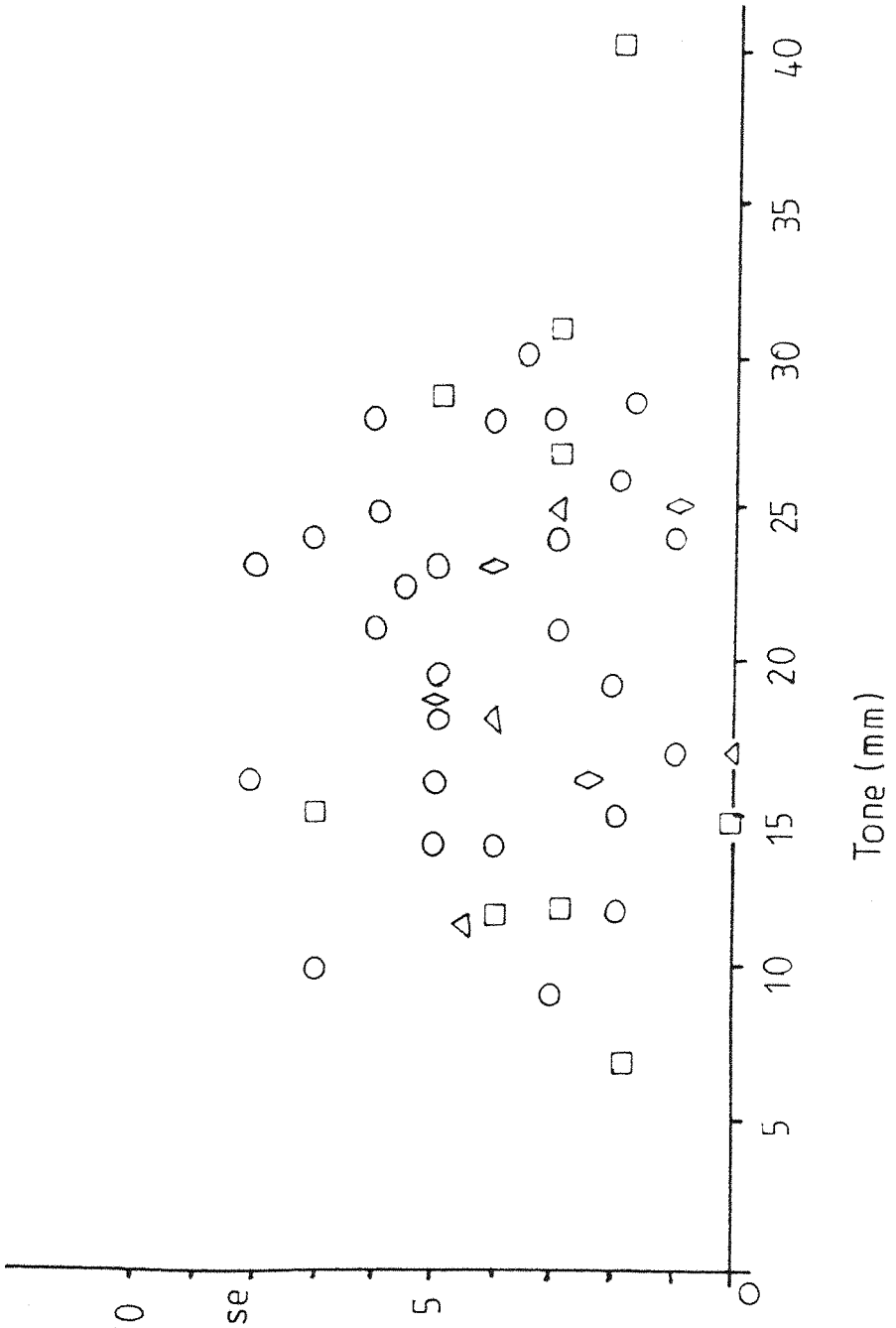

n=4
n=32
n=8
n=8
30
25
20
15
10
5
R (% Ti)
10
30
50
70
Pressure (Bar)

FIGURE 3.28 The effect of pressure on inherent tone of tracheal smooth muscle.

Precompression tone (in mm) is shown on the x axis and the decrease in tone due to compression is shown on the y axis. The results and conditions are given in Table 3.22. There is no obvious relationship between precompression tone and the response of tracheal smooth muscle to compression.

◊ = 13.1 bar.
□ = 31 bar.
○ = 43 bar.
△ = 67 bar.


Response
(mm)
10
5
0
5
10
15
20
25
30
35
40
Tone (mm)

Recovery

Following decompression a slow recovery of tone began. Recovery was considered to be complete when a stable level of tone had been reached and maintained for at least 15 minutes. The maximum time from decompression to "recovery" was never greater than 20 minutes. The recovery from compression was often incomplete. However, occasionally the tone recorded after decompression was greater than that prior to compression.

The tone after recovery was compared with that of the matched control which had been maintained at 1 bar throughout.

There was no significant difference in tone between experimental and control groups after the same time had elapsed (Tables 3.23-3.26 inclusive).

Tone before Compression (mm)	Tone after Decompression (mm)	$\%T_i$
Chain: Control		
20.0	13.0	65.0
19.0	19.0	100.0
23.0	22.0	95.0
17.0	16.0	94.1
Mean		88.5
SD		15.9
SE		7.9
Chain: Experimental		
12.0	13.0	108.0
31.0	27.0	87.1
40.0	31.0	77.5
15.0	18.0	120.0
Mean		98.2
SD		19.2
SE		9.7

TABLE 3.23 Recovery from 31 Bar ($He:O_2:CO_2$ mixture).

Paired tracheal chains were prepared in the usual way and allowed to take up tone. One chain of each pair (the experimental chain) was compressed to 31 bar using a $He:O_2:CO_2$ mixture. The inherent tone before compression and after decompression are recorded in this table. The tone after decompression was expressed as a percentage of the precompression tone and is shown in the final column ($\%T_i$).

The second chain of each pair was maintained at atmospheric pressure for the same period of time and the inherent tone noted as above.

There was no significant difference in inherent tone between the experimental and control groups after the same time had elapsed.

Tone before Compression (mm)	Tone after Decompression (mm)	$\%T_i$
Chain: Control		
36.0	35.0	97.2
21.0	21.0	100.0
23.0	20.0	86.9
18.0	18.0	100.0
22.0	23.0	104.5
Mean		97.7
SD		6.5
SE		2.9
Chain: Experimental		
26.0	25.0	96.1
28.0	31.0	110.7
28.0	24.0	85.0
21.0	19.0	90.5
24.5	23.0	93.8
Mean		95.4
SD		9.4
SE		4.2

TABLE 3.24 Recovery from 43 Bar (He alone).

Paired tracheal chains were prepared in the usual way and allowed to take up tone. One chain of each pair was compressed to 43 bar using He alone. Precompression tone and post decompression tone are recorded in this table. Also shown is the post decompression tone, expressed as a percentage of the precompression tone ($\%T_i$).

The inherent tone recorded in the control chain at the same times is also shown.

There was no significant difference in tone between experimental and control groups after the same time had elapsed.

Tone before Compression (mm)	Tone after Decompression (mm)	$\%T_i$
Chain: Control		
15.0	13.0	86.6
24.0	19.0	79.2
11.0	11.0	100.0
17.0	15.0	88.2
16.0	16.0	100.0
30.0	28.0	93.3
17.0	16.0	94.1
6.0	5.0	83.3
15.0	15.0	100.0
Mean		91.6
SD		7.7
SE		2.5
Chain: Experimental		
14.0	17.0	121.4
23.0	17.0	73.9
16.0	12.0	75.0
25.0	20.0	80.0
22.5	20.0	88.8
28.5	28.0	98.2
18.0	19.0	105.0
21.0	18.0	85.7
17.0	19.0	111.0
Mean		93.2
SD		16.6
SE		5.6

TABLE 3.25 Recovery from 43 Bar ($He:O_2:CO_2$ mixture).

Paired tracheal chains were prepared in the usual way and allowed to take up tone. One chain of each pair was compressed to 43 bar using $He:O_2:CO_2$ mixture. Pre-compression tone and post decompression tone are recorded in this table. Also shown is the post decompression tone, expressed as a percentage of the precompression tone ($\%T_i$).

The inherent tone recorded in the control chain at the same times is also shown.

There was no significant difference in tone between experimental and control groups after the same time had elapsed.

Tone before Compression (mm)	Tone after Decompression (mm)	$\%T_i$
Chain: Control		
13.0	10.0	76.9
23.0	19.0	82.6
15.0	15.0	100.0
18.0	16.0	88.8
Mean		87.1
SD		9.8
SE		4.9
Chain: Experimental		
11.5	9.0	78.2
18.0	20.0	111.1
17.0	17.0	100.0
25.0	22.0	88.0
Mean		94.3
SD		14.3
SE		7.1

TABLE 3.26 Recovery from 67 Bar He:O_2:CO_2 mixture.

Paired tracheal chains were prepared in the usual way and allowed to take up tone. One chain of each pair was compressed to 67 bar using He:O_2:CO_2 mixture. Pre-compression tone and post decompression tone are recorded in this table. Also shown is the post decompression tone, expressed as a percentage of the pre-compression tone ($\%T_i$).

The inherent tone recorded in the control chain at the same times is also shown.

There was no significant difference in tone between experimental and control groups after the same time had elapsed.

(iii) Effects of repeat compression

The response to an initial compression to 43 bar was a relaxation of 26.0 ± 4.7% (SE). A second (identical) compression resulted in relaxation of 28.8 ± 5.3% (SE). There is no significant difference between these two results (see Table 3.27).

(iv) The effect of Propranolol

The effect of propranolol on the response to 31 bar is shown in Table 3.28. No significant effect was observed. Propranolol had no significant effect on the recovery of the inherent tone following decompression (Table 3.29).

(v) The effect of Atropine

At 31 bar and in the presence of atropine the mean relaxation response was 26.2 ± 8.6% (Table 3.30). This was not significantly different from the response recorded for untreated preparations which was 18.7 ± 5.6% (n = 8), (Table 3.22).

At 43 bar in the presence of atropine, the mean response was a relaxation of 20.2 ± 2.5% (Table 3.31). This was not significantly different from the response of 31 untreated preparations at the same pressure, which was 23.5 ± 2.6% (Table 3.22).

From the data presented in Tables 3.32 and 3.33 it can be seen that the tone recovered after decompression was not significantly different from that observed in the 1 bar control preparations at the same time interval.

(vi) Effects of Tetrodotoxin on the response to 43 bar

The relaxation recorded in response to 43 bar was 15.5 ± 4.6%. When the same preparations were recompressed in the presence of TTX the mean response was 13.6 ± 1.5% (Table 3.34).

The presence of TTX had no significant effect on the tone and was recovered after decompression (Table 3.35).

Tone before Compression$_1$ (mm)	Decrease (mm)	% Decrease	Tone before Compression$_2$ (mm)	Decrease (mm)	% Decrease
15.0	7.0	46.6	10.0	5.0	50.0
19.0	5.0	26.3	11.0	4.0	36.0
21.0	3.0	14.3	25.0	6.0	24.0
12.0	4.0	33.3	12.0	5.0	41.6
26.0	2.0	7.7	24.0	3.0	12.5
24.0	7.0	29.1	24.0	5.0	20.8
12.0	3.0	25.0	12.0	2.0	16.6
Mean		26.0			28.8
SD		12.6			13.9
SE		4.7			5.3

TABLE 3.27 Effect of repeat compression.

Single tracheal chains were prepared and allowed to take up tone in the usual way. Each chain was compressed to 43 bar using He alone. The inherent tone recorded prior to compression (compression$_1$) is shown. The response to compression (in mm) is given and the response expressed as a percentage of precompression tone. The preparations were decompressed, and when inherent tone had recovered, the compression was repeated. Tone prior to the second compression (compression$_2$) is recorded, as is the response to the compression, and the response expressed as a percentage of precompression tone.

There was no significant difference between the response to compression$_1$ and the response to compression$_2$.

TABLE 3.28 The effect of propranolol on the response to 31 bar.

Four single tracheal chains were prepared in the usual way and allowed to take up inherent tone. Each chain was compressed to 31 bar using He alone. The precompression tone, the response in mm, and percentage decrease in tone are shown in the table. Following decompression inherent tone was allowed to recover and each chain was treated with propranolol (10^{-6} g/ml). A second identical compression was then initiated. Precompression tone, the response (in mm) and the percentage decrease in tone are recorded.

The presence of propranolol in a concentration sufficient to block the maximal response to noradrenaline, had no significant effect on the response to compression to 31 bar.

TABLE 3.29 The effect of propranolol on the recovery from 31 bar.

In the experiments described in Table 3.30 the tone after decompression was also recorded and expressed as a percentage of inherent tone prior to $compression_1$ and $compression_2$ (%T).

The presence of propranolol had no significant effect on the recovery from compression to 31 bar.

Tone before Compression$_1$ (mm)	Decrease (mm)	% Decrease	Tone before Compression$_2$ (mm)	Decrease (mm)	% Decrease
29.0	5.0	17.2	29.0	7.5	24.0
26.0	3.0	11.5	26.0	3.0	11.5
15.0	7.0	46.0	14.0	6.0	42.8
7.0	2.0	28.0	5.0	3.0	60.0
Mean		25.6			34.6
SD		15.1			21.2
SE		8.9			10.6

TABLE 3.28 Effect of Propranolol on the response to 31 Bar.

Tone before Compression$_1$ (mm)	Tone after Decompression$_1$ (mm)	%T_i	Tone before Compression$_2$ (mm)*	Tone before Decompression$_2$ (mm)*	%T_i
29.0	30.0	104.0	29.0	21.0	72.0
26.0	26.0	100.0	26.0	26.0	100.0
15.0	14.0	107.0	14.0	14.0	100.0
7.0	5.0	71.4	5.0	3.0	60.0
Mean		95.6			83.0
SD		16.3			20.2
SE		9.6			10.1

TABLE 3.29 Effect of Propranolol on recovery from 31 Bar. *10^{-6}g/ml Propranolol

TABLE 3.30 Paired tracheal chains were prepared in the usual way and allowed to take up tone. Atropine (10^{-6} g/ml) was added to the bath fluid of both chains prior to the compression. The precompression tone, response to compression and the percentage decrease are shown. Compression to 31 bar was achieved using a $He:O_2:CO_2$ mixture.

The mean response was not significantly different from that recorded in similar untreated preparations (Table 3.32). The control chain was maintained at 1 bar throughout.

TABLE 3.31 Paired tracheal chains were prepared in the usual way. After inherent tone had been established atropine (10^{-6} g/ml) was added to the bathing fluid and one preparation of each pair was compressed to 43 bar using the appropriate $He:O_2:CO_2$ mixture. The precompression tone, the response (in mm) and the percentage decrease are recorded in the table.

The mean response was not significantly different from that recorded in similar untreated preparations (Table 3.33).

The control chain was maintained at 1 bar throughout.

Tone before Compression (mm)	Decrease (mm)	% Decrease
14.0	2.0	14.3
15.0	5.0	33.3
25.5	0.0	0.0
18.0	6.0	33.3
22.0	11.0	50.0
Mean		26.2
SD		19.3
SE		8.6

TABLE 3.30 Effect of Atropine pretreatment on the response to 31 Bar.

Tone before Compression (mm)	Decrease (mm)	% Decrease
9.0	2.0	22.0
11.0	3.0	27.3
32.0	5.0	15.6
19.0	3.0	15.7
Mean		20.2
SD		5.6
SE		2.5

TABLE 3.31 Effect of Atropine pretreatment on the response to 43 Bar.

TABLE 3.32 The effect of atropine on the recovery from 31 bar.

During the experiments described in Table 3.32 the inherent tone achieved after decompression was expressed as a percentage of the precompression tone and is shown as $\%T_i$ in this table. Although the control chains were not compressed, the inherent tone was recorded at the same time as that in the experimental chain. In the table, E denotes the experimental chain, compressed to 31 bar using the $He:O_2:CO_2$ mixture. C denotes the control chain which was maintained at 1 bar throughout.

There was no significant difference between the $\%T_i$ recorded from control and experimental chains after the same time had elapsed. Furthermore, there was no significant difference between $\%T_i$ recovered here and in similar untreated preparations (Table 3.25).

TABLE 3.33 The effect of atropine on the recovery from 43 bar.

During the experiments described in Table 3.33, inherent tone recorded post decompression and expressed as a percentage of precompression tone was recorded and is shown in this table. Inherent tone was recorded simultaneously in the control chains and is also shown here. E denotes the experimental chain which was compressed to 43 bar, using the $He:O_2:CO_2$ mixture. C denotes the control chain which was maintained at 1 bar throughout.

There was no significant difference in $\%T_i$ recorded from control and experimental chains after the same time had elapsed. Furthermore, the $\%T_i$ was not significantly different from the tone recovered in similar untreated chains (Tables 3.27, 3.26).

Chain	Tone before Compression (mm)	Tone after Decompression (mm)	$\%T_i$	Chain	Tone before Compression (mm)	Tone after Decompression (mm)	$\%T_i$
E	14.0	11.0	78.5	C	15.0	10.0	66.0
E	15.0	8.0	53.3	C	9.0	5.0	55.0
E	25.5	23.0	90.5	C	21.0	22.0	104.0
E	18.0	17.0	94.4	C	25.0	20.0	80.0
E	22.0	16.0	72.7	C	10.0	8.0	80.0
Mean			77.8				77.0
SD			16.3				18.3
SE			7.3				8.2

TABLE 3.32 Effect of Atropine pretreatment on recovery from compression to 31 Bar.

Chain	Tone before Compression (mm)	Tone after Decompression (mm)	$\%T_i$	Chain	Tone before Compression (mm)	Tone after Decompression (mm)	$\%T_i$
E	9.0	9.0	100.0	C	15.0	15.0	100.0
E	11.0	17.0	150.0	C	15.0	10.0	67.0
E	32.0	26.0	81.2	C	25.0	24.0	96.0
E	19.0	17.0	89.0	C	15.0	14.0	93.0
Mean			105.0				89.0
SD			31.0				15.0
SE			15.5				7.5

TABLE 3.33 Effect of Atropine pretreatment on recovery from compression to 43 Bar.

Tone before Compression$_1$ (mm)	Decrease$_1$	% Decrease	Tone before Compression$_2$ (mm)*	Decrease$_2$*	% Decrease
22.5	5.5	24.4	23.5	3.5	14.8
28.5	1.5	5.2	27.5	2.5	9.0
18.0	5.0	27.7	12.0	2.0	16.6
21.0	3.0	14.2	18.0	2.0	11.0
17.0	1.0	5.8	18.0	3.0	16.6
Mean		15.5			13.6
SD		10.4			3.4
SE		4.6			1.5

*TTX 10-6g/ml

TABLE 3.34 Effect of TTX on the response to 43 Bar.

Paired tracheal chains were prepared in the usual way and allowed to take up tone. One chain of each pair was compressed to 43 bar using $He:O_2:CO_2$ mixtures (1) and the precompression tone, response (in mm) and the percentage decrease are recorded in the table. After decompression (see Table 3.37) and recovery, both chains were treated with TTX (10^{-6} g/ml) and stimulated at 32 Hz to ensure that all response to electrical stimulation had been inhibited. Subsequently the experimental chain was recompressed (2) to the same depth and the response recorded as before. The control chain was maintained at 1 bar throughout.

TTX had no significant effect on the response of tracheal chain to compression to 43 bar.

TABLE 3.35 The effect of TTX on the recovery of inherent tone from compression to 43 bar.

In the experiments described in Table 3.36 the recovery of inherent tone following compression in untreated and TTX treated cases was compared with the inherent tone recorded in the paired control preparations after the same time had elapsed.

The tone recorded after decompression (1 and 2) was expressed as a percentage of precompression tone ($\%T_i$). The tone recorded after the same time had elapsed was recorded for the control chains, and expressed similarly.

TTX had no effect on the rate of change of inherent tone in the control chains, or on the response of the experimental chains to compression to 43 bar.

Furthermore, the inherent tone recovered after decompression was not significantly different from that recorded in control chains after the same time had elapsed.

Tone before Compression (mm)	Tone after Decompression (mm)	$\%T_i$
Chain: Control, no drug		
16.0	16.0	100.0
30.0	28.0	93.3
17.0	16.0	94.1
11.0	10.0	90.9
15.0	15.0	100.0
Mean		95.6
SD		4.1
SE		1.8
: Control, TTX (10^{-6}g/ml)		
14.0	14.0	100.0
27.0	27.0	100.0
11.0	8.0	72.7
6.0	5.0	83.3
14.0	13.5	96.4
Mean		90.5
SD		12.0
SE		5.4
: Experimental, no drug		
22.5	20.0	88.8
28.5	28.0	98.2
18.0	19.0	105.0
21.0	18.0	85.7
17.0	19.0	111.0
Mean		98.0
SD		10.9
SE		4.8
: Experimental, TTX (10^{-6}g/ml)		
23.5	19.0	80.8
27.5	28.0	102.0
12.0	12.0	100.0
18.0	19.0	105.0
18.0	16.0	88.8
Mean		95.3
SD		10.2
SE		4.5

TABLE 3.35 Effect of TTX on recovery from 43 Bar.

(vii) Effect of Ca^{2+} on the response to pressure

(a) Effect of reduced $[Ca^{2+}]_o$ on the response to 13.1 bar.

Tracheal chains were compressed on three consecutive occasions to 13.1 bar (He alone). There was no significant difference between the responses recorded after each compression. Before the second compression $[Ca^{2+}]_o$ was halved to 1.2 mM $[Ca^{2+}]_o$. The tone of the preparations was reduced by 49% (Table 3.36).

(b) Effect of raised $[Ca^{2+}]_o$ on the response to 31 bar.

Tracheal chains were compressed three times to 31 bar. Before the second compression the chains were bathed in Krebs' solution containing three times the usual $[Ca^{2+}]_o$ (7.2 mM). A mean increase in tone of 4.1 ± 2.9% was recorded. The response to pressure in the presence of 7.2 mM $[Ca^{2+}]_o$ was significantly less than the response recorded in unmodified Krebs' solution ($p < 0.005$). After $[Ca^{2+}]_o$ was restored to 2.4 mM, the compression was repeated and no significant difference was observed between the response to $compression_1$ and $compression_3$ (Table 3.37).

(c) Effect of raised $[Ca^{2+}]_o$ on the response to 43 bar.

Tracheal chains were compressed three times to 43 bar. Before the second compression the tissues were bathed in Krebs' solution containing 14.4 mM Ca^{2+}. A rise in tone of 14.4 ± 5.5% was recorded. The response to compression to 43 bar was significantly reduced by the presence of 14.4 mM Ca^{2+} ($p < 0.01$). By contrast there was no significant difference between the responses to pressure recorded in unmodified Krebs' solution (Table 3.38).

(viii) The effect of Ouabain on the response to pressure

The presence of ouabain (11 µM) and propranolol (1 µg/ml) in the bathing fluid resulted in a sustained increase in inherent tone of five tracheal chain preparations. The mean increase in tone was 32.8 ± 14.0%. The presence of the drugs had no significant effect on the response to compression (Table 3.39).

Ouabain had no effect on the recovery from 43 bar (Table 3.40).

(ix) Effect of Quinidine on the responses to pressure

Tracheal chains treated with quinidine (50 µg/ml) underwent a sustained contraction. The mean increase in inherent tone was 44.4 ± 17.7% (see also p.113). Quinidine had no significant effect on the response to compression to 43 bar (Table 41). Furthermore, the recovery from compression was unaltered by the presence of quinidine (Table 42).

Pressure	$[[Ca^{2+}]_o$ = 2.4mM]			$[[Ca^{2+}]_o$ = 1.2mM]			$[[Ca^{2+}]_o$ = 2.4mM]		
	Tone (mm)	Decrease (mm)	% Decrease	Tone (mm)	Decrease (mm)	% Decrease	Tone (mm)	Decrease (mm)	% Decrease
13.1	23.0	4.0	17.4	13.0	4.0	30.7	-	-	-
	19.0	5.0	26.3	11.0	3.0	27.2	-	-	-
	25.0	1.0	4.0	15.0	0.0	0.0	16.0	2.0	12.5
	16.5	3.0	18.1	12.0	0.0	0.0	14.0	3.0	21.4
Mean			16.4			14.5			16.5
SD			9.2			16.7			6.4
SE			4.6			8.3			3.2

TABLE 3.36 The effect of reduced $[Ca^{2+}]_o$ on the response to 13.1 bar.

Tracheal chains were prepared in the usual way and allowed to take up tone. The tracheal chains were compressed to 13.1 bar (He alone). The response (in mm) was expressed as a percentage of precompression tone (% Decrease). After decompression and recovery, the bathing fluid was replaced with Krebs' solution containing 1.2 mM Ca^{2+} and the compression repeated. Following recovery the bathing fluid was replaced with normal Krebs' solution and the compression repeated. There was no significant change in the percentage decrease in inherent tone when $[Ca^{2+}]_o$ was halved. Furthermore, when $[Ca^{2+}]_o$ was restored to normal, the response to compression was not significantly different from the response to the first compression.

Pressure	Response in 2.1mM Ca^{2+}			Response in 7.2mM Ca^{2+}			Response in 2.1mM Ca^{2+}		
	Tone (mm)	Decrease (mm)	% Decrease	Tone (mm)	Decrease (mm)	% Decrease	Tone (mm)	Decrease (mm)	% Decrease
31	24.0	5.0	20.8	25.0	2.0	8.0	24.7	7.0	29.0
	21.0	6.0	28.5	19.0	4.0	21.0	18.0	0.0	0.0
	24.0	4.0	16.6	27.0	1.5	5.5	-	-	-
	21.0	5.0	23.8	23.0	0.0	0.0	23.5	3.5	14.8
	12.0	4.0	33.3	12.0	3.0	25.0	12.0	6.0	50.0
	24.0	3.0	12.5	26.0	2.0	7.6	-	-	-
Mean	21.0	4.5	22.6	22.0	2.08	11.2	19.4	4.1	23.4
SD	4.6	1.04	7.6	5.65	1.35	9.6	5.6	3.1	21.0
SE	1.8	0.42	3.1	2.3	0.5	3.9	2.3	1.3	-

TABLE 3.37 The effect of raised $[Ca^{2+}]_o$ on the response to 31 bar.

Tracheal chains were prepared in the usual way and allowed to take up tone. Each chain was compressed on three consecutive occasions to 31 bar. On the second occasion the $[Ca^{2+}]_o$ was trebled. This treatment significantly reduced the response to compression ($p < 0.005$); student's paired T-test). When $[Ca^{2+}]_o$ was restored to normal, the response was not significantly different from the response to the first compression.

In each case the precompression tone, the change in tone (in mm) and the change expressed as a percentage of precompression tone are recorded.

Pressure	Response in 2.1mM Ca^{2+}			Response in 14.4mM Ca^{2+}			Response in 2.1mM Ca^{2+}		
	Tone (mm)	Decrease (mm)	% Decrease	Tone (mm)	Decrease (mm)	% Decrease	Tone (mm)	Decrease (mm)	% Decrease
43	15.5	5.0	32.0	20.0	4.0	20.0	23.0	6.0	26.0
	26.5	6.5	4.5	30.0	2.0	6.6	29.0	5.0	17.2
	21.0	6.0	28.5	27.0	2.0	7.4	24.5	3.0	12.2
	29.0	5.0	17.2	27.0	1.0	3.7	23.0	4.0	17.4
	21.0	6.0	28.5	24.0	5.0	20.8	25.0	6.0	24.0
	24.0	1.0	4.0	26.0	2.0	7.6	22.0	2.0	9.1
Mean	22.8	4.9	22.5	25.6	2.6	11.0 *	24.4	4.3	17.6
SD	4.8	2.0	10.3	3.3	1.5	7.3	2.5	1.6	6.5
SE	1.9	0.8	4.2	1.3	0.6	2.9	1.0	0.6	

TABLE 3.38 The effect of raised $[Ca^{2+}]_o$ on the response to 43 bar.

Tracheal chains were prepared in the usual way and allowed to take up tone. Each chain was compressed to 43 bar on three consecutive occasions. Prior to the second compression $[Ca^{2+}]_o$ was raised six fold. This treatment was accompanied by a significant reduction in the response to compression and was completely reversed when $[Ca^{2+}]_o$ was restored to normal values ($p < 0.01$, student's T-test (paired)).

In each case the precompression tone (mm), the response to compression (mm) and the decrease expressed as a percentage of precompression tone are recorded.

TABLE 3.39 The effect of ouabain (11 μM) on the response to 43 bar.

Tracheal chains were prepared and allowed to take up tone in the usual way. Each chain was compressed to 43 bar (He alone) and the results recorded as before. After decompression and recovery (Table 3.42), ouabain (11 μM) and propranolol (10^{-6} g/ml) were added to the bath fluid of each chain. Inherent tone was significantly increased. After 30 minutes the preparations were recompressed to 43 bar (He alone).

In each case the decrease in tone recorded following compression ($_1$ and $_2$) is expressed as a percentage of the tone recorded prior to each compression.

TABLE 3.40 The effect of ouabain on the recovery from 43 bar.

In the experiments described in Table 3.42 the tone recovered after each compression was recorded, expressed as a percentage of the tone prior to each compression.

Ouabain (11 μM) had no significant effect on the recovery of inherent tone following decompression.

Tone before Compression$_1$ (mm)	Decrease	% Decrease	Tone before * Compression$_2$ (mm)	Decrease	% Decrease
10.0	7.0	70.0	18.0	6.0	33.3
23.0	8.0	34.7	25.0	7.0	28.0
30.0	3.5	11.6	33.0	1.5	4.5
12.0	2.0	16.6	18.0	2.0	11.1
19.0	2.0	10.5	22.0	1.5	6.8
Mean		28.7			15.4
SD		25.0			14.6
SE		11.2			6.5

TABLE 3.39 Effect of Ouabain on the response to 43 Bar.

Tone before Compression$_1$ (mm)	Tone after Decompression$_1$ (mm)	% Decrease	Tone before * Compression$_2$ (mm)	Tone after Decompression$_2$ (mm)	% Decrease
10.0	3.0	30.0	18.0	12.0	66.0
23.0	18.5	80.4	25.0	23.0	92.0
30.0	30.0	100.0	33.0	30.0	91.0
17.0	15.0	88.2	18.0	17.0	94.0
19.0	15.0	79.0	22.0	20.0	91.0
Mean		75.52			86.8
SD		26.7			11.7
SE		11.9			5.2

* Ouabain

TABLE 3.40 Effect of Ouabain on the recovery from 43 Bar.

TABLE 3.41 The effect of quinidine on the response to compression.

Tracheal chains were prepared in the usual way and allowed to take up inherent tone. Each chain was compressed to 43 bar (He alone) and the decrease in tone recorded (both in mm) and expressed as a percentage of the precompression tone. Following decompression and recovery (Table 3.42) quinidine (50 μg/ml) was added to the bath fluid of each preparation. After 20 minutes inherent tone had risen significantly and the tissue was recompressed to 43 bar. There was no significant difference between the responses to compresion_1 (no drug) and compression_2 (quinidine, 50 μg/ml).

TABLE 3.42 The effect of quinidine on the recovery from compression to 43 bar.

In the experiments described in Table 3.41 the inherent tone recorded after decompression was noted and expressed as a percentage of the precompression tone in the absence and presence of quinidine (50 μg/ml). Quinidine had no significant effect on the recovery of inherent tone following decompression.

Tone before Compression	Decrease	% Decrease	Tone before Compression [Quinidine (50µg/ml)]	Decrease	% Decrease
14.0	2.0	14.3	24.0	5.0	20.8
28.0	4.0	14.3	30.0	3.0	10.0
9.0	3.0	33.3	16.0	4.0	25.0
14.0	4.0	28.5	17.0	8.0	47.0
Mean		22.5			25.7
SD		9.6			15.5
SE		4.8			7.7

TABLE 3.41

Tone before Compression	Decrease	% Decrease	Tone before Compression [Quinidine (50µg/ml)]	Decrease	% Decrease
14.0	12.0	85.7	24.0	9.0	37.5
28.0	24.0	85.7	17.0	15.0	88.3
9.0	9.0	100.0	16.0	16.0	100.0
14.0	10.0	71.4	17.0	9.0	53.0
Mean		85.7			69.7
SD		11.6			29.3
SE		5.8			14.6

TABLE 3.42

(x) Effects of pressure on guinea pig lung strip

Of the five lung parenchyma strips compressed to 43 bar, two responded with an abrupt relaxation in a manner similar to the majority of tracheal chain preparations. However, the other three preparations underwent slow relaxation, which continued until tone had been completely abolished. An example of each type of response is shown in Fig. 3.29.

The effect of compression to 43 bar on the lung strip are shown in Table 3.43. While the tone recovered after decompression it was not significantly different from that recorded in the control chain after the same time interval (Table 3.44).

(xi) The effect of compression to 43 bar Ar

The effect of He compression and Ar compression were compared and the results are shown in Tables 3.45 and 3.46. The use of Ar as a compression gas has no significant effect on the response to compression.

(xii) The effect of pressure on the response to field stimulation

Using the Harvard smooth muscle transducer, the contraction phase of the response to field stimulation at both ambient and high pressure was usually small or absent. Thus, only the effect of pressure on the relaxation phase has been recorded here. The effect of compression to 67 bar is shown in Table 3.51, Fig. 3.34. In each case tone is recorded as mm deflection of the pen, while each response is recorded as a percentage of the tone observed immediately before stimulation. The effect of compression to 31 and 43 bar, in the presence and absence of atropine are shown in Tables 3.47-50(II), Fig. 3.30-3.33 (II).

Despite the reduction in tone recorded after each compression, the response to field stimulation at 31, 43 or 67 bar were not significantly different from those recorded at atmospheric pressure either pre or post compression. Furthermore, there were no significant differences recorded between control and experimental preparations at any time during the experiments. In four experiments at 31 bar and four at 43 bar the tracheal chains were treated with 10^{-6} g/ml atropine. No significant changes were recorded in these experiments.

TABLE 3.43 Five paired lung strip preparations were set up as described in the text and allowed to take up tone. One strip of each pair was compressed to 43 bar (He alone), the control strip remaining at 1 bar throughout. The table shows the precompression tone (mm) and the decrease in tone following compression in mm and expressed as a percentage of the precompression tone. Two types of response were observed: either a reduction in tone of similar magnitude to that seen in tracheal chain, or a slow relaxation until all inherent tone had been lost. Both effects were fully reversed after decompression. Because of the variation in response the mean response size has not been computed.

TABLE 3.44 The recovery of inherent tone after compression of the guinea pig lung strip to 43 bar.

In the experiments described in Table 3.45 the inherent tone following decompression and recovery was noted, and expressed as a percentage of precompression tone ($\%T_i$) The control preparations were maintained at 1 bar throughout and inherent tone was noted at the same times as compression (Time O) and full recovery (Time D) in the experimental chains. There was no significant difference in the $\%T_i$ recorded in the control and experimental preparations.

Expt. No.	Tone (mm)	Decrease (mm)	% Decrease
1	14.0	4.0	28.5
2	19.0	4.0	21.0
3	12.0	12.0	100.0
4	20.0	20.0	100.0
5	26.0	26.0	100.0

TABLE 3.43 Effect of compression to 43 Bar He on tone of guinea pig lung strip.

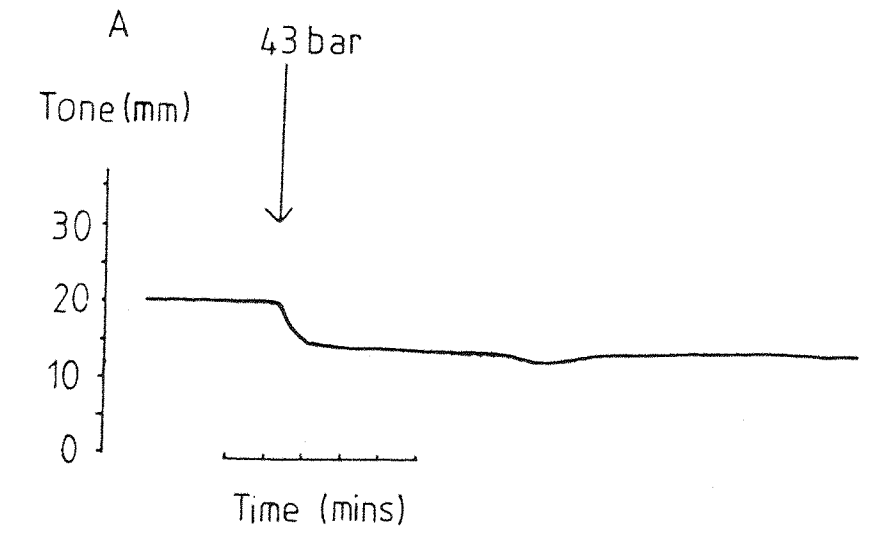
Experimental			Control		
Tone before Compression (Time O) (mm)	Tone after Decompression (Time D) (mm)	$\%T_i$	Tone (Time O) (mm)	Tone (Time D) (mm)	$\%T_i$
14.0	19.0	135.0	14.0	11.0	78.0
19.0	19.0	100.0	14.0	14.0	100.0
12.0	11.0	92.0	15.0	14.0	107.0
20.0	20.0	100.0	17.0	21.0	81.0
26.0	15.0	57.6	24.0	24.0	100.0
Mean	96.9				93.2
+SD	27.5				12.8
SE	12.3				5.7

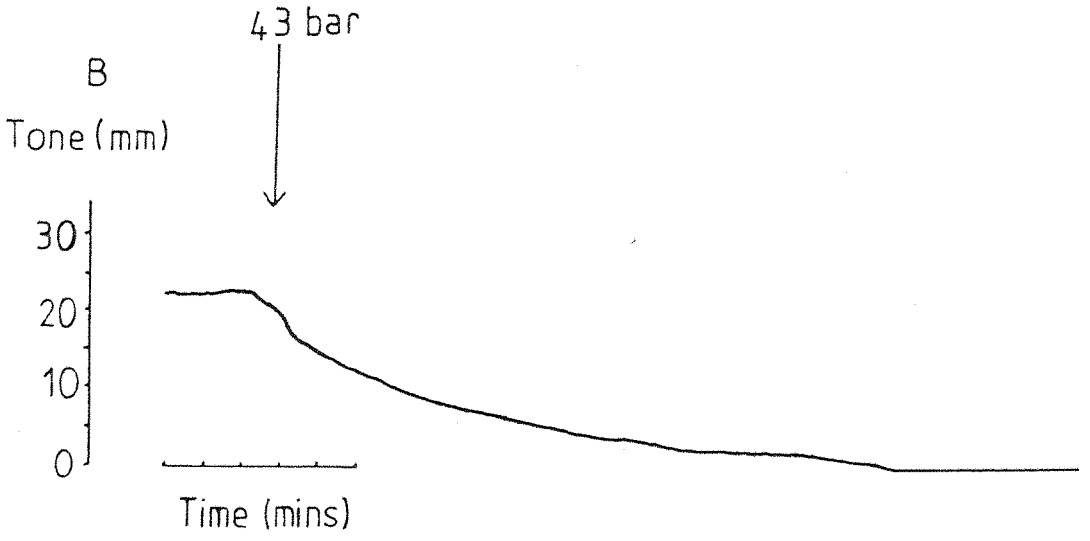
TABLE 3.44 Recovery from 43 Bar He (lung strip).

FIGURE 3.29 Typical responses of guinea pig lung strip to compression to 43 bar.

A. Two preparations responded in a similar manner to the majority of guinea pig tracheal chain preparations with an abrupt reduction in tone which was maintained throughout the compression.

B. Three preparations responded with gradual decline to zero tone. This response was never observed with tracheal chain preparations.


A
43 bar
Tone (mm)
30
20
10
0
Time (mins)


43 bar
B
Tone (mm)
30
20
10
0
Time (mins)

He_1			Ar_2			Ar_1			He_2		
Tone	Decrease	% Decrease	Tone	Decrease	% Decrease	Tone	Decrease	% Decrease	Tone	Decrease	% Decrease
11.0	5.0	45.0	9.0	3.0	33.3	22.0	5.0	22.7	21.0	6.0	28.5
17.0	4.0	23.5	13.0	4.0	30.7	14.0	3.0	21.0	11.0	2.0	18.8
15.0	2.0	13.3	12.0	3.0	25.0	17.0	4.0	23.5	18.0	2.0	11.0
Mean		27.3			29.6			21.6			20.1
SD		16.1			4.2			2.5			8.7
SE		9.3			2.4			1.4			5.0

TABLE 3.45 Six tracheal chains were prepared in the usual way and allowed to take up tone. Three chains were compressed to 43 bar He (He_1) and the same preparations were recompressed to 43 bar Ar (Ar_2). The remaining three tracheal chains were compressed first with Ar (Ar_1) followed by compression to 43 bar He (He_2). In each case the precompression tone, the decrease in tone due to compression and the decrease as a percentage of precompression tone are given in the table. There is no significant difference between the response to He compression and the response to Ar compression whether He compression preceded Ar compression or vice versa.

% Decrease in tone due to He compression		% Decrease in tone due to Ar compression	
1	45.0	33.3	2
	23.5	30.7	
	13.3	25.0	
2	28.5	22.7	1
	18.8	21.0	
	11.0	23.5	
Mean	23.35	26.0	
SD	12.4	4.86	
SE	5.1	1.98	

TABLE 3.46 The data from Table 3.45 has been retabulated in order to combine the responses to He compression and Ar compression. Once again, it can be seen that the response to compression with Ar was not significantly different from that observed as a result of compression with He.

FIGURE 3.34 The effect of 67 bar on the response to field stimulation.

Paired tracheal chains were prepared in the usual way and allowed to take up tone. Each chain was stimulated at 4, 8, 12 and 16 Hz in three consecutive frequency response tests. Results were recorded using the Harvard Smooth Muscle Transducer.

The data shown in Tables 3.51a and 3.51b are represented graphically in this figure.

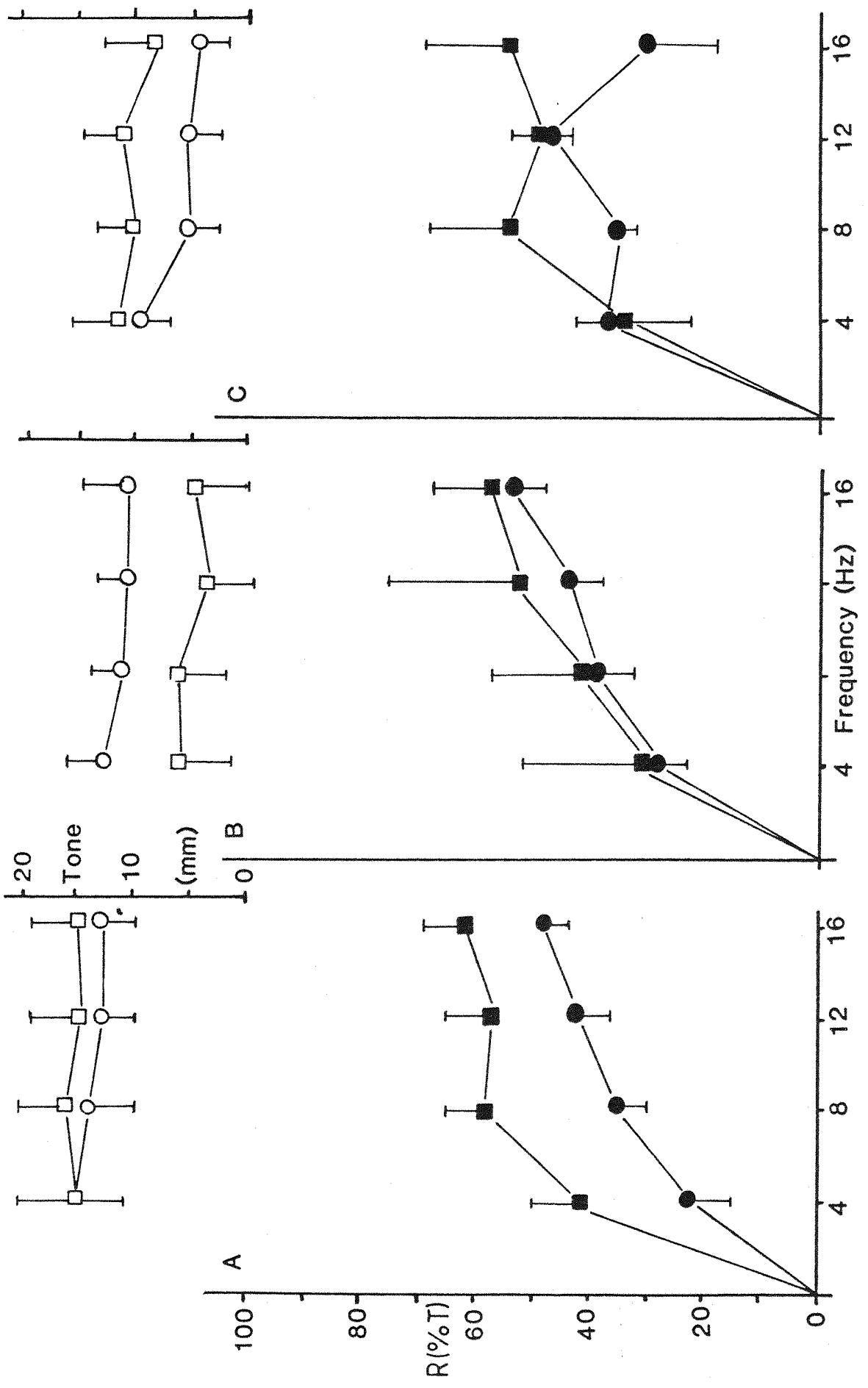
■◧ The experimental chains

●○ The control chains

The frequency of stimulation is shown on the x axis.

On the left-hand y axis is the response to field stimulation, expressed as a percentage of inherent tone recorded just prior to field stimulation (R(%T)).

On the right-hand y axis is the inherent tone (in mm) recorded immediately prior to field stimulation.


A
B
C
R(%T)
100
80
60
40
20
0
4
8
12
16
Frequency (Hz)
Tone
20
10
0
(mm)

Recovery from field stimulation

In some preparations the recovery from field stimulation was reduced markedly when compared with that of the same chain recorded at atmospheric pressure. Although individual cases of poor recovery were quite striking and were seen at each pressure, the variability in recovery times tended to mask those results when recorded as a group. Figs. 3.35 and 3.36 show the percentage recovery of prestimulation tone 5 minutes after stimulation had ceased (% $R_{/5}$) for each recovery frequency of stimulation applied to two groups of preparations compressed to 43 and 67 bar respectively. At 43 bar no significant reduction in % $R_{/5}$ was observed. However, at 67 bar there was a marked and significant reduction in % $R_{/5}$ at each frequency ($p < 0.01$). This effect was only partially reversible (Tables 3.52 and 3.53(II).

FIGURE 3.35 The effect of 43 bar on the recovery of tracheal chain from field stimulation.

The data from Tables 3.52a and 3.52b (II) are represented graphically in this figure.

■ The experimental chains.
● The control chains.

The frequency of stimulation is shown on the x axis.

On the y axis is the percentage of precompression tone, recovered 5 minutes after stimulation (%R/5).

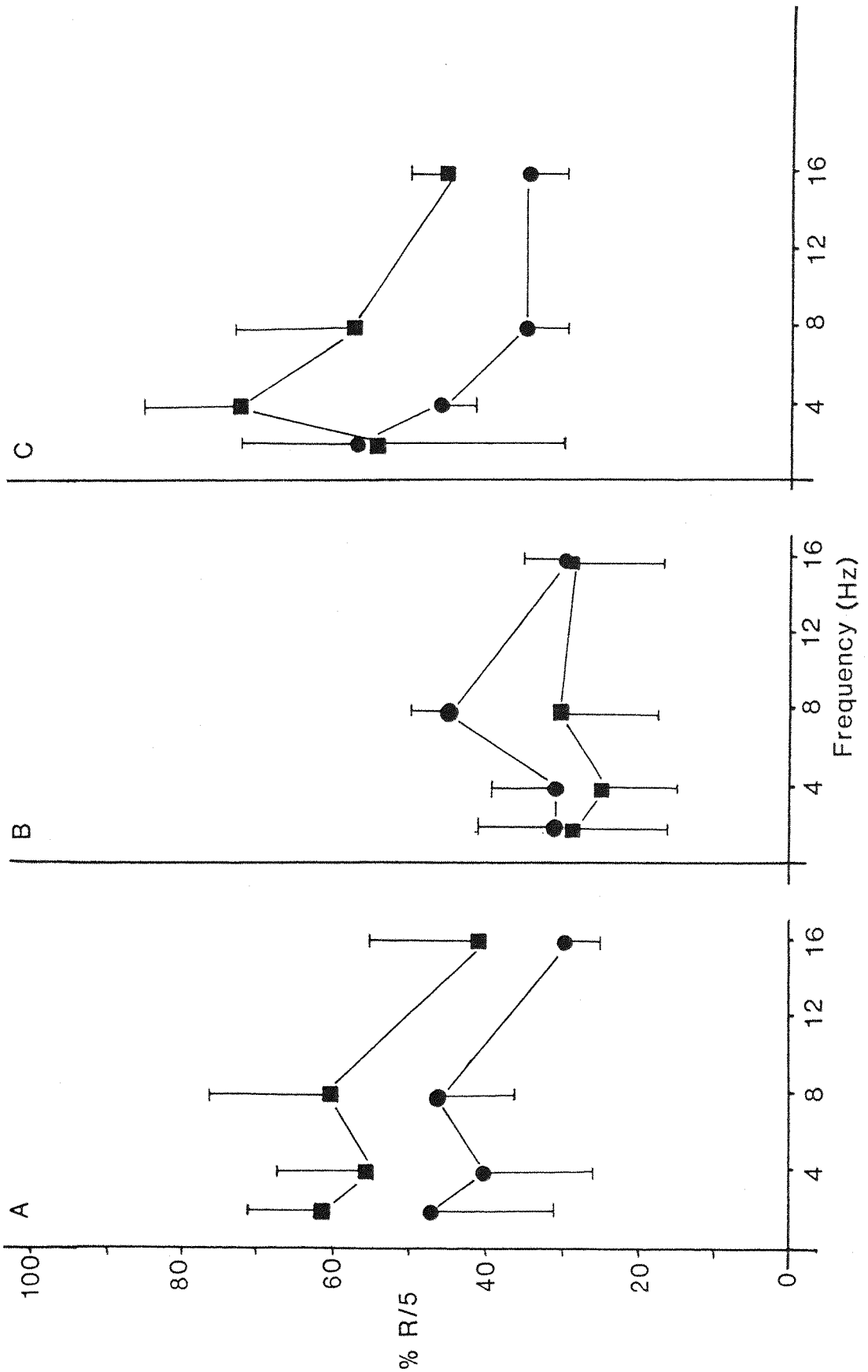

A
B
C
100
80
60
40
20
0
% R/5
4
8
12
16
Frequency (Hz)

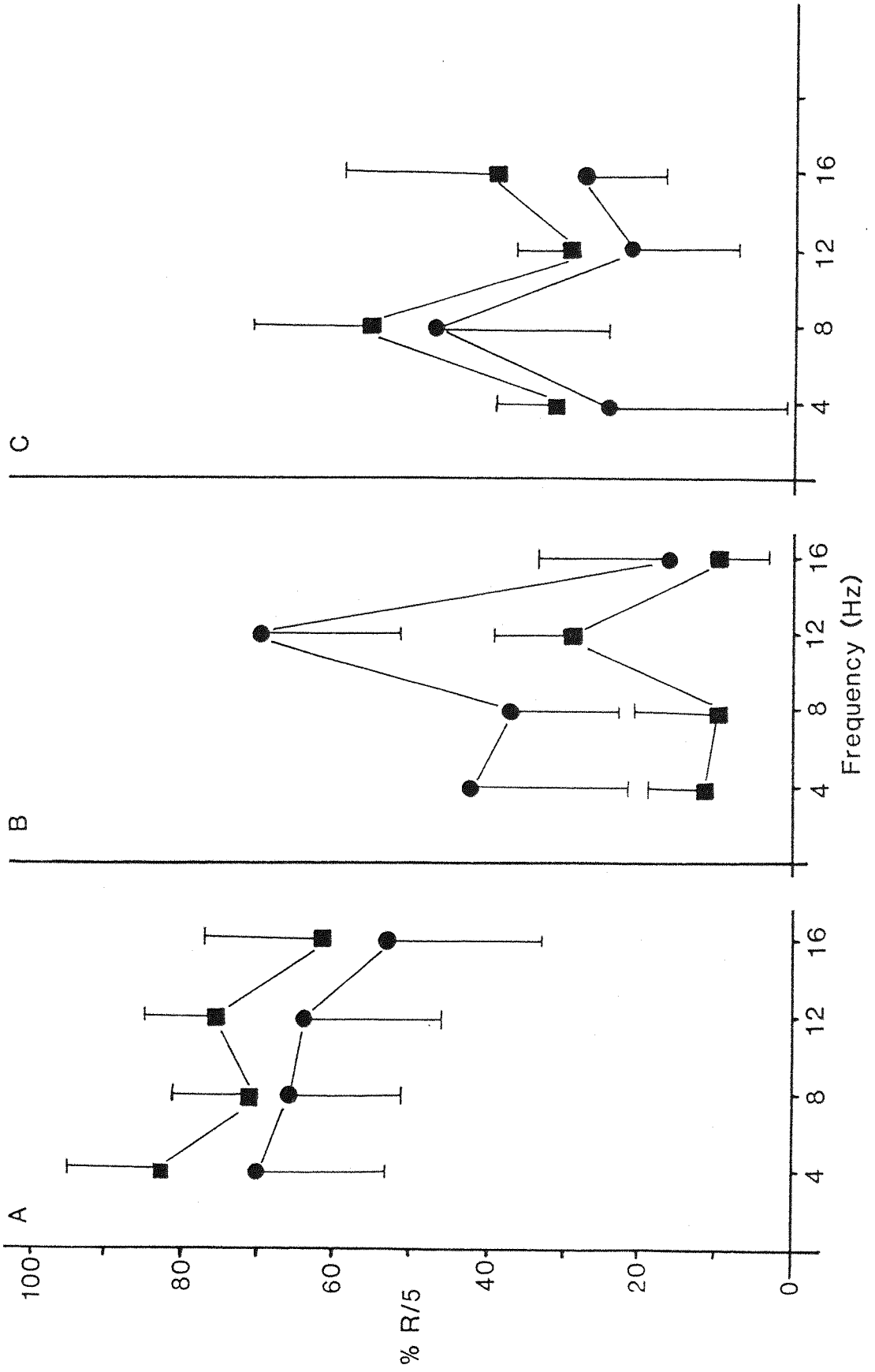
FIGURE 3.36 The effect of 67 bar on the recovery of tracheal chain from field stimulation.

The data from Tables 3.53a and 3.53b (II) are represented graphically in this figure.

■ The experimental chains.
● The control chains.

The frequency of stimulation is shown on the x axis.

On the y axis is the percentage of precompression tone, recovered 5 minutes after stimulation (%R/5).


A
B
C
100
80
60
40
20
0
% R/5
4
8
12
16
Frequency (Hz)

4. DISCUSSION

Despite advances in submarine escape technology and careful training, accidents, both fatal and non-fatal, still occur. Attempts have been made to correlate these accidents with factors such as the smoking habits, and apparent nervousness of the trainees and their ability to follow instructions, but no clear aetiology has been defined (Greene, 1978).

Numerous studies have been conducted using both animal and human subjects (Chyryssanthou, Teichner, Goldstein, Kalberer and Antopol, 1970; Schaeffer, McNulty, Carey and Liebow, 1958; Denney and Glas, 1964; Malhotra and Wright, 1960; Florio, 1980). Furthermore, respiratory indices have been assessed under conditions which simulate the immersion of submarine escape (see McWhirter, Harrison and Florio, 1979 for references), without revealing any clue as to the possible cause of the incidents apart from "airway obstruction, leading to air trapping and subsequent air embolism".

One possible cause of air trapping which has been neglected until now, is that of a direct effect of pressure on smooth muscle of the airways. There have been several attempts to investigate the effects of hydrostatic or He pressure on other smooth muscle preparations, but the majority of those studies were carried out at extremely high pressures and were complicated by changes in temperature, gas tension and pH. The experiments described in this thesis represent the first attempt to investigate the effect of pressure on the smooth muscle of the airways. During these experiments the temperature of the bathing fluid and gas tension were rigorously controlled. The pH of the Krebs' solution was monitored and the effect of pH on tracheal smooth muscle has been assessed.

It was intended originally to study the compression/decompression profile of submarine escape training. However, this proved impossible due to problems of instrumentation (see Appendix D). Nevertheless, the effect of high, static pressure on tracheal smooth muscle has been studied over the range of 13-67 bar. These pressures include those encountered in modern submarine escape, which is considered practical from a maximum of 600 fsw (21 bar) and saturation diving, the limit of which has been recently

extended to 67 bar.

In this discussion, the following points are considered:

A. The validity of the guinea pig tracheal chain as a model for human bronchial muscle.

B. The effects of changes in the gas tension, ionic content of the bathing fluid, of drugs and of field stimulation on tracheal smooth muscle under normobaric conditions.

C. The effects of high pressure on the inherent tone of tracheal smooth muscle, in the light of the normobaric results.

D. The significance of the findings of the study to the pathophysiology of submarine escape and saturation diving.

A. The validity of the guinea pig tracheal chain as a model for human bronchial muscle.

In order to investigate the effects of pressure on airway smooth muscle itself, it is necessary to isolate the tissue from the neural and humoral control to which it is normally subject. By incubating the tissue in an organ bath it is possible to control the micro-environment with respect to ionic content, temperature and gas tension, and this greatly facilitates the interpretation of data.

However, it should be noted that while the isolated tissue preparation allows the study of discrete events under closely controlled conditions, the response of the same tissue *in situ* may be modified or even negated by the influences of the higher centres, or by events secondary to the principal stimulus, such as the increased gas density, which accompanies high pressure. Thus, the response of the isolated tissue may be of greater or lesser significance, than anticipated to the whole animal.

In the present study the guinea pig tracheal chain was chosen as a model for human bronchial muscle. There is a wide range of tracheal preparations from which to choose: the chain of intact rings of Castillo and de Beer (1947), the open chain form of Akacsu (1952, 1959) and the paired chains described by Foster (1960). In addition, tracheal smooth muscle spirals and zig-zag strips have been described and following the development of high resolution strain gauge transducers, single tracheal segments have been used (Stephens, Kroeger and Mehta, 1969). The treachea may also be used as a tube of variable diameter in which the effect of drugs is reflected by changes in fluid pressure (Farmer and Coleman, 1969).

Of these methods, the most appropriate for this study was a modification of the method described by Foster (1960), where two chains were prepared from each guinea pig trachea. This strategy resulted in close matching of inherent tone, response to drugs and to field stimulation so that experimental and control data could be obtained from the same animal. The interpretation of the data relies on this matching in many instances. Several authors (Coburn and Tomita, 1973; J. Russell, 1978; Richardson and Beland, 1976) have noted

regional variations in the innervation and receptor population of the respiratory tracts of different species. The value of Foster's method is that any such variation is confounded. However, Harding and McWhirter, 1980 (unpublished observations) were unable to demonstrate any regional variation in the response of guinea pig tracheal chain to field stimulation.

The role of bronchial smooth muscle is two fold: it regulates dead space volume and controls regional ventilation. Thus, an understanding of the control of airway calibre is of vital importance in the treatment of obstructive and restrictive airways disease. The literature contains a wealth of data on the control of airway smooth muscle from a variety of preparations and species. By far the most common tissue to be studied in this context is the guinea pig trachea. Hawkins and Paton (1958) first demonstrated the similarities between guinea pig trachealis and human bronchial muscle. Their studies have been extended by many authors who have shown that with respect to inherent tone, responses to drugs (e.g. Collier, Holgate, Schachter and Shorley, 1960 and Newball, Keiser and Pisano, 1975) and distribution of nerve endings (e.g. Coburn and Tomita, 1973 and Richardson and Beland, 1976), guinea pig tracheal chain is a good pharmacological model for human bronchus. However, some of the results obtained in this study differ radically from those obtained in other species.

The disagreement between animal models is particularly marked when considering the effect of hypoxia on airway smooth muscle tone. For example, Kroeger and Stephens (1971) demonstrated that single segments of canine trachealis muscle were contracted by hypoxia. They argued that this was advantageous to the dog since inspired air would be diverted to better perfused regions of the respiratory tract. The present study has shown that guinea pig tracheal smooth muscle relaxes in response to hypoxia (see below). According to Kroeger's argument this would lead to an increase in respiratory dead space and an exacerbation of the respiratory failure.

Data from other preparations are unhelpful in resolving this contradiction. Nissel (1950) used the isolated cat lung preparation to investigate the effects of hypoxia and hypercapnia on airway dynamics. He found that bronchioles which had been constricted previously by carbamyl choline or histamine dilated during hypoxia,

but that hypoxia had no effect on untreated lungs. Widdicombe (1966) recorded that in intact artificially ventilated dogs, inhalation of hypoxic gas caused bronchoconstriction. This response was mediated by peripheral chemoreceptors and was abolished by vagotomy. The picture is also confused in humans since some authors recorded bronchoconstriction, and others bronchodilation, in response to hypoxia (see Butler and Caro (1960) for review).

Hence it is not clear, which if any, of the animal models described in the literature is comparable with the human respiratory tract. The main difficulty arises from the wide variety of experimental conditions and analytical techniques which have been used. However, the important clue to solving this confusion may be found in Nissel's discovery that only smooth muscle previously constricted by drugs responded with a relaxation during hypoxia. Thus, it is possible that the wide variability in response in all preparations is due to differing states of contraction of the tissue prior to the hypoxic challenge. This view is endorsed by Stein and Widdicombe (1975) who showed that the effects of both chemo and mechanoreceptor stimulation on artificially ventilated dogs were highly dependent upon initial airway "tone". Initial airway constriction favoured dilator responses and vice versa. The results of the present study also demonstrated that constrictor responses were large in the presence of low inherent tone and that dilator responses were large in the presence of high inherent tone.

In the human lung *in vivo* it is likely that under normal conditions some airways will be constricted, some dilated and some completely closed. The net effect of hypoxia on airway resistance would therefore depend on the conditions prevailing before the hypoxic challenge.

Tone versus Tonus

Some of the confusion in the literature undoubtedly arises from the use of the word tone, or sometimes tonus to mean two different things. In this thesis tone or inherent tone refers to a spontaneous, slowly developing myogenic contracture which is stable for several hours. This tone is independent of nervous influence since neither atropine, propranolol or TTX have any effect on it in

concentrations which inhibit the response to field stimulation. Two so-called purinergic antagonists, PIT and quinidine, cause temporary and sustained increases in tone respectively. However, these effects are probably not associated with their antagonism of the adenine nucleotides (see p.173). Furthermore, the H_1 and H_2 antagonists, mepyramine (7 μM) and cimetidine (8 μM) have no effect on inherent tone (Harding and McWhirter, unpublished observations).

Other authors (see Widdicombe, 1966) have used the term respiratory tone or tonus to refer to a property of the respiratory tract of animals with intact nervous systems. This respiratory tonus is reduced by atropine (Widdicombe, 1966) and increased by propranolol (MacDonald, Ingram and McNeill, 1975) suggesting that respiratory tonus is of nervous origin. Respiratory tonus is the outcome of the overall eleasticity of resistance to stretch of the lungs to which inherent tone may be a small contributory factor.

A comparison of guinea pig tracheal smooth muscle with airway smooth muscle from other species reveals a number of similarities, but also a striking difference in their response to hypoxia. These differences may be explained by taking the inherent tone of the preparation into account.

In this study inherent tone was assessed and the effects of each variable on inherent tone have been described. Appropriate analytical techniques have been used throughout the collaboration of the data in order to demonstrate clearly where changes in inherent tone alter the interpretation of the data.

Other authors have shown that the guinea pig tracheal chain is a valid pharmacological model for human bronchial muscle. However, its validity as a physiological model has not really been tested. In the absence of data from human tissue itself, it is possible to say that both guinea pig and human airways exhibit smooth muscle tone, and that responses to drugs and hypoxia appear to vary according to the value of this tone. Hence guinea pig trachea is probably a better model than that from cat or goat which exhibit little or no inherent tone.

B. The effect of gas tension, ions, drugs and field stimulation on tracheal smooth muscle under normobaric conditions

2B(i) The effect of $[Ca^{2+}]_o$ on inherent tone.

It is clear from the results summarised in Fig. 3.1, that tracheal smooth muscle tone is roughly proportional to $[Ca^{2+}]_o$ over the range 0.3-1.2 mM. Further increases in $[Ca^{2+}]_o$ did not result in a linear increase in inherent tone.

It was not possible to measure $[Ca^{2+}]$ during this study, and no values for $[Ca^{2+}]_i$ appear in the literature. Therefore it must be assumed that, as in other tissues, $[Ca^{2+}]_i$ is maintained at a low level ($\simeq 10^{-6}$ M; see Hasselbach, 1960). If this is true, then there will be a steep electrochemical gradient for Ca^{2+} from outside the cell to inside.

There are several possible reasons why inherent tone does not increase linearly above $[Ca^{2+}]_o$ of 2.4 mM.

(a) Ca^{2+} enters the cell passively and saturates all the Ca^{2+} binding sites on the contractile proteins.

For this to be true, initial tone would be equivalent to the maximum possible contraction. However, tissues with normal tone respond to spasmogens with a contraction; hence, maximum inherent tone is not equivalent to maximum contraction.

(b) As Ca^{2+} enters the cell, $[Ca^{2+}]_i$ rises until it reaches a critical value. Ca^{2+} uptake by mitrochondria and sarcoplasmic reticulum is stimulated. Ca^{2+} efflux is also enhanced, via the Ca^{2+} pump or by cation exchange mechanisms. Both Ca^{2+}/Ca^{2+} exchange and Na^{+}/Ca^{2+} exchange have been described in a variety of tissues (see Baker, 1973). It can be proposed that at a particular level of inherent tone, passive Ca^{2+} influx and total Ca^{2+} efflux are in equilibrium.

(c) The ion channels through which Ca^{2+} enters the cell are saturated at 2.4 mM $[Ca^{2+}]_o$. The rate of Ca^{2+} entry is exactly balanced by Ca^{2+} efflux or uptake into intracellular organelles.

In the simplest view of tracheal smooth muscle, inherent tone is a straightforward indicator of $[Ca^{2+}]_i$. Resting tone is the net effect of several influences and may be modified by altering one or more of the factors which contribute to that equilibrium (see Ruegg, 1971).

2B(ii) The effect of hypoxia on tracheal smooth muscle tone.

Tracheal smooth muscle loses approximately 50% of its inherent tone when the usual bubbling gas (95% O_2:5% CO_2) is replaced by 95% N_2:5% CO_2. Some preparations show a transient small increase in tone which is quickly reversed to a relaxation (Fig. 3.3).

Relaxation in response to hypoxia has been observed in other smooth muscle preparations, for example, rat stomach strip, rabbit duodenum (Eckenfels and Vane, 1972) and guinea pig taenia coli (Bauer, Goodford and Huter, 1965). However, some smooth muscles contract in response to hypoxia, notably pulmonary artery (Lloyd, 1968).

The effect of hypoxia on canine airway smooth muscle has been studied in detail (see Kroeger and Stephens, 1971). The study demonstrated that canine bronchial smooth muscle responded to hypoxia with a gradual increase in resting tension, which was maintained and accompanied by a fall in maximum active tension development. The effect was enhanced by the presence of phosphate in the bathing medium, and by the absence of a sugar substrate, which was replaced by NaCl. The rise in resting tension could be correlated with a rise in total cell Ca. ^{45}Ca efflux was enhanced during hypoxia, even in the absence of Ca^{2+} from the bathing medium. They proposed the model described below and shown in Fig. 4.1 to account for their findings.

In the absence of O_2 and exogenous substrate the cell ATP was depleted. This prevented the action of the Ca^{2+} pump at the sarcolemma, and in the sarcoplasmic reticulum. Therefore, $[Ca^{2+}]_i$ rose and was accompanied by a rise in resting tension.

It appears from the work of Stephens and co-workers that resting tension reflects the $[Ca^{2+}]_i$ in a simple manner. However, the results of Bauer et al (1965) conflict with this concept. They showed that guinea pig taenia coli exposed to hypoxia underwent a transient contraction, followed by a relaxation. Total cell Ca began to rise during the first 10 minutes of hypoxia, but did not reach a peak until all mechanical activity had ceased, some 5 hours later.

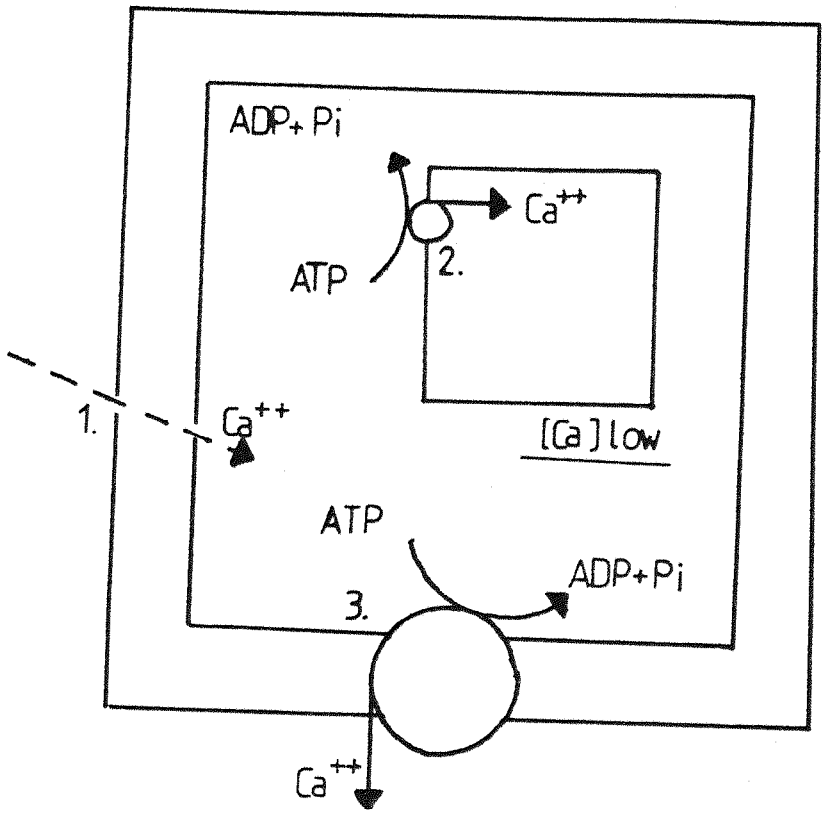
Intracellular $[Ca^{2+}]$ has been shown to rise in guinea pig uterine smooth muscle in response to hypoxia (Van Breeman et al, 1966). However, no measurements of tension were made during that study.

FIGURE 4.1 Mechanism of action of hypoxia, proposed by Kroeger and Stephens 1971.

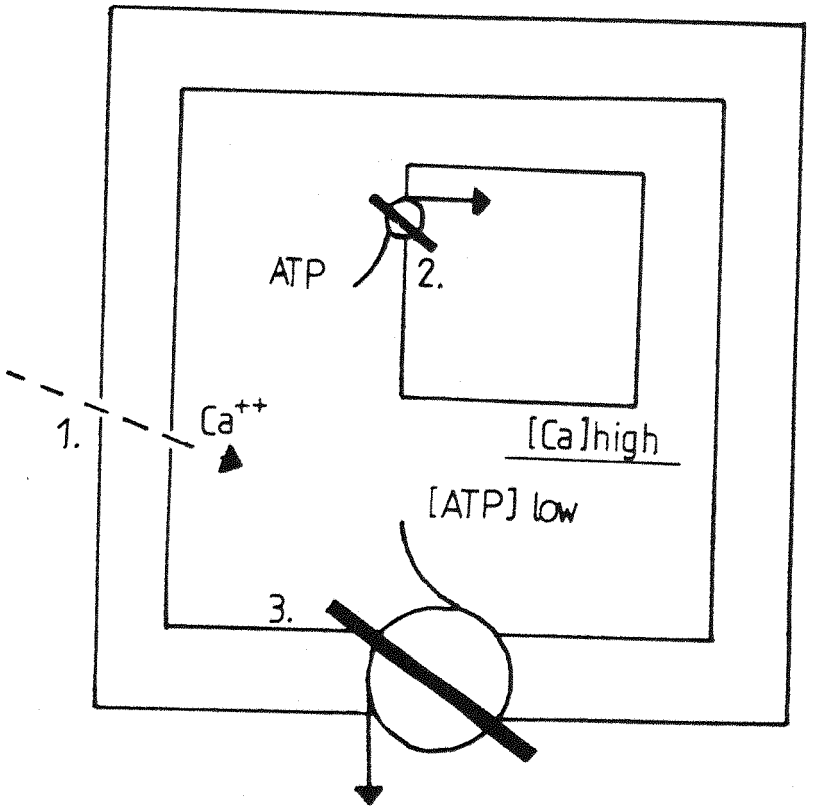
A. Under normoxic conditions $[Ca^{++}]_i$ is maintained at a low level by the action of the Ca^{++} pump (3) at the sarcolemma, and by the uptake of Ca^{++} into intracellular organelles (2). Ca^{++} enters the cell by passive leak mechanism (1).

B. In the absence of O_2 both Ca pumps are inhibited, Ca^{++} continues to enter the cell down its electrochemical gradient and $[Ca^{++}]_i$ rises.

A. Normoxia



B. Hypoxia



The Ca content of a variety of smooth muscle preparations rises during hypoxia. This is accompanied in some tissues by a maintained rise in smooth muscle tone, in others by a fall in tone, and in some by a transient rise, followed by a progressive fall in tone. Thus, it is clear that smooth muscle tone does not always reflect $[Ca^{2+}]_i$ in the simple way proposed by Kroeger et al (1971).

It might be that guinea pig tracheal smooth muscle tone falls during hypoxia because $[Ca^{2+}]_i$ falls and without a direct measure of $[Ca^{2+}]_i$ this cannot be ruled out. However, in view of the results from other studies, which are summarised above, such a mechanism seems unlikely.

The results shown in Fig. 3.5 support the view that $[Ca^{2+}]_i$ does not fall during hypoxia. If $[Ca^{2+}]_i$ were reduced by hypoxia then one might expect that raising extracellular Ca^{2+} would oppose, if not abolish, the effect of hypoxia. This is evidently not so: indeed, as $[Ca^{2+}]_o$ is increased, the effect of hypoxia appears to be enhanced. Such a result might be expected if hypoxia were in fact accompanied by a rise in intracellular Ca.

2B(iii) The effect of substrate withdrawal on the response to hypoxia.

It is interesting to note that in the present study and in the investigations of Stephens and others, the effect of hypoxia was enhanced by the absence of exogenous substrate from the bathing fluid. Guineapig tracheal smooth muscle takes up and maintains normal tone for up to 2 hours when bathed in sorbitol Krebs' solution. However, when exposed to hypoxia, the tissue loses all tone within 20 minutes.

In the absence of exogenous substrate the tissue must rely for its energy on endogenous substrates such as glycogen and free fatty acids. Under anaerobic conditions glycogenolysis can yield a small amount of ATP. However, the catabolism of fatty acids is dependent on the presence of O_2 and cannot continue anaerobically. The results of this study indicate that in the absence of glucose tracheal smooth muscle tone can be maintained by the oxidation of free fatty acids to yield ATP. During hypoxia this is inhibited and inherent tone is reduced. Stephens, Meyers and Cherniak (1968) demonstrated that the elevated resting tension recorded in canine trachealis muscle during hypoxia was associated with increased glycogenolysis and lactate production. If guinea pig tracheal smooth muscle contains any glycogen store, it is apparently not used to maintain tone during hypoxia, since tone is abolished by hypoxia when exogenous substrate is absent.

Orehek, Douglas, Lewis and Bouhuys (1973) demonstrated that guinea pig tracheal smooth muscle tone was associated with a continuous release of prostaglandins from the tissue. Similar results had been obtained in other tissues. Prostaglandin synthesis occurs only in aerobic conditions. The role of prostaglandins in the maintenance of airway smooth muscle tone will be considered later.

2B(v) Effect of drugs on tracheal smooth muscle.

Several of the drugs used in this study have been shown to alter inherent tone of tracheal smooth muscle. Some of these have been investigated in greater detail on other tissues, from which studies their mechanism of action has been proposed. Each drug which alters tracheal smooth muscle tone has been shown to change $[Ca^{2+}]_i$ in other tissues.

(a) Ouabain

The classical model to explain the action of ouabain is shown in Fig. 4.2. The Na^+ pump restores the Na^+ and K^+ gradients which have been reduced by a change in the plasma membrane permeability, e.g. after an action potential. If the Na^+ gradient is not maintained, the cell becomes inexcitable or unresponsive to extracellular messengers.

In some tissues a Na^+/Ca^{2+} exchange has been demonstrated (see Baker, 1973). In such tissues the cell membrane is said to be leaky to Na^+. As Na^+ enters the cell it loses potential energy which is coupled or harnessed to the extrusion of Ca^{2+}. Ca^{2+} moves out of the cell against its electrochemical gradient and $[Ca^{2+}]_i$ is kept at a low level. According to the model the rate of Ca^{2+} loss is dependent on the electrochemical gradient for Na^+. Thus, when the Na^+ pump is inhibited by the presence of ouabain (or by hypoxia) $[Ca^{2+}]_i$ rises. This indirect action of ouabain is thought to account for the increase in force of contraction of the heart which accompanies ouabain treatment (see Akera and Brody, 1977) and for the enhanced evoked and spontaneous release of neurotransmitters which have been recorded (Baker, 1973). However, there has been considerable argument about the existence of a Na^+/Ca^{2+} exchange mechanism in smooth muscle. The controversy has been reviewed recently by van Breeman, Aaronson and Loutzenhiser (1979).

In the present study, ouabain (11 μM) has been shown to have a biphasic effect. Tracheal chains undergo an initial contraction (Phase I), which is quickly reversed to a relaxation (Phase II),

FIGURE 4.2 Classical model of the action of ouabain.

Ouabain inhibits the action of the Na^+ pump (Na^+/K^+ ATPase). Na^+ accumulates within the cell. The leak of Na^+ into the cell from the extracellular fluid is reduced due to the diminished electrochemical gradient. Since Na^+ entry is coupled to Ca^{++} extrusion, Ca^{++} also accumulates within the cell.

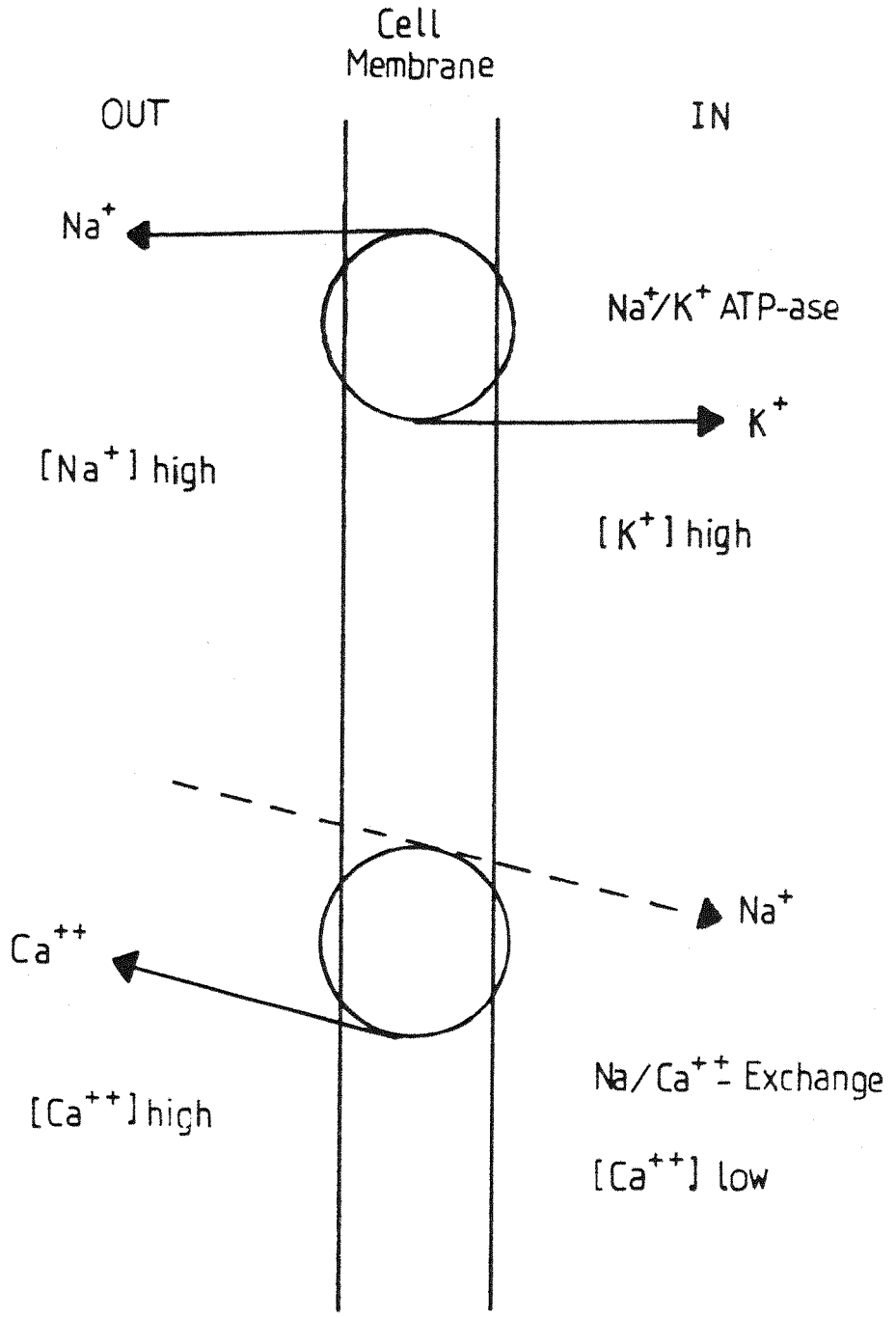


Fig 42

Proposed mechanism of action of ouabain.

(see Fig. 3.6). Ouabain exerts a biphasic effect, with a similar time course, in guinea pig taenia coli (Casteels, 1966).

There has been no detailed study of ion fluxes and tension changes during onabain treatment of guinea pig tracheal smooth muscle. However, ion fluxes have been studied in ouabain treated taenia coli from the same species. This tissue is similar to guinea pig tracheal smooth muscle in its response to hypoxia. Thus, it is possible that the mechanisms which maintain $[Ca^{2+}]_i$ are similar to those in trachealis muscle.

In taenia coli, the contraction, or Phase I of the response to ouabain, is accompanied by a rise in $[Ca^{2+}]_i$ (Casteels, Raeymaekers, Goffin and Wuytack, 1973). This result supports the model described earlier and shown in Fig. 4.2. If this response is due to an indirect action on the Na^+/Ca^{2+} exchange mechanism, then other manoeuvres which reduce the Na^+ gradient, should cause $[Ca^{2+}]_i$ to rise. Casteels et al further demonstrated that K^+ depletion of taenia coli caused both $[Na^+]_i$ and $[Ca^{2+}]_i$ to rise and that $[Ca^{2+}]_i$ increased during hypoxia and substrate withdrawal. However, they could not demonstrate any change in the Na^+ gradient during hypoxia.

In a similar study, Van Breeman, Farinas, Casteels, Gerba, Wuytack and Deth (1973) showed that during hypoxia and substitution of sorbitol for glucose $[Ca^{2+}]$ did not differ from normoxic controls. However, they did demonstrate a significant reduction in the Na^+ gradient. Incubation of taenia coli in ouabain (2×10^{-5}M) for one hour also significantly reduced the Na^+ gradient but $[Ca^{2+}]_i$ was unchanged.

Van Breeman et al (1979) were unable to demonstrate that ouabain induced a rise in $[Ca^{2+}]_i$ in taenia coli, but they did record a reduction in the Na^+ and K^+ gradients. $[Ca^{2+}]_i$ was raised by metabolic inhibition induced by iodoacetate and Dinitrophenol (DNP).

Thus, an increase in $[Ca^{2+}]_i$ is not always recorded after incubation of smooth muscle in solutions containing ouabain. Even when a $[Ca^{2+}]_i$ increase can be demonstrated it does not seem to be related to changes in the Na^+ gradient as might be expected, and the same authors report contradictory results.

It is possible that ouabain itself increases membrane permeability to Ca^{2+}. Burton and Godfraind (1974) incubated taenia coli in $^{45}Ca^{2+}$ labelled Krebs' solution. Ouabain enhanced both Na^+ and $^{45}Ca^{2+}$ uptake compared with untreated preparations. Later, James and Roufougalis (1977) obtained similar results using guinea pig ileum.

Many of the difficulties in interpreting the results of the studies outlined above lie in the methods used to measure $[Ca^{2+}]_i$ and Ca^{2+} fluxes, which are both cumbersome and slow. In particular, measurement of ^{45}Ca efflux may overestimate net Ca^{2+} efflux since some of the $^{45}Ca^{2+}$ will exchange for unlabelled Ca^{2+}, with no net effect on $[Ca^{2+}]_i$. Furthermore, Phase I of the response of taenia coli to ouabain is complete before the end of the incubation period for these methods. Thus the $[Ca^{2+}]_i$ recorded may reflect events in Phase II of the response, when $[Ca^{2+}]_i$ may be falling. In support of this view are the results of Casteels et al (1973) and James and Roufougalis (1977) who used comparatively short incubation times and who were able to record increases in $[Na^+]_i$ and $[Ca^{2+}]_i$.

Guinea pig tracheal smooth muscle shows a biphasic response in the presence of ouabain. Phase I (contraction) is not abolished by atropine and is unlikely to be due to α-receptor stimulation for which no evidence was found here (see p. 172). Other workers have been unable to confirm that $[Ca^{2+}]_i$ increases due to a reduction in Na^+/Ca^{2+} exchange and so the classic mechanism for the action of ouabain is in doubt in smooth muscle (p. 159). It is possible that $[Ca^{2+}]_i$ may be raised directly via an increase in membrane permeability to Ca^{2+}, rather than indirectly via the inhibition of the Ca^{2+} pump.

Phase II (relaxation) of the response is blocked by propranolol and is probably the result of noradrenaline released from nerve terminals or of adrenaline released from chromaffin tissue. Both of these effects could be explained by a rise in $[Ca^{2+}]_i$ in the relevant cells.

2(Bvb) Indomethacin

Since their discovery in bovine seminal fluid, the prostaglandins and their relatives the thromboxanes and endoperoxides have been implicated in a variety of cellular functions. They have been shown

to participate in platelet aggregation, hormone secretion, to modulate neurotransmitter release and smooth muscle tone. Specific binding of labelled prostaglandins has been demonstrated and receptors for prostaglandins have recently begun to be characterised (Coleman, 1980). A few prostaglandin antagonists have been studied, but have been found to be largely non-specific (Bennett and Posner, 1971).

By far the most useful class of drugs for the investigation of prostaglandin effects has been the non-steroidal anti-inflammatory drugs (NSAID's). The NSAID's have been shown to inhibit prostaglandin synthetase (Vane, 1971; Flower, 1974). Since prostaglandins are not stored in cells, but are synthesised 'on demand', the NSAID's exert rapid, widespread effects in all the tissues studied.

In 1973, Orehek, Douglas, Lewis and Bouhuys demonstrated that indomethacin reduced the inherent tone of guinea pig tracheal spirals. They also demonstrated that indomethacin (6 μg/ml) altered the response of the tissue to the spasmogens, Ach and histamine.

It had previously been shown (Sweatman and Collier, 1968; Smith, 1973) that the E series of prostaglandins exerted an inhibitory action on airway smooth muscle while the F prostaglandins caused contraction. Therefore, Orehek et al proposed that continuous intramural synthesis of prostaglandin $F_{2\alpha}$, in vitro, was responsible for the maintenance of inherent tone. Although prostaglandin $F_{2\alpha}$ is said to be a spasmogen and the E prostaglandins spasmolytic in tracheal smooth muscle, it has been shown (Coleman, 1980) that in tissues without tone the E prostaglandins cause contraction. Thus, the E prostaglandins may actually be responsible for initiating, if not for maintaining, inherent tone in this tissue.

Continuous prostaglandin synthesis has been shown to regulate inherent tone in other smooth muscle preparations, for example: rabbit isolated jejunum (Ferreira, Herman and Vane, 1976); rat stomach strip (Splawinski, Nies, Sweatman and Oates, 1973) and chick rectum (Eckenfels and Vane, 1972).

In the present study indomethacin was added to the bathing fluid of tracheal chains before inherent tone was established. Uptake of tone was normal during the first 5 minutes of incubation. However, after one hour the indomethacin treated chains had only 18% of the

tone recorded in the untreated chains. Thus, prostaglandin synthesis is required both to establish and to maintain inherent tone.

Prostaglandin synthesis is highly dependent on molecular oxygen (see Hillier, 1978 for review). Thus, removal of O_2 from the tissue should have the same effect as indomethacin. As we have seen in section 2B(ii), replacement of O_2 by N_2 reduces tracheal smooth muscle tone by 50% while indomethacin has a more profound effect. Hypoxia has been shown to inhibit smooth muscle tone in rat stomach strip and chick rectum and this has been correlated with a parellel decrease in prostaglandin synthesis (Ferreira, Herman and Vane, 1976). In a similar study Eckenfels and Vane (1972) demonstrated a decreased release of prostaglandin during hypoxic incubation of rabbit jejunum. Unfortunately, in these and other studies the tone itself was not assessed quantitatively, so that a comparison between the maximum relaxation observed with indomethacin and with hypoxia cannot be made. However, in the present study a discrepancy between the effects of hypoxia and indomethacin has been demonstrated, hypoxia reducing tone by 50% while indomethacin caused a reduction of about 80%.

Indomethacin is known to have a number of other effects, apart from its inhibition of prostaglandin synthetase. At a concentration of 60 µg/ml it is thought to uncouple oxidative phosphorylation. Furthermore, it has been shown to inhibit binding of Ca^{2+} to intracellular membranes (Northover, 1973) and to decrease Ca^{2+} uptake (Famaey, 1977) by guinea pig ileum. Also, indomethacin has been shown to inhibit phosphodiesterase (Zor, 1976).

Thus, non-specific anti-spasmogenic effects of indomethacin might account for the differences between the effects of hypoxia and indomethacin on tracheal smooth muscle.

The precise mechanism of action of prostaglandins is currently the subject of intense investigation. The prostaglandins have been described as Ca^{2+} ionophores, acting at the cell membrane to increase Ca^{2+} influx. However, this proposal has been challenged by Hertelendy et al, 1979, who incubated rat pituitary glands with prostaglandin E and with the Ca^{2+} ionophores X537A. Both agents caused an increase in Growth Hormone (GH) secretion, associated with a rise in ^{45}Ca efflux. However, only the effect of

prostaglandin was accompanied by a rise in cAMP. Thus, Hertelendy et al concluded that prostaglandins did not act merely as Ca^{2+} ionophores, but since their effects were dependent on the extracellular Ca^{2+} concentration they must modulate Ca^{2+} influx and thus $[Ca^{2+}]_i$ via an effect on the cell membrane. This view is supported by the recent description of prostaglandin receptors (Coleman, 1980).

2(Bvc) PIT

PIT has been demonstrated to oppose the relaxant effects of ATP on guinea pig taenia caecum (Hooper, Spedding and Weetman, 1974; Foster, Hooper, Spedding, Sweetman and Weetman, 1978). However, its usefulness as a specific purinergic antagonist has been hampered by relaxant effects which have been demonstrated in several tissues. The antagonism of ATP by PIT has been demonstrated largely on tissues treated with Ach, histamine or carbachol.

In the present study PIT (50 μM) induced a biphasic response when applied to the tracheal chain. Immediately after addition of the drug, a short-lived contraction was initiated. There was a mean increase in tension of about 15%, but this was quickly reversed to a relaxation. A similar response was recorded by Burnstock, Cocks, Crowe and Kasakov (1978) in guinea pig urinary bladder, where the contraction phase lasted 20-40 minutes. In guinea pig taenia caecum, however, the response appears to be monophasic, since only relaxation is observed (Foster et al, 1978), at the same concentration of PIT as used here.

Foster et al and Burnstock et al demonstrated that the PIT induced relaxation was not altered by phentolamine, propranolol, TTX or guanethidine. Although similar in time course to the relaxation invoked by hypoxia, a reduction in prostaglandin synthesis was not thought to be the cause of PIT induced relaxation. They further concluded that PIT was not a partial agonist.

Both groups compared the spasmolytic effect of PIT to that of papaverine, since PIT and papaverine relaxed K^+ induced Ca^{2+} contracture. The mechanism of action of papaverine is itself obscure. It may

inhibit phosphodiesterase but some workers have been unable to demonstrate increases in cAMP in taenia coli during incubation with papaverine (see Spedding et al, 1978).

2(Bvd) Verapamil

Verapamil and its methoxy derivative, D600, has been shown to block Ca^{2+} channels. Colatsky and Hogan (1980) have reported that verapamil blocks both outward and inward Ca^{2+} fluxes in cardiac muscle. Smooth muscle preparations such as vascular smooth muscle and guinea pig ureter, which are spontaneously active, are relaxed by 10^{-5} M verapamil (Van Breeman et al, 1979). Verapamil has also been shown to inhibit Ca^{2+} influx in isolated nerve preparations (Baker, 1973).

In this study verapamil (5×10^{-4} M) has been shown to induce 100% relaxation in tracheal smooth muscle when added to preparations in which tone has been established. This supports the view that tracheal smooth muscle tone is maintained by a continuous Ca^{2+} influx (See Fig. 4.3). However, verapamil pretreatment had no significant effect on uptake of tone. It can be seen from Fig. 3.9 that both the tone achieved and the rate of uptake of tone were normal in the presence of 5×10^{-4} verapamil. This result was somewhat unexpected since the data discussed so far, suggested that tone is dependent on extracellular Ca^{2+}, and on continuous synthesis of prostaglandins. A suggested role for prostaglandins was that they increase the permeability of the cell membrane to Ca^{2+}.

There are several possible explanations for this unexpected result.

1. It takes 20-30 minutes for verapamil to exert its effect.
2. A higher dose of verapamil is required to inhibit the Ca^{2+} influx during uptake of tone.
3. The prostaglandin sensitive Ca^{2+} channel is not blocked by verapamil.
4. Initiation of inherent tone is due to release of Ca^{2+} from intracellular sites, and not to increased Ca^{2+} influx as previously suggested.

It can be seen from Fig. 3.9 that tone begins to fall within 5 minutes of its addition to the bathing fluid of a preparation with

normal tone. Thus, it is unlikely that short contact time can explain the lack of effect of verapamil on uptake of tone.

During their experiments on the release of Growth Hormone from rat pituitary gland, Hertelendy et al (1978) noted that they could obtain only 25% inhibition of PGE_1 stimulated Growth Hormone release with a high dose of verapamil. However, release of GH stimulated by Ca^{2+}, ionophore (X537A) was abolished by verapamil. This supports the view that PGE_1 mediated Ca^{2+} influx is not sensitive to verapamil. However, Hertelendy et al measured $^{45}Ca^{2+}$ efflux. The Ca^{2+} could be arising from cytoplasmic Ca^{2+} or from recently formed Ca^{2+} stores.

If verapamil is truly a Ca^{2+} channel blocker, then the increase in tone may not be due to an increased influx but to a release of Ca^{2+} bound in intracellular stores. In support of this view are the results of two recent studies (Ishizawa, Nitta, Miyazaki, 1980, Wheeler and Weiss, 1980).

Ishizawa et al reported that La inhibited contraction of guinea pig stomach muscle induced by $PGF_{2\alpha}$, but not that induced by isotonic K^+. They referred to a previous study where they had shown that verapamil had little effect on the response of the same tissue to $PGF_{2\alpha}$, while blocking the effect of isotonic K^+. They suggest that high concentrations of La^{3+} prevents the release of Ca^{2+} from intracellular sites and that $PGF_{2\alpha}$ acts to release sequestered Ca^{2+}.

Wheeler and Weiss (1980) demonstrated that subthreshold doses of PGE_1 potentiated the response of submaximal concentrations of noradrenaline and angiotensin III on rabbit aortic strips. These effects were not altered by reduced $[Ca^{2+}]_o$, lowered temperature, the presence of phentolamine or D600. They also concluded that PGE_1 increased Ca^{2+} mobilisation from an intracellular site.

Recent evidence suggests that the contractile effects of prostaglandins in smooth muscle are due to the release of Ca^{2+} from sites within the cell. In tracheal smooth muscle both uptake and maintenance of tone require prostaglandin synthesis. However, the maintenance of tone is related to $[Ca^{2+}]_o$. Experience has revealed also that if a tracheal chain is bathed in Ca^{2+}-free Krebs' solution, it never takes up tone. However, under such drastic circumstances Ca^{2+} may actually diffuse out of cells.

PGE_1 seems to have a dual effect on tracheal smooth muscle tone. In the absence of tone, PGE_1 causes contraction while in the presence of tone it causes relaxation. It has been suggested that Ca^{2+} influx is partly balanced by the stimulation of Ca^{2+} uptake by intracellular organelles.

Fig. 4.3 represents tracheal smooth muscle cell suggested by the effects of indomethacin and verapamil.

Prostaglandin, synthesised within the cell, causes Ca^{2+} to be released from the sarcoplasmic reticulum and/or mitochondria. $[Ca^{2+}]_i$ rises rapidly. Permeability of the cell to Ca^{2+} may be enhanced by prostaglandins acting extracellularly and there is a high inward electrochemical gradient for Ca^{2+}. The stability and normal function of the surface membrane will depend on the presence of Ca^{2+} in the bathing fluid. Tone reaches a critical level which is dependent on the prostaglandin concentration. Ca^{2+} ATP-ase in the membranes of intracellular organelles is stimulated. Ca^{2+} is gradually removed from the cytoplasm, but this loss is exactly balanced by Ca^{2+} entering from the extracellular fluid. Thus, a stable tone is achieved. If at this stage Ca^{2+} entry is blocked by verapamil, tone begins to fall since Ca^{2+} ATP-ase continues to operate normally.

Since indomethacin reduces tone which is already established (Orehek et al, 1973) then at least 80% of Ca^{2+} entry during maintained tone must be due to an action of prostaglandins. Thus, the newly identified prostaglandin receptors may be the means via which Ca^{2+} permeability is controlled.

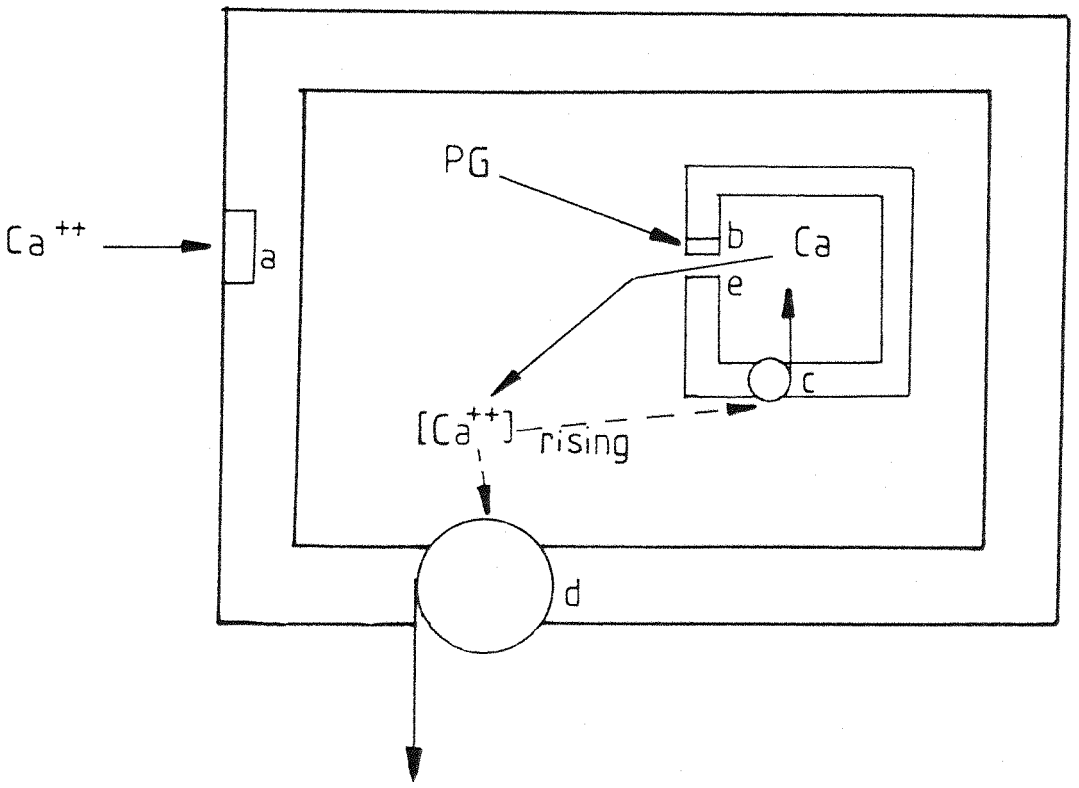
The purpose of such a complicated mechanism is a matter for speculation. Prostaglandin synthesis is intimately linked to the availability of O_2 and the metabolic status of the cell. As prostaglandin synthesis is reduced by hypoxia total cell Ca^{2+} has been shown to rise. However, this is often associated with a fall in tension. If sarcoplasmic reticulum ATP-ase activity were to continue during hypoxia then even when other pump mechanisms fail, the Ca^{2+} would be removed from the vicinity of the contractile proteins, thus conserving ATP generally. Sarcoplasmic reticulum

FIGURE 4.3 The control of $[Ca^{2+}]_i$, suggested by the effects of indomethacin and verapamil on tracheal smooth muscle.

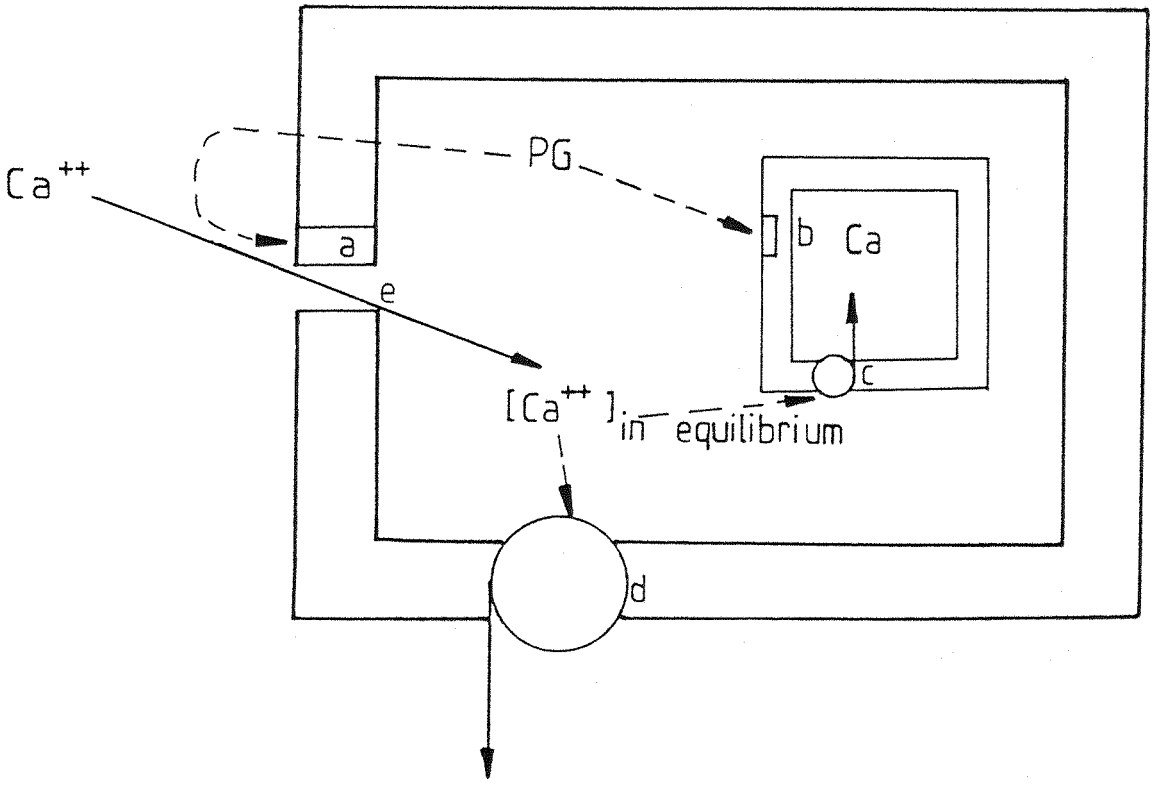
1. Initially intracellular prostaglandin levels are low. PGs act on the intracellular organelles, (b) to release Ca^{++} (e). As the cytoplasmic Ca concentration rises Ca^{++} is pumped back into the intracellular organelles (c) and is pumped out of the cell (d).

2. As PG level rises and begins to appear in the extracellular fluid, PG receptors on the plasma membrane are activated (a) and Ca^{++} enters via prostaglandin sensitive channels (e). $[Ca^{++}]_i$ is the result of the equilibrium between Ca^{++} uptake (c), Ca^{++} extrusion (d) and Ca^{++} entry (e).

1.



2.



ATP ase has a very high affinity for ATP and such a differential effect is feasible.

2(Bve) Quinidine

Quinidine is a non-specific drug which has a variety of actions. In this study it has been shown that it is a muscarinic antagonist and an effective, non-specific, neurone blocker. It has also been proposed as a purinergic nerve blocker. One unexpected result was that it caused an increase in tone which was larger than that evoked by ouabain.

Quinidine has been shown to release Ca^{2+} from intracellular organelles in skeletal muscle (Batra, 1974). Using suspensions of disrupted skeletal muscle cells, Carvalho, 1968) demonstrated that the mitochondrial fraction is more sensitive to quinidine than the microsomal or SR fraction.

Although the mitochondria are not thought to contribute greatly to the control of $[Ca^{2+}]_i$ in skeletal muscle, their role may be of greater importance in smooth muscle where the SR is less well developed.

The increase in tone of trachealis muscle recorded in the presence of quinidine is probably due to the release of Ca^{2+} from mitochondria and/or SR into the cytoplasm.

It would be interesting to continue the investigation of the spasmogenic effects of ouabain and quinidine. Verapamil is thought to block Ca^{2+} influx into a variety of cells. If the rise in tone due to ouabain is a result of increased Ca^{2+} influx, then verapamil should inhibit the response. Likewise, if quinidine induces an increase in release of Ca^{2+} from intracellular sites, then verapamil should be without effect.

3(Bvi) Field stimulation

In this part of the study field stimulation was used to release neurotransmitters from nerve endings present in the tissue. The response of the tissue was recorded in the absence and presence of drugs whose mechanisms of action are known to a greater or lesser extent. Field stimulation under the conditions stated did not result in direct stimulation of the smooth muscle, since TTX completely abolished the response to field stimulation (Bvia).

The response of guinea pig tracheal smooth muscle to field stimulation is biphasic. The initial contractile phase was abolished by atropine and therefore is due to the stimulation of parasympathetic cholinergic neurones. This result is in agreement with numerous previous studies, for example: Carlyle, 1964; Widdicombe, 1966; Farmer and Coleman, 1970; Rikimaru and Sudoh, 1971; and seems to be the only aspect of tracheal smooth muscle about which there is no debate. Furthermore, acetylcholinesterase containing fibres have been demonstrated in the bronchial tree of mammals (Mann, 1971).

The relaxation response to field stimulation was not abolished by a concentration of propranolol which blocked the response of other preparations to adrenaline (Bvid). This result is in agreement with Coleman (1973); Coburn and Tomita (1973) and Richardson and Bouchard (1975). Furthermore, reserpine pretreatment (Bvic) did not abolish the response of the tracheal chain to field stimulation confirming the findings of Coburn and Tomita (1973) and Bando, Shindo, Shimo (1973). These results suggest that there is a non-adrenergic inhbitory nervous pathway in guinea pig trachea. However, agreement is by no means universal on this as Foster (1963) and Rikimaru and Sudoh (1971) showed that adrenergic neurone blockers (guanethidine and bretylium respectively) completely blocked the inhibitory response to field stimulation in guinea pig tracheal chain. It is possible that guanethidine and bretylium also block the release of a non-adrenergic inhibitory transmitter in the concentrations chosen (Burnstock, 1975), although this was not the case in guinea pig intestinal smooth muscle (Small and Weston, 1979(b)).

Catecholamine (CA) containing nerve fibres have been identified in guinea pig trachea (Rikimaru and Sudoh, 1971) and in the trachea of sheep, calf, goat, pig and rabbit (Mann, 1971). However, many of the CA fibres were found close to blood vessels and may not have innervated the trachealis muscle. Field stimulation would not discriminate between these two sites. In addition, Mann (1971) failed to demonstrate any CA fibres in the bronchial muscle of the same species listed above.

There is little doubt as to the presence of β-adrenoreceptors in the smooth muscle of guinea pig trachea, since adrenaline, a "mixed" adrenoreceptor agonist initiated a dose related relaxation

in guinea pig trachea which was completely abolished by propranolol (Bvii), see footnote.

Although it is conventional to describe the innervation of tracheal smooth muscle as parasympathetic-cholinergic and sympathetic-noradrenergic (for example, Ganong, 1971), recently this view has been contested. Several papers suggest that the major inhibitory nervous pathway in guinea pig tracheal smooth muscle is non-adrenergic in nature. This view is supported by the results of the present study.

Similar non-adrenergic inhibitory pathways have been described in other tissues, for example: the guinea pig gastrointestinal tract (Small and Weston, 1979); guinea pig urinary bladder (Burnstock, Cocks, Crowe and Kasakov, 1978) and others (see Burnstock, 1975 for review). A strong lobby supports the hypothesis that the transmitter substance released from these nerve endings is ATP or a closely related purine nucleotide (Burnstock, 1972). ATP and adenosine both caused a dose-related relaxation of guinea pig trachea (Bviii, ix), mimicking the effects of nerve stimulation and fulfilling one of the criteria for positive identification of a neurotransmitter. Very high concentrations of ATP and adenosine were required to achieve relaxation of tracheal smooth muscle and this might indicate that their action was non-specific. However, Small and Weston (1979) argue that this could be due to very effective inactivation of the transmitter which is another criterion for neurotransmitter identification.

The major hindrance to the confirmation or denial of the purinergic nerve hypothesis is the lack of adequate purinergic receptor blockers. Burnstock (1972) suggested that quinidine was

Footnote. Some preparations were less sensitive than others to adrenaline (Table 3.19) and this might indicate the presence of α-adrenoreceptors to oppose the relaxant effects of β-adrenoreceptor stimulation. Foster (1966) found no evidence to suggest that the presence of α-adrenoreceptors in guinea pig trachea, but experiments on anaesthetised dogs (Castro de la Mata, M Penna and D Aviado, 1962) and on guinea pig isolated lung (Nagasaka, Bauckaert, Shaepdryver and Heymanns, 1964) indentified α-adrenoreceptor mediated contractions in the presence of high concentrations of β-blockers. Everitt and Cairncross (1971) confirmed this in guinea pig trachea and α-adrenoreceptors have been demonstrated in human lung in vivo (Anthracite, Vachon and Knapp, 1971). The question of α-receptor mediated responses was not pursued in this study.

such an antagonist. While quinidine blocked the response to field stimulation remaining after treatment with propranolol (Bvid), it also blocked the cholinergic response to field stimulation (Bvie) and had no effect on the response to exogenous ATP, adenosine or adrenaline (Bvii, viii, ix). Thus, quinidine probably acted as a local anaesthetic to block nerve mediated responses.

Similarly, it was claimed that theophylline was a specific purinergic antagonist (Ally and Nakatsu, 1976) but Small and Weston (1979b) could not confirm this in guinea pig trachea.

PIT is the most promising purinergic receptor blocker to date (Hooper et al, 1974; Foster et al, 1978). However, PIT exerts a non-specific relaxant effect on smooth muscle (see Bvc) and this complicates its use. Several authors have applied PIT to tissues where the "tone" has been restored with carbachol or histamine. The validity of this technique has been questioned recently. O'Donnell and Wanstall (1980) showed that the selectivity of tracheal smooth muscle for β-adrenoceptor agonists determined on preparations with intrinsic tone was not the same as that determined on preparations contracted with carbachol. Carlyle (1964) and Kirkpatrick and Tomita (1980) have shown that tracheal smooth muscle bathed in Ca^{2+} free solutions continued to respond to carbachol and histamine in the absence of inherent tone. This suggests that while inherent tone is maintained by the entry of Ca^{2+} from the extracellular fluid, agonist induced contraction is the result of Ca^{2+} released from intracellular stores.

Since the relaxant effect of ATP or field stimulation cannot be demonstrated in the absence of tone, the efficacy of PIT as a purinergic receptor blocker on smooth muscle preparations cannot be established except by electrophysiological techniques.

Further investigation into the nature of the non-adrenergic inhibitory transmitters was not attempted in this study.

B(x-xii) Effect of hypoxia on the response to field stimulation

It has been seen that inherent tone of tracheal smooth muscle is reduced by hypoxia (Table 3.3) and that when inherent tone is changed it can alter the apparent response of the tissue to a standard stimulus. This is clearly demonstrated by the effect of hypoxia on the response to field stimulation and the dose response curves of two putative neurotransmitters.

The relaxation response to field stimulation was significantly reduced by hypoxia. However, the dose response curve to adrenaline was unaltered by hypoxia. The same concentration of adrenaline can reduce inherent tone by the same proportion whether inherent tone has been reduced by hypoxia or not. This suggests that hypoxia acts presynaptically to reduce inhibitory neurotransmitter release.

Hypoxia causes an increase in the contractile response to field stimulation at all frequencies. In Fig. 3.21it can be seen that the response of the tissue to a standard dose of Ach increases as inherent tone decreases, whether that decrease is due to the effects of hypoxia or not. It appears from these data that the capacity of the preparation to contract is determined by the level of inherent tone which prevails. In order to understand the effect of hypoxia it is useful to introduce a measure of the "contraction potential". Throughout this study the relaxation responses have been referred to a maximum relaxation (p. 47). It is not practical to standardise the maximum contraction in a similar way since many agonists such as Ach, nicotine or histamine produce irreversible changes in tone, or exhibit tachyphylaxis. However, by adding together the tone (in grams) and the contractile response (in grams) recorded after a standard dose of Ach it is possible to obtain a measure of the maximum contraction for each preparation under the given conditions.

In control experiments (Table 3.20b) this value is relatively constant and is maintained during hypoxia. However, during field stimulation experiments, the maximum contraction is significantly less during hypoxia, than that of the same preparations during normoxia. Thus despite the apparent increase in size of the contractile response to field stimulation, hypoxia does reduce the maximum contraction recorded at each frequency. Since the effect of exogenous Ach is not similarly reduced, this is probably due to a reduction of Ach release, and not to a change in post synaptic sensitivity.

It has been demonstrated that the same dose of an agonist can have strikingly different effects, depending on the value of inherent tone. It is also remarkable that spasmolytic agents such as adrenaline should abolish a fixed proportion of inherent tone, rather than reduce inherent tone by the same amount (in grams)

for the same concentration of drug. It has been argued (Carlyle, 1964) that the cellular mechanisms which maintain inherent tone, and those which are responsible for contraction in response to spasmogens are separate. This is demonstrated easily by removing $[Ca^{2+}]_o$ from the bathing fluid and then challenging the tissue with histamine. The tissue will continue to respond to histamine in the absence of inherent tone (Carlyle, 1964; Kirkpatrick and Tomita, 1980). However, the results obtained here suggest that there must be some feedback mechanism between the uptake and release of Ca^{2+} from the contractile elements as a result of neurotransmitter action at the cell membrane, and the existing state of contraction of the tissue. In comparison with skeletal muscle, very little is understood about the mechanism of smooth muscle contraction and its control. The nature of the intracellular organelles which form Ca^{2+} sinks is also not known in smooth muscle, although mitochondria and a limited sarcoplasmic reticulum probably play a role. It is beyond the scope of the present study to speculate in detail on the complex relationship between tone and response, but the results obtained emphasise that such a relationship exists. Many authors apparently fail to recognise the importance of this link and studies on the effect of drugs on tracheal smooth muscle often appear without stating if the tissue exhibits inherent tone under the stated conditions, or if the drugs used alter inherent tone. These ommissions could seriously undermine the conclusions drawn by the authors.

C. The effects of pressure on inherent tone

Tracheal smooth muscle underwent a reversible relaxation in response to compression to 13-67 bar. The response was usually rapid in onset, but took several minutes to recover after decompression. The size of the response differed from one preparation to another, but this variability was not related to the tone prior to compression. Furthermore, on recompression, the response was not significantly different from that observed after the first compression. A similar relaxation was observed when guinea pig lung strips were compressed to 43 bar. However, some of these preparations lost inherent tone throughout the exposure to high pressure. This was in contrast to the tracheal chain where, after the initial relaxation, tone was maintained until decompression. The response of lung strip to compression was also reversible on return to normal atmospheric pressure.

There are a number of possible causes of the relaxation which follows compression; they include:

(i) Artefact: Electrical, mechanical or physiological.
(ii) A change in temperature.
(iii) Inappropriate gas mixtures - leading to hypoxia, or changes in pH.
(iv) Toxic effect of He.
(v) Increased spontaneous release of an inhibitory neuro-transmitter; alternatively a decreased spontaneous release of excitatory transmitter.
(vi) Direct effect on $[Ca^{2+}]_i$.

(i) Artefact

It is unlikely that the results obtained were due to mechanical or electrical artefact (Appendix D). Artefactual changes of this nature are easy to recognise and tend to occur during compression or decompression and indicate a fault such as movement of the lever arm or "shorting" of electrical connections. In practice any experiments which produced such errors were either abandoned, or restarted after the fault had been rectified.

It is also unlikely that the compliance of the "inert" components of the preparation was altered by pressure. On several occasions single pieces of thread, or knotted chains prepared only from cartilage were set up. No changes in transducer output were ever recorded under such circumstances.

(ii) Temperature

Appendix B shows that the temperature of the bath fluid was constant during compression to and decompression from a maximum of 1000 psig (69 bar). In many previous studies (see Introduction) of the effects of high pressure on isolated tissues, compression and decompression were accompanied by adiabatic temperature changes of which the authors were unaware or took no account. He gas has a high thermal capacity and hence is an excellent heat conductor, but in the present study the rapid flow of water at 37°C in the water jacket was sufficient to prevent any changes occurring in the bath fluid temperature.

(iii) Inappropriate gas mixtures

The problem of maintenance of P_{O_2}, P_{CO_2} and pH at high pressure has been tackled in a variety of ways by different authors. For example, it is possible to oxygenate Ringers solution (see Edwards, 1939) or liquid paraffin (Harper, MacDonald and Wann, 1978) prior to hydrostatic compression. Ornhagen (1976) took similar precautions when using Tyrodes' solution to bathe the mouse isolated sinus node preparations. Little and Paton (1980) and Kendig (1975) who superfused their preparations with Krebs' solution flushed the chamber with 95% O_2:5% CO_2 at atmospheric pressure, before raising the total pressure by adding pure N_2 or He.

Initially, it was though that this method would be suitable for use with tracheal chains. However, it was demonstrated that

the small bath volume of MacI was inadequate to maintain inherent tone in experiments of even short duration. As the dissolved O_2 was consumed inherent tone began to decline. Continued bubbling with 95% O_2:5% CO_2 at high pressure also resulted in serious reduction of inherent tone. Since the responses of tracheal chains are dependent on inherent tone it was important to ensure that the preparation was not affected by altered partial pressure of O_2 and CO_2 during compression. When MacI was in use the Krebs' solution was bubbled continuously with the gas mixtures shown in Table 2.1. At each depth the calculated partial pressures of O_2 and CO_2 were 0.95 bar and 0.05 bar respectively and were equivalent to conditions at atmospheric pressure.

It was not possible to measure the dissolved oxygen content of the Krebs' solution in these experiments. The possibility that the response observed on compression was due to hypoxia is considered in a later section.

However, attempts were made to mueasure the pH of the bathing fluid, and the difficulties of pH measurement at pressure, and of the interpretation of the results are considered in Appendix C.

The effect of pressure on the pH of Krebs' solution will depend on two factors:

a. The effect of pressure on the dissociation of the buffer components.
b. The effect of pressure on the CO_2 dissolved in the bathing fluid.

a. The effect of pressure on the ionisation of dilute salt solutions and the dissociation of weak acids is of major interest to physical chemists and oceanographers (see Sleigh and MacDonald, 1972). The effects of very high pressure on dilute solutions have been summarised by Hills (1972). The effect of pressure in the physiological range (< 70 bar) is less well documented. Disteche (1959, 1972) has made a particular study of the effect of pressure on the dissociation of weak acids. Using specially constructed pressure compensated pH electrodes at pressures up to 1000 ata, Disteche was able to show that a number of theoretical predictions about the effect of pressure on aqueous solutions are valid. For example, it was known that water is relatively incompressible,

so that at 1000 ata dilute salt solutions undergo an increase in concentration of only 4%. However, an equilibrium process such as the dissociation or ionisation of a solute is governed by a dissociation constant (K) which is pressure dependent according to the relationship

$$RT\left(\frac{\delta \log K}{\delta P}\right)_T = -\Delta V^o$$

where R is the gas constant

T is the absolute temperature (^oK)

P is the total pressure (ata)

ΔV^o is the partial molar volume of dissociation ($cm^3\ mole^{-1}$) when

$$\Delta \bar{V}^o = V_i^o - \Sigma_j \bar{V}_j^o$$

and

V_i^o and V_j^o are the partial molar volumes of the undissociated and dissociated components, respectively.

High pressure favours the dissociation of non-electrolytes and the dissolution of all electrolytes in water since these processes occur with a decrease in partial molar volume, ie

$$\bar{V}_j^o < \bar{V}_i^o$$

This volume decrease is due to the "electrostriction" or tighter packing of water around the ionised solutes which lowers the free energy of dissociation (see Horne, 1969) and leads to an increase in the conductance, density and $[H^+]$ of dilute solutions at high pressure.

The extent to which pressure affects the dissociation of any species in aqueous solution depends on the direction and magnitude of the molecular volume change which occurs during dissociation.

Krebs' solution is a phosphate/bicarbonate buffer and is a poor choice for investigations into the effect of pressure, since the partial molar volume changes are large (HCO_3^- : -25.4; $CO_3^=$ -25.6 $cm^3\ mole^{-1}$. Phosphate, K_i - 15.7; phosphate, K_2 = - 24.0 $cm^3\ mole^{-1}$) (see Disteche, 1972 for sources). Thus, the pH of a phosphate/bicarbonate buffer decreases as pressure is increased.

The values given above were obtained at 22°C and 1 ata using pure solutions of the salts. The presence of other ions tends to complicate the result. For example, the equilibrium

$$CaCO_3 \rightleftharpoons Ca^{2+} + CO_3^{=}$$

is shifted to the right by increased pressure and this tends to mask the enhanced dissociation of HCO_3^- (Disteche, 1972). Such effects make the magnitude of pH change in complex solutions such as sea water or Krebs' solution difficult to predict. However, the change in pH of Krebs' solution has been measured by Kendig (1975) using a "modified glass electrode". The pH fell from 7.4 at 1 ata to 7.2 at 20.5 ata, a change which Kendig considered to be without significant effect on rat superior cervical ganglion.

In their investigations into the effect of pressure on Helix neurons, Wann and co-workers used Tris and Hepes buffers. These amines undergo a small positive volume change on dissociation (Tris $\Delta\bar{V}^o$ = + 2.5 cm^3 mole, Disteche, 1972) and their aqueous solutions become slightly more alkaline at high pressure. Because of the small molecular volume change these buffers are less sensitive to pressure than conventional buffers. However, these may not be suitable for use with smooth muscle preparations (see Appendix A).

b. When the salts which comprise Krebs' solution are dissolved in distilled water the pH is 8.3. Physiological pH is 7.4 and this is achieved by equilibrating the aqueous solution with CO_2 at a partial pressure of 0.05 bar. According to Henry's Law the solubility (s) of a gas in solution is

$$s = K \alpha P$$

where K is a constant.

α is Bunsen's coefficient of solubility.

P is the partial pressure of the gas.

Note that α is dependent on the prevailing temperature and pressure.

Henry's Law is an example of an ideal gas relationship. This means that when it is applied at low pressures and therefore dilute solutions of a real gas, it gives a good approximation to the actual behaviour of the gas. However, at high pressure the term P

should be replaced by the fugacity (f), or better still, the activity (a) of the gas, since the individual molecules can no longer be considered to be independent of one another. The extent to which each gas deviates from ideal behaviour is characteristic of the gas itself and depends on the absolute temperature and the total pressure.

At 100 bar the fugacity of O_2 is only 1% less than the partial pressure (Glasstone, 1956) and this is unlikely to affect significantly the inherent tone of tracheal smooth muscle. Similarly, the fugacity of He is only 1% greater than the partial pressure at a total pressure of 100 bar, so that the estimation of the He pressure in Table 4.1 is not seriously at fault.

However, CO_2 departs more noticeably from the ideal gas laws, particularly at pressures less than 100 bar, as shown in Table 4.1.

The values in Table 4.1 were estimated from the critical pressure (P_c) and critical temperature (T_c) of CO_2 obtained from International Critical Tables and the values of f/p determined from the generalised fugacity curves (Glasstone, 1956).

P_c = 72.9 ata

T_c = 304.1 ^oK

reduced pressure (π)

$$\pi = \frac{\text{total pressure}}{P_c}$$

reduced temperature Θ

$$\Theta = \frac{\text{ambient temperature}}{T_c}$$

Total pressure (ata)	π	Θ	f/P	P	f
13	0.18	1.02	0.9	0.05	0.045
31	0.42	1.02	0.8	0.05	0.040
43	0.59	1.02	0.7	0.05	0.035
67	0.92	1.02	0.6	0.05	0.030

TABLE 4.1

Thus, at each pressure employed in this study the fugacity of CO_2 was significantly less than its partial pressure and would result in the pH of the Krebs' solution becoming more alkaline.

Thus, two effects appear to be occurring simultaneously: there is an increased dissociation of carbonate, bicarbonate and phosphate which tends to increase the $[H^+]$. However, the divergence of CO_2 from the ideal gas laws suggests that fCO_2 is less than PCO_2 and this will tend to result in alkalinisation of Krebs' solution. Measurements of pH at 31 and 43 bar revealed an alkaline shift of 0.2 and 0.4 pH units respectively. It has been demonstrated (Appendix C) that inherent tone is relatively insensitive to pH in the alkaline range and it is unlikely, therefore, that the response to compression is a result of the pH changes recorded.

A comparison of the response to compression with the response to hypoxia.

Both hypoxia and high pressure cause a reversible reduction of inherent tone of similar magnitude. PO_2 was not measured in this study. However, a number of experimental results indicate that the compression-relaxation is not a result of hypoxia.

Firstly, two methods of compression were employed. When using MacI, O_2 along with CO_2 and He was bubbled through the bathing fluid, whereas, with MacII O_2 was only in contact with the surface of the bathing fluid. Although this might be expected to lead to differing states of oxygenation, no significant difference in the response to compression was recorded.

Secondly, the time course of the response to compression was more rapid in onset than the response to hypoxia.

Thirdly, the response to hypoxia was slightly enhanced by increasing the $[Ca^{2+}]_o$. The response to compression to 43 bar was significantly reduced by raising the $[Ca^{2+}]_o$.

Fourthly, during hypoxia the relaxation response to field stimulation was reduced. When the response was expressed as a proportion of the tone recorded immediately prior to the stimulation (R/T(%)), this value was also reduced. During compression to 31, 43 and 67 bar, the response to field stimulation was reduced. However, in this case the value of R/T(%) was not reduced.

From a consideration of the experimental results and some theoretical predictions of the effect of pressure on dilute solutions it is unlikely that the results obtained were due to the choice of gas mixtures used for compression.

iv. Toxic effect of He

He is a diluent gas in breathing mixtures employed in saturation diving, but despite a suggestion in 1939 that He might also be useful in submarine escape (see p. 27), this has never been pursued, probably due to the lack of storage space. He is also used at 1 bar in the diagnosis and treatment of respiratory disorders. In each case advantage is taken of the physical properties of He - e.g. its poor solubility and low density; in addition, He is regarded as an inert gas.

However, several studies indicate that even at 1 bar, He can exert pharmacological effects (Raymond, Sode, Langworthy, Blosser and Johnsonbaugh, 1974; Raymond, Weiskopf, Halsey, Goldfien, Eger, Severinghaus, 1972). It has been shown that bardycardia due either to immersion or to 'dry' compression is exacerbated by the use of He as a breathing gas (Hong, Moore, Lally and Morlock, 1973; Raymond et al, 1972) and that arrhythmias in isolated hearts made hypoxic by coronary ligation could be counteracted by the presence of as little as 0.2 bar He (Pifarre, Cox, Jasuja, and Neville, 1969; Toltzis and Scott, 1972). In one study (Raymond et al, 1972) on dogs, the bradycardia due to He was accompanied by a reduction in the plasma concentrations of adrenaline and noradrenaline in the peripheral venous blood. Thus, He was thought to exert its effect via a reduction in the sympathetic control of the heart. This was followed (Raymond et al, 1974) by a study on human subjects of the effect of He breathing on plasma growth hormone (GH) concentrations. In this study the plasma concentration of GH was higher after exercise and raised further by He breathing. From this Raymond concluded that He exerted its effect via a central depression of sympathetic output. Changes in the excretory pattern of catecholamine derivatives in human subjects in a pressure chamber at 3 bar have also been recorded (Hempleman, 1979). Similar increases in GH were noted by Raynand, Varene and Durand (1980).

Nevertheless, the toxicity of He at 1 bar has not been confirmed by all workers (see for example Holland, Wolfe and Kylstra, 1973). Ornhagen (1977) using mouse isolated sinus mode preparations could show no significant effect of He up to 140 bar on the bradycardia induced by compression. However, Ornhagen also noted that none of the preparations exposed to He showed any arrhythmia, while several preparations compressed with N_2 gas or compressed hydrostatically did. Enclosing the tracheal

chain preparation in a chamber of He (at 1 bar) had no effect on inherent tone, or on its loss due to the removal of 95% O_2: 5% CO_2 (p. 115). Furthermore, simultaneous bubbling of He and 0.95 bar $O_2$0.05 bar CO_2 had no effect on inherent tone over a three hour period. Additional support for the view that He is not exerting a toxic effect on tracheal smooth muscle can be obtained from the observation that the method of compression (either in MacI or MacII) had no effect on the response to pressure. Since He is poorly soluble in aqueous solution it is likely that these two methods would lead to significantly different quantities of He being dissolved in the bathing fluid.

Hydrostatic compression could not be accomplished with this preparation. However, the response to He was compared with the response to compression with Ar, another "inert" gas. Argon is known to have more toxic effects than He. It inhibits glycolysis in mouse isolated tissue slices (South and Cook, 1950) and is an anaesthetic at pressures greater than 100 bar. The response to He compression was not significantly different from that recorded during Ar compression.

From the experimental results discussed above it does not seem likely that the response to compression is due to the use of He as the compression medium. However, anomalous results have been obtained in other isolated tissue preparations and in human studies and these effects should be systematically investigated.

v. Changes in transmitter release

Neither atropine nor propranolol have any effect on inherent tone of tracheal smooth muscle and it is therefore unlikely that spontaneous release of Ach or noradrenaline contributes to the normal control of inherent tone. Similarly, neither atropine nor propranolol had a significant effect on the response of tracheal chain after compression to 31 and 43 bar. It is concluded that neither an increased release of noradrenaline nor a decreased release of Ach could account for the relaxation recorded immediately after compression. Quinidine, a proposed purinergic receptor blocker, also had no effect on the response to compression. However, it has been shown that quinidine is not a specific ATP antagonist and an increased release of ATP or other putative purinergic transmitter cannot be excluded.

The presence of TTX had no effect on the response of tracheal chains to compression. Spontaneous firing of nerve action potentials has been recorded (Kendig, Schneider and Cohen, 1978) and TTX should abolish any such effect. Finally, there is no evidence to suggest that evoked release of neurotransmitter from tracheal chain is altered by He pressure up to 67 bar. Although the absolute magnitude of the response to field stimulation was reduced, this was accompanied by a reduction in inherent tone and R/T(%) was unaffected.

Other studies have recorded significant changes in neurotransmitter release or evidence of altered synaptic transmission in response to high pressure (see Wann and MacDonald, 1980). For example, Campenot (1975) reported that hydrostatic pressure (50-200 atm) reduced the amplitude of excitatory junction potentials at crustacean neuromuscular junctions, which he attributed to a reduction in quantal release. Similarly, Kendig (1975) found that at 69 ata and above, He pressure reversibly depressed excitatory synaptic transmission in rat superior cervical ganglia. Excitatory post synaptic potentials were slowed and synaptic fatigue was enhanced in squid giant synapse by as little as 35 ata He pressure (Henderson and Lowenhaupt, 1977). Wann (1979) demonstrated that both excitatory and inhibitory synaptic transmission was depressed by hydrostatic compression of Helix neurones.

Although the studies described above all suggest that synaptic transmission is altered by pressure in the physiological range, they cannot identify the site at which pressure is acting. It is not clear if the presynaptic release of the neurotransmitter is reduced, or if the transduction of the stimulus by the post-synaptic membrane is altered. Indeed, Campenot (1975) recorded that the input conductance of the crustacean muscle cell was reduced by hydrostatic compression. However, this was not confirmed in Helix neurones by Wann, MacDonald and Harper (1979), where a non-specific increase in membrane conductance was recorded over the range 50-300 atm. Some studies indicate that receptor-agonist interactions are slowed by high pressure (Akers and Carlsen, 1972; Athey and Akers, 1978) and this would also complicate the interpretation of data obtained by conventional electrophysiological techniques.

Recently, two studies have attempted to measure transmitter release by less ambiguous techniques. Ashford, MacDonald and Wann

(1979) measured the frequency of spontaneous miniature end plate currents (mepc) at pressures up to and including 50 atm. Mepc frequency was reduced significantly and at higher pressures (100-150 atm) the decay time of the mepc was prolonged, showing that both pre and post synaptic events could be influenced by pressure.

There is only one published study which records the effect of pressure on the release of neurotransmitter. Little and Paton (1980) collected the superfusate from guinea pig ileum which had been exposed to high He pressure. Spontaneous Ach release was increased by 20% at 136 atm, but was not significantly altered at 68 atm. Evoked release of Ach was variable and was not significantly altered by 136 atm.

There is no evidence to suggest that spontaneous or evoked transmitter release from tracheal chain is affected by He pressure in the range 13-67 bar, or that such changes are responsible for the relaxation observed on compression. The results suggest that pressure is acting directly on the muscle cell. Similar conclusions were reached by Miki (1960) who studied frog intestinal muscle, and Ebbecke (1914) in studies on frog skeletal muscle.

vi. Direct effect on $[Ca^{2+}]_i$

The evidence discussed thus far suggests that the relaxation of tracheal smooth muscle which follows compression is due to a direct effect of pressure on smooth muscle. A review of the literature reveals that all cells contain multiple sites which might be susceptible to pressure, so that the relaxation reported here may be the net result of several opposing influences. This is because all cellular reactions occur with a molecular volume increase or decrease and pressure retards the former but favours the latter, (see also, Zimmerman, 1970; MacDonald and Sleigh, 1972 and MacDonald, 1975). Pressure acts in a relatively non-specific way and discussion of the numerous known (and unknown) variables is unlikely to be profitable. However, it has been shown that the effect of pressure on inherent tone is ameliorated by raising $[Ca^{2+}]_o$ and furthermore, that raising intracellular Ca^{2+} by the use of drugs has no significant effect on the response to compression. Thus, the pressures studied here appear to be exerting a relatively specific effect on inherent tone which is likely to be mediated via the cell membrane.

It has been shown that both an increase and decrease in $[Ca^{2+}]_i$ can accompany relaxation of smooth muscle (p. 156). The experimental evidence reviewed on p. 197 indicates that the responses to hypoxia (accompanied by an increase in intracellular Ca) and to compression are not alike. Hence, it is possible to conclude that compression results in a reduction in $[Ca^{2+}]_i$.

The $[Ca^{2+}]_i$ is controlled by three major mechanisms: Ca^{2+} influx from the extracellular fluid, whether passive or modulated by prostaglandins; active Ca^{2+} extrusion at the cell membrane and active Ca^{2+} uptake into intracellular organelles.

A decrease in $[Ca^{2+}]_i$ could be accounted for by one or both of the following: a decrease in Ca^{2+} influx or an increase in the activity of the Ca^{2+} pump, either at the level of the cell membrane or intracellular membranes. In addition, it has been proposed recently that passive binding of Ca^{2+} to cellular membranes may play an important role in the control of $[Ca^{2+}]_i$ and hence in the control of inherent smooth muscle tone (Kolber and Van Breeman, 1981). An increase in passive binding of Ca^{2+} to cell membranes would also result in a reduction $[Ca^{2+}]_i$.

Each of the Ca^{2+} control mechanisms outlined above have one feature in common - they are all located in the cell membrane. The cell membrane is a principal target for the effects of high pressure. Firstly, it is one of the most important organelles which control cellular function since it maintains ion gradients, contains protein receptors linking extracellular events to intracellular responses and physically protects the cell from damage. Secondly, according to the critical volume hypothesis, pressure antagonises the actions of local anaesthetics by reversing the expansion and fluidisation of membranes caused by these diverse agents. Thirdly, a variety of cell membranes can be purified, eg erythrocyte ghosts, sarcoplasmic reticulum fragments and artificial or model membranes may be made from phosphlipids in the form of black lipid films, bilayers and liposomes which provide a simplified system on which to test the predicted effects of high pressure (and anaesthetics).

Pressure has four major effects on artificial membranes, it reduces the fluidity, decreases the surface area and increases the surface charge density (Trudell, Hubel and Cohen, 1973). Furtheremore, pressure raises the transition temperature (T_t) ie the temperature at

which the membrane changes from a gel to a liquid crystalline state (MacDonald, 1978). Conversely, anaesthetics fluidise membranes, increase their surface area, decrease the surface charge density and lower T_t.

It has been proposed that these effects are of particular significance to annular lipid, ie the lipid in the immediate vicinity of membrane proteins, whether they are receptors, ion channels, pump proteins or membrane bound enzymes (A.G. Lee, 1976).

Thus, pressure could alter Ca^{2+} permeability Ca^{2+} extrusion and/or Ca^{2+} binding, all of which could lead to a reduction in inherent tone.

Cation permeability is reduced by He at high pressure: an hypothesis

I am unaware of any studies in which Ca^{2+} flux has been measured at high pressure. However, several workers have investigated the effect of pressure on the permeability of cell membranes and lipid bilayers to monovalent cations. Brouha, Pequeux, Schoffeniels and Disteche (1970) showed that the potential difference recorded between the external and internal surfaces of frog skin, mounted in an Ussing chamber, was increased by compression to 100 atm. They proposed that hydrostatic pressure had increased the permeability of the frog skin to Na^+ ions. (Okada, 1954) reported that pressure of up to 1000 atm increased electrical conductivity of erythrocytes and bladder tissue. Hall (1981) showed that passive Na^+ influx into erythrocytes was increased by pressures as low as 30 atm, and this effect was enhanced by low $[Ca^{2+}]_i$. By contrast, studies with liposomes containing valinomycin, a non-specific monovalent cation ionophore, have indicated that cation permeability is reduced by high pressure up to 300 atm (Johnson, Miller and Bangham, 1973).

In view of reports of both increases and decreases in cation permeability in membrane fragments and model membranes it is possible that pressure could reduce the permeability of the tracheal smooth muscle membrane to Ca^{2+}. Although direct evidence for this view is lacking, the hypothesis is supported by the evidence that raising $[Ca^{2+}]_o$, hence increasing the inwardly directed Ca^{2+} gradient, appears to counteract the effect of pressure. Reducing $[Ca^{2+}]_o$ has a variable effect on the response to pressure. At atmospheric pressure increasing $[Ca^{2+}]_o$ above 2.4mM has no significant effect on inherent tone. This suggests that Ca^{2+} uptake and Ca^{2+} extrusion are increased to negate

the effect of raised Ca^{2+} influx, or that at $[Ca^{2+}]_o$ of 2.4 mM, the membrane sites responsible for Ca^{2+} permeability are maximally activated. If the latter were true it would be possible to decrease Ca^{2+} permeability (and hence inherent tone) but not to increase it.

It has been shown that at least 50% of inherent is mediated by prostaglandin synthesis since 50% of inherent tone is abolished by hypoxia, and about 70% of inherent tone is lost during indomethacin treatment. The remaining 30-50% of inherent tone may be due to passive Ca^{2+} permeability. It is interesting to note that a tracheal chain loses 20-30% of inherent tone following compression and that this response does not increase linearly with pressure. The mean relaxation recorded levels out at 43-67 bar. This could indicate that passive Ca^{2+} influx is particularly susceptible to pressure in the range 13-67 bar, but that other mechanisms are unaffected. This proposal could be tested easily by compression of indomethacin treated tracheal chains.

A feature of the response to compression, noted earlier, was that the response to compression was very variable. It is possible that the contribution of passive permeability to the inherent tone varies from one preparation to another. If pressure was acting solely on the passive Ca^{2+} influx, the response to pressure would be similarly variable. It is not clear why passive Ca^{2+} influx should vary from one preparation to another, but evidence to suggest that it does can be found in Tables 3.1 and 3.2, from which it can be seen that changing Ca^{2+}_o alters the inherent tone of some tracheal chains more than others.

The activity of Ca^{2+} pump may be enhanced by He pressure

There is only one report of the effects of high pressure on Mg^{2+} dependent Ca^{2+} ATPase. Heremans and Wuytack (1980) demonstrated a significant decrease in specific activity of Ca^{2+} ATPase at pressures greater than 200 bar. Na^+/K^+ ATPase has been studied more thoroughly at high pressure. Gershfield and Shanes (1958) reported that compression of toad sciatic nerve to 167 and 670 atm resulted in Na^+ loading and K^+ depletion but this result may have been due to hypoxia. However, Pequeux (1972) demonstrated that hydrostatic compression to 500 atm inhibited Na^+/K^+ ATPase activity in isolated gills from sea water adapted eels under normoxic conditions. Also, Hall (1981) reported that the Na^+ pump in human erythrocytes was inactivated at high pressure, but that prolonged incubation at pressure accompanied by Na^+ loading led to reactivation. Hall has

reviewed the proposed mechanism of the Na^+ pump and the reported effects of high pressure on the enzyme and proposed that pressure reduced the affinity of the enzyme for Na^+ and K^+.

It is possible that high pressure causes a decrease in the fluidity of the annular lipid around the Ca^{2+} pump initiating a conformational change in the pump protein, but no evidence can be found in the literature to support the view that pressure either accelerates the pump, or increases the affinity of the pump for Ca^{2+}. In either case one might expect that the recovery of inherent tone following contraction would be accelerated and the recovery from relaxation to be prolonged. In fact, recovery from relaxation was prolonged in the present experiments and was sometimes incomplete: this effect was most marked at 67 bar. This result could arise from other effects of pressure, such as prolonged receptor-agonist interactions (Akers and Carlsen, 1972) or delayed removal or inactivation of neurotransmitters from the synaptic cleft. Thus, this observation is not adequate support for the view that the Ca^{2+} pump is accelerated by high pressure.

Furthermore, this hypothesis is not supported by the results of experiments with local anaesthetic agents, which might be expected to exert opposite effects to pressure on a membrane process. Propranolol used in this study primarily for its β-adrenoreceptor antagonist action, also has local anaesthetic properties but had no effect on the response of tracheal chain to pressure. Similarly, quinidine at a dose which was almost certainly exerting local anaesthetic effects, did not alter the response to compression. Furthermore, although raising $[Ca^{2+}]_i$ might be expected to induce an increase in Ca^{2+} pump activity, it is difficult to see how raising $[Ca^{2+}]_o$ could reverse the effect of pressure if pressure were acting solely to increase the activity of the pump.

The feasibility that He pressure increases passive Ca^{2+} binding to cell membranes

Kolber et al (1981) have suggested that changes in passive cation binding could account for the effect of some drugs and ions on smooth muscle tone. Cell membranes are known to have an excess of negative charge on their surface due to the presence of hydrophillic phospholipid head groups, and glycoproteins which bear a net negative change at physiological pH. This is particularly well documented for erythrocytes (Passow, 1964), but has been demonstrated in unicellular organisms such as tetrahymena (e.g. Murakami and

Zimmerman, 1970) and for sarcoplasmic reticulum (Chiu, et al, 1980) by their electrophoretic mobility. Kolber et al (using data derived from taenia coli) demonstrated that increasing $[Na^+]_i$ caused an increase in smooth muscle tone and stated that this could be accounted for by a displacement of Ca^{2+} bound previously to the inside of the cell membrane, without causing an alteration in Ca^{2+} flux or in total cell Ca^{2+} content. Other monovalent cations had a similar effect. In addition, they demonstrated that raising the $[Ca^{2+}]_i$ raised the threshold concentration of Na^+ required to produce this effect. Van Breeman et al have argued that the cell membrane (particularly its intracellular surface) has been neglected as a potential Ca^{2+} pool. It is clear that where drugs (e.g. ouabain) alter $[Na^+_i]$ they may render this pool more labile.

Kolber's hypothesis predicts that positively charged drugs which can bind to the membrane should have a similar effect. Okumura and Denborough (1980) reported the effects of a variety of intravenous and local anaesthetics on tracheal smooth muscle. Procaine and lignocaine, both positively charged, evoked contracture, but the concentrations used were very high (0.1-1 mM). Thiopentone caused contracture at low doses, while diazepam which has no net charge at physiological pH, caused a relaxation at concentrations in the μM range.

An analogy may be drawn here with the erythrocyte which Sheetz and Singer (1974) used as the basis for their bilayer couple theory of membrane function. They proposed that membrane proteins and phospholipids were distributed assymetrically in the two halves of membrane bilayers and that these two halves could respond differently to a perturbation, such as the introduction of a drug molecule. They based this theory on some interesting experimental results which showed that positively charged drugs which could enter the cell, e.g. chlorpromazine produced a change in shape of the erythrocyte which they termed "cupping". Sheetz and Singer proposed that once inside the cell chlorpromazine was attracted to the inside of the membrane surface which bore a net negative charge. When bound to this half of the bilayer, chlorpromazine caused assymetrical fluidisation and expansion of the bilayer so that the inside of the membrane had a greater surface area than the outside - hence the cupping effect. Intact erythrocytes were less permeable to methochlorpromazine, a quaternary amine analogue of chlorpromazine. Interestingly, this

drug caused crenation of intact erythrocytes, but cupping of unsealed erythrocyte ghosts. Sheetz and Singer estimated that these effects could be produced by a differential expansion of one half of the bilayer by as little as 1%.

Experimental evidence in widely diverse tissues suggests that membrane surface charge may be important to the normal function of the cell. It has been reported that the surface charge density of phospholipidbilayers is increased by high pressure. To my knowledge there is no report of similar measurements on biological membranes. Van Breeman et al have suggested that the negative charge on the inner surface of the smooth muscle cell membranes binds Ca^{2+} and that this Ca^{2+} is displaced by high concentrations of monovalent cations. It may be proposed that high pressure increases the surface charge density leading to an increase in passively bound Ca^{2+} and that this in turn leads to a decrease in inherent tone.

Increased membrane bound Ca^{2+} can stabilise phospholipid bilayers and raise T_t (Trauble and Eibl, 1974). It is interesting that crenated erythrocytes have been identified in the blood of divers after a stay at 31 and 43 bars (Carlyle, Rowles, Nicholls and Paciorek, 1979) and this perhaps indicates that the inner surface of the erythrocyte has bound Ca^{2+} irreversibly, causing permanent shape changes in some cells. It should be noted, that an increase in permeability of the red cell to Ca^{2+} could also produce this effect, since treatment with ionophores results in identical shape changes

Although this hypothesis is attractive (not least because it could be tested experimentally by measuring electrophoretic mobility of cells or membrane fragments at high pressure) it raises several problems. Firstly, if passive Ca^{2+} binding causes a reduction in inherent tone, why does the inherent tone not recover spontaneously during the compression phase, as the passive Ca^{2+} influx restores $[Ca^{2+}]_i$? Secondly, why does raising $[Ca^{2+}]_o$ oppose an effect which is apparently occurring on the cytoplasmic side of the membrane?

It may be proposed that by increasing the amount of Ca^{2+} bound to the membrane that the Ca^{2+} gradient "seen" by the passive Ca^{2+} channel is reduced, so that this has an indirect effect on passive Ca^{2+} permeability. This would then be counteracted by raising $[Ca^{2+}]_o$ and restoring both passive Ca^{2+} influx and $[Ca^{2+}]_i$.

Other authors have reported that changes in Ca^{2+} permeability and/or Ca^{2+} binding could account for the effect of pressure on a variety of preparations. For example, Ponat (cited by MacDonald, 1975) reported that Mytilus gill tissue exhibited increased "pressure tolerance" when bathed in Ca^{2+} rich sea water. Furthermore, Murakami and Zimmerman (1970) demonstrated that Tetrahymena, which usually moves towards the cathode when suspended in an electric field, tended to migrate towards the anode at high pressure. This "galvanotaxis" is thought to be related to Ca^{2+} permeability. Harper (1978) showed that many of the effects of hydrostatic pressure on helix neurones could be duplicated by bathing the ganglia in media containing low $[Ca^{2+}]_o$. Finally, Hall (1981) showed that Na^+ loading of erythrocytes at high pressure was enhanced by incubation of the cells in medium containing low $[Ca^{2+}]_o$.

The experiments reported here support the view that pressure in the range 13-67 bar can alter $[Ca^{2+}]_i$ in smooth muscle. However, the observations are unable to distinguish which of the control mechanisms is responsible for the change. It seems unlikely that pressure is causing an activation of the Ca^{2+} pump since at pressures only slightly in excess of those used here activity of both the Ca^{2+} pump and the Na^+ pump are reduced. Circumstantial evidence suggests that Ca^{2+} permeability is reduced by high pressure and this may be due to an effect of pressure on membrane surface charge density. Passive Ca^{2+} binding to the cell membrane may also be increased.

Ca^{2+} plays an essential and universal role in the control of cell function and hence an understanding of the mechanism of the effect of pressure on $[Ca^{2+}]_i$ may be fundamental to an understanding of other responses to pressure, such as dividing bradycardia, spontaneous contracture of skeletal muscle, changes in transmitter release and the more complex phenomenon known as HPNS.

A range of techniques are available for determining Ca^{2+} flux and intracellular Ca^{2+} concentrations. Passive Ca^{2+} binding can also be determined using fluorescent indicators. Finally, the activity of the Ca^{2+} pump can be measured spectrophotometrically. The application of pressure to these techniques would be a challenge but should yield valuable data and increase our understanding of pressure phenomena generally.

Although in this discussion I have placed considerable emphasis on the possible role of Ca^{++} in the pressure-induced relaxation, there are

many other target sites which lie between the stimulus (pressure) and the effect (muscle lengthening). Since all molecular reactions are accompanied by a volume change, most, if not all, aspects of cell metabolism and excitation-contraction coupling will be affected by pressure. For example, the viscosity of both undifferentiated and differentiated cytoplasm is reduced by pressures of only 67 ata (MacDonald, 1975). This may be considered an important component of muscle elasticity and hence contractility.

Actin, a contractile protein, undergoes an increase in volume during the transition from globular to filamentous in the presence of Mg.ATP. Thus Actin would be depolymerised by high pressure (probably in excess of 100 ata) and this could lead to a reduced efficiency in the contractile apparatus (Ikkai and Ooi, 1966). Finally, although detailed biochemical studies at high pressure are scarce, enzymes from the glycolytic and gluconeogenic pathways have been studied in both shallow water and deep sea adapted fishes (Mustafa, Moon and Hochachka, 1971). These studies revealed that the velocity of some enzymic reactions is increased by pressure, while that of others is reduced. For example, the activity of muscle pyruvate kinase was reduced by pressures from 200-700 ata, while that of liver Fructose 1,6-diphosphatase (FDP-ase) was doubled at 533 ata. These and other aspects of cellular metabolism at high pressure have been reviewed by MacDonald (1975).

It has been the cherished hope of many physiologists that pressure might exert specific and quantifiable effects on cellular processes, and thus prove valuable as a research tool. This hope is unlikely to be fulfilled since pressure acts at many cellular sites. However, in an attempt to understand the effects of pressure on tracheal smooth muscle tone, this study has been extended to cover aspects of tracheal pharmacology which were unclear or not reported. Thus, indirectly the study of the effect of pressure on tracheal smooth muscle has increased our understanding of the mechanisms by which inherent tone is initiated and maintained and has highlighted, in particular, the importance of membrane surface charge density to the control of cell function.

D. The significance of the findings of the study to the patho-physiology of submarine escape and saturation diving

It has been shown that guinea pig tracheal smooth muscle undergoes a reversible relaxation at depths which are typical of submarine escape and saturation diving. As a result of this finding, several questions may be asked:

a. What are the implications of the result for the submarine escapee?

b. What are the implications of the result for the diver?

c. How might the direct effects of pressure on airway smooth muscle be overcome in the practical situation?

a. It was proposed originally that spontaneous contracture of airway smooth muscle, similar to that recorded in skeletal muscle (see Cattell, 1935) would lead to air embolism during rapid decompression. No evidence of such contracture has been obtained in this study. On the contrary airway smooth muscle from both the large conducting airways and the lung parenchyma underwent a relaxation which was reversed on decompression. However, there is no lack of evidence that pulmonary air embolism does occur during submarine escape training and that it can lead to dizziness, confusion, loss of consciousness and death following ascent from as little as 9 m (Liebow, Stark, Vogel and Schaeffer, 1959; Greene, 1978).

Pulmonary air embolism has been provoked in animal experiments by decompressing dogs (Schaeffer, McNulty, Carey and Liebow, 1958), guinea pigs (Denney and Glas, 1964) and rabbits (Malhotra and Wright, 1960), whose trachea had been ligated surgically. Such extreme measures are only comparable with voluntary breathholding in human trainees and there is no evidence to suggest this in any of the British or Amercian case studies (see Greene, 1978 for review). Thus, it seems that air trapping does occur during submarine escape but on a smaller scale than in animal experiments and not as a result of spontaneous airway contracture.

It was a basic assumption of the original hypothesis that an increase in airway smooth muscle would lead to air trapping. However, some authors consider that one role of airway smooth muscle tone is to resist closure of the airway (Macklem, 1971; Knudson and Knudson, 1975). If this is true, then a reduction of inherent tone might exacerbate the tendency for airways to close.

Knudson et al (1975) demonstrated that canulated isolated trachea (from dogs) collapsed at a critical transmural pressure, but that this pressure was significantly greater following the administration of Ach. The transmural pressure is analagous to transpulmonary pressure of the intact lung which is the sum of the negative pressure tending to keep the airways inflated, the elastic recoil of the lung tending to close the airways and the mechanical influences of surrounding airways, alveoli and capillaries. Macklem (1971, 1975) argues that bronchioles are remarkably stable and that airways of 1mm diameter will remain open when the transpulmonary pressure is zero for two reasons: firstly, because of respiratory "tone" (not defined) and secondly, because of the low surface tension due to the presence of surfactant. Thus, in the normal healthy adult the majority of airways are inflated and only after maximal expiration (ie at residual volume) will there be a significant number of closed airways.

The reduction in inherent tone recorded here could contribute to air trapping in the human lung by reducing the ability of the airways to resist closure, ie by increasing the compliance of the airways at a given transpulmonary pressure. Support for this view may be obtained by considering the results of animal experiments. Both Schaeffer et al (1958) and Malhotra et al (1960) recorded the transpulmonary pressure during rapid decompression of animals with obstructed air flow. Malhotra and Wright noted that the pressure at which lung rupture appeared to occur was higher in their experiments than in those of Liebow et al. Although this may be a species difference Malhotra and Wright aruged that respiratory tonus was higher in their unanaesthetised rabbits, than in the anaesthetised dogs in the study by Liebow. They proposed that increased compliance of the respiratory tract during anaesthesia reduced the critical inflation pressure required to rupture the lung. Malhotra and Wright demonstrated that by strapping the thorax of the rabbits the critical transpulmonary pressure was raised, and this, they stated, was due to a further decrease in the compliance of the lung. Liebow et al also demonstrated that chest strapping could raise the transpulmonary pressure sustained before lung rupture.

It was noted earlier that inherent tone may be only a small factor in respiratory "tonus", and hence the effect of compression on the intact lung may be very small. However, a number of other

factors, summarised in the Introduction, also lead to air trapping during submarine escape. Of these contributory factors is immersion itself, which causes the displacement of blood from the legs into the abdomen and thorax (see Dahlback, 1978). The presence of engorged capillaries close to small airways increases residual volume, ie the volume of air trapped after airway closure. If those airways are already more compliant due to a direct effect of pressure, the residual volume could be further enlarged.

An additional feature of submarine escape procedure, not discussed previously, may exacerbate the problem of air trapping in the intact human lung. The trainees are instructed to exhale as hard as they can all the way to the surface. However, the gas expands most rapidly during the last 10m of the ascent, when the pressure is halved. Before reaching 10m many recruits report that they have "run out of air", but that this sensation is soon replaced by the need to breathe out again as they approach the surface (see Liebow et al, 1958). It has been suggested that during forced exhalation the trainees are reaching residual volume and that the gas in their lungs is not expanding rapidly enough to keep the airways open. Once the airways are closed, the pressure required to re-open them may be greater than that required to rupture the alveolar membrane. Similar effects are recorded in patients with chronic asthma where following airway closure, emphysema may develop due to the relatively large negative pressure produced in the thorax by forced inhalation.

It is proposed that pressure has a direct effect on airway smooth muscle tone, leading to an increase in the compliance of the lung. This effect, although small in itself exacerbates the effects of other features of submarine escape procedure, which tend to cause air trapping and may lead to pulmonary air embolism during rapid decompression.

b. There have been many studies on the effects of real and simulated dives on respiratory performance of divers (see Symposia on Underwater Physiology 1-7, The Underwater Handbook, Schilling, Werts and Schandelmeier). One of the major problems is that of gas density, which is partly overcome by breathing He. However, in the most recent deep dives, N_2 has been re-introduced into the gas mixture to ameliorate the symptoms of HPNS. Although this has allowed greater

depths to be achieved, the capacity of the divers for work while at 47 bar appears to be impaired by inspiratory dyspnoea (Salzano, Camporesi, Stolp, Saltzman, Bell and Shelton, 1980). Thus, respiratory insufficiency may be a limiting factor preventing useful work at depths greater than 460m.

It is interesting to note that despite the divers reports of inspiratory insufficiency, they did not exceed the maximum voluntary ventilation recorded at atmospheric pressure, and the partial pressures of O_2 and CO_2 in the arterial blood samples were normal. Salzano et al suggest the "inappropriate breathlessness" experienced by the divers may be due to a form of mismatching between the peripheral chemo- and mechanoreceptors and the central responsiveness to these afferents. However, it can be argued that the breathlessness was not inappropriate since arterial concentrations of O_2 and CO_2 were maintained within normal values. Indeed one diver whose ventilation rate was less at depth than at atmospheric pressure, for a given work load, did show mild hypercapnia.

It is not clear if a reduction in inherent tone could contribute to the apparent mismatching of respiratory load to respiratory response reported by Salzano et al. Inspiratory dyspnoeais unlikely to be associated with an increase in compliance, or a decrease in airway resistance.

The results of a recent study of airway resistance at a simulated depth of 67 bar are not yet available. However, it seems that the techniques presently feasible in a hyperbaric chamber would be unable to detect the small change in resistance anticipated from the results of the present study (J. Florio, personal communication). Furthermore, there is no evidence to suggest that airways in intact humans are more prone to collapse at depth than at atmospheric pressure. Indeed, the reverse seems to be true since Matsuda, Hong, Nakayama, Arita, Lin, Claybaugh, Lundgren and Smith (1980) report that the expiratory reserve volume was increased during a saturation dive to 31 bar, suggesting that airways remain open at lower total lung volume. Furthermore, Ohta, Arita, Nakajama, Tamaya, Lundgren, Lin, Smith, Morin and Matsuda (1980) report that air trapping was not changed by compression to 31 bar.

Experiments on human divers during saturation dives to depths in excess of 300m have revealed no evidence of an increase in static

compliance or a reduction in airway resistance. This could be due to the relatively slow compression rate, allowing time for the tissues to adapt, or to reflex counterconstriction, which clearly cannot occur in the isolated tissue. Furthermore, the hyperexcitability of HPNS may have a greater influence at other levels of respiratory control, so that inherent tone of the airways is of negligible concern.

c. Submarine escape training accidents are rare, and the major problem seems to be the identification of individuals who are prone to such accidents. At present vitallograph measurements are made on every trainee and chest x-rays are obtained before he begins training. These"safety-nets"are clearly not catching all the trainees who are at risk. In view of the results of this study it can be proposed that the "at-risk-trainees" have a higher than normal residual volume either before or after immersion or both, and that this possibility should be investigated. Clearly the more airways which close during forced exhalation, the greater the risk of lung rupture during the rapid ascent. It would be impractical to train escapees not to exhale to residual volume, since the dangers of breatholding are far greater than those of over-exhaling. In addition, much of the success of present techniques lies in their simplicity.

However, it may be worthwhile to reconsider the question of chest strapping, since this reduces airway closure and increases maximum expiratory flow at low lung volumes (Sybrecht, Garrett and Anthonisen, 1975) and reduces air trapping due to immersion (Dahlback, 1978). Since the present study suggests that static airway compliance may be reduced by pressure per se then any measures which decrease airway compliance might be valuable.

It is worth noting that during SEIE ascents, the expansion of the chest may be partially restricted by the presence of gas expanding in the hood (which escapes at the waist level) and in the quilted suit, and these effects may be inadvertently mimicking the effect of chest strapping.

The conclusions of the study are:-

1. Inherent tone of guinea pig airway smooth muscle is reduced by high He pressure in the range 13-67 bar.
2. This may be due to a reduction in $[Ca^{2+}]_i$.
3. The decrease in tone could exacerbate other mechanisms leading to air trapping during submarine escape.
4. Present screening techniques are not adequate to determine if trainees at risk have a higher than normal volume of air trapped at residual volume particularly during immersion.
5. The decrease in inherent tone could contribute to the "inappropriate breathlessness" reported during saturation diving. However, there is no evidence to suggest that air trapping is increased during a stay at depth (without immersion) or that airway resistance is reduced.

APPENDIX A.

CHOICE OF PHYSIOLOGICAL BUFFER

It is conventional to bathe mammalian isolated tissue preparations in solutions of inorganic salts in similar concentrations to those found in plasma. Tracheal chain preparations are bathed most commonly in a modified Krebs' solution. Krebs' solution is based on a phosphate/bicarbonate buffer and its composition is shown in Table AA1. In order to achieve a pH of 7.4, this solution is bubbled with 0.05 bar, 0.95 bar O_2 at atmospheric pressure. At pressures greater than atmospheric, the solution must be bubbled with the same partial pressure of CO_2 and O_2 and this means that mixtures of different proportions of CO_2, O_2 and He must be available for each depth to be studied. In order to reduce the cost and complexity of this procedure alternative bathing solutions were sought, which could be bubbled with O_2 alone. A physiological buffer based on phosphate salts was tried and its composition is also shown in Table AA1.

A number of differences were noticed between preparations bathed in Krebs' solution and in phosphate buffer. Firstly, the inherent tone of the tracheal chain preparations bathed in phosphate buffer was only 20% of that recorded in Krebs' solution. Secondly, the threshold frequency required to initiate a response to field stimulation was higher in chains bathed in phosphate buffer than in those bathed in Krebs' solution and often chains bathed in phosphate buffer were completely inexcitable. Thirdly, chains bathed in Krebs' solution could sustain a higher load and recovered from stimulation more completely.

Other authors have noted discrepancies between the responses of isolated tissues in different "physiological" buffers. For example, Standford (1978) demonstrated that the response of arterial smooth muscle strips to hypoxia was determined by the use of phosphate or Krebs' solutions as bathing fluid.

It was concluded that Krebs' solution was preferable to phosphate buffer for two reasons:

1. Inherent tone recorded in Krebs' solution was higher than that in phosphate buffer.
2. Since other authors had used Krebs' solution to bathe tracheal chains their data provided a standard against which to compare the results of the present study.

It should be noted that Krebs' solution may not mimic true extracellular fluid particularly with regard to the ionised free Ca^{2+} concentration. The concentration of Ca^{2+} employed in Krebs' solution reflects total plasma Ca^{2+} content, some of which will be bound to plasma protein and membrane fragments. Thus the Ca^{2+} concentration used here and in other studies is probably higher than that found in extracellular fluid.

The use of phosphate/bicarbonate buffer in high pressure studies has been questioned by Disteche (1972) who pointed out that these salts undergo large volume changes during dissociation and that their buffering capcity will be altered by pressure. The use of Tris (Tris [hydroxymethyl] aminomethane) was considered since this compound is a well established physiological buffer which undergoes only a small molecular volume change during dissociation and is unlikely to be affected by the pressures used in this study. However, there is a growing body of evidence to suggest that Tris exerts pharmacological effects at atmospheric pressure.

For example, Tris has been shown to protect against oxygen toxicity (Hill and Ray, 1976) and to enhance spontaneous release of neurotransmitters (Gillespie, 1975). Most recently Altura, Carella and Altura (1980) have shown that 5mm Tris depressed the responses of arterial and venous smooth muscle to prostaglandins. This effect was reversed by replacing the tissues in Krebs' buffer. Since Tris is apparently not pharmacologically inert, it was decided not to employ it as a buffer in this study.

Composition	Krebs' (g/L)	Phosphate (g/L)
NaCl	5.54	8.00
KCl	0.35	0.20
Na_2HPO_4	-	1.15
NaH_2PO_4	0.16	0.20
$NaHCO_3$	2.10	-
$CaCl_2$	0.28	0.20
$MgCl_2$	0.29	0.10
Glucose	2.10	1.00
Bubbling gas	5% CO_2 95% O_2	O_2

TABLE AA1

APPENDIX B.

TEMPERATURE CONTROL AND MONITORING

Since many pressure studies have been complicated by changes in temperature during compression and decompression it was essential to avoid this problem in the present study. A constant temperature in the vicinity of the tissue was achieved by circulating water, at $37^{o}C$ and at 1 bar, in a stainless steel water jacket around the organ bath mounted inside the hyperbaric chamber. The temperature inside the organ bath was monitored using a thermistor.

A number of unidentified thermistors were tested. Each one was tested at pressures up to 69 bar and calibrated against a conventional mercury glass thermometer inserted into the closed circuit loop close to the water exit from the chamber. Each thermistor was found to be reliable up to this pressure.

The criteria for the final choice of thermistor were as follows:

a. Small size - the volume of the organ bath in MacI was only 10mls and clearly the less fluid displaced by apparatus placed in the bath, the better.

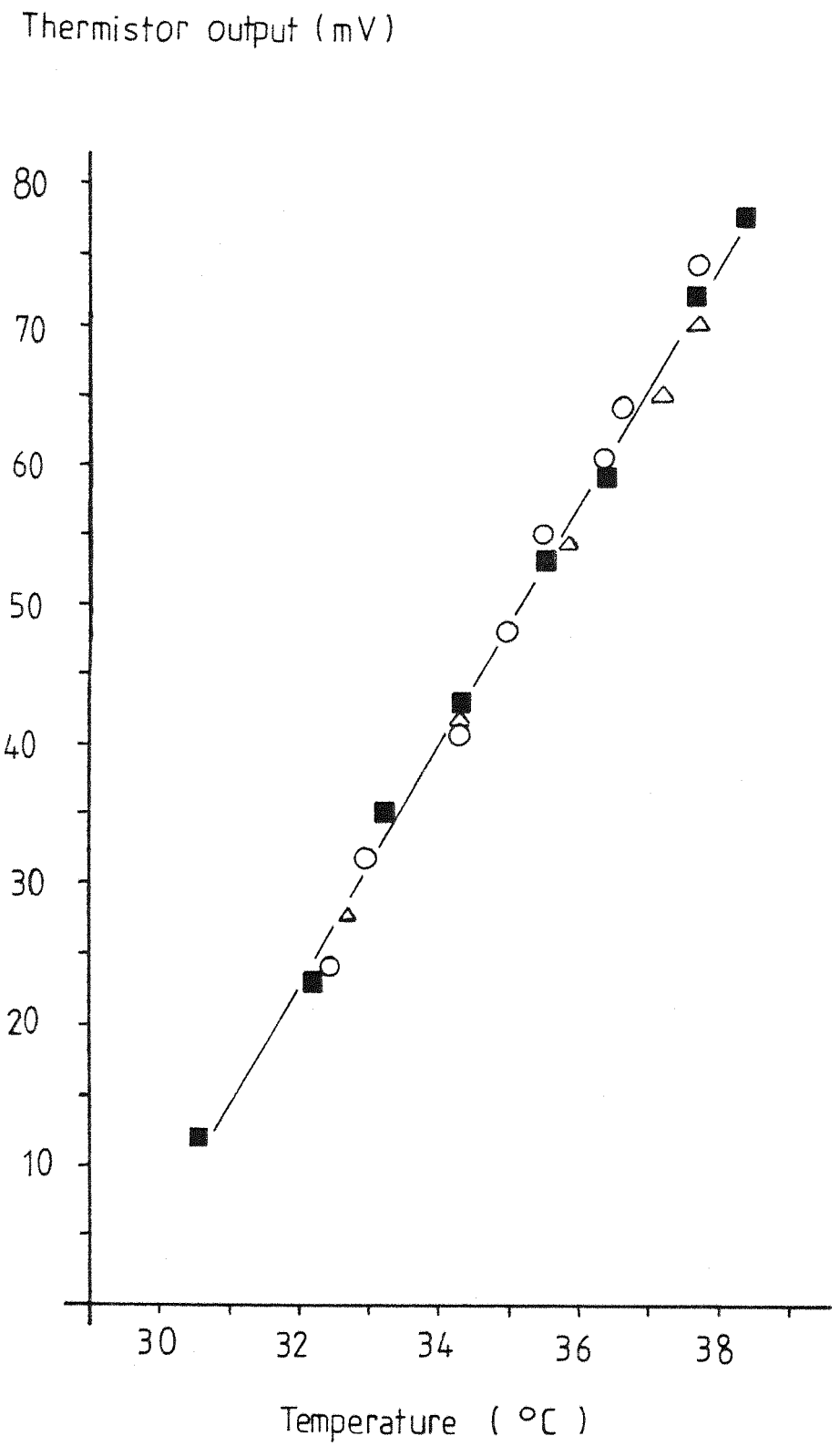
b. Non-toxic - one thermistor tested had a copper surface which caused a consistent deficiency in the inherent tone of isolated tracheal chain preparations.

c. Smooth surface - it was observed that the temperature of the bathing fluid was constant during compression and decompression even though that of the chamber atmosphere increased and decreased. Occasionally a rapid decrease in temperature of the bathing fluid was reversed on reaching atmospheric pressure. It became apparent that this was due to the formation of a gas bubble around the thermistor and that the temperature recorded during the pressure decrease was that of the expanding (and therefore, cooling) gas bubble. This problem could be overcome by gently filing off any rough edges on the thermistor.

d. Fast response time - this is necessary to follow the fast compression and decompression phases.

The final choice of thermistor was a YSI series III probe normally used to monitor temperature in blood vessels. Its diameter is 0.55cm and it has a smooth polythene surface. Its response time is 0.2 seconds. The calibration curve of the YSI thermistor is shown in Fig AB1.

FIGURE AB1 Calibration curve of the YSI thermistor at 1 bar and 69 bar.


Thermistor output (mV)
80
70
60
50
40
30
20
10
30
32
34
36
38
Temperature (°C)

APPENDIX C.

MAINTENANCE AND MONITORING OF pH

PARTIAL PRESSURE (bar)			TOTAL PRESSURE (bar)	%		DEPTH	
O_2	CO_2	He		O_2	CO_2	m	ft
0.95	0.05	Nil	1.0	95.00	5.00	Surface	
0.95	0.05	0.914	1.914	49.63	2.60	9.14	30) Submarine
0.95	0.05	1.829	2.829	33.58	1.76	18.29	60) escape
0.95	0.05	2.804	3.804	24.97	1.31	28.04	92) training depth
0.95	0.05	6.096	7.096	13.380	0.704	60.96	200)
0.95	0.05	12.192	13.192	7.201	0.379	121.92	400) Sea
0.95	0.05	18.288	19.286	4.925	0.259	182.88	600) escape
0.95	0.05	22.860	23.860	3.981	0.209	(228	750)* depth
0.95	0.05	30.0	31.0	3.06	0.16	300)	
0.95	0.05	42.0	43.0	2.209	0.118	420) Saturation diving	
0.95	0.05	66.0	67.0	1.418	0.074	660) depth	
0.95	0.05	100.0	101.0	0.94	0.04	(1000)	
				Balance He			

* Deepest possible attempt likely, 600-630 fsw deepest operational ascent.

TABLE AC1.

The pH, PCO_2 and PO_2 of the bathing fluid were maintained by bubbling with gas mixtures selected for each depth. The gas mixtures were supplied by Air Products Limited and their composition is shown in Table AC1.

There are a number of problems associated with the measurement of pH at high pressure which have not been fully resolved. Several trials were carried out with conventional combined glass electrodes which proved unsatisfactory, largely due to the formation of bubbles in the electrolytes during decompression. Although the electrode was nominally sealed, it was clearly not pressure-tight. It was possible to ensure more rapid equilibration of pressure by drilling a hole in the wall of the electrode. However, this lead to mixing of the two electrolytes by overflow during decompression. Alternative electrodes were sought with the following criteria in mind:

a. Pressure insensitive.

b. Smooth surface.

c. Small diameter.

The reasons for the final two criteria have already been discussed with respect to the choice of thermistor.

A number of manufacturers were approached, but few could offer any assistance with respect to the first criterion. Eventually a Pye Ingold electrode was selected. It is 6.0cm long and has a maximum diamter of 0.5cm. Furthermore, it was compatible with the meter already available. It differed from other pH electrodes considered in that it had a solid electrolyte and thus should not suffer from "decompression sickness". Its recommended use is in the measurement of stomach pH, and therefore it is mechanically strong.

There is one serious disadvantage in the use of this electrode at high pressure. After compression and decompression the pH reading was 0.2 units higher than that measured with a conventional glass electrode which had not been subjected to high pressure. This error could still be observed up to 12 hours after the decompression, but was reversed after 24 hours. This error could be corrected for by recalibrating after decompression. Disteche (1972) noted changes in the output of pH electrodes at pressure which were not fully reversed on decompression, but offered no explanation for the phenomenon.

Since the electrolytes used in the pH electrode described above, was different from that used by Disteche, it is reasonable to exclude the possibility that the effect was mediated via a change in the electrolyte dissociation. It is possible that pressure alters the properties of the ion selective glass membrane.

In view of the difficulties in the measurement of pH at pressure outlined above, the values recorded below should be considered as qualitative rather than quantitative, i.e. the direction of pH shift recorded is probably reliable, but the magnitude of the shift may not be.

A number of different compression protcols were investigated with respect to their effect on the pH of the bath fluid.

(i) Fig. AC1 shows how compression to 31 bar He in MacI in the absence of bubbling gas results in a rapid rise in pH to 8.0 which is not fully reversed after 60 minutes. This illustrates the need to equilibrate the gas mixture with the bath fluid when using MacI.

(ii) An alternative protocol is also shown in Fig. AC1. The solution was bubbled with the gas mixture, appropriate for a final pressure of 31 bar, before the onset of compression. The pH rose to 8.0 but was restored to normal values within 10 minutes of reaching 31 bar.

(iii) Bubbling with the appropriate gas for the final depth was initiated after compression was complete. During compression the pH rose to 8.0 but fell to 7.6 (31 bar) or 7.8 (67 bar) within 60 seconds of bubbling. This method was adopted for all experiments conducted in MacI.

The effect of pH on the inherent tone of tracheal chain was investigated and the results of a typical experiment are shown in Fig. AC2. It can be seen that the inherent tone of tracheal smooth muscle is unaltered by pH over the range 7.3 → 7.8.

FIGURE AC1 The effect of compression to 31 bar He in the absence of bubbling gas on the pH of Krebs' solution.

The effect of compression to 31 bar using 30 bar He:0.95 bar O_2:0.05 bar CO_2 on the pH of Krebs' solution.

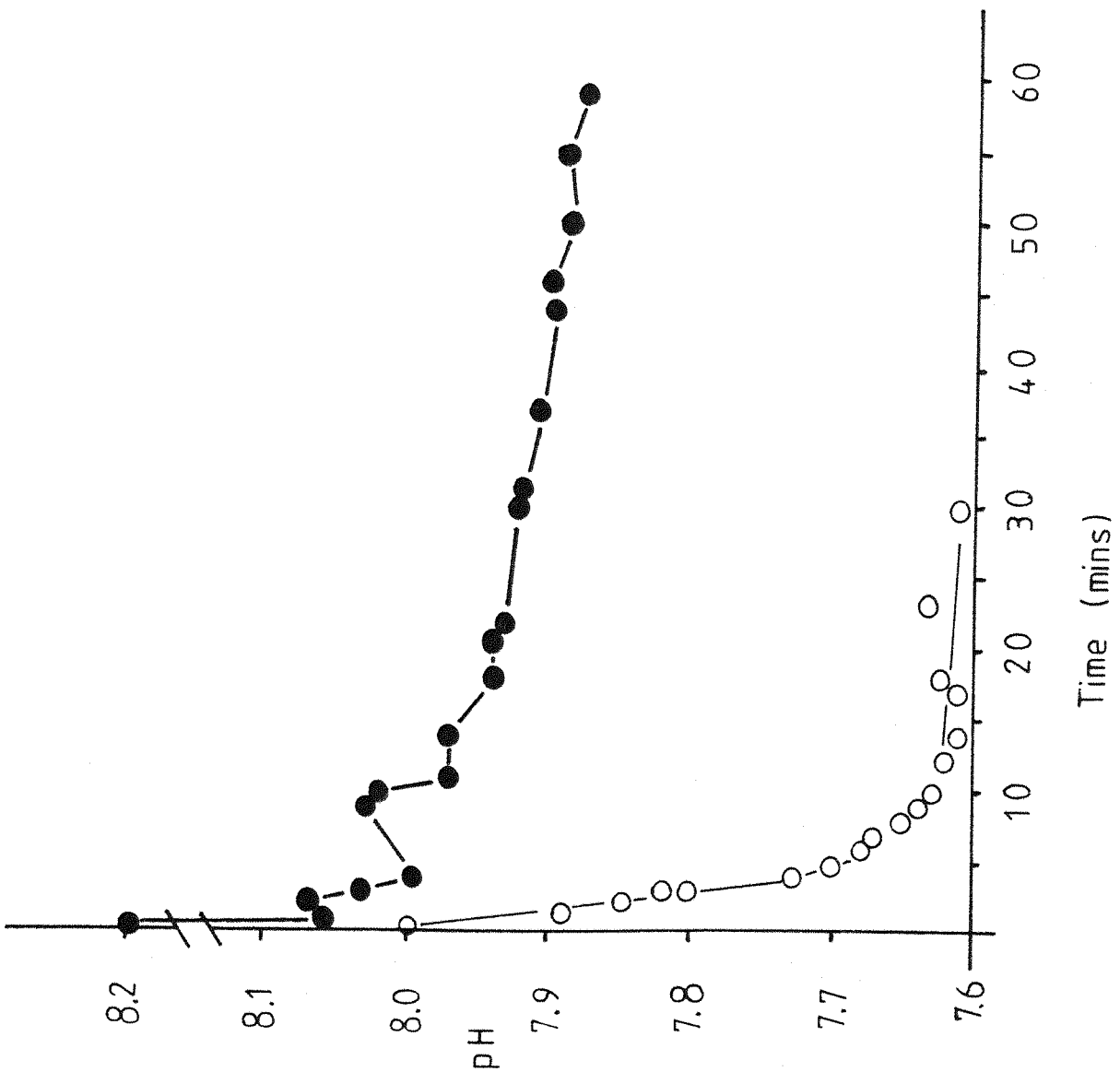
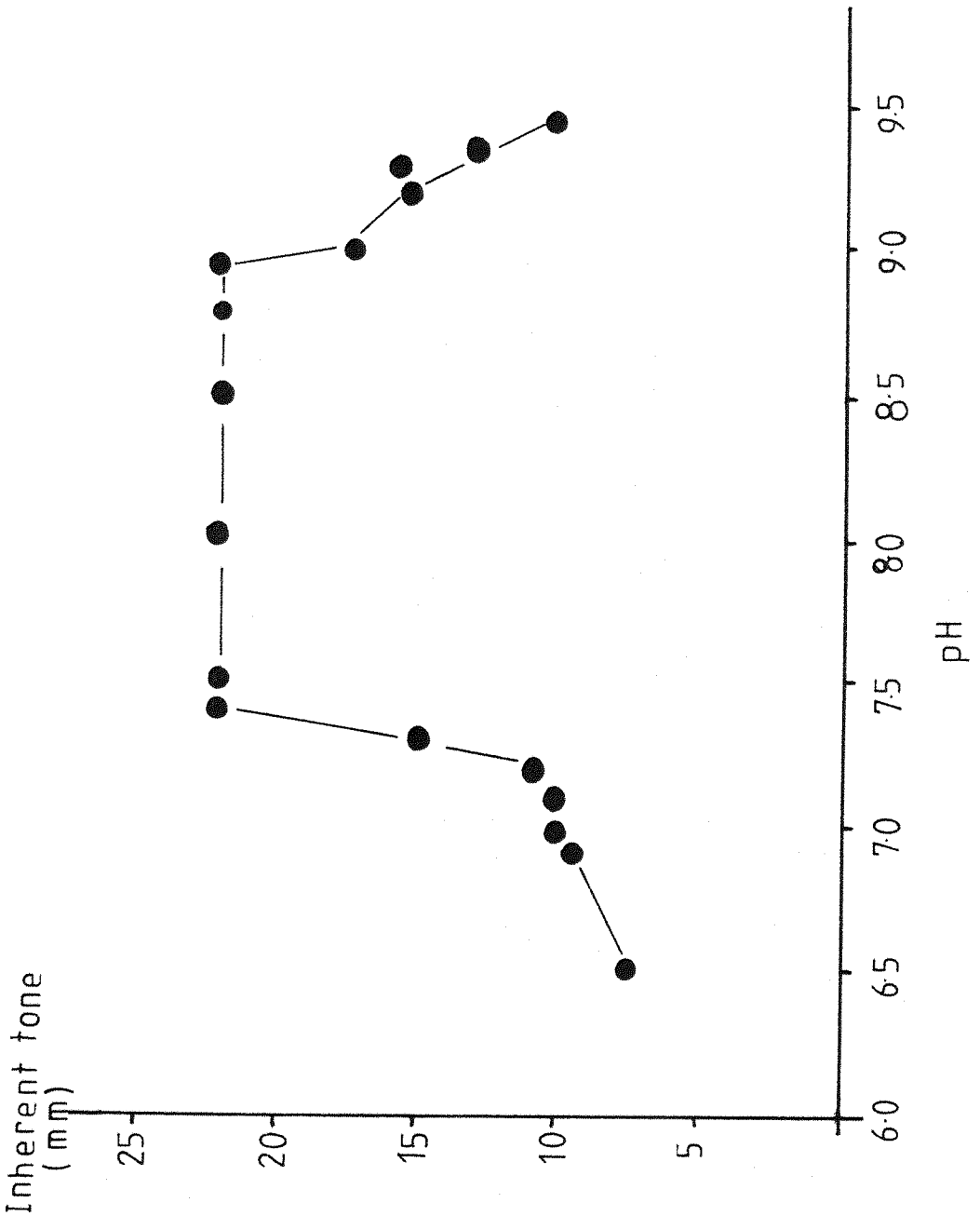

8.2
8.1
8.0
7.9
7.8
7.7
7.6
pH
10
20
30
40
50
60
Time (mins)

FIGURE AC2 The effect of pH on the inherent tone of tracheal smooth muscle.


Inherent tone
(mm)
25
20
15
10
5
6·0
6·5
7·0
7·5
8·0
8·5
9·0
9·5
pH

APPENDIX D.

SELECTION OF TRANSDUCER

Several transducers were screened for their suitability for use with tracheal smooth muscle. Those which proved appropriate were subjected to two tests of their sensitivity to pressure, see Table AD1. Firstly, the effect of compression and decompression on the basal output of the transducer was monitored. Secondly, a coil operated switch was attached by a rigid bar to the transducer lever arm. Opening and closing of the switch moved the lever arm in a reproducible way. The effect of compression maintained high pressure and decompression on the transducer output during the test deflection was recorded.

Transducer Make	Isometric/Isotonic	Principle
a. Dynamometer	Isometric)	Variable resistance in
b. Grass FTO3C	Isometric or Isotonic)	Wheatstone bridge
c. Palmer Photo-transducer	Isotonic	Variable density wedge
d. Jackson	Isotonic	Capacitance
e. Harvard Smooth Muscle	Isotonic	Moving coil

TABLE AD1.

a. Dynamometer

Only a few trials were conducted with this transducer. The output was erratic during compression and decompression. In retrospect this may have been due to a "dry joint" in the Wheatstone bridge which became apparent during gas flow. However, trials with this transducer were abandoned.

b. Grass FTO3C

These transducers were unreliable in either the isometric or isotonic mode. Different transducers of the same model gave different responses, some being stable throughout an experiment, others not. Therefore, it was decided to discontinue experiments with the Grass FTO3C at high pressure.

c. Palmer Phototransducer

The output from this transducer increased by 10% on compression. However, the major difficulty with this transducer was the frequent failure of the U2 battery required to power it. It is probable that as the transducer was in use for 6-7 hours continuously, sufficient

current was drawn from the battery to disable it, and always at the most inconvenient time. An independent power source controlled from outside the pressure chamber could have been used to overcome this problem if another, more suitable transducer, had not been found.

d. Jackson

This transducer which comprised a moving armature inside a capacitor was modified for our purposes by the manufacturers, so that all the electronic components (except the capacitor) were placed outside the chamber. Unfortunately this transducer gave changes in base line during decompression and the size of a standard test deflection was altered unpredictably at high pressure. Furthermore, its output was not linear over the range most suitable for use with tracheal chain.

e. Harvard Smooth Muscle Transducer

This transducer was adopted for all the experiments reported here. As with other commercial transducers, small unpredictable changes in base line could occur during compression and decompression, although this was not always observed. There was no significant difference between the standard test deflections recorded at 1 bar and at pressures up to 100 bar He or Air. However, the output during test deflections varied during compression and decompression. It was decided to adopt this transducer with the following reservations:

(i) Changes in base line observed during compression and decompression should not be related to a physiological process without independent evidence.

(ii) The transducer was reliable up to and including the maintained pressure of 100 bar.

It is suspected that the major problem with all of the transducers tested was the movement of gas into and out of confined spaces within electronic components, or the formation of tiny gas bubbles in the resins in which the components were embedded. This would account for the erratic and unpredictable responses of many of the transducers during pressure swings.

A further problem, common to all the transducers was that of corrosion. The chamber atmosphere was warm and humid and contained a high P_{O_2}. Under these adverse conditions even "stainless" steel was liable to rust. The bearings of the Harvard transducer were

particularly affected by corrosion since they were close to the surface of the Krebs' solution and thus tended to be splashed with salt spray. Replacement bearings made of Teflon were tested, but these had greater inertia than the originals and thus were unsuitable. It was found that a weekly, liberal spray with WD40 retarded the development of serious corrosion and this is recommended to other users.

APPENDIX I

TABLE 3.4a The effect of substrate withdrawal on the response to hypoxia

Paired tracheal chains were bathed in Krebs' solution containing sorbitol instead of glucose and allowed to take up tone in the usual way. The results were recorded on Grass FTO3C transducers.

After 60 minutes one chain of each pair was made hypoxic by bubbling with 5% CO_2:95% N_2. The responses are recorded in grams and as a percentage of initial tone, ie the inherent tone recorded after 60 minutes. When O_2 was restored, inherent tone was recorded for a further 65 minutes.

The bathing fluid was then replaced by Krebs' solution containing glucose and the inherent tone noted after 15 minutes when it had become steady.

TIME FROM START OF EXPT.		EXPT. NO. 1	2	3	4	5	6	7	Mean	SD	SE
SORBITOL KREBS'											
60 mins	Initial tone(g)	2.1	2.0	1.2	1.5	1.7	2.8	2.5	2.0	0.5	0.2
	% initial tone	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	-	-
HYPOXIC											
80 mins	Tone (g)	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.04	0.1	0.03
	% initial tone	0.0	0.3	0.0	0.0	0.0	0.0	0.0	1.8	4.9	1.8
95 mins	Tone (g)	1.7	1.8	1.6	1.0	1.7	2.1	2.0	1.7	0.4	0.1
	% initial tone	83.8	93.3	13.0	68.0	100.0	74.4	78.9	89.7	21.0	7.9
110 mins	Tone (g)	1.0	1.6	1.6	1.0	1.6	1.4	0.3	1.2	0.5	0.2
	% initial tone	48.0	83.0	126.0	68.0	92.0	48.0	105.0	67.9	37.1	14.0
125 mins	Tone (g)	0.6	1.2	1.3	1.0	1.6	1.4	0.0	1.0	0.5	0.2
	% initial tone	32.0	60.0	105.0	68.0	57.0	30.0	0.0	50.3	33.4	12.6
GLUCOSE KREBS'											
15 mins	Tone (g)	1.9	2.2	1.7	1.4	2.1	2.7	3.0	2.1	0.5	0.2
	% initial tone	93.5	110.0	136.0	95.4	123.0	95.0	118.0	110.1	16.4	6.2

TABLE 3.4a. Effect of replacement of glucose by sorbitol in Krebs' solution tone: Experimental chains, 5% CO_2:95% N_2 introduced at 60 minutes.

TABLE 3.4b The paired control chains were bathed in sorbitol Krebs' solution and maintained under normoxic conditions for 125 minutes. Inherent tone is recorded in grams and as a percentage of initial tone. The bathing fluid was replaced with Krebs' solution containing glucose and the inherent tone noted after 45 minutes.

TIME FROM START OF EXPT.		EXPT. NO. 1	2	3	4	5	6	7	Mean	SD	SE
SORBITOL KREBS'											
60 mins	Initial tone(g)	2.3	2.3	1.5	2.0	2.0	2.8	1.9	2.1	0.4	0.2
	% initial tone	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	-	-
80 mins	Tone (g)	2.3	2.1	1.6	0.6	2.2	2.3	1.8	1.8	0.6	0.2
	% initial tone	100.0	94.0	109.0	66.0	110.0	81.4	93.0	93.3	15.8	5.9
95 mins	Tone (g)	2.3	2.1	1.6	1.3	2.2	2.2	1.8	1.9	0.4	0.15
	% initial tone	97.1	91.1	109.0	63.0	110.0	76.0	93.1	91.3	17.0	6.4
110 mins	Tone (g)	2.1	2.1	1.6	1.26	2.2	2.1	1.6	1.8	0.3	0.1
	% initial tone	91.4	96.8	109.0	63.3	110.0	72.1	82.7	89.3	17.7	6.7
125 mins	Tone (g)	2.1	2.0	1.6	1.3	2.3	1.9	1.7	1.8	0.37	0.14
	% initial tone	91.4	88.2	109.0	63.3	113.0	67.4	89.6	88.8	18.7	7.1
GLUCOSE KREBS'											
15 mins	Tone (g)	2.3	2.6	2.0	1.8	2.2	2.3	2.3	2.2	0.2	0.1
	% initial tone	100.0	114.7	136.0	90.0	110.0	81.4	117.2	107.0	18.2	6.9

TABLE 3.4b. Effect of replacement of glucose by sorbitol in Krebs' solution on inherent tone: Control chains, normoxic throughout.

TABLE 3.5a The effect of $[Ca^{2+}]_o$ on the response to hypoxia.

Tracheal chains were prepared and allowed to take up tone (T) in the usual way. 5% CO_2:95% O_2 was replaced by 5% CO_2:95% N_2. When tone was again steady the response (R) to hypoxia was noted and expressed as a percentage of tone recorded prior to hypoxia. This procedure was repeated after the tone had recovered and the bathing fluid replaced with Krebs' solution containing 4.8, 9.6 and finally 14.4 mM Ca^{2+}. In each case the tone and the response to hypoxia were noted and the response expressed as a percentage of the tone prior to hypoxia.

TABLE 3.5b Two tracheal chains were prepared and allowed to take up tone as above. Inherent tone was recorded on Harvard smooth muscle transducers. These chains were subjected to hypoxia on four consecutive occasions. After tone had recovered, the bathing fluid was replaced with Krebs' solution containing 2.4 mM Ca^{2+}.

$[Ca^{2+}]_o$ = 2.4mM			$[Ca^{2+}]_o$ = 4.8mM			$[Ca^{2+}]_o$ = 9.6mM			$[Ca^{2+}]_o$ = 14.4mM		
T (mm)	R (mm)	%	T (mm)	R (mm)	%	T (mm)	R (mm)	%	T (mm)	R (mm)	%
7.0	2.5	35.0	7.5	6.0	80.0	8.5	5.5	64.0	9.5	7.0	73.6
4.0	2.3	57.5	3.5	1.5	42.8	3.0	2.0	66.0	4.0	2.0	50.0
6.0	1.5	25.0	9.0	4.5	50.0	8.0	5.0	62.5	7.0	5.2	74.0
17.0	6.0	35.0	17.0	8.0	47.0	17.0	9.0	53.0	-	-	-
Mean		38.1			54.9			61.3			65.8
SD		13.7			16.9			5.7			13.7
SE		7.9			9.8			3.3			7.9

TABLE 3.5a

$[Ca^{2+}]_o$ = 2.4mM			$[Ca^{2+}]_o$ = 2.4mM			$[Ca^{2+}]_o$ = 2.4mM			$[Ca^{2+}]_o$ = 2.4mM		
T (mm)	R (mm)	%	T (mm)	R (mm)	%	T (mm)	R (mm)	%	T (mm)	R (mm)	%
10.0	5.0	50.0	10.0	6.0	60.0	10.0	6.0	60.0	10.0	3.0	30.0
12.0	4.5	37.5	12.0	4.8	40.0	12.0	5.0	41.6	12.0	4.8	28.2
Mean		44.0			50.0			51.0			29.0
SD		8.8			14.1			13.0			1.4

TABLE 3.5b

EXPT. NO.	TIME (MINS) 0		5		10		15	
	Initial tone (T_i) (g)	$\%T_i$	Tone (g)	$\%T_i$	Tone (g)	$\%T_i$	Tone (g)	$\%T_i$
1	1.6	100.0	2.0	124.0	0.46	29.0	0.46	29.0
2	2.6	100.0	2.9	110.0	2.3	87.5	1.8	67.5
3	2.1	100.0	2.0	94.0	1.8	87.5	1.5	72.0
4	1.9	100.0	2.1	141.0	2.1	110.0	2.1	79.0
5	2.6	100.0	3.0	115.0	2.5	95.0	2.4	92.0
6	2.4	100.0	2.7	110.0	1.3	54.0	0.5	21.6
7	1.5	100.0	1.7	118.0	1.8	127.0	1.3	90.9
8	2.2	100.0	2.9	133.0	2.6	90.9	1.26	43.0
9	2.1	100.0	2.5	118.7	2.8	134.0	2.1	100.0
Mean		100.0		118.1		90.5		66.1
SD				13.7		33.0		28.5
SE				4.5		11.0		9.5

TABLE 3.6a The effect of ouabain on inherent tone of tracheal smooth muscle

Tracheal chains were prepared and inherent tone recorded on Grass FTO3C transducers. Atropine (1 µg/ml) was added to the bath fluid followed by ouabain (11 µM). The tone was noted for a further 15 minutes. The response of the tissue is recorded in grams and as a percentage of initial tone (% T_i).

EXPT. NO.	TIME (MINS) 0		5		10		15	
	Initial tone (g)	$\%T_i$	Tone (g)	$\%T_i$	Tone (g)	$\%T_i$	Tone (g)	$\%T_i$
1	2.3	100.0	2.9	129.0	2.9	129.0	2.8	123.0
2	1.3	100.0	1.8	135.0	2.0	150.0	2.0	150.0
3	2.3	100.0	2.6	111.0	2.6	114.0	2.4	103.0
4	1.6	100.0	1.8	108.0	1.8	108.0	1.7	104.0
5	1.8	100.0	2.2	122.0	2.4	133.0	2.3	126.0
6	1.5	100.0	1.9	126.0	2.5	161.0	2.3	152.0
7	0.8	100.0	1.0	115.0	1.1	115.0	1.1	115.0
8	1.3	100.0	2.4	180.0	2.4	180.0	2.3	175.0
9	2.1	100.0	2.3	109.0	2.6	125.0	2.4	115.0
Mean		100.0		126.1		135.0		129.2
SD				22.3		24.0		24.5
SE				7.4		8.0		8.2

TABLE 3.6b The effect of ouabain on inherent tone of tracheal smooth muscle.

The paired chains were prepared as in Table 3.6a. Atropine (1 µg/ml) and propranolol (1 µg/ml) were added to the bath fluid followed by ouabain (11 µM). Tone was recorded for 15 minutes.

Time (mins)	Experiment No. 1	2	3	4	5	6			
	Tone (g)	Tone (g)	Tone (g)	Tone (g)	Tone (g)	Tone (g)	Mean	SD	SE
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-
5	0.1	0.1	0.1	0.1	0.6	0.2	0.2	0.2	0.1
10	0.9	1.1	0.6	0.5	1.2	0.7	0.8	0.3	0.1
15	1.1	1.6	0.8	0.7	1.4	0.9	1.1	0.4	0.2
20	1.1	1.5	0.8	0.7	1.5	1.0	1.1	0.4	0.1
25	0.9	1.5	0.7	0.6	1.5	0.8	1.0	0.4	0.2
30	0.7	1.4	0.7	0.5.	1.5	0.7	0.9	0.4	0.2
35	0.6	1.1	0.6	0.3.	1.4	0.5	0.7	0.4	0.2
40	0.5	1.1	0.5	0.3	1.4	0.4	0.7	0.4	0.2
45	0.4	0.9	0.4	0.3	1.3	0.3	0.6	0.4	0.2
50	0.4	0.7	0.4	0.3	1.3	0.3	0.5	0.4	0.2
55	0.3	0.7	0.3	0.3	1.2	0.2	0.5	0.4	0.2
60	0.3	0.6	0.3	0.3	1.1	0.2	0.5	0.3	0.1
65	0.3	0.5	0.4	0.3	1.0	0.2	0.4	0.3	0.1
70	0.3	0.5	0.3	0.3	0.9	0.2	0.4	0.3	0.1
75	0.3	0.4	0.3	0.3.	0.9	0.2	0.4	0.2	0.1
80	0.3	0.4	0.3	0.3	0.8	0.2	0.4	0.2	0.1
85	0.3	0.4	0.4	0.3	0.8	0.1	0.4	0.2	0.1
90	0.3	0.4	0.3	0.3	0.7	0.1	0.4	0.2	0.1

TABLE 3.7a The effect of indomethacin on the uptake of inherent tone in tracheal smooth muscle.

Paired tracheal chains were prepared and bathed in Krebs' solution. Indomethacin in ethanol (0.2% v/v) was added to one chain of each pair after aminophylline had been washed out and before tone had begun to rise. These data are shown in Fig 3.7a. The responses were recorded on Grass FT03C transducers and are given in grams. The inherent tone was recorded for 90 minutes and is recorded here at 5 minute intervals.

Time (mins)	Experiment No. 1	2	3	4	5	6			
	Tone (g)	Tone (g)	Tone (g)	Tone (g)	Tone (g)	Tone (g)	Mean	SD	SE
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-
5	0.5	0.1	0.1	0.9	0.1	0.1	0.3	0.3	0.1
10	1.0	1.0	0.5	2.6	0.5	1.2	1.1	0.8	0.3
15	1.3	1.9	0.9	3.0	1.7	1.7	1.7	0.7	0.3
20	1.2	2.2	1.9	3.0	2.3	2.0	2.1	0.6	0.3
25	1.1	2.3	2.2	2.9	2.7	1.9	2.2	0.6	0.3
30	1.3	2.2	2.3	2.9	2.7	1.5	2.1	0.6	0.3
35	1.5	2.3	2.3	2.8	2.7	2.2	2.3	0.5	0.2
40	1.6	2.3	2.3	2.8	2.7	2.3	2.3	0.4	0.2
45	1.7	2.3	2.2	2.8	2.7	2.5	2.3	0.4	0.2
50	1.6	2.2	2.1	2.6	2.7	2.6	2.3	0.4	0.2
55	1.5	2.1	2.0	2.7	2.7	2.7	2.3	0.5	0.2
60	1.5	2.0	1.9	2.6	2.8	2.7	2.2	0.5	0.2
65	1.4	1.9	1.9	2.6	2.9	2.7	2.2	0.6	0.3
70	1.3	1.8	1.8	2.5	2.9	2.7	2.2	0.6	0.3
75	1.3	1.7	1.7	2.5	2.9	2.7	2.1	0.6	0.3
80	1.2	1.7	1.9	2.5	2.8	2.8	2.1	0.7	0.3
85	1.1	1.6	1.9	2.5	2.8	2.7	2.1	0.7	0.3
90	1.1	1.5	1.9	2.5	2.7	2.7	2.0	0.6	0.3

TABLE 3.7b The effect of indomethacin on the uptake of inherent tone in tracheal smooth muscle.

The matched chains were prepared and allowed to take up tone in the usual way. Ethanol (0.2% v/V) was present in the bath fluid. Inherent tone was recorded for 90 minutes and is noted here at 5 minute intervals.

Time (mins)	Experiment No. 1	2	3	4			
	Tone (mm)	Tone (mm)	Tone (mm)	Tone (mm)	Mean	SD	SE
0	16.0	17.0	20.0	13.0	16.5	2.8	1.4
5	18.0	20.0	22.0	15.0	18.7	2.9	1.4
10	18.0	18.0	24.0	15.0	18.7	3.7	1.8
15	17.0	17.0	20.0	14.0	17.0	2.4	1.2
20	14.0	16.0	18.0	12.0	15.0	2.6	1.3
25	11.0	15.0	13.0	10.0	12.2	2.2	1.1
30	9.0	14.0	10.0	9.0	10.5	2.4	1.2
35	7.0	13.0	8.0	7.0	8.7	2.8	1.4
40	5.0	11.0	6.0	6.0	7.0	2.7	1.3
45	5.0	9.0	5.0	5.0	6.0	2.0	1.0
50	4.0	7.0	5.0	4.0	5.0	1.4	0.8
55	2.0	5.0	4.0	3.0	3.5	1.3	0.6
60	1.0	4.0	3.0	2.0	2.5	1.3	0.6

TABLE 3.8a Paired tracheal chains were prepared in the usual way and allowed to take up tone, which was recorded on Harvard smooth muscle transducers. When tone was established PIT (50 μM) was added (Time 0) and tone recorded for a further 60 minutes. The responses are summarised in Table 3.8a by noting the tone recorded at 5 minute intervals.

Time (mins)	Experiment No. 1	2	3	4			
	Tone (mm)	Tone (mm)	Tone (mm)	Tone (mm)	Mean	SD	SE
0	21.0	15.0	12.0	10.0	14.5	4.8	2.4
5	21.0	15.0	12.0	10.0	14.5	4.8	2.4
10	21.0	15.0	12.0	10.0	14.5	4.8	2.4
15	21.0	15.0	12.0	10.0	14.5	4.8	2.4
20	21.0	15.0	12.0	10.0	14.5	4.8	2.4
25	20.0	15.0	12.0	10.0	14.2	4.3	2.1
30	20.0	15.0	12.0	10.0	14.2	4.3	2.1
35	20.0	15.0	12.0	10.0	14.2	4.3	2.1
40	20.0	15.0	12.0	10.0	14.2	4.3	2.1
45	20.0	15.0	12.0	10.0	14.2	4.3	2.1
50	20.0	15.0	12.0	10.0	14.2	4.3	2.1
55	20.0	15.0	12.0	10.0	14.2	4.3	2.1
60	20.0	15.0	12.0	10.0	14.2	4.3	2.1

TABLE 3.8b Paired control chains were prepared as above and maintained in the absence of any drug. Inherent tone was recorded for 60 minutes after PIT was added to the experimental preparations.

Time (mins)	1 Tone (mm)	2 Tone (mm)	3 Tone (mm)	4 Tone (mm)	Mean	SD	SE
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	10.0	8.0	7.0	7.0	8.0	1.4	6.7
10	17.0	14.0	10.5	9.0	12.6	3.6	1.8
15	23.0	16.0	12.0	12.0	15.7	5.2	2.6
20	24.0	18.0	16.0	15.0	18.2	4.0	2.0
25	24.0	18.0	20.0	17.0	19.7	3.1	1.5
30	23.0	17.0	20.0	17.0	19.2	2.8	1.4
35	22.0	16.0	19.0	16.0	18.2	2.8	1.4
40	21.0	15.0	18.0	15.0	17.2	2.8	1.4
45	19.0	14.0	16.5	15.0	16.1	2.2	1.1
50	17.0	12.0	15.0	14.0	14.5	2.1	1.0
55	16.0	11.0	14.0	13.0	13.5	2.1	1.0
60	15.0	10.0	13.0	13.0	12.7	2.1	1.0
65	14.0	8.5	12.0	13.0	11.8	2.4	1.2
70	12.0	7.5	11.0	12.0	10.6	2.1	1.0
75	12.0	7.0	11.0	11.5	10.4	2.3	1.1
80	11.0	6.0	10.0	11.0	9.5	2.4	1.2
85	10.0	6.0	9.0	11.0	9.0	2.2	1.1
90	10.0	5.0	8.0	10.0	8.2	2.4	1.2
95	9.0	4.0	8.0	9.0	7.5	2.4	1.2
100	8.0	2.0	7.0	8.0	6.2	2.8	1.4

TABLE 3.9a Paired tracheal chains were prepared and the inherent tone recorded on Harvard smooth muscle transducers. To one chain of each pair verapamil (5 x 10^{-5} M) was dissolved in methanol (0.1% v/V) and added to the bath fluid at time 0. Tone was recorded (in mm) for 100 minutes, shown here at 5 minute intervals.

Time (mins)	1 Tone (mm)	2 Tone (mm)	3 Tone (mm)	4 Tone (mm)	Mean	SD	SE
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	8.0	6.0	7.0	9.0	9.5	1.3	0.6
10	16.0	12.0	11.0	14.0	13.2	2.2	1.1
15	19.0	13.0	15.0	18.0	16.2	2.7	1.3
20	20.0	14.0	17.0	21.0	18.0	3.2	1.6
25	20.0	15.0	19.0	23.0	19.2	3.3	1.6
30	20.0	15.0	19.0	23.0	19.2	3.3	1.6
35	20.0	15.0	19.0	23.0	19.2	3.3	1.6
40	20.0	15.0	19.0	23.0	19.2	3.3	1.6
45	18.0	14.0	18.5	22.0	18.1	3.3	1.6
50	16.0	13.0	17.0	21.0	16.7	3.3	0.9
55	15.0	12.5	16.0	19.0	15.6	2.7	1.3
60	14.0	12.0	15.0	17.0	14.5	2.1	1.0
65	13.0	11.0	13.5	16.0	13.4	2.0	1.0
70	11.5	10.5	12.0	16.0	12.5	2.4	1.2
75	10.5	10.5	11.0	15.0	11.7	2.2	1.1
80	8.0	10.0	9.0	14.0	11.0	2.4	1.2
85	7.0	10.0	8.0	12.0	10.0	2.9	1.5
90	5.0	9.5	7.0	12.0	8.4	3.0	1.5
95	5.0	9.0	7.0	11.0	8.0	2.6	1.3
100	4.0	9.0	6.0	10.0	7.2	2.7	1.4

TABLE 3.9b The second chain of matched tracheal chains was allowed to take up tone in the usual way except that methanol 0.01% v/V was present in the bath fluid from time 0. At time 30 minutes, when inherent tone was steady, verapamil 5 x 10^{-5} M was added and the inherent tone recorded for a further 70 minutes.

TABLE 3.10 A. Five tracheal chains (unpaired) were and allowed to take up tone in the usual way. Tone and the response to field stimulation were recorded on Harvard smooth muscle transducers. Inherent tone (T), the contraction response (C) and the relaxation response (R) are recorded in Table 3.10. Both contraction and relaxation are expressed as a percentage of inherent tone recorded just prior to field stimulation.

B. Atropine (10^{-6} g/ml) was added to the bath fluid of each of the chains shown in Table 3.10A. The contractile response (C) was abolished at all frequencies, but the relaxation (R) was unaffected.

EXPT. NO.	4 Hz					8 Hz					FREQUENCY 12 Hz					12 Hz				
	T (mm)	C (mm)	% T	R (mm)	% T	T (mm)	C (mm)	% T	R (mm)	% T	T (mm)	C (mm)	% T	R (mm)	% T	T (mm)	C (mm)	% T	R (mm)	% T
1	16.0	0.5	3.1	2.0	12.5	16.0	0.5	3.1	3.5	21.8	16.0	1.0	6.2	5.0	31.2	13.0	2.0	15.4	7.0	53.8
2	18.0	1.5	8.3	6.0	33.3	18.0	1.0	5.5	8.0	44.4	17.0	2.0	11.7	10.0	58.8	17.0	5.0	29.4	15.0	88.2
A 3	17.0	1.0	5.8	1.0	5.8	17.0	0.0	0.0	3.0	17.6	17.0	1.0	5.8	5.0	29.4	17.0	4.0	23.5	11.0	64.7
4	18.0	1.0	5.5	0.0	0.0	18.0	2.0	11.1	0.0	0.0	18.0	3.0	16.6	4.0	22.2	18.0	6.0	33.3	10.0	55.5
5	19.0	2.0	10.5	0.0	0.0	21.0	3.0	14.3	0.0	0.0	24.0	5.0	20.8	3.0	12.5	23.0	9.0	39.1	11.0	47.8
Mean			6.6		10.3			6.8		16.8			12.2		30.8			28.1		62.0
SD			2.8		13.8			5.8		18.4			6.5		17.3			9.1		15.8
SE			1.2		6.2			2.6		8.2			2.9		7.7			4.1		7.1
1	14.0	0.0	-	1.0	7.1	14.0	0.0	-	2.0	14.3	13.0	0.0	-	4.0	30.7	13.0	0.0	-	5.0	38.4
2	17.0	0.0	-	5.0	29.4	16.0	0.0	-	8.0	50.0	16.0	0.0	-	9.0	56.2	17.0	0.0	-	16.0	94.1
B 3	17.0	0.0	-	1.0	5.8	17.0	0.0	-	4.0	23.5	17.0	0.0	-	5.0	29.4	17.0	0.0	-	10.0	58.8
4	17.0	0.0	-	1.0	5.8	18.0	0.0	-	1.0	5.5	17.0	0.0	-	2.0	11.7	17.0	0.0	-	7.0	41.1
5	23.0	0.0	-	2.0	8.6	22.0	0.0	-	2.0	9.1	22.0	0.0	-	4.0	18.2	21.0	0.0	-	4.0	19.0
Mean					11.4					20.5					29.2					50.3
SD					10.1					17.8					17.0					28.2
SE					4.5					7.9					7.6					12.6

TABLE 3.10 Effect of Atropine (1 µg/ml) on the response to transmural stimulation.

Expt. No.	Frequency H_z											
	4			8			16			32		
	T (mm)	R (mm)	%T	T (mm)	R (mm)	%T	T (mm)	R (mm)	%T	T (mm)	R (mm)	%T
1	30.0	1.0	03.0	30.0	4.5	15.0	30.0	8.0	26.0	30.0	14.0	46.6
2	23.0	0.0	00.0	23.0	1.0	04.3	22.0	2.0	09.1	22.0	04.0	18.2
3	24.0	0.0	00.0	23.0	0.0	00.0	24.0	1.0	04.1	24.0	01.0	04.1
4	31.0	0.0	00.0	29.0	0.0	00.0	28.0	2.0	07.1	27.0	04.0	14.8
5	27.0	0.0	00.0	27.0	0.0	00.0	27.0	4.0	14.8	27.0	04.0	14.8
6	22.0	0.0	00.0	22.0	0.0	00.0	22.0	0.0	00.0	21.0	01.0	04.7
Mean			00.5			00.9			10.2			17.2
SD			01.2			01.8			09.2			15.5
SE			00.5			00.7			03.7			06.3

TABLE 3.11 Tracheal chains were prepared from 6 animals which were pretreated with reserpine (1 mg/kg) on two consecutive days prior to sacrifice. The relaxation response (R) is shown in Table 3.10. The response is also expressed as a percentage of the inherent tone prior to stimulation (%T). The data were recorded using a Harvard smooth muscle transducer.

TABLE 3.12 A. Five tracheal chains were prepared and set up in the usual way. The results were recorded on Harvard smooth muscle transudcers. Transmural stimulation at 4, 8, 16 and 32 Hz evoked relaxation responses (R) which increased in size with increasing frequency. The responses were expressed as a percentage of inherent tone (T) recorded just prior to stimulation (%T).

B. Atropine (10^{-6} g/ml) and propranolol (10^{-6} g/ml) were added to the bath fluid and the frequency response tests were repeated. The results are summarised as in A.

C. Finally, quinidine (0.05 mM) was added to the bath fluid and the frequency response tests repeated. The results are summarised as in A.

Expt. No.	Frequency Hz											
	4			8			16			32		
	T (mm)	R (mm)	%T	T (mm)	R (mm)	%T	T (mm)	R (mm)	%T	T (mm)	R (mm)	%T
1	34.0	0.0	00.0	34.0	7.0	20.5	33.0	14.0	42.4	31.0	23.0	74.2
2	23.0	0.0	00.0	22.0	3.0	13.6	21.0	08.0	38.1	19.0	14.0	73.7
A 3	19.0	0.0	00.0	21.0	0.8	00.0	24.0	02.5	10.4	23.0	11.0	47.8
4	16.0	3.0	18.7	15.0	5.0	33.3	16.0	09.0	56.2	16.0	14.0	87.5
5	18.0	5.0	27.7	19.0	8.0	42.1	17.0	10.0	58.8	17.0	15.0	88.2
Mean			09.3			21.9			41.2			74.3
SD			13.1			16.5			19.3			16.3
SE			05.9			07.5			08.6			07.4
1	33.0	2.0	06.1	35.0	6.0	17.1	35.0	08.0	22.8	36.0	12.0	33.3
2	15.0	1.0	06.6	17.0	2.5	14.7	18.0	05.0	27.7	18.0	07.0	38.8
B 3	18.0	0.0	00.0	18.0	1.0	05.5	18.0	00.0	00.0	18.0	02.0	11.1
4	18.0	1.0	05.0	19.0	5.0	26.3	19.0	06.0	31.5	17.0	12.0	70.5
5	18.0	4.0	22.0	18.0	6.0	33.3	18.0	09.0	50.0	17.0	09.0	52.9
Mean			08.1			19.4			26.4			74.3
SD			08.3			10.7			17.9			16.3
SE			03.7			04.8			08.0			07.4

TABLE 3.12

Continued ...../over

TABLE 3.12 continued ...../

Expt. No.	Frequency H_z											
	4			8			16			32		
	T (mm)	R (mm)	%T	T (mm)	R (mm)	%T	T (mm)	R (mm)	%T	T (mm)	R (mm)	%T
1	37.0	0.0	-	36.0	0.0	-	35.0	0.0	-	34.0	0.0	-
2	19.0	0.0	-	19.0	0.0	-	18.0	0.0	-	18.0	0.0	-
C 3	18.0	0.0	-	18.0	0.0	-	18.0	0.0	-	18.0	0.0	-
4	22.0	0.0	-	23.0	0.0	-	23.0	0.0	-	23.0	0.0	-
5	20.0	0.0	-	20.0	0.0	-	20.0	0.0	-	20.0	0.0	-
Mean												
SD												
SE												

TABLE 3.13 The effect of quinidine on the contractile response of tracheal chain to field stimulation.

Inherent tone is recorded prior to stimulation (T_i) and throughout the frequency response tests (T). The contractile response (C) is noted as a percentage of the tone recorded immediately prior to stimulation (%T).

A Control period.
B In the presence of quinidine (0.05 mM).

	Frequency (Hz)														
	2			4			8			16			32		
	T (g)	R (g)	R (%T)	T (g)	R (g)	R (%T)	T (g)	R (g)	R (%T)	T (g)	R (g)	R (%T)	T (g)	R (g)	R (%T)
A 1	2.6	0.0	0.0	2.6	0.0	0.0	2.6	0.1	0.3	2.6	-	0.2	2.6	0.1	3.8
2	1.4	0.0	0.0	1.5	0.0	0.0	1.4	0.0	0.0	1.3	0.0	0.0	1.3	0.1	7.7
3	1.7	0.0	0.0	1.5	0.0	0.0	1.4	0.1	7.1	1.3	0.1	7.7	1.3	0.2	15.3
4	1.7	0.1	5.8	1.6	0.1	6.2	1.6	0.1	6.2	1.6	0.1	6.2	1.6	0.1	6.2
Mean			1.4			1.5			3.4			3.5			8.2
SD			2.9			3.1			3.7			4.0			4.9
SE			1.4			1.5			1.8			2.0			2.5
B 1	3.0	0.0	0.0	3.0	0.0	0.0	3.0	0.0	0.0	3.0	0.0	0.0	3.0	0.0	0.0
2	1.6	0.0	0.0	1.6	0.0	0.0	1.6	0.0	0.0	1.6	0.0	0.0	1.5	0.0	0.0
3	1.8	0.0	0.0	1.8	0.0	0.0	1.8	0.0	0.0	1.9	0.0	0.0	1.9	0.0	0.0
4	2.1	0.0	0.0	2.1	0.0	0.0	2.1	0.0	0.0	2.1	0.0	0.0	2.1	0.0	0.0
Mean			0.0			0.0			0.0			0.0			0.0
SD															
SE															

TABLE 3.13

TABLE 3.14 The effect of quinidine on the relaxation response of tracheal chain to field stimulation. Inherent tone is recorded prior to stimulation (T_i) and throughout the frequency response tests (T). The relaxation response (R) is recorded as a percentage of inherent tone recorded immediately prior to stimulation (%T).

A Control period.
B In the presence of quinidine (0.05 mM).

EXPT. NO.		Frequency (Hz)														
		2			4			8			16			32		
		T (g)	R (g)	R (%T)	T (g)	R (g)	R (%T)	T (g)	R (g)	R (%T)	T (g)	R (g)	R (%T)	T (g)	R (g)	R (%T)
A	1	2.6	-	-	2.6	0.0	0.0	2.6	0.1	5.0	2.6	0.3	10.0	2.6	0.8	30.7
	2	1.4	0.1	16.6	1.5	0.3	22.7	1.4	0.5	38.0	1.3	0.6	50.0	1.3	0.9	73.6
	3	1.7	0.5	28.0	1.5	0.6	40.9	1.4	0.7	52.4	1.3	1.1	80.0	1.3	1.3	100.0
	4	1.7	0.1	3.8	1.6	-	-	1.6	-	-	1.6	-	-	1.6	-	-
Mean				16.1			21.2			31.8			46.6			68.1
SD				12.1			20.4			24.0			35.1			34.9
SE				6.0			12.0			14.0			20 6			20.6
B	1	3.0	0.0	0.0	3.0	0.0	0.0	3.0	0.0	0.0	3.0	0.0	0.0	3.0	0.0	0.0
	2	1.6	0.1	4.0	1.6	0.1	3.8	1.6	0.1	3.8	1.6	0.1	3.7	1.5	0.1	3.7
	3	1.8	0.1	7.4	1.8	0.1	6.8	1.8	0.1	3.4	1.9	0.1	3.4	1.9	0.0	0.0
	4	2.2	0.0	0.0	2.1	0.0	0.0	2.1	0.0	0.0	2.1	0.0	0.0	2.1	0.0	0.0
Mean				2.8			2.6			1.8			1.7			0.9
SD				3.5			3.3			2.1			2.0			1.8
SE				1.8			1.6			1.0			1.0			0.9

TABLE 3.14

TABLE 3.15a Experimental

A dose response curve to adrenaline was obtained over the range 1.0-8.0 mM on four pairs of tracheal chains. The inherent tone at the start of each dose cycle (T_s) is recorded in grams, as is the relaxation response (R). R is also given as a percentage of T_s ($\%T_s$).

A Control dose cycle.
B In the presence of 0.05 mM quinidine.

TABLE 3.15b Controls

The second chain of each pair was subjected to two consecutive dose cylces of adrenaline, in the absence of any other drug.

A Control dose cycle.
B Control dose cycle.

EXPT NO	Dose of adrenaline (mM)								
	1.0		2.0		4.0		8.0		
	T_S(g)	R(g)	T_S(g)	R(g)	T_S(g)	R(g)	T_S(g)	R(g)	T_S(g)
A 1	2.8	1.7	61.9	2.4	85.7	2.7	97.6	2.8	100.0
A 2	2.5	1.1	44.7	1.6	65.7	2.2	86.8	2.5	100.0
A 3	2.1	0.3	12.5	0.7	34.4	1.5	68.7	2.1	100.0
A 4	1.5	0.3	21.7	0.6	39.0	0.8	56.5	1.3	86.9
Mean			35.2		56.2		77.4		96.7
SD			22.4		24.0		18.3		6.5
SE			11.2		12.0		9.1		3.2
B 1	2.6	1.3	50.0	2.1	80.0	2.6	100.0	2.6	100.0
B 2	2.7	1.0	36.6	2.0	73.2	2.5	92.7	2.5	92.7
B 3	2.0	0.4	20.0	0.8	40.0	1.5	76.6	1.9	96.6
B 4	1.5	0.0	0.0	0.0	0.0	0.3	22.7	0.6	45.4
Mean			35.3		64.4		73.0		83.6
SD			15.0		21.4		34.9		25.7
SE			7.5		10.7		17.4		12.8

TABLE 3.15a Effect of quinidine on the response to adrenaline

EXPT NO	Dose of adrenaline (mM)								
	1.0		2.0		4.0		8.0		
	T_S(g)	R(g)	T_S(g)	R(g)	T_S(g)	R(g)	T_S(g)	R(g)	T_S(g)
A 1	2.3	1.6	71.0	2.1	88.5	2.3	100.0	2.3	100.0
A 2	2.8	1.6	55.8	2.1	72.1	2.5	88.4	2.8	100.0
A 3	1.7	0.3	19.4	0.7	42.3	1.6	69.2	1.6	96.0
A 4	2.4	0.2	8.0	0.7	30.5	2.2	56.1	2.2	91.0
Mean			36.5		58.3		73.4		96.7
SD			29.7		26.6		27.9		4.2
SE			14.8		13.3		13.9		2.1
B 1	1.9	1.3	65.5	1.5	79.0	1.8	93.0	1.9	100.0
B 2	2.6	0.9	35.0	1.4	55.0	2.1	80.0	2.5	95.0
B 3	1.4	0.3	19.0	0.5	38.0	0.9	66.0	1.4	90.4
B 4	2.5	0.1	5.4	0.4	16.2	1.0	40.5	2.4	75.6
Mean			31.2		47.0		69.8		90.2
SD			25.8		26.5		22.4		10.5
SE			12.9		13.2		11.2		2.2

TABLE 3.15b Effect of quinidine on the response to adrenaline (Controls)

TABLE 3.16a Experimental

A dose response curve to ATP was obtained over the range 0.01-3.0 mM on five pairs of tracheal chains. The inherent tone at the start of each dose cycle (T_s) is recorded in grams, along with the relaxation response (R). R is also given as a percentage of T_s ($\%T_s$).

A Control dose cycle.
B In the presence of 0.05 mM quinidine.

TABLE 3.16b Controls

The second chain of each pair was subjected to two consecutive dose cycles of ATP, in the absence of any other drug.

A Control dose cycle.
B Control dose cycle.

EXPT. No.	Dose of ATP (mM) 0.01 T_S (g)	0.01 R (g)	0.01 $\%T_i$	0.1 R (g)	0.1 $\%T_S$	1.0 R (g)	1.0 $\%T_S$	3.0 R (g)	3.0 $\%T_S$
A 1	0.8	0.1	12.5	0.1	12.5	0.5	66.6	0.8	100.0
A 2	2.5	0.5	21.0	1.3	50.0	1.8	71.0	2.5	100.0
A 3	1.2	0.1	11.0	0.2	35.2	0.9	77.7	1.2	100.0
A 4	1.5	0.2	13.6	0.3	18.2	0.7	47.7	1.3	90.9
A 5	1.8	0.2	11.1	0.5	25.9	1.2	66.6	1.6	88.9
Mean			13.8		28.4		65.9		95.9
SD			4.1		14.8		11.2		5.6
SE			1.8		6.6		4.9		2.5
B 1	0.9	0.0	0.0	0.1	11.1	0.4	44.4	0.9	100.0
B 2	2.3	0.3	13.0	1.8	78.2	1.8	78.3	2.3	100.0
B 3	1.1	0.1	9.1	0.8	72.7	0.9	81.8	1.1	100.0
B 4	2.1	0.1	4.7	1.2	57.1	1.2	57.1	2.0	95.2
B 5	1.2	0.1	8.3	0.8	38.1	0.9	42.8	1.2	100.0
Mean			7.0		51.4		60.8		99.0
SD			4.9		27.4		18.4		2.1
SE			2.2		12.2		8.2		0.9

TABLE 3.16a

EXPT. NO.	Dose of ATP (mM) 0.01 T_S (g)	0.01 R (g)	0.01 $\%T_S$	0.1 R (g)	0.1 $\%T_S$	1.0 R (g)	1.0 $\%T_S$	3.0 R (g)	3.0 $\%T_S$
A 1	1.2	0.1	8.3	0.4	33.3	1.2	100.0	1.2	100.0
A 2	2.0	0.5	25.0	0.5	25.0	1.1	65.5	2.0	100.0
A 3	1.5	0.3	20.0	0.3	20.0	0.9	60.0	1.5	100.0
A 4	1.5	0.5	33.3	0.5	33.3	1.0	66.6	1.5	100.0
A 5	1.1	0.2	18.2	0.3	27.3	0.7	63.6	1.1	100.0
Mean			20.9		27.8		71.1		100.0
SD			9.2		5.7		16.3		
SE			4.1		2.5		7.3		
B 1	0.8	0.0	0.0	0.1	12.5	0.6	33.3	0.8	100.0
B 2	1.4	0.1	7.1	0.2	14.3	1.1	78.6	1.4	100.0
B 3	1.5	0.2	13.3	0.3	20.0	1.0	66.6	1.5	100.0
B 4	1.1	0.1	9.1	0.3	27.3	0.6	54.5	1.1	100.0
B 5	0.9	0.0	0.0	0.4	44.4	0.6	66.6	0.9	100.0
Mean			5.9		23.7		59.9		100.0
SD			5.8		12.9		17.1		
SE			2.6		5.7		7.6		

TABLE 3.16b

TABLE 3.17a Experimental

A dose response curve to adenosine was obtained over the range 0.2-1.5 mM on four pairs of tracheal chains. The inherent tone at the start of each dose cycle (T_S) is recorded in grams, along with the relaxation response (R). R is also given as a percentage of T_S ($\%T_S$).

A Control dose cycle.
B In the presence of 0.05 mM quinidine

TABLE 3.17b Control

The second chain of each pair was subjected to two consecutive dose cycles of adenosine in the absence of any other drug.

A Control dose cycle.
B Control dose cycle.

EXPT. NO.	Dose of Adenosine (mM)								
	0.2			0.6		1.0		1.5	
	T_s (g)	R (g)	$\%T_s$	R (g)	$\%T_s$	R (g)	$\%T_s$	R (g)	$\%T_s$
A 1	1.1	0.2	18.0	0.7	63.6	1.0	90.9	1.1	100.0
A 2	0.8	0.1	7.5	0.4	50.0	0.6	75.0	0.6	75.0
A 3	2.8	0.1	4.6	0.8	28.5	1.7	60.7	2.1	75.0
A 4	2.6	0.6	23.0	1.8	69.2	2.2	84.6	2.3	88.5
Mean			13.3		52.8		77.8		84.6
SD			8.6		18.1		13.1		12.1
SE			4.3		9.0		6.5		6.0
1	1.1	0.2	18.2	0.6	54.5	0.9	81.8	1.1	100.0
2	0.7	0.0	4.3	0.3	42.8	0.5	71.4	0.6	85.7
3	3.0	0.0	0.0	0.5	16.6	1.4	46.6	2.0	66.6
4	3.0	0.6	20.0	1.8	60.0	2.5	80.0	2.6	86.6
Mean			10.6		43.5		69.9		84.7
SD			9.9		19.3		16.2		13.7
SE			4.9		9.6		8.1		6.8

TABLE 3.17a

EXPT. NO.	Dose of Adenosine (mM)								
	0.2			0.6		1.0		1.5	
	T_s (g)	R (g)	$\%T_s$	R (g)	$\%T_s$	R (g)	$\%T_s$	R (g)	$\%T_s$
A 1	2.0	0.3	15.0	0.8	40.0	1.3	65.0	1.6	80.0
A 2	0.8	0.2	25.0	0.6	75.0	0.7	87.5	0.8	100.0
A 3	2.4	0.5	20.8	1.4	58.3	2.0	83.3	2.3	95.8
A 4	2.6	0.4	15.3	1.2	46.1	1.2	46.1	2.3	88.5
Mean			19.0		54.8		70.4		91.1
SD			4.8		15.4		18.9		8.7
SE			2.4		7.7		9.4		4.3
B 1	1.8	0.5	27.0	1.1	61.1	1.3	72.2	1.5	83.3
B 2	1.0	0.3	30.0	0.6	60.0	0.9	90.0	1.0	100.0
B 3	2.5	0.2	8.0	0.6	24.0	1.4	56.0	1.7	68.0
B 4	3.3	0.5	15.1	1.4	42.4	2.5	75.7	2.6	80.0
Mean			20.0		46.8		73.5		82.8
SD			10.3		17.5		13.9		13.2
SE			5.1		8.7		6.9		6.6

TABLE 3.17b

TABLE 3.18a Paired tracheal chains were prepared in the usual way and the relaxation response to field stimulation was recorded using Grass FTO3C transducers.

The relaxation response (R) is expressed as a percentage of inherent tone (T) recorded just prior to stimulation (R(%T)).

Three consecutive frequency response tests over the range 2-32 Hz were conducted under normoxic conditions (A-C).

EXPT. NO.	FREQUENCY (Hz) 2			4			8			16			32		
	T (g)	R (g)	%T	T (g)	R (g)	%T	T (g)	R (g)	%T	T (g)	R (g)	%T	T (g)	R (g)	%T
A 1	1.6	0.1	40.0	1.6	0.2	12.5	1.4	0.3	21.4	1.5	0.5	33.3	1.5	0.9	60.0
2	1.5	0.3	20.0	1.4	0.6	42.8	1.3	0.8	61.5	1.3	1.1	84.6	1.3	1.2	92.3
3	2.1	0.1	2.8	1.9	0.3	15.7	1.8	0.6	33.3	1.7	1.0	58.8	1.6	1.2	75.0
4	1.6	0.3	18.7	1.5	0.5	33.3	1.4	0.7	50.0	1.5	0.9	60.0	1.5	1.1	73.3
5	1.4	0.5	35.7	1.4	0.8	57.1	1.5	1.0	66.6	1.4	1.1	78.5	1.4	1.3	92.8
6	1.4	0.3	21.4	1.4	0.6	42.8	1.3	0.7	53.8	1.3	0.9	69.2	1.4	1.2	85.7
Mean			17.1			34.0			47.7			64.1			79.8
SD			12.2			17.2			17.2			18.1			12.8
SE			4.9			7.0			7.0			7.4			5.2
B 1	1.5	0.0	0.0	1.5	0.1	8.6	1.5	0.3	20.0	1.4	0.5	35.7	1.4	0.8	57.1
2	1.4	0.3	21.4	1.3	0.6	46.1	1.3	0.8	61.5	1.3	1.1	84.6	1.3	1.2	92.3
3	1.3	0.3	23.1	1.3	0.3	23.0	1.3	0.5	38.5	1.3	0.8	61.5	1.3	1.0	76.9
4	1.5	0.3	20.0	1.5	0.6	40.0	1.3	0.7	53.8	1.4	0.8	57.1	1.3	1.1	84.6
5	1.5	0.5	33.3	1.3	0.8	61.5	1.3	1.0	76.9	1.2	1.0	83.3	1.2	1.2	100.0
6	1.6	0.3	18.7	1.6	0.6	37.5	1.5	0.8	53.3	1.4	0.9	64.3	1.4	1.1	78.5
Mean			19.4			36.1			50.6			64.4			81.5
SD			10.8			18.4			19.5			18.2			14.8
SE			4.4			7.5			7.9			7.4			6.0
C 1	1.3	0.1	4.6	1.3	0.1	10.0	1.3	0.3	23.1	1.3	0.5	38.4	1.2	0.8	66.6
2	1.4	0.4	28.5	1.3	0.7	53.8	1.3	0.9	69.2	1.3	1.1	84.6	1.3	1.1	84.6
3	1.3	0.1	10.0	1.3	0.3	23.1	1.3	0.5	38.5	1.3	0.8	61.5	1.2	0.9	75.0
4	1.3	0.3	23.1	1.3	0.5	38.5	1.2	0.7	58.3	1.2	0.8	66.6	1.2	1.0	83.3
5	1.3	0.7	53.8	1.3	0.7	53.8	1.3	0.9	69.2	1.3	1.1	84.6	1.3	1.1	84.6
6	1.6	0.6	37.5	1.4	0.6	42.8	1.3	0.6	46.1	1.3	0.7	53.8	1.3	1.0	76.9
Mean			26.2			37.0			50.7			64.9			76.9
SD			18.1			17.4			18.3			17.9			7.1
SE			7.4			7.1			7.5			7.3			2.9

TABLE 3.18a

TABLE 3.18b Tracheal chains were prepared and monitored as in Table 3.18a. During period A, the tissue was stimulated at 2-32 Hz under normoxic conditions. During period B the 5% CO_2:95% O_2 was replaced by 5% CO_2:95% N_2 and the tests repeated. During period C normoxic conditions were restored and the frequency response tests repeated once more.

EXPT. NO.	FREQUENCY (Hz) 2			4			8			16			32		
	T (g)	R (g)	%T	T (g)	R (g)	%T	T (g)	R (g)	%T	T (g)	R (g)	%T	T (g)	R (g)	%T
1	1.3	0.1	4.6	1.3	0.1	10.0	1.2	0.3	25.0	1.2	0.5	41.6	1.2	0.6	50.0
2	1.6	0.1	8.1	1.5	0.3	22.0	1.4	0.5	35.7	1.4	0.8	57.1	1.5	1.0	66.6
A 3	1.8	0.1	3.3	1.7	0.3	19.4	1.6	0.6	37.5	1.5	0.9	60.0	1.5	1.1	73.3
4	1.8	0.1	7.2	1.7	0.4	23.5	1.6	0.6	37.5	1.6	0.9	56.2	1.6	1.3	81.2
5	1.2	0.3	27.5	1.2	0.5	38.3	1.2	0.66	55.0	1.2	0.8	66.6	1.2	1.0	83.3
6	1.7	0.2	11.7	1.7	0.5	27.0	1.6	0.7	43.7	1.6	1.1	68.7	1.5	0.9	60.0
Mean			10.4			23.4			39.1			58.4			69.1
SD			8.8			9.3			9.9			9.6			12.8
SE			3.6			3.8			4.0			3.9			5.2
1	0.5	0.0	0.0	0.6	0.0	0.0	0.5	0.0	0.0	0.5	0.1	26.0	0.5	0.2	40.0
2	0.8	0.0	0.0	0.9	0.1	6.6	0.9	0.2	22.2	0.9	0.4	44.4	1.0	0.6	60.0
B 3	0.6	0.0	0.0	0.8	0.1	16.2	0.6	0.1	21.6	0.6	0.3	50.0	0.6	0.5	83.3
4	0.8	0.0	3.7	0.8	0.1	12.5	0.8	0.1	16.2	0.8	0.3	37.5	0.8	0.6	75.0
5	0.5	0.0	0.0	0.6	0.2	33.3	0.5	0.2	40.0	0.5	0.3	60.0	0.4	0.4	100.0
6	0.7	0.1	8.5	0.6	0.2	33.3	0.6	0.1	21.6	0.5	0.3	60.0	0.5	0.5	100.0
Mean			2.0			16.9			20.3			46.3			76.4
SD			3.5			13.7			12.8			13.3			23.5
SE			1.4			5.6			5.2			2.4			9.6
1	1.0	0.0	0.0	1.0	0.2	16.0	0.9	0.3	33.3	0.9	0.4	44.4	1.0	0.5	50.0
2	1.1	0.1	11.8	1.2	0.4	33.3	1.2	0.5	41.6	1.3	0.7	53.8	1.5	1.1	73.3
C 3	1.5	0.1	8.6	1.5	6.3	20.0	1.5	0.5	33.3	1.4	0.8	57.1	1.4	1.1	78.6
4	1.4	0.1	4.3	1.6	0.4	25.0	1.7	0.6	35.3	1.7	1.0	58.8	1.7	1.3	76.5
5	0.9	0.1	11.1	1.0	0.3	30.0	1.1	0.6	54.5	1.1	0.7	63.6	1.1	0.8	72.7
6	1.4	0.1	4.3	1.8	0.5	27.7	1.6	0.6	37.5	1.5	0.7	46.6	1.5	1.2	80.0
Mean			6.7			25.3			39.2			54.0			71.8
SD			4.6			6.4			8.1			7.4			11.1
SE			1.8			2.6			3.3			3.0			4.5

TABLE 3.18b

TABLE 3.19a Paired tracheal chains were prepared in the usual way and the relaxation response to adrenaline recorded using Grass FT03C transducers. In the tables which follow the responses have been expressed as a percentage of tone recorded prior to the first dose of adrenaline.

During period A the tissues were maintained under normoxic conditions.
During period B the 5% CO_2:95% O_2 was replaced by 5% CO_2:95% N_2 and the dose response tests were repeated.
During period C, normoxic conditions were restored and the dose response tests repeated a further time.

The concentration of adrenaline found to relax the tracheal chain varied from one preparation to another.

	[Adrenaline] µg/ml T_i (g)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.5	2.0	3.0
							RESPONSE ($\%T_i$)							
1	2.0		6.6			26.6					56.6		96.6	100.0
2	1.7	15.4	26.9	38.5	46.1		61.5		65.4		73.1			
3	1.6	25.0	54.2	79.2	87.5	91.6								
A 4	1.5		9.1	27.3	54.5	72.7		86.4		100.0				
5	2.1			22.6		35.5		45.2			61.3	83.8		
6	1.8	21.4	50.0	67.8	82.1	92.8		100.0						
1	1.0		40.0			80.0					100.0		100.0	100.0
2	1.1	41.2	64.7	82.3	88.2		94.1		100.0		100.0			
3	1.5	60.8	100.0	100.0	100.0	100.0								
B 4	1.1		68.7	93.7	100.0									
5	0.5			0.0		0.0					12.5	62.5	100.0	
6	0.9	14.8	44.4	74.1	81.5	96.3								
1	1.7		46.1			76.9					100.0		100.0	100.0
2	2.3	17.6	38.2	61.7	73.5		82.3		88.2		88.2			
3	1.5	36.4	59.1	68.2	81.8	100.0								
C 4	1.5	50.0	77.2	90.9	95.4	100.0								
5	1.3						21.0	36.8				68.4	84.2	
6	1.2	27.7	33.3	55.5		72.2		100.0						

TABLE 3.19a

TABLE 3.19b The second chain of each pair was set up as in Table 3.21a and maintained under normoxic conditions during three consecutive dose-response tests as a control.

	[Adrenaline] µg/ml T_i (g)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.5	2.0	3.0
							RESPONSE ($\%T_i$)							
	1	1.2		11.1			33.3				66.6		88.8	100.0
	2	2.2	33.3	54.5	63.6	69.7		78.8			84.8	100.0		
A	3	1.8	44.4	66.6	77.7	100.0								
	4	1.5					21.7		43.5		65.2		100.0	
	5	2.6			17.5		30.0		35.0			45.0	60.0	65.0
	6	2.1	25.0	53.1	75.0	84.4	87.5		100.0					
	1	1.3					42.1				63.1		89.5	100.0
	2	2.3	28.6	51.4	68.6	82.8		97.1			100.0			
B	3	2.1	51.6	58.1	93.5	100.0								
	4	1.1		35.3	52.9	76.5	88.2		100.0					
	5	1.5												
	6	1.5	26.1	47.8	69.5	86.9			95.6					
	1	0.9					57.1				85.7		100.0	100.0
	2	2.5	23.7	47.4	65.8	78.9		92.1						
C	3	-												
	4	1.3	35.0	65.0	90.0	100.0								
	5	1.3												
	6	1.0	25.0	50.0	70.0	80.0	90.0		100.0					

TABLE 3.19b

APPENDIX II

TABLE 3.47 The effect of 31 bar on the response to field stimulation.

Paired tracheal chains were prepared in the usual way and allowed to take up tone. Each chain was stimulated at 2, 4, 8 and 16 Hz in three consecutive frequency response tests.

The table records the inherent tone of the preparations (in mm) and the response to field stimulation expressed as a percentage of inherent tone recorded just prior to stimulation (R(%T)).

a. Experimental

A. at 1 bar 0.95 bar O_2:0.05 bar CO_2.

B. at 31 bar 30 bar He:0.95 bar O_2: 0.05 bar CO_2.

C. at 1 bar 0.95 bar O_2:0.05 bar CO_2.

b. Controls

The paired control chains were maintained at 1 bar throughout (A, B and C). Neither inherent tone nor R(%T) were significantly altered during these control experiments.

EXPT. NO.	FREQUENCY (Hz) 2		4		8		16	
	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	R(%T)
A 1	16.0	-	16.0	-	16.0	50.0	14.0	57.0
A 2	30.0	30.0	31.0	48.0	31.0	64.0	30.0	80.0
A 3	20.0	-	20.0	15.0	20.0	40.0	15.0	20.0
A 4	20.0	35.0	18.0	44.0	17.0	64.0	16.0	62.5
Mean	21.5	32.5	21.2	35.6	21.0	54.5	18.7	54.8
SD	5.9	3.5	6.7	18.0	6.8	11.7	7.5	25.2
SE	2.9	2.5	3.3	10.4	3.4	5.8	3.7	12.6
B 1	6.0	-	6.0	-	6.0	100.0	7.0	71.0
B 2	28.0	32.0	24.0	37.5	25.0	40.0	25.0	60.0
B 3	17.0	-	17.0	-	17.0	29.0	15.0	12.0
B 4	12.0	41.0	8.0	37.5	5.0	60.0	3.0	66.6
Mean	15.7	36.5	13.7	37.5	13.2	57.2	12.5	52.4
SD	9.3	6.3	8.3	-	9.5	31.2	9.7	27.3
SE	4.6	4.5	4.2	-	4.7	15.6	4.8	13.6
1	15.0	-	15.0	-	15.0	53.0	11.0	63.0
2	27.0	18.5	23.0	26.0	22.0	54.0	23.0	74.0
3	16.0	-	16.0	-	16.0	18.7	17.0	29.0
4	8.0	50.0	6.0	66.0	6.0	92.0	7.0	85.0
Mean	14.0	34.2	15.0	46.0	14.7	54.4	14.5	62.7
SD	9.8	22.7	6.9	28.3	6.6	29.9	3.8	24.2
SE	4.9	15.9	3.5	20.0	3.3	14.9	1.9	12.1

a. Experimental Chains

TABLE 3.47 Effect of 31 bar on the response to field stimulation.

EXPT. NO.	FREQUENCY (Hz) 2		4		8		16	
	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	R(%T)
A 1	15.0	-	15.0	-	15.0	33.0	13.0	38.0
A 2	19.0	10.0	20.0	22.5	20.0	57.5	19.0	57.8
A 3	15.0	26.0	16.0	37.5	15.0	40.0	15.0	40.0
A 4	15.0	36.0	12.0	41.0	11.0	54.5	11.0	59.0
Mean	16.0	24.0	15.7	33.6	15.2	46.2	14.5	48.7
SD	2.0	13.1	3.3	9.8	3.7	11.6	3.4	11.2
SE	1.0	7.7	1.6	5.8	1.8	5.8	1.7	5.6
B 1	22.0	-	22.0	-	22.0	23.0	20.0	30.0
B 2	19.0	16.0	19.0	26.0	19.0	31.0	19.0	52.0
B 3	17.0	-	17.0	35.0	16.0	31.0	17.0	41.0
B 4	9.0	38.0	5.0	60.0	4.0	50.0	3.0	100.0
Mean	16.7	27.0	15.7	40.3	15.2	33.7	14.7	55.7
SD	5.5	15.0	7.4	17.6	7.8	11.4	7.9	30.8
SE	2.8	11.1	3.7	10.4	3.9	5.7	3.9	15.4
C 1	20.0	-	20.0	25.0	19.0	23.0	15.0	16.0
C 2	18.0	27.0	17.0	35.0	17.0	52.0	17.0	70.0
C 3	15.0	26.0	14.0	36.0	15.0	40.0	14.0	40.0
C 4	3.0	66.0	12.0	100.0	0.0	0.0	0.0	0.0
Mean	14.0	39.6	15.7	49.0	12.7	28.7	11.5	31.5
SD	7.6	22.8	3.5	34.3	8.6	22.5	7.7	30.4
SE	3.8	13.4	1.7	17.2	4.3	11.2	3.8	15.2

b. Control Chains

TABLE 3.47 Effect of 31 bar on the response to field stimulation.

FIGURE 3.30 The effect of 31 bar on the response to field stimulation.

The data shown in Tables 3.47a and 3.47b are represented graphically in this figure.

■ □ The experimental chains.

● ○ The control chains.

The frequency of stimulation is shown on the x axis.

On the left-hand y axis is the response to field stimulation, expressed as a percentage of inherent tone recorded just prior to field stimulation (R(%T)).

On the right-hand y axis is the inherent tone (in mm) recorded immediately prior to field stimulation.

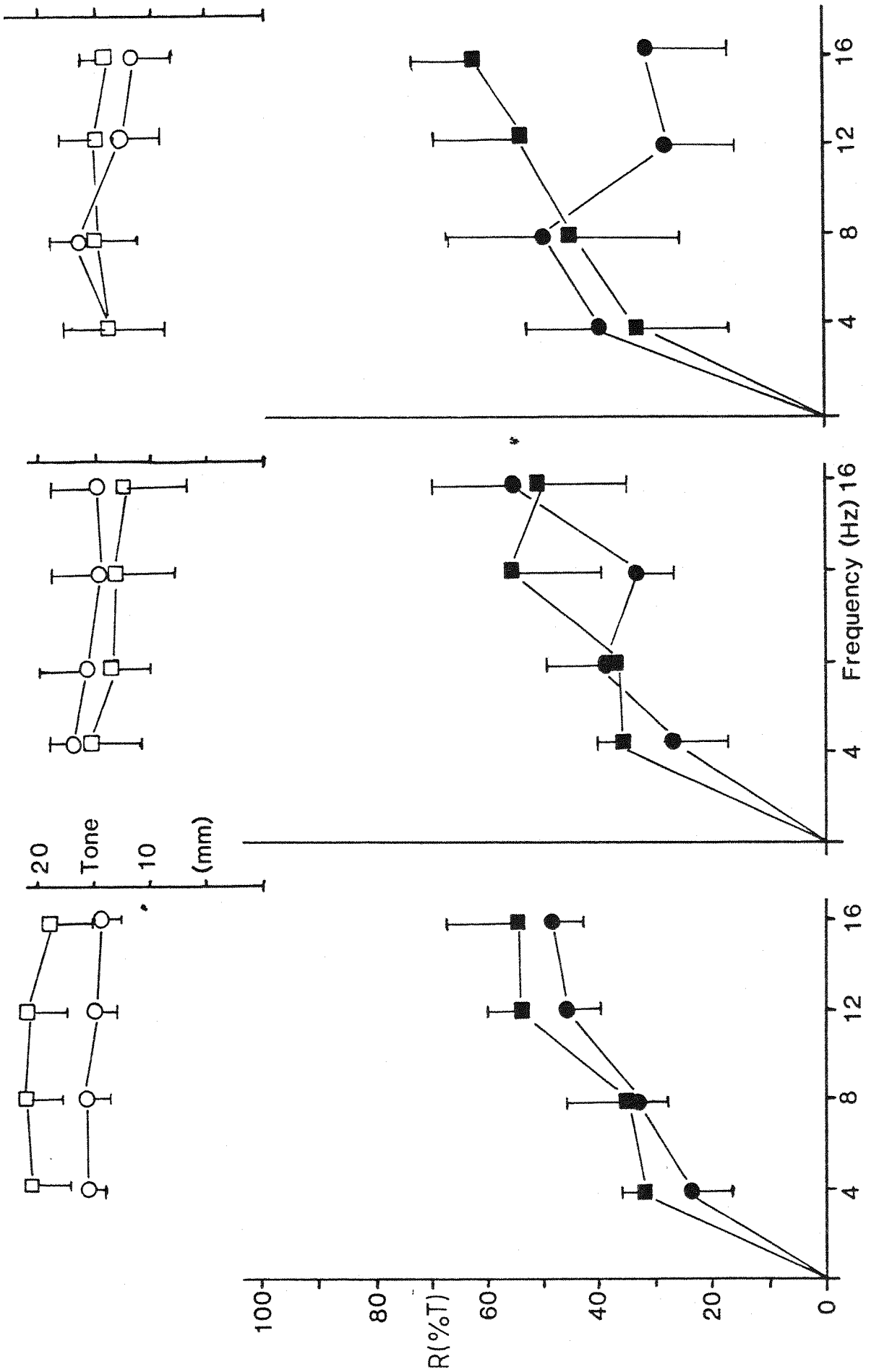

R(%T)
100
80
60
40
20
0
4
8
12
16
Frequency (Hz)
Tone
20
10
(mm)

TABLE 3.48 The effect of 31 bar on the response to field stimulation (atropine (10^{-6} g/ml) present throughout).

Paired tracheal chains were prepared in the usual way and allowed to take up tone. Atropine was added to the bath fluid of each chain to a final concentration of 10^{-6} g/ml. Each chain was stimulated at 2, 4, 8 and 12 Hz in three consecutive frequency response tests.

The table shows the inherent tone of the preparations (in mm) and the response to field stimulation expressed as a percentage of inherent tone recorded just prior to stimulation (R(%T)).

a. Experimental

A. at 1 bar 0.95 bar O_2:0.05 bar CO_2.

B. at 31 bar 30 bar He:0.95 bar O_2: 0.05 bar CO_2.

C. at 1 bar 0.95 bar O_2:0.05 bar CO_2.

Inherent tone was reduced by compression to 31 bar but (R(%T)) was not significantly altered.

b. Controls

Control Chains: The paired control chains were maintained at 1 bar throughout (A, B and C). Neither inherent tone nor R(%T) were significantly altered during these control experiments.

EXPT. NO.	FREQUENCY (Hz) 2		4		8		12	
	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	(%T)	Tone (mm)	R(%T)
1	23.0	34.7	22.0	45.0	21.0	52.4	19.0	47.4
2	14.0	39.0	12.0	41.6	12.0	50.0	11.0	59.1
A 3	15.0	20.0	14.0	28.6	13.0	30.7	12.0	41.6
4	25.0	8.0	24.0	12.5	22.0	18.0	21.0	28.5
5	27.0	18.5	25.0	44.0	27.0	48.1	22.0	59.0
Mean	20.8	24.0	19.4	34.3	19.0	39.8	17.0	47.1
SD	5.9	12.6	5.9	13.8	6.4	14.9	5.1	12.8
SE	2.6	5.6	2.7	6.2	2.8	6.6	2.3	5.7
1	15.0	30.0	11.0	36.0	9.0	44.0	9.0	33.0
2	9.0	38.0	5.0	60.0	4.0	50.0	3.0	100.0
B 3	10.0	20.0	10.0	20.0	10.0	30.0	9.0	33.0
4	17.0	17.0	15.0	26.0	12.0	25.0	11.0	54.0
5	21.0	33.0	20.0	50.0	19.0	58.0	20.0	70.0
Mean	14.4	21.6	12.2	38.4	10.8	41.4	10.4	52.0
SD	4.9	14.9	5.6	16.6	3.3	13.7	6.1	36.7
SE	2.2	6.6	2.5	7.4	1.5	6.1	2.7	16.4
1	-	-	-	-	-	-	-	-
2	3.0	60.0	2.0	100.0	0.0	100.0	0.0	100.0
C 3	9.0	16.0	8.0	18.7	8.0	25.0	8.0	33.0
4	18.0	22.0	18.0	27.0	18.0	33.0	18.0	33.0
5	22.0	34.0	21.0	40.0	21.0	52.0	20.0	80.0
Mean	13.0	33.0	12.2	21.4	11.7	52.5	11.5	59.5
SD	8.6	19.4	8.8	16.7	9.6	33.6	9.3	36.3
SE	4.3	9.7	4.4	8.4	4.8	16.8	4.6	18.1

TABLE 3.48a Effect of 31 bar on the response to field stimulation. 10^{-6}g/ml atropine present throughout.

Experimental chains.

EXPT. NO.	FREQUENCY (Hz) 2		4		8		16	
	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	R(%T)
1	17.0	41.2	15.0	73.3	15.0	86.6	15.0	86.6
2	20.0	35.0	18.0	44.0	18.0	61.1	16.0	62.5
A 3	21.0	38.1	16.0	50.0	13.0	69.2	14.0	92.8
4	32.0	28.1	28.0	50.0	24.0	62.5	18.0	61.1
5	28.0	0.0	28.0	10.7	28.0	32.1	27.0	40.7
Mean	23.6	28.5	21.0	45.6	19.6	62.3	18.0	68.7
SD	6.2	16.6	6.5	22.5	6.3	19.7	5.2	21.1
SE	2.7	7.4	2.9	10.0	2.8	8.8	2.3	9.6
1	11.0	72.0	7.0	100.0	5.0	100.0	5.0	100.0
2	12.0	41.0	8.0	37.5	5.0	60.0	6.0	33.0
B 3	12.0	16.0	12.0	41.0	12.0	58.0	10.0	90.0
4	12.0	50.0	13.0	61.0	12.0	66.0	14.0	78.0
5	25.0	16.0	26.0	34.0	24.0	41.0	24.0	58.0
Mean	14.4	39.0	13.2	54.7	11.6	65.0	11.8	71.8
SD	5.9	23.8	7.6	27.4	7.7	21.6	7.7	26.7
SE	2.6	10.6	3.4	12.2	3.5	9.6	3.4	11.9
1	11.0	82.0	10.0	100.0	9.0	100.0	9.0	100.0
2	8.0	50.0	6.0	66.0	6.0	91.6	6.0	100.0
C 3	16.0	18.7	16.0	25.0	15.0	53.0	15.0	64.0
4	11.0	27.0	12.0	58.0	12.0	58.0	12.0	91.0
5	30.0	25.0	29.0	29.0	28.0	39.0	28.0	55.0
Mean	15.2	40.5	11.0	55.6	14.0	68.3	12.0	82.0
SD	8.7	26.0	4.2	30.5	8.5	26.2	9.3	21.1
SE	3.9	11.6	1.8	13.6	3.8	11.7	4.2	9.4

TABLE 3.48b Control chains.

FIGURE 3.31 The effect of 31 bar on the response to field stimulation (atropine (10^{-6} g/ml) present throughout).

The data shown in Tables 3.48a and 3.48b are represented graphically in this figure.

■ □ The experimental chains.

● ○ The control chains.

On the left-hand y axis is the response to field stimulation, expressed as a percentage of inherent tone recorded just prior to field stimulation (R(%T)).

On the right-hand y axis is the inherent tone (in mm) recorded immediately prior to field stimulation.

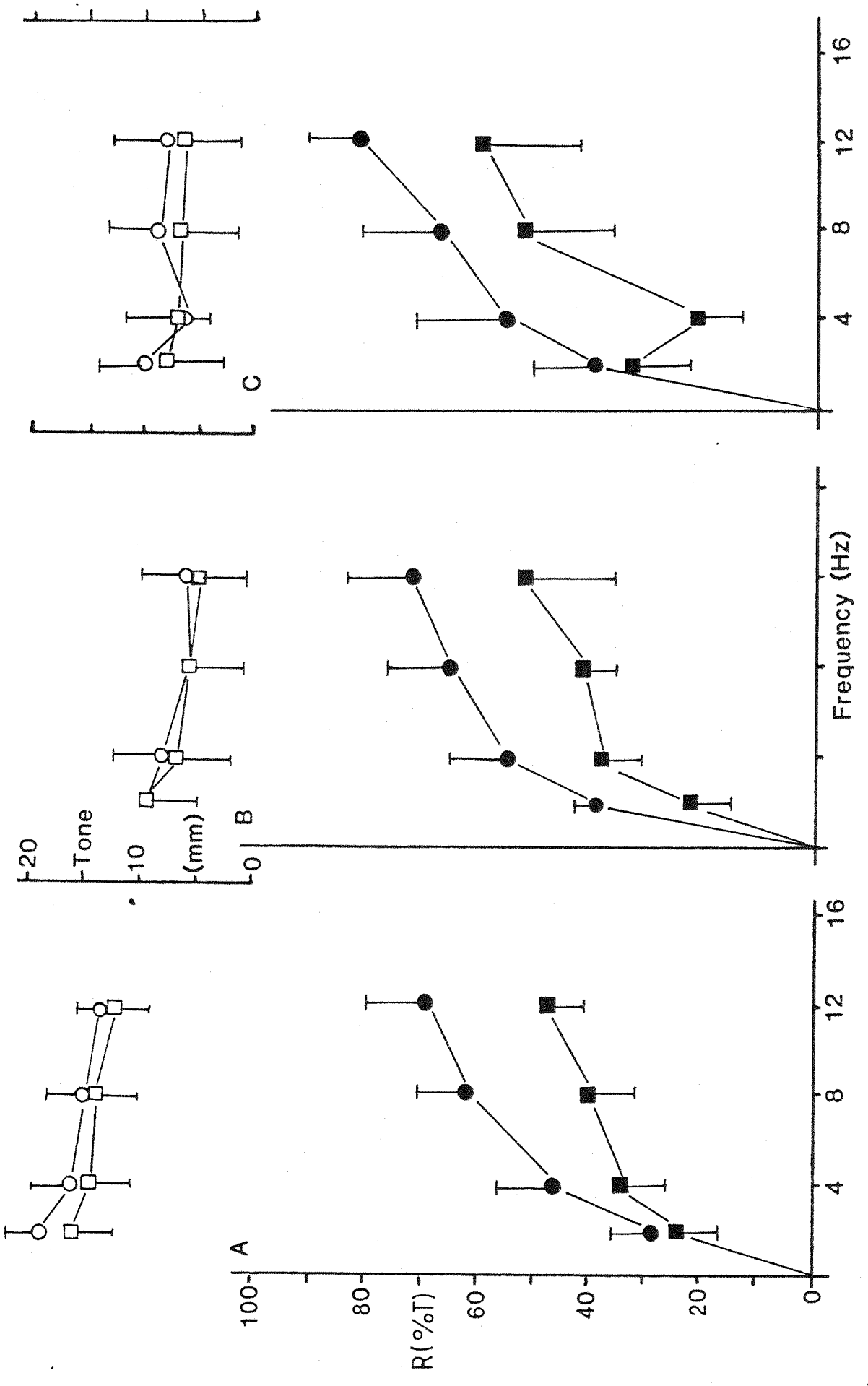

A
B
C
100
80
60
40
20
0
R(%T)
4
8
12
16
Frequency (Hz)
20
10
0
Tone
(mm)

TABLE 3.49 The effect of 43 bar on the response to field stimulation.

Paired tracheal chains were prepared in the usual way and allowed to take up tone. Each chain was stimulated at 4, 8, 16 and 20 Hz in three consecutive frequency response tests.

The table shows the inherent tone of the preparations (in mm) and the response to field stimulation expressed as a percentage of inherent tone recorded just prior to stimulation (R(%T)).

a. Experimental

A. at 1 bar, 0.95 bar O_2:0.05 bar CO_2.

B. at 43 bar, 42 bar He:0.95 bar O_2: 0.05 bar CO_2.

C. at 1 bar, 0.95 bar O_2:0.05 bar CO_2.

b. Controls

The paired control chains were maintained at 1 bar throughout (A, B and C). Neither inherent tone, nor R(%T) were significantly altered during these control experiments.

EXPT. NO.	FREQUENCY (Hz) 4		8		16		20	
	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	R(%T)
A 1	16.0	25.0	16.0	37.0	16.5	66.0	13.0	69.0
A 2	30.0	16.0	30.0	26.0	39.0	36.0	25.0	40.0
A 3	22.0	36.0	22.0	40.0	21.0	38.0	20.0	60.0
A 4	8.0	18.0	8.0	31.0	8.0	37.5	-	-
Mean	19.0	23.7	19.0	33.5	21.1	44.4	19.3	56.3
SD	9.3	9.0	9.3	6.2	13.1	14.4	6.0	14.8
SE	4.6	4.5	4.6	3.1	6.5	7.2	3.0	8.5
B 1	11.0	27.0	11.0	80.0	12.0	75.0	11.0	54.0
B 2	33.0	15.0	32.0	18.0	30.0	23.0	28.0	32.0
B 3	15.0	33.0	16.0	37.0	16.0	50.0	14.0	57.0
B 4	8.0	18.0	7.5	33.0	7.0	28.0	6.5	-
Mean	16.7	23.2	16.6	42.0	16.2	44.0	14.8	41.6
SD	11.2	8.3	10.8	26.6	9.8	23.7	9.3	13.6
SE	5.6	4.1	5.4	13.3	4.9	11.8	4.6	7.8
C 1	13.0	26.0	13.0	42.0	11.0	45.0	11.0	54.0
C 2	13.0	30.0	13.0	38.0	12.0	50.0	12.0	50.0
C 3	16.0	37.5	16.0	37.5	16.0	37.5	14.0	57.0
C 4	4.5	22.0	5.0	30.5	5.0	30.0	-	-
Mean	11.6	28.8	11.7	37.0	11.0	40.6	12.3	53.6
SD	4.9	6.6	4.7	4.7	4.5	8.7	1.5	3.5
SE	2.5	3.3	2.3	2.4	2.3	4.4	0.8	2.0

TABLE 3.49a Effectof compression to 43 bar on the response to field stimulation.

Experimental chains.

EXPT. NO.	FREQUENCY (Hz) 4		8		16		20	
	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	R(%T)
A 1	19.0	5.0	19.0	15.0	20.0	50.0	16.0	50.0
A 2	33.0	18.0	32.0	34.0	30.0	46.0	30.0	46.0
A 3	30.0	36.0	30.0	46.0	-	-	30.0	63.0
A 4	19.5	25.0	19.0	47.0	18.0	57.0	19.0	-
Mean	25.4	21.0	25.0	35.5	22.6	51.0	23.7	53.0
SD	7.2	12.9	6.9	14.8	6.4	5.6	7.3	8.8
SE	3.6	6.5	3.5	7.4	3.8	3.2	3.6	5.1
B 1	13.0	11.5	11.0	18.0	11.0	45.0	8.0	37.0
B 2	9.0	22.0	6.0	16.0	5.0	20.0	3.0	33.0
B 3	19.0	26.0	16.0	31.0	17.0	52.0	15.0	60.0
B 4	6.0	50.5	5.5	54.0	4.5	44.0	-	-
Mean	11.7	27.5	9.6	29.1	9.4	40.2	8.6	43.3
SD	5.6	16.5	4.9	17.4	5.8	13.9	6.0	14.5
SE	2.8	8.2	2.5	8.7	2.9	6.9	3.5	8.4
C 1	12.0	8.0	16.0	18.0	15.0	26.0	16.0	43.0
C 2	6.0	50.0	7.0	28.0	6.5	53.0	7.0	57.0
C 3	16.0	31.0	20.0	30.0	21.0	33.0	20.5	58.0
C 4	9.0	22.0	10.0	30.0	9.0	66.0	9.0	-
Mean	10.7	21.7	13.2	26.5	12.8	44.5	13.1	52.6
SD	4.3	17.6	5.8	5.7	6.5	18.3	6.2	8.4
SE	2.1	8.8	2.9	2.8	3.2	9.2	3.1	4.2

TABLE 3.49b Control chains.

FIGURE 3.32 The effect of 43 bar on the response to field stimulation.

The data shown in Tables 3.49a and 3.49b are represented graphically in this figure.

■ □ The experimental chains.

● ○ The control chains.

The frequency of stimulation is shown on the x axis.

On the left-hand y axis is the response to field stimulation, expressed as a percentage of inherent tone recorded just prior to field stimulation (R(%T)).

On the right-hand y axis is the inherent tone (in mm) recorded immediately prior to field stimulation.

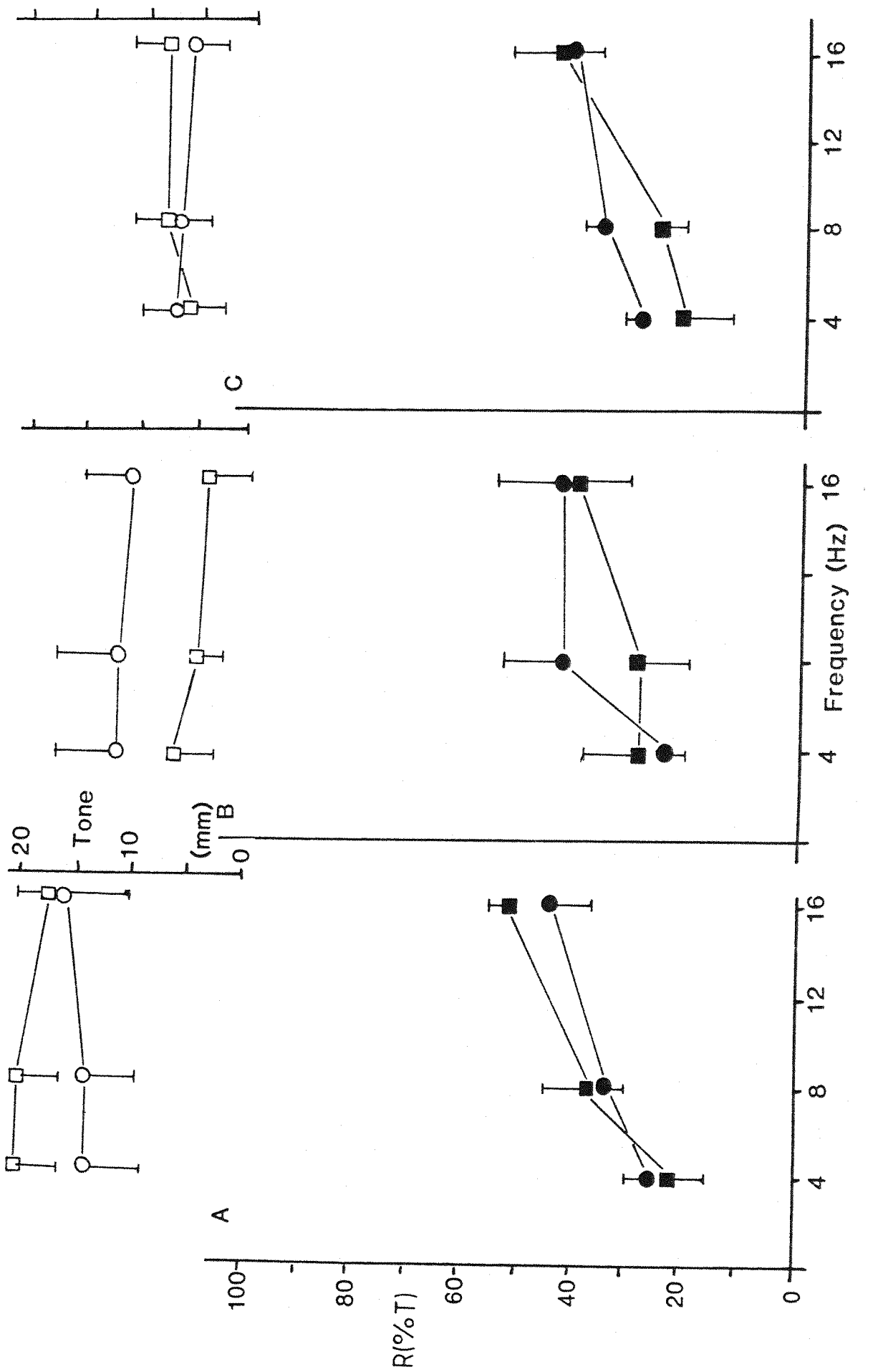

A
B
C
R(%T)
100
80
60
40
20
0
4
8
12
16
Frequency (Hz)
Tone
20
10
0
(mm)

TABLE 3.50 The effect of 43 bar on the response to field stimulation (atropine (10^{-6} g/ml) was present throughout).

Paired tracheal chains were prepared in the usual way and allowed to take up tone. Each chain was stimulated at 2, 4, 8 and 16 Hz in three consecutive frequency tests. Atropine was added to the bath fluid of both preparations.

The table shows the inherent tone of the preparations (in mm) and the response to field stimulation expressed as a percentage of inherent tone recorded just prior to stimulation (R(%T)).

a. Experimental

A. at 1 bar, 0.95 bar O_2:0.05 bar CO_2.

B. at 43 bar, 42 bar He:0.95 bar O_2:0.05 bar CO_2.

C. at 1 bar, 0.95 bar O_2:0.05 bar CO_2.

Inherent tone was reduced by compression to 43 bar but R(%T) was not significantly altered.

b. Controls

The paired control chains were maintained at 1 bar throughout (A, B and C). Neither inherent tone, nor R(%T) were significantly altered during these control experiments.

EXPT. NO.	FREQUENCY (Hz) 2		4		8		16	
	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	R(%T)
A 1	17.0	32.0	16.0	40.0	15.5	45.0	15.0	50.0
A 2	26.0	0.0	26.0	19.2	26.0	13.4	25.0	30.0
A 3	21.5	46.5	19.0	55.0	17.0	53.0	17.0	70.5
A 4	14.0	21.4	14.0	35.0	14.0	43.0	14.0	52.0
Mean	19.6	24.9	18.7	37.3	18.1	38.6	17.7	50.6
SD	5.2	19.6	5.2	14.7	5.4	17.3	4.9	16.5
SE	2.6	9.8	2.6	7.4	2.7	8.6	2.5	8.3
B 1	15.0	30.0	14.0	28.0	14.0	39.0	14.0	53.5
B 2	26.0	11.5	26.0	19.2	25.0	20.0	25.0	28.0
B 3	16.0	56.0	14.0	57.0	13.0	61.0	14.0	71.0
B 4	14.0	35.0	13.0	46.0	11.0	59.0	10.0	60.0
Mean	17.7	33.1	16.7	37.5	15.7	44.7	15.7	53.1
SD	5.6	18.3	6.2	11.1	6.3	19.2	6.4	18.2
SE	2.8	9.1	3.1	8.5	3.1	9.6	3.2	9.1
C 1	15.0	36.0	13.0	46.0	13.0	57.0	11.0	59.0
C 2	24.0	12.5	25.0	18.0	25.0	28.0	22.0	32.0
C 3	15.0	53.0	13.0	61.5	12.0	58.0	12.0	66.0
C 4	11.0	36.0	11.0	54.5	9.0	66.6	8.0	75.0
Mean	16.2	34.4	15.5	45.0	14.7	52.4	13.2	58.0
SD	5.5	16.6	6.4	19.1	7.0	16.8	6.1	18.5
SE	2.7	8.3	3.2	9.5	3.5	8.4	3.0	9.2

TABLE 3.50a Effect of 43 bar on the response to field stimulation. 10^{-6}g/ml, Atropine present throughout.

Experimental Chains.

EXPT. NO.	FREQUENCY (HZ) 2		4		8		16	
	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	R(%T)
A 1	20.0	30.0	17.0	35.0	15.0	50.0	13.0	69.0
A 2	33.0	10.6	33.0	18.0	32.0	25.0	31.0	38.0
A 3	20.0	40.0	20.0	90.0	20.0	100.0	20.0	100.0
A 4	20.0	20.0	17.0	35.0	16.0	43.0	14.0	57.0
Mean	18.2	25.1	21.7	44.5	20.7	42.0	19.5	66.0
SD	13.6	12.6	7.6	31.3	7.8	42.5	8.3	26.0
SE	6.8	6.3	3.8	15.7	3.9	21.2	4.1	13.0
B 1	6.0	30.0	5.0	40.0	5.0	40.0	6.0	50.0
B 2	26.0	11.5	26.0	19.0	25.0	20.0	25.0	28.0
B 3	11.0	0.0	9.0	0.0	10.0	30.0	9.0	11.0
B 4	11.0	72.0	8.0	87.0	7.0	85.0	9.0	88.0
Mean	13.5	28.4	12.0	36.5	11.7	43.7	10.7	44.2
SD	8.6	31.6	9.5	37.4	9.0	28.6	10.4	33.2
SE	4.3	15.8	4.7	18.7	4.5	14.3	5.2	16.6
C 1	9.0	27.7	10.0	40.0	10.0	50.0	9.0	44.4
C 2	24.0	4.0	24.0	12.5	25.0	18.0	25.0	28.0
C 3	15.0	66.6	14.0	71.0	16.0	62.5	18.0	77.7
C 4	12.0	0.0	17.0	17.6	23.0	21.7	23.0	21.7
Mean	15.0	24.6	16.2	35.3	18.5	38.0	18.7	42.9
SD	6.5	30.5	5.9	26.6	6.8	21.6	7.1	25.0
SE	3.2	15.3	2.9	13.3	3.4	10.8	3.5	12.5

TABLE 3.50b Control chains.

FIGURE 3.33 The effect of 43 bar on the response to field stimulation (atropine (10^{-6} g/ml) present throughout).

The data shown in Tables 3.50a and 3.50b are represented graphically in this figure.

■ □ The experimental chains.

● ○ The control chains.

The frequency of stimulation is shown on the x axis.

On the left-hand y axis is the response to field stimulation, expressed as a percentage of inherent tone recorded just prior to field stimulation (R(%T)).

On the right-hand y axis is the inherent tone (in mm) recorded immediately prior to field stimulation.

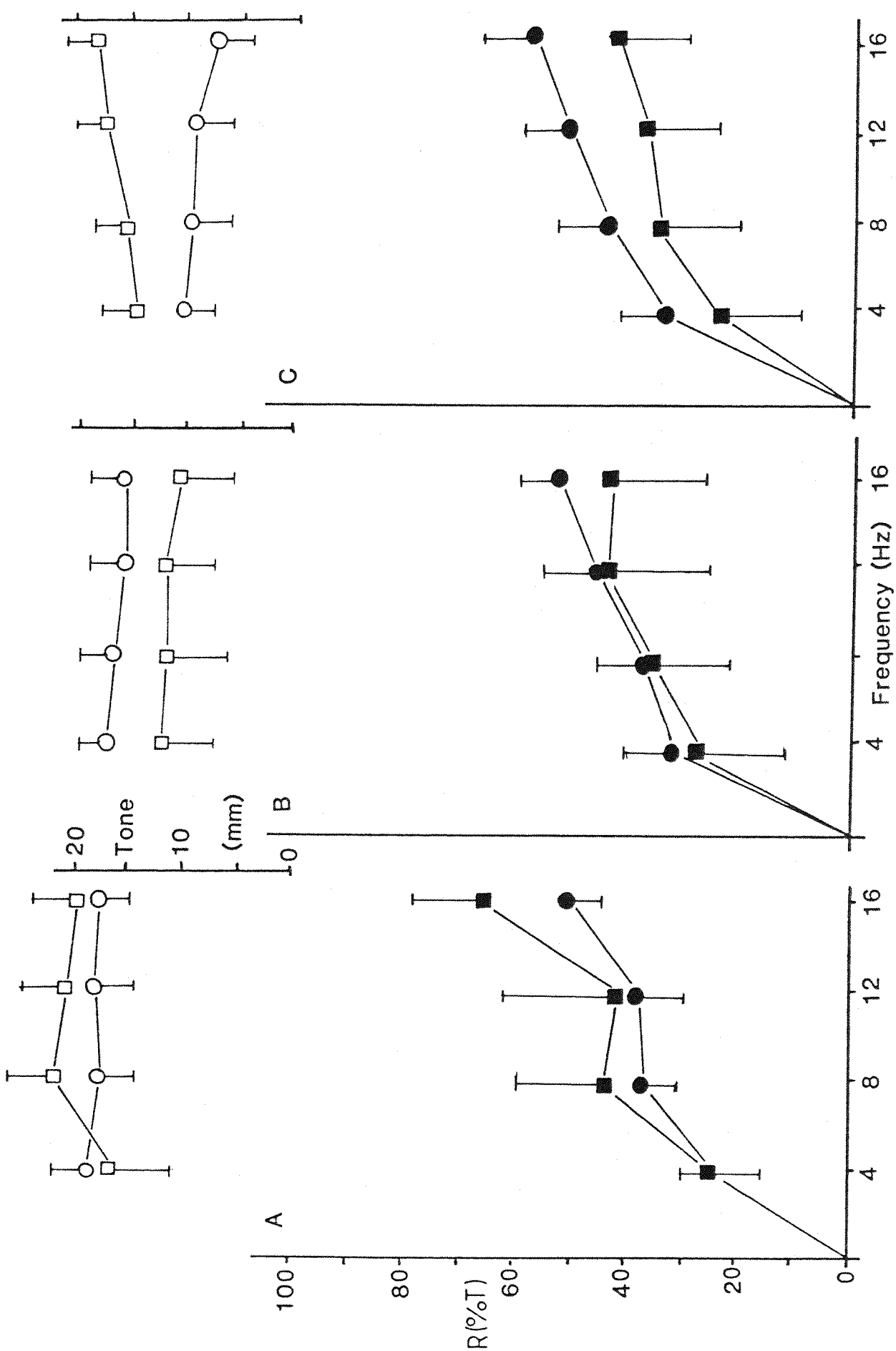

A
B
C
R(%T)
100
80
60
40
20
0
4
8
12
16
Frequency (Hz)
Tone
20
10
0
(mm)

TABLE 3.51 The effect of 67 bar on the response to field stimulation.

Paired tracheal chains were prepared in the usual way and allowed to take up tone. Each chain was stimulated at 4, 8, 12 and 16 Hz in three consecutive frequency response tests.

The table shows the inherent tone (in mm) of the preparations and the response to field stimulation expressed as a percentage of inherent tone recorded just prior to stimulation (R(%T)).

a. Experimental

A. at 1 bar, 0.95 bar O_2:0.05 bar CO_2.

B. at 67 bar, 66 bar He:0.95 bar O_2: 0.05 bar CO_2.

C. at 1 bar, 0.95 bar O_2:0.05 bar CO_2.

b. Controls

The paired control chains were maintained at 1 bar throughout (A, B and C). Neither inherent tone, nor R(%T) were significantly altered during these control experiments.

EXPT. NO.	FREQUENCY (Hz) 4		8		12		16	
	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	R(%T)
A 1	14.0	10.9	12.5	44.0	12.0	50.0	13.0	53.0
A 2	18.5	35.0	18.0	34.0	18.0	43.0	18.0	52.0
A 3	27.0	22.0	26.0	27.0	23.0	33.0	23.0	38.0
Mean	19.8	22.6	18.8	35.0	17.6	42.0	18.0	47.6
SD	6.6	12.0	6.8	8.5	5.5	8.5	5.0	8.4
SE	3.8	6.9	3.9	4.9	3.2	4.9	2.9	4.2
B 1	13.0	38.0	11.0	54.0	10.5	57.0	9.0	66.6
B 2	23.0	26.0	22.0	36.0	20.0	35.0	23.0	52.0
B 3	17.5	20.0	17.0	29.0	17.0	41.0	16.5	45.0
Mean	17.8	28.0	16.6	39.6	15.8	44.3	16.2	54.5
SD	5.0	9.2	5.5	12.9	4.8	11.4	7.0	11.0
SE	2.9	5.3	3.2	7.4	2.4	6.6	4.0	6.4
C 1	16.0	30.0	13.0	41.0	12.0	33.0	11.0	66.0
C 2	19.0	31.0	15.0	34.0	15.0	41.0	14.0	45.0
C 3	10.0	50.0	6.0	33.0	6.0	40.0	6.0	42.0
Mean	15.0	37.0	11.3	36.0	11.0	38.0	10.3	31.0
SD	4.6	11.3	4.7	4.3	4.6	4.3	4.0	21.7
SE	2.6	6.5	2.7	2.5	2.6	2.5	2.3	12.5

TABLE 3.51a Effect of 67 bar on the response to field stimulation.

Experimental chains.

EXPT. NO.	FREQUENCY (Hz) 4		8		12		16	
	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	R(%T)	Tone (mm)	R(%T)
A 1	10.0	60.0	11.0	72.0	12.0	75.0	13.0	76.0
A 2	25.0	36.0	28.0	54.0	26.0	47.0	25.0	50.0
A 3	25.0	28.0	24.0	50.0	23.0	50.0	24.0	60.0
Mean	20.0	41.3	21.0	58.6	20.3	57.3	20.6	62.0
SD	8.6	16.6	8.8	11.7	7.4	15.3	6.6	13.1
SE	5.0	9.6	4.4	6.7	4.3	8.8	3.9	7.7
B 1	20.0	75.0	20.0	75.0	16.0	100.0	18.0	75.0
B 2	10.0	15.0	10.0	20.0	9.0	22.0	11.0	54.0
B 3	5.0	4.0	2.0	30.0	2.0	37.0	2.0	44.0
Mean	11.6	31.3	11.6	41.6	9.0	53.0	10.3	57.6
SD	7.6	38.2	7.6	29.3	7.0	41.4	8.0	15.8
SE	4.4	22.1	4.4	16.9	4.0	23.9	4.6	9.1
C 1	9.0	55.0	6.0	80.0	7.0	57.0	6.0	66.0
C 2	22.0	30.0	22.0	50.0	21.0	45.0	18.0	50.0
C 3	20.0	18.0	20.0	36.0	20.0	47.0	19.0	50.0
Mean	17.0	34.3	16.0	55.3	16.6	49.6	14.3	55.3
SD	7.0	18.8	4.0	22.4	8.4	6.4	7.2	9.2
SE	4.0	10.9	2.3	12.9	4.2	3.7	4.2	5.3

TABLE 3.51b Control chains.

TABLE 3.52 The effect of 43 bar on the recovery from field stimulation.

The recovery from each response to field stimulation was recorded as the percentage of prestimulation tone recovered 5 minutes after stimulation had ceased (%R/5).

The table shows the recovery from field stimulation in the experiments recorded in Table 3.49.

a. Experimental.

A 1 bar, 0.95 bar O_2:0.05 bar CO_2.
B 43 bar, 42 bar He:0.95 bar O_2:0.05 bar CO_2.
C 1 bar, 0.95 bar O_2:0.05 bar CO_2.
$\%R/5$ was significantly reduced by compression to 43 bar.

b. Controls.

The paired control chains were maintained at 1 bar throughout (A, B and C).

EXPT. NO.		FREQUENCY (Hz) 2	4	8	16
		%R/5	%R/5	%R/5	%R/5
A	1	50.0	33.3	14.3	12.5
	2	85.0	50.0	75.0	66.6
	3	50.0	83.3	66.0	44.4
	4	-	-	-	-
Mean		61.6	55.5	60.0	41.2
SD		20.2	25.4	32.8	27.1
SE		10.1	12.7	16.4	13.5
B	1	0.0	0.0	0.0	0.0
	2	50.0	42.8	55.0	54.4
	3	0.0	0.0	50.0	33.3
	4	-	-	-	-
Mean		28.8	24.7	30.4	27.4
SD		24.0	20.6	25.3	22.8
SE		12.0	10.3	12.6	11.4
C	1	0.0	66.6	40.0	40.0
	2	100.0	100.0	92.8	54.5
	3	60.0	50.0	40.0	40.0
	4	-	-	-	-
Mean		53.3	72.2	57.6	44.8
SD		50.3	25.4	30.4	8.4
SE		25.2	12.7	15.2	4.2

a. Experimental Chains

EXPT. NO.		FREQUENCY (Hz) 2	4	8	16
		%R/5	%R/5	%R/5	%R/5
A	1	66.6	60.0	41.6	25.0
	2	0.0	0.0	28.5	26.6
	3	50.0	38.0	44.4	25.0
	4	73.0	1.5	71.4	40.0
Mean		47.4	39.8	46.4	29.1
SD		33.0	28.6	18.0	7.3
SE		16.5	14.3	9.0	3.6
B	1	40.0	25.0	30.7	41.6
	2	0.0	20.0	40.0	28.5
	3	44.0	56.2	50.0	20.0
	4	40.0	25.0	54.5	26.6
Mean		31.1	31.5	43.8	29.2
SD		20.8	16.6	10.6	9.0
SE		10.4	8.3	5.3	4.5
C	1	50.0	50.0	33.3	33.3
	2	100.0	33.3	22.2	21.4
	3	50.0	50.0	57.0	37.5
	4	27.2	50.0	33.0	46.1
Mean		56.8	45.8	34.5	34.6
SD		30.7	8.4	10.2	10.2
SE		15.3	4.2	5.1	5.1

b. Control Chains

TABLE 3.52

TABLE 3.53 The effect of 67 bar on the recovery from field stimulation.

The recovery from each response to field stimulation was recorded as the percentage of prestimulation tone recovered 5 minutes after stimulation had ceased (%R/5).

The table shows the recovery from field stimulation in the experiments recorded in Table 3.51.

a. Experimental.

A 1 bar, 0.95 bar O_2:0.05 bar CO_2.
B 67 bar, 66 bar He:0.95 bar O_2:0.05 bar CO_2.
C 1 bar, 0.95 bar O_2:0.05 bar CO_2.
%R/5 was significantly reduced by compression to 67 bar.

b. Controls.

The paired control chains were maintained at 1 bar throughout (A, B and C).

EXPT. NO.		FREQUENCY (Hz) 4	8	12	16
		%R/5	%R/5	%R/5	%R/5
A	1	83.0	57.0	55.0	66.0
	2	100.0	64.0	75.0	50.0
	3	100.0	100.0	100.0	100.0
	4	44.0	61.5	72.0	31.0
Mean		81.7	70.6	75.5	61.7
SD		26.4	19.8	18.5	29.2
SE		13.2	9.9	9.3	14.6
B	1	33.0	0.0	0.0	0.0
	2	0.0	0.0	50.0	16.0
	3	0.0	0.0	33.0	0.0
	4	12.5	4.3	33.0	25.0
Mean		11.4	10.7	29.0	10.2
SD		15.5	21.5	20.9	12.4
SE		7.7	10.7	10.4	6.2
C	1	20.0	25.0	25.0	0.0
	2	50.0	60.0	44.0	37.5
	3	-	100.0	-	100.0
	4	25.0	37.5	20.0	22.0
Mean		31.6	55.6	29.6	39.8
SD		16.1	32.9	12.6	42.9
SE		22.5	15.4	18.0	17.4

a. Experimental Chains

EXPT. NO.		FREQUENCY (Hz) 2	4	8	16
		%R/5	%R/5	%R/5	%R/5
A	1	25.0	40.0	33.0	28.0
	2	57.0	44.0	40.0	27.0
	3	100.0	100.0	100.0	100.0
	4	100.0	80.0	83.0	28.0
Mean		70.5	66.0	64.0	53.2
SD		36.4	28.9	32.6	34.3
SE		18.2	14.5	19.1	20.2
B	1	14.0	0.0	16.0	16.0
	2	0.0	25.0	28.0	0.0
	3	100.0	66.0	66.0	75.0
	4	57.0	60.0	66.6	57.0
Mean		42.7	37.7	69.1	37.0
SD		45.0	30.9	36.0	34.8
SE		22.5	15.4	18.0	17.4
C	1	0.0	50.0	0.0	25.0
	2	0.0	0.0	0.0	0.0
	3	100.0	100.0	50.0	50.0
	4	0.0	40.0	40.0	40.0
Mean		25.0	47.5	22.5	28.7
SD		50.0	41.1	26.3	21.7
SE		25.0	20.5	13.1	10.8

b. Control Chains

TABLE 3.53

ANON (1974). The report of the Submarine Escape Training Review Committee. August 1974.

A. AKACSU (1952). The action of drugs on the isolated trachea. J. Pharm. Pharmacol. 4 671.

A. AKACSU (1959). The physiologic and pharmacologic characterisation of the tracheal muscle. Arch. int. Pharmacodyn CXXII No. 1-2 201-207.

T. AKERA, T.M. BRODY (1977). Role of Na^+/K^+ ATPase in the inotropic action of digitalis. Pharm. Revs. 29 (3) Sept. 1977.

T.K. AKERS & L.C. CARLSEN (1972). The changes in smooth muscle receptor coupling of acetylcholine and norepinephrine at high pressure. Fifth Symposium on Underwater Physiology 88.

A.I. ALLY & K. NAKATSU (1976). Adenosine inhibition of isolated rabbit ileum and antagonism by theophylline. J. Pharmacol. Exp. Therm. 199 208-215.

B.M. ALTURA, A. CARELLA & B.T. ALTURA (1980). Adverse effects of Tris HEPES and MOPS buffers on contractile responses of arterial and venous smooth muscle induced by prostaglandins. Prostaglandins Med. 5 123-130.

W.H. ANDERSON, J.K. KRZENOWSKI, J.B. POLSON & A. SZVENTANYI (1979). Increased synthesis of prostaglandin like material during histamine tachyphylaxis in canine tracheal smooth muscle. Biochem. Pharmacol. 28 2223-2226.

R.F. ANTHRACITE, L. VACHON & P.H. KNAPP. (1971). Alpha adrenergic receptors in the human lung. Psychosomatic Medicine 33 (6) 481-489.

M. ASHFORD, A.G. MACDONALD & K.T. WANN (1979). Moderate hydrostatic pressures reduce the spontaneous release of transmitter in the frog. J. Physiol. 292 44P.

G.R. ATHEY & T.K. AKERS (1978). Analysis of frog neuromuscular function at hyperbaric pressures. Undersea Biomedical Research 5 (2) 199-208.

P.F. BAKER ET AL (1969). Influence of Ca^{2+} on sodium efflux in squid axons. J. Physiol. 200 431-58.

P.F. BAKER (1973). Transport and metabolism of calcium ions in nerve. Progress in Mol. Biol. and Biophysics 24 177-223.

T. BANDO, N. SHINDO & Y. SHIMO (1973). The nonadrenergic inhibitory nervesin the tracheal smooth muscle of the guinea pig. Proc. J. Physiol. Soc. Japan 508-509.

S. BATRA (1974). The effects of drugs on calcium uptake and calcium release by mitochondria and sarcoplasmic reticulum of frog skeletal muscle. Biochem. Pharmacol. 23 89-101.

H. BAUER, P.J. GOODFORD & J. HUTER (1965). The calcium content and calcium uptake of the smooth muscle of the guinea pig taenia coli. J. Physiol. 176 163-179.

H.J. BEIN (1956). The pharmacology of rauwolfia. Pharmac. Rev. 8 435-483.

A. BENNET & J. POSNER (1971). Studies on prostaglandin antagonists. Br. J. Pharmac. 42 584-594.

P.B. BENNETT & E.J. TOWSE (1971). The high pressure nervous syndrome during a simulated oxygen-helium dive to 1500 ft. Electroencephalography and Clinical Neurophysiology 31 383-393.

J.W. BLACK, W.A.M. DUNCAN & R.G. SHANKS (1965). Comparison of some properties of pronethalol and propranolol. Br. J. Pharmacol. 25 577-591.

R.W. BRAUER & B.W. BEAVER ET AL. (1977). Time, rate and kmp factors in the onset of high pressure convulsions. J. App. Physiol. 43 (2) 173-182.

A. BROUHA, A. PEQUEX, E. SCHOFFENIELS & A. DISTECHE (1970). The effects of high hydrostatic pressure on the permeability characteristics of the isolated frog skin. B.B.A. 219 455-462.

G. BURNSTOCK (1972). Purinergic nerves. Pharm. Revs. 24, 509-581.

G. BURNSTOCK (1975). Comparative studies of purinergic nerves. J. Exp. Zool. 194 103-134.

G. BURNSTOCK, T. COCKS, R. CROWE & L. KASAKOV (1978). Purinergic innervation of the guinea pig urinary bladder. Br. J. Pharmac. 63 125-138.

J. BURTON & T. GODFRAIND (1974). Sodium-calcium sites in smooth muscle and their accessibility to lanthanum. J. Physiol. 241 287-298.

J. BUTLER, C. CARO, R. ALCALA & A. DUBOIS (1960). Physiological factors affecting airway resistance in normal subjects. J. Clin. Invest. 39 584-591.

R.B. CAMPENOT (1975). The effects of high hydrostatic pressure on transmission at the crustacean neuromuscular junction. Comp. Biochem. Physiol (B) 52 133-140.

R.F. CARLYLE (1964). The responses of the guinea pig isolated intact trachea to transmural stimulation and the release of an Ach-like susbtance under conditions of rest and stimulation. Brit. J. Pharm. 22, 126-136.

R.F. CARLYLE, G. NICHOLS, J.A. PACIOREK, P.M. ROWLES & N. SPENCER (1979a). J. Physiol. 292 34P.

A.P. CARVAHLO (1968). Calcium binding properties of sarcoplasmic reticulum as influenced by ATP caffeine quinine and local anaesthetics. J. Gen. Physiol. 52 622-42.

R. CASTEELS (1966). The action of ouabain on the smooth muscle cells of the guinea pig's taenia coli. J. Physiol. 184 131-142.

R. CASTEELS, L. RAEYMAEKERS, J. GOFFIN & F. WUYTACK (1973). A study of factors affecting the cellular calcium content in the smooth muscle cells. Arch. Int. Pharmacodyn. Ther. 201 191-192.

J.C. CASTILLO & E.J. DE BEER (1947). The tracheal chain: a preparation for the study of antispasmodics with particular reference to bronchodilator drugs. J. Pharmacol. Exp. Ther. 90 104-109.

R. CASTRO DE LA MATA, M. PENNA & D. AVIADO (1962). Reversal of sympathetic bronchodilation by dichloroisoproterenol. J. Pharmacol. and Exp. Therap. 135 197-203.

Mc.K. CATTELL & D.J. EDWARDS (1932). Conditions modifying the influence of hydrostatic pressure on striated muscle with special reference to the role of viscosity changes. J. Cell. Comp. Physiol. 1 11.

Mc.K. CATTELL (1936). The physiological effects of pressure. Biol. Revs. 11 441-476.

Mc.K. CATTELL & J.W. DRAPER (1936). In: the physiological effect of pressure. Biol. Revs. 11 441-476.

Mc.K. CATTELL & D.J. EDWARDS (1936). Cited by Mc.K. Cattell (1936). The physiological effects of pressure. Biol. Revs. 11 441-476.

V.K. CHIU, D. MOURING, B. WATSON & D. HAYNES (1980). Measurement of surface potential and surface change densities of narcoplasmic reticulum membranes. J. Membrane Biol. 56 121-132.

C.F. CHRYSSANTHOU, F. TEICHNER, G. GOLDSTEIN, KALBERER & ANTOPOL (1970). Studies in Dysbarism III. A smooth muscle activating factor (SMAF) in mouse lungs and its increase in decompression sickness. Aerospace Med. 41 43-48.

R.F. COBURN & T. TOMITA (1973). Evidence for noradrenergic inhibitory nerves in the guinea pig trachealis muscle. Am. J. Physiol. 224 (5) 1072-1080.

T.J. COLATSKY & P.M. HOGAN (1980). Effects of external calcium, calcium-blocking agents, and stimulation frequency on cycle length dependent changes in canine cardiac action potential duration. Circ. Res. 46 453-552.

R.A. COLEMAN (1980). Purine antagonists in the identification of adenosine-receptors in the guinea pig trachea and the role of purines in nonadrenergic inhibitory neurotransmission. Br. J. Pharmac. 69, 359-366.

R.A. COLEMAN, P.P. HUMPHREY, I. KENNEDY, G.P. LEVY & P. LUMLEY (1980). Are there two types of prostaglandin receptor mediating vasodilation in the dog? Br. J. Pharmacol. 68 114P-115P.

H.O.J. COLLIER, J.A. HOLGATE, M. SCHACHTER & P.G. SHORLEY (1960). The bronchostrictor action of bradykinin in the G Pig. Brit. J. Pharmacol. 15 290.

G. DAHLBACK (1978). Lung mechanics during immersion with special reference to pulmonary air trapping. PhD Thesis Lund, Sweden.

M.K. DENNEY & W.W. GLAS (1964). Experimental studies in barotrauma. J. Trauma 4 791-796.

A. DISTECHE (1959). pH measurements with a glass electrode withstanding 1500 kg (cm^2) hydrostatic pressure. Rev. Sc. Instr. 30 (6) 474-8.

A. DISTECHE (1972). Effects of pressure on the dissociation of weak acids. Symp. Soc. Exph. Biol. Ed. A.G. MacDonald.

C.W. DOMVILLE-FIFE (1910). Submarines of the World's Navies. Pub: Francis Griffiths (London).

U. EBBECKE (1914). Wirkung allseitiger Kompression auf den Froschmuskel. Pflug. Arch. ges. Physiol. 157 79.

A. ECKENFELS & J.R. VANE (1972). Prostaglandins, oxygen tension and smooth muscle tone. Br. J. Pharmacol. 45 451-462.

D.J. EDWARDS (1935). The action of pressure on the tension response of smooth muscle. Am. J. Physiol. 113 37.

D.J. EDWARDS & Mc.K. CATTELL (1930). The action of compression on the contraction of heart muscle. Am. J. Physiol. 93 90.

B.J. EVERITT & K.D. CAIRNCROSS (1969). Adrenergic receptors in guinea pig trachea. J. Pharm. Pharmacol. 21 97-102.

J.P. FAMAEY, J. FONTAINE & J. REUSE (1977). The effects of non-steroidal anti-inflammatory drugs on cholinergic and histamine induced contractions of guinea pig ileum. Brit. J. Pharmacol. 60 165-171.

J.B. FARMER & R.A. COLEMAN (1970). A new preparation of the isolated intact trachea of the guinea pig. J. Pharm. Pharmacol. 22 46-50.

S.H. FERREIRA, A. HERMAN & J.R. VANE (1976). Prostaglandin generation maintains of the smooth muscle tone of the rabbit isolated jejunum. Proceedings of Brit. Pharm. Soc. Jan. 1976 328-330P.

J.T. FLORIO, E.J. TOWSE & D.A.R. MACKENZIE (1979). Breathing pattern, respiratory gas flow and heart rate during simulated

submarine escape. Report: AMTE(E) R79 402.

R.J. FLOWER (1974). Drugs which inhibit prostaglandin biosynthesis. Pharmacol. Rev. 26 33.

H.E. FOSTER, M. HOOPER, M. SPEDDING, A.J. SWEETMAN & D.F. WEETMAN (1978). Antagonism of the inhibitory effects of adenosine S-triphosphate on the isolated taenia of the guinea pig caecum: structure activity relationships within a series of isatogen derivatives. Brit. J. Pharmacol. 63 309-314.

R.W. FOSTER (1960a). The paired tracheal chain preparation. J. Pharm. Pharmacol. 12 189-191.

R.W. FOSTER (1963). PhD Thesis, Kings College, London.

R.W. FOSTER (1966). The nature of the adrenergic receptors of the trachea of the guinea pig. J. Pharm. Pharmacol. 18 1-12.

W.F. GANONG (1971). Review of Medical Physiology. 5th Edition. Pub. Lange USA.

N.L. GERSHFIELD & A.M. SHANES (1959). The influence of high hydrostatic pressure on cocaine and veratrine action in vertebrate nerve. J. Gen. Physiol. 42 647-653.

J.S. GILLESPIE & A.T. McKNIGHT (1975). Effect of Tris on the response to various isolated mammalian preparations to nerve and drug stimulation. J. Physiol. 248 (1) 12-13P.

GLASSTONE (1956). Thermodynamics for Physical Chemists.

K. GREENE (1978). Causes of death in submarine escape training casualties, analysis of gases and review of the literature. AMTE(E) R78 402.

L. GRODZINSKA, B. PANCZENKO & R.J. GRYGLEWSKI (1975). Generation of prostaglandin E-like material by the guinea pig trachea contracted by histamine. J. Pharm. Pharmacol. 27 88-91.

A.C. HALL (1980). The effects of high hydrostatic pressure on red blood cells. PhD Thesis, Aberdeen.

A.A. HARPER, A.G. MACDONALD & K.T. WANN (1977). The action of high hydrostatic pressure on voltage clamped Helx neurones. J. Physiol. 273 70-71P.

A.A. HARPER (1978). The effects of high pressure on the electrical characteristics of molluscan neurones. PhD Thesis, University of Aberdeen.

J. HARDING & J. McWHIRTER (1980) (unpublished). A comparison of the pharmacological properties of the cervical and thoracic portions of the trachea and of the lung parenchyma from the guinea pig. BSc Thesis, Portsmouth Polytechnic.

W. HASSELBACH (1969). Relaxing factor and the relaxation of muscle. Prog. Biophys. Molec. Biol. 14 169-222.

D.F. HAWKINS & W.D.M. PATON (1958). Responses of isolated bronchial muscle to ganglionically active drugs. J. Physiol. 144 193-219.

K. HAYASHI (1961). Biological action of high hydrostatic pressure. Jap. J. Med. Progr. 48 292-305 (Jap.) Translation available.

K. HAYASHI (1961). Cell stimulation effects of high hydrostatic pressures and characteristics thereof. J. Physiol. Soc. Japan 23 527-546.

H.V. HEMPLEMAN, ET AL (1979). Human physiological studies at high pressure. AMTE Rept. No. R80 402.

J.V. HENDERSON, M.T. LOWENHAUPT & D.L. GILBERT (1977). Helium pressure alteration of function in squid giant synapse. Undersea Biomedical Research 4 (1) 19-25.

K. HEREMANS & F. WUYTACK (1980). Pressure effect on the arrhenius discontinuity in Ca^{2+} ATP-ase from sarcoplasmic reticulum. Febs Lett. 117 161-163.

F. HERTELENDY, H. TODD & R.J. NARCONIS (1978). Studies on growth hormone secretion. IX Prostaglandins do not act like ionophores. Prostaglandins 15 575-590.

L. HILL (1912). Caisson sickness and the physiology of work in compressed air. Publishers: Arnold.

K. HILLIER (1978). Prostaglandins and Thromboxanes: Pharmacologic and Biosynthetic aspects. Seminars in Perinatology 2 (3) 197-210.

B.A. HILLS & D.E. RAY (1977). Modification of hyperbaric oxygen toxicity in rats by Tris buffer and Li. Proc. Phys. Soc. 18.6.77. J. Physiol. 271 26-27P.

G.J. HILLS (1972). Physics and chemistry of high pressures. In Sleigh and MacDonald 1972. "The Effects of Pressure on Organisms."

J.A. HOLLAND, W.G. WOLFE & J.A. KYLSTRA (1973). Helium: Absence of antiarrhythmic effect in anesthetised dogs. J. Thorac. Cardiovas. Surg. 66 478-480.

S.K. HONG, T.O. MOORE, D.A. LALLY & F. MORLOCK (1973). Heart rate response to apreic face immersion in hyperbaric heliox environment. J. Appl. Physiol. 34 770-774.

M. HOOPER, M. SPEDDING, A.J. SWEETMAN & D.F. WEETMAN (1974). 2-2' Pyridylisatogen tosylate: an antagonist of the inhibitory effects of ATP on smooth muscle. Proc. BPS Jan. 1974 458-459P.

R.A. HORNE (1969). Marine Chemistry. The structure of water and the chemistry of the hydrosphere. New York, Wiley - Interscience.

T. IKKAI & T. OOI (1966). Actin: volume change on transformation of G-form to F-form. Science, NY, 152 1756-7.

I.R. INNES & M. NICKERSON (1975). Atropine, scopolamine and related anti-muscarinic drugs. Ch. 25, The pharmacological basis of therapeutics, eds. L.S. Goodman and A. Gilman, MacMillan Press.

M. ISHIZAWA, H. NITTA & E. MIYAZAKI (1980). Inhibitory action of lanthanum (La) on PGF_2 -induced contraction of guinea pig stomach muscle. Prostaglandins 19 407-413.

M.R. JAMES & B.D. ROUFOGALIS (1977). The effect of ouabain on the guinea pig ileum longitudinal muscle I and II. Can J. Physiol. Pharmacol. 55, 1190-1196.

S.M. JOHNSON, K.W. MILLER & A.D. BANGHAM (1973). The opposing effects of pressure and general anaesthetics on the cation permeability of liposomes of varying lipid composition. BBA 307 42-57.

C.Y. KAO (1966). Tetrodotoxin saxitoxin and their significance in the study of excitation phenomena. Pharmac. Rev. 18, 997-1049.

J. KENDIG, J. TRUDELL & E.N. COHEN (1975). Effects of pressure and anaesthetics on condution and synaptic transmission. J. Pharmacol and Exptl. Ther. 195 216-224.

J. KENDIG, T.M. SCHNEIDER & E.N. COHEN (1978a). Pressure temperature repetitive impulse generation in crustacean axon. J. Appl. Physiol. 45(5) 742-746.

C.T. KIRKPATRIC, R.J. MORROW & T. TOMITA (1980). The contractile response of smooth muscle to immersion in hypertonic solutions. Clin. Expt. Pharmac. Physiol. 7 147-158.

R.J. KNUDSON & D.C. KNUDSON (1975). Effect of muscle constriction on flow limiting collapse of isolated canine trachea. J. Appl. Physiol. 38, 125-131.

M.A. KOLBER & C. VAN BREEMAN (1981). Competitive membrane absorption of Na^+/K^+ and Ca^{2+} in smooth muscle cells. J. Membr. Biol. 58 115-121.

H.A. KREBS & K. HENSELEIT (1932). Untersuchungen uber die Harnstoff bildung im Tierkorper. Hoppe Seylers Z. Physiol. Chem. 210, 1932 33-66.

E. KROEGER & N.L. STEPHENS (1971). Effect of hypoxia on energy and calcium metabolism in airway smooth muscle. Am. J. Physiol. 220 1199-1204.

J.A. KYLSTRA (1967). Hydraulic compression of mice to 166 atm. Science 155 793-794.

A.G. Lee (1976). Model for action of local anaesthetics. Nature 262 545-548.

A. LIEBOW, J.E. STARK, J. VOGEL & K.E. SCHAEFFER (1959). Intrapulmonary air trapping in submarine escape training casualties. United States Armed Forces Medical Journal X(3) 265-289.

H.J. LITTLE & W.D.M. PATON (1979). The effects of high pressure helium and nitrogen on the release of acetylcholine from the guinea pig ileum. Br. J. Pharmacol. 67 221-228.

T.C. LLOYD JR. (1968). Hypoxic pulmonary vasoconstriction: role of perivascular tissue. J. Appl. Physiol. 25 560-565.

K.M. LULITCH, H.W. MITCHELL & M.P. SPARROW (1976). The cat lung strip as an in vitro preparation of peripheral airways: a comparison of β adrenoceptor agonists, antacoids and anaphylactic challenge on the lung strip and trachea. Br. J. Pharmacol. 58 71-79.

A.G. MACDONALD (1972). The effects of pressure on organisms. Ed. M.A. Sleigh, A.G. MacDonald.

A.G. MACDONALD (1978). A dilatometric investigation of the effects of general anaesthetics, alcohols and hydrostatic pressure on the phase transition in smectic mesophases of dipalmitoyl phosphatidyl choline. BBA 507 26-37.

A.G. MACDONALD & K.T. WANN (1978). Physiological aspects of anaesthetics and inert gases. Academic Press.

A.G. MACDONALD, C.G. INGRAM & R.S. McNEILL (1975). The effect of propranolol on airway resistance. Br. J. Anaesth. 39 919-925.

P.T. MACKLEM (1971). Airway obstruction and collateral ventilation. Physiol. Rev. 51, 368-436.

P.T. MACKLEM & L.A. ENGEL (1975). The physiological implications of airways smooth muscle constriction. Postgrad. Med. J. 51(7) Suppl. 45-52.

M.S. MALHOTRA & H.C. WRIGHT (1960). Arterial air embolism during decompression and its prevention. Proc. Roy. Soc. B 154 418-427.

S.P. MANN (1971). The innervation of mammalian bronchial smooth muscle: the localisation of catecholamines and cholinesterases. Histochemical Journal 3 319-331.

M. MATSUDA, S.K. HONG, H. NAKAYAMA, H. ARITA, Y.C. LIN, J. CLAYBAUGH, C. LUNDGREN & R.M. SMITH (1980). Physiological responses to immersion at 31 ATA (Seadragon IV). The Proceedings of the 7th Symposium on Underwater Physiology, 5-10 August 1980.

J. McWHIRTER, P. HARRISON & J.T. FLORIO (1979). The effect of immersion on FEV_1/FVC. Report AMTE(E) R79 404.

H. MIKI (1960). Effect of high hydrostatic pressure on smooth muscle. Okayama Igakkai Zasshi 72 1613-1634. (Journal of Okayama Medical Society.).

T. MURAKAMI (1958). Effects exerted by high hydrostatic pressures on ATPase activities. Cell Chemistry Symposium 8, 71-77.

T.H. MURAKAMI & A.M. ZIMMERMAN (1970). A pressure study of galvanotaxis in tetrahymena. In: High pressure effects on cellular processes 139-154. Ed. A.M. Zimmerman, N.Y. and London, Acad. Press.

T. MUSTAFA, T.W. MOON & P.W. HOCHACHKA (1971). Effects of pressure and temperature on the catalytic and regulatory properties of muscle pyruvate kinase from an offshore benthic fish. Am. Zool. 11 451-66.

M. NAGASAKA, J. BOUCKAERT, A.F. De SHAEPDRYVER & C. HEYMANS (1964). Adrenergic constriction in isolated guinea pig lung revealed by Nethalide. Arch. Int. Pharmacodyn. 149 237-242.

H.H. NEWBALL, A. KEISER & B. PISANO (1975). Bradykinin and human airways. Respn. Physiol. 24 139-146.

O. NISSELL (1950). The action of oxygen and carbon dioxide on the bronchioles and vessels of the isolated perfused lungs. Acta Physiol. Scanda. 21 Suppl. 73.

B.J. NORTHOVER (1973). Effect of anti-inflammatory drugs on the binding of calcium to cellular membranes in various human and guinea pig tissues. Br. J. Pharmacol. 48 496.

S. O'DONNELL & J. WANSTALL (1980). Are the pA_2 values of selective β adrenoceptor antagonists valid when obtained on guinea pig tracheal preparations contracted with carbachol. J. Pharm. Pharmacol. 32 413-416.

K. OKADA (1954). Effects of high hydrostatic pressure on the permeability of plasma membranes. Okayama Igakkai Zasshi 66 2094.

F. OKUMURA & M.A. DENBOROUGH (1980). Effects of anaesthetics on guinea pig tracheal smooth muscle. Br. J. Anaesth. 52 199-204.

J. OREHEK, J.S. DOUGLAS, A.J. LEWIS & A. BOUHUYS (1973). Prostaglandin regulation of airway smooth muscle tone. Nature New Biol. 245 84-85.

H. ORNHAGEN (1977). Hyperbaric Bradycardia and Arrythmia. Ph.D. Thesis Lund, Sweden.

H. ORNHAGEN & P. HOGAN (1976). Effects of high ambient pressure on the spontaneous frequency of the mammalian sinus node. Fed. Proc. 35 237.

H. ORNHAGEN & S. SIGURDSON (1978). Effects of increased ambient pressure on spontaneous activity in the portal vein. Soc. for Exptl. Biol. Brighton, January 1978.

H. PASSOW (1964). Ion and water permeability of the red blood cell. In: "The red blood cell" 71-145. Eds. C. Bishop and D.McN. Surgenor. Academic Press N.Y.

A. PEQUEUX (1972). Hydrostatic pressure and membrane permeability. Symp. Soc. exp. Biol. 26, 483-4.

R. PIFARRE, W.D. COX, M. JASUJA & W.E. NEVILLE (1969). Helium in the prevention of ventricular fibrillation. Dis. Chest. 56 135-138.

H.P. RANG & U.M. RITTER (1970). On the mechanism of desensitisation at cholinergic receptors. Molec. Pharmacol. 6 357-383.

L. RAYMOND, R.B. WEISKOPF, M.J. HALSEY, A. GOLDFIEN, E.I. EGER & J.W. SEVERINGHAUS (1972). Possible mechanisms for the anti-arrhythmic effect of helium in anaesthetised dogs. Science 176 1250-1252.

L.W. RAYMOND, J. SODE, H. LANGWORTHY, J. BLOSSER & R. JOHNSONBAUGH (1974). Increase in plasma growth hormone concentration: a new effect of helium breathing. Undersea Biomedical Research 1 (1) 91-97.

J. RAYNAND, P. VARENNE & D. DURAND (1980). Comparison of metabolic responses and growth hormone release during submaximal exercise in man breathing heliox or air at normal barometric pressure. Proceedings of the 7th Symposium on Underwater Physiology (5-10 July 1980).

P. REGNARD (1891). Recherches experimentales sur les condition physiques de la vie dans les eau. Pub: Masson (Paris).

J. RICHARDSON & J. BELAND (1976). Non-adrenergic inhibitory nervous system in human airways. J. Appl. Physiol. 41 764-771.

J.B. RICHARDSON & T. BOUCHARD (1975). Demonstration of a non-adrenergic inhibitory nervous system in the trachea of the guinea pig. J. Allergy Clin. Immunol. 56(6) 473-480.

A. RIKIMARU & M. SUDOH (1971). Innervation of the smooth muscle of the guinea pig trachea. Jap. J. Smooth Muscle Res. 7 35-44 (1971).

A.P. RINFRETT & G.F. DOEBLER (1961). Biological effects and applications in He, Ar and the rare gases. Ed. J.R. Cook 1961.

J.C. RUEGG (1971). Smooth muscle tone. Phys. Revs. 51 (1) 201-248.

J.A. RUSSELL (1978). Responses of isolated canine airways to electric stimulation and acetyl choline. J. Appl. Physiol. 45 (5) 690-698.

J. SALZANO, E.M. CAMPORESI, B. STOLP, H. SALTZMAN, W. BELL & D. SHELTON (1980). Inspiratory dyspnea during exercise at 47 ATA. Proceedings of the 7th Symposium on Underwater Physiology, 5-10 July 1980.

K.E. SCHAEFFER, W.P. McNULTY, C. CAREY & A.A. LIEBOW (1958). Mechanisms in development of intestitial emphysema and airway embolism on decompression from depth. J. Appl. Physiol. 13 15-29.

C.W. SCHILLING, M.F. WERTS & N.R. SCHANDELMEIER (1976). The Underwater Handbook. A guide to Physiology and Performance for the Engineer. Plenum Press: New York.

M.P. SHEETZ & S.J. SINGER (1974). Biological membranes as bilayer couples. A molecular mechanism of drug-erythrocyte interactions. Proc. Natl. Acad. Sci. USA 71 (11) 4457-4461.

W.O. SHELFORD (1960). "Subsunk". Publishers Harrap, London.

M.A. SLEIGH & A.G. MACDONALD (1972). The effects of pressure on organisms. Cambridge University Press.

R.C. SMALL & A.H. WESTON (1979a). Intramural Inhibition in Rabbit and Guinea Pig Intestine. Physiological and regulatory functions of adenosine and adenine nucleotides. Eds. H.P. Baer and G.I. Drummond. Raven Press, N.Y. 45-60.

R.C. SMALL & A.H. WESTON (1979b). Theophylline antagonises some effects of purines in the intestine but not those of intramural inhibitory nerve stimulation. Brit. J. Pharmac. 67 301-308.

A.P. SMITH (1973). "Lungs". In: The Prostaglandins p203. Ed. P.W. Ramwell, Plenum Press.

F.E. SOUTH & S.F. COOK (1953). Effect of He on the respiration and glycolysis of mouse liver slices. J. Gen. Physiol. 36 513.

F.E. SOUTH, Jr. & S.F. COOK (1953). Argon, Xenon, Hydrogen and the O_2 consumption and glycolysis of mouse tissue slices. J. Gen. Physiol. 37 335.

M. SPEDDING & D.F. WEETMAN (1978). The mechanism of the relaxant effect of 2-2' pyridyl isatogen on the isolated taenia of the guinea pig caecum. Br. J. Pharmac. 63 659-664.

J.A. SPLAWINSKI, A.S. NIES, B.S. WEETMAN & J.A. OATES (1973). The effects of arachidonic acid prostaglandin E_2 and prostaglandin $F_{2\alpha}$ on the longitudinal stomach strip of the rat. J. Pharm. and Exp. Ther. 187 501-510.

J.F. STEIN & J.G. WIDDICOMBE (1975). The interaction of chemo and mechanoreceptor signals in the control of airway calibre. Respiration Physiology 25 363-376.

N.L. STEPHENS (1976). Airway smooth muscle. In: Lung Cells in Disease. Ed. A. Bonhuys, Amsterdam, North Holland 113-138.

N.L. STEPHENS, E. KROEGER & J.A. MEHTA (1969). Force-velocity characteristics of respiratory airway smooth muscle. J. Appl. Physiol. 26 685-692.

N.L. STEPHENS, J.L. MEYERS & R.M. CHERNIAK (1968). Oxygen, carbon dioxide, H^+ ion and bronchial length-tension relationships. J. Appl. Physiol. 25 376-383.

W.J. SWEATMAN & H.O. COLLIER (1968). Effects of prostaglandins on human bronchial muscle. Nature (London) 217 69.

G.W. SYBRECHT, L. GARRETT & N.R. ANTHONISEN (1975). Effect of chest strapping on regional lung function. J. Appl. Physiol. 39 707-713.

H. TRAUBLE & H. EIBL (1974). Electrostatic effects on lipid phase transitions. Membrane structure and ionic environment. Proc. Natl. Acad. Sci. USA 71 214-219.

J.R. TRUDELL, W.L. HUBELL & E.N. COHEN (1973). The effect of two inhalation anaesthetics on the order of spin labelled phospholipid vesicles. BBA 291 321-327.

C.V. VAN BREEMAN, P. AARONSON & R. LOUTZENHISER (1979). Sodium calcium interactions in mammalian smooth muscle. Pharm. Rev. 30 167-208.

C.V. VAN BREEMAN & E.E. DANIEL (1966). The influence of high potassium depolarisation and acetylcholine on calcium exchange in the rat uterus. J. Gen. Physiol. 49 1299-1317.

C.V. VAN BREEMAN, B. FARINAS, R. CASTEELS, P. GERBA, F. WUYTACK & R. DETH (1973). Factors controlling cytoplasmic Ca^{++} concentration. Phil. Trans. Roy. Soc. London Ser. B. Biol. Sci. 265 57-71.

J.R. VANE (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin like drugs. Nature (New Biol.) 231 232-235.

J. van ROSSUM (1963). Cumulative Dose Response Curves. Arch. int. pharamcodyn. 143 (3-4) 299-330.

VON ANITA PONAT (1967). Untersuchungen zur zellularen Druckresistenz ver schiedener Evertbraten der Nord-und Ostsee. Kielen Meeresforsch 23 21-47.

K.T. WANN & A.G. MACDONALD (1980). The effects of pressure on excitable cells. Comp. Biochem. Physiol. 66A 1-12.

K.T. WANN, A.G. MACDONALD & A.H. HARPER (1979). The effects of high hydrostatic pressure on the electrical characteristics of helix neurones. Comp. Biochem. Physiol. 64A 149-159.

E.S. WHEELER & G.B. WEISS (1980). Effects of prostaglandin E_1 on contractility and ^{45}Ca release in rabbit aortic smooth muscle. Prostaglandins 19 761-778.

J.G. WIDDICOMBE (1963). Regulation of tracheobronchial smooth muscle. Phys. Revs. 43 (1) 1-37.

J.G. WIDDICOMBE (1966). The regulation of bronchial calibre. Advances in respiratory physiology. Ed. C.G. Caro, Edward Arnold.

S. YEANDLE (1977). Neuromuscular transmission in the frog at 31 ATA helium pressure. Undersea Biomedical Res. 4 (2) 159-168.

A.M. ZIMMERMAN (1970). High pressure effects on ce-lular processes. Academic Press, New York, London.

U. ZOR, R. KOCH & Z. NAOR (1976). Differential effects of prostaglandin synthetase inhibitors on prostaglandin E_2 binding and on prostaglandin - or cholera toxin-induced cyclia AMP accumulation in the rabbit uterus. Adv. in Prostaglandin and Thromboxane Res. 1 331-335.