A METHOD FOR THE STUDY OF DRUG ACTION ON THE SPINAL REFLEXES OF THE FROG

by

ARTHUR BUNZEL

A Thesis submitted to the
Faculty of Graduate Studies and Research
of McGill University
in partial fulfilment of the requirements
for the degree of Master of Science

Montreal

August 1953

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Arthur Bunzel

I. A C K N O W L E D G M E N T S

First and foremost, I wish to express my deepest appreciation to Dr. F.C. McIntosh, the man and the teacher who, with sincere interest and inexhaustible patience, has given me so generously of his time. To his graduate students, he is a warm friend and a source of inspiration.

My acknowledgments are due to Dr. A.S.V. Burgen, for the suggestion of the problem; and to Drs. B.D. Burns and K. Terroux, for their stimulating guidance and for most valuable criticism given me throughout this investigation. I must thank Dr. B.D. Burns, specifically, for all I have learnt from my discussions with him.

I am most grateful to Miss N. Pedley - my partner in the project - for continual cooperation and moral support; to Miss L. Azoulay and Mr. S. Deitcher, for reading parts of the manuscript; to Mr. D. Cameron, for helping me with the photography, and to Miss E.M. Lamprecht, for the excellent typing of this thesis.

This research project was made possible by a grant from the National Research Council of Canada.

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A. STATEMENT OF THE PROBLEM.

The main purpose of this paper is to describe in detail a new vertebral perfusion method:

1) in which the spinal cord is maintained without its natural blood supply, on gaseous oxygen perfused through the heart;
2) that can serve as a pharmacological test object for the demonstration of the effects of drugs on spinal (excitatory and inhibitory) reflexes, and
3) in which the perfusate through the active spinal cord can be conveniently collected and reperfused to test for the possibility of active substance(s) therein.

B. PROBLEMS IN THE DESIGN OF AN "IDEAL PREPARATION" ARE:

1) Neurological isolation of the spinal cord from upper centres.
2) Physiological isolation of the cord (through interruption of its blood supply) from other organs of the body to exclude homeostatic actions on the reflexes.

3) Maintenance of the cord without its blood supply.

4) Pharmacological isolation of the spinal cord to ensure that the drugs' effects on the spinal cord alone are recorded.

5) Collection of effluents of sufficient concentration, so that if spinal reflex activity be associated with the release of chemical products, their detection is possible.

C. DISCUSSION OF THE LITERATURE ON METHODS HITHERTO USED TO TEST THE EFFECTS OF DRUGS ON THE SPINAL CORD.

The literature will be discussed under the following headings:

1) Experiments in which drugs reach the spinal cord through the vascular route in the "whole animal".

2) Experiments on the "spinal animal"; drugs still administered through the intravascular route.

3) Experiments in which precautions were taken to ensure that the spinal cord was the only site of drug action in the reflex arc. The blood supply to the cord is still intact.

4) Experiments in which the circulation to the spinal cord has been interrupted, and the spinal cell survived in an artificial Ringer environment into which drugs could be introduced.

A. STATEMENT OF THE PROBLEM

The mechanism of synaptic transmission in the central nervous system has been the focus of attention of many neuro-physiologists in recent years. Even the most ardent upholders of the electrical theory of propagation of impulses from one cell to the other have by now conceded to a chemical mediation of excitation or inhibition of one cell by another. Previously chemically biased workers, on the other hand, now admit that electro-physiological techniques are also important in that they throw light on the electrical signs which accompany transmission at a junction.

The problem then arose as to whether the methods and inferences used, e.g., in proving that Acetylcholine was the transmitter at the neuro-muscular junction, could be adapted to an investigation of the mechanism of synaptic transmission of spinal reflexes. For any interpretation of results would be complicated by the facts that -

a) the spinal cord may be heterogeneous as to transmitter substance releasing cells, and

b) in the spinal cord, both excitation and inhibition by the same presynaptic cell is possible.

The main methods used to elucidate the details of transmission process at the neuro-muscular junction were:

a) Recording electrical signs concomitant with impulse propagation at the junction.

b) Content analysis of the chemical transmitter and of the enzymes concerned with its synthesis and destruction, in tissues suspected of the transmitter's participation.

c) Close arterial injection of the suspected transmitter (ACh) which in the proper dosage was expected to result in imitation of normal activity of the post-synaptic cell.

d) Injection of drugs which inhibit the enzymes concerned with the synthesis or destruction of the chemical transmitter.

e) The collection and assay of the transmitter substance in the effluent from the active junctional region, provided that precautions have been taken to prevent rapid destruction of the chemical.

Several investigators (for references, see Eccles, 1953) have adapted electro-physiological techniques to the spinal cord, amongst which Eccles' (1952) technique of introducing a micro-electrode into a single anterior horn cell has been, by virtue of experimental design, most fruitful in illuminating the mechanism of synaptic transmission.

Biochemical analysis of spinal cord content of suspected transmitters and associated enzymes is also in progress (Kwiatowski, 1943; Feldberg, 1945; Burgen, 1951).

In the past, few experiments were sufficiently well designed to permit conclusions as to the real effect of a drug

on spinal reflexes; fewer still, are the preparations thus far proposed, which allow the collection and testing of a spinal cord effluent.

This group of workers (Dr. B.D. Burns, Dr. K.Terroux, Miss N. Pedley, and the author) has, therefore, attempted to overcome the difficulties inherent in perfusing a spinal cord, at room temperature, without its vascular supply in such a way that the perfusion fluid does not reach the muscle from which reflexes are recorded.

The cord of the spinal frog was perfused with Ringer through the vertebral canal and the perfusate was collected in the brachial region. Drugs could reach the spinal cord through this perfusion line. Perfusion of gaseous oxygen, through the vascular system was chosen as a method of maintaining a high O_2-tension in the spinal cord. The reflex response of a small flexor muscle (the biceps femoris) to homolateral stimulation of the foot, and its alternate inhibition due to simultaneous stimulation of both feet, were used as indicators of the "excitatory" and "inhibitory" states of the spinal cord. These responses (and the effects of various experimental conditions thereon) were recorded on a slowly revolving kymograph.

It is the main purpose of this paper to describe, in detail, this new preparation for testing the effects of drugs on spinal reflexes (Methods).

To illustrate the use of the method, figures of the effects of some drugs on this preparation will be presented,

(Results) but full discussion of the theoretical importance of our findings as related to synaptic transmission, will be given elsewhere (Pedley, 1953).

B. PROBLEMS TO BE DEALT WITH IN DESIGNING AN "IDEAL PREPARATION" FOR STUDYING DRUG ACTION ON SPINAL REFLEXES.

In order to investigate the possibility of chemical transmission in the spinal cord, a preparation has to be so designed that it permits

a) the survival of the spinal cord in an artificial medium;

b) the perfusion of the spinal cord with drugs to demonstrate their possible effect on reflexes, and

c) the collection of products of reflex activity in the perfusate.

In such a preparation the spinal cord would have to be isolated neurologically, physiologically, and pharmacologically from the rest of the body.

1. Neurological Isolation of the Spinal Cord

The spinal cord should be completely separated from upper centres, since they are known to modify spinal reflexes, and it is widely recognized that the same drug may act differently and in different concentrations on higher and lower centres. If the reflexes are recorded from the lower limbs, they should preferably be unmodified of brachial reflexes.

Ideally, the spinal cord should be completely de-afferented up to the entrance of the sciatic nerves so that stimuli arising from points other than the stimulated site are excluded.

2. Physiological Isolation of the Spinal Cord.

If the circulation to the spinal cord remained intact, drugs carried through the blood could exert secondary effects on reflexes by action on other organs. For instance, as a result of injection of a drug, the chemical composition of the blood may conceivably be changed through endocrine discharge (e.g., of the adrenal medulla or of other glands if not interfered with by the operations) which in itself might affect reflexes in a complex manner. Products of activity of excitable tissues other than the spinal cord may secondarily affect the reflexes. If the cord is dependent for its oxygen supply on the normal circulatory system, drugs causing vaso-constriction or vaso-dilation may alter the direct effect of the drugs on reflexes. Therefore, before it could be concluded that drug A had X effects on the reflexes, one would have to execute control experiments to see if X was not the resultant of X_1, X_2, ... X_n effects. Since the effects of changes in the composition of the blood, and in its supply to the cord in response to drugs are themselves unknowns, it is best to deprive the spinal cord of its normal blood supply.

3. Maintenance of the Cord.

Once the circulation is interrupted, the functions of the blood with respect to the spinal cord have to be substituted:

a) a milieu of a proper temperature, pH, osmosity, and relative concentration of ions have to be provided for the spinal cell;

b) oxidation, which yields energy for nervous and synaptic activity, has to be supported by the supply of oxygen and glucose, and the removal of metabolites.

4. Pharmacological Isolation of the Cord.

Since the direct effects of drugs on the spinal cord per se are to be investigated, it is essential that the drugs do not reach the peripheral part of the reflex arc(s) involved, i.e., the skin receptors, afferent and efferent nerves (dorsal and ventral roots) and, most important, the muscle which records the spinal reflexes. A separate perfusion of the spinal cord is thus most desirable.

Arrangements will have to be made for the administration of different concentrations of drugs through the spinal perfusion line.. Moreover, since metabolism of the drugs by the liver, etc., is no longer possible (after removal of the circulation), the drugs should be easily removable so that their effects on the spinal cord may be reversible.

5. Effluent Collection.

Since the ultimate goal of this field of research is demonstration of the presence of possible chemical products of synaptic activity in the perfusion fluid, the spinal cord would have to be maintained on a slow perfusion rate, so that active substances can reach sufficient concentration to be assayed on the same or another preparation.

C. LITERATURE ON THE METHODS USED TO STUDY DRUG ACTION ON SPINAL REFLEXES.

Review of the literature will be restricted to a discussion of some of the methods that various investigators employed to demonstrate the effects of drugs on spinal reflexes, in an attempt to elucidate or offer evidence for a chemical nature of synaptic transmission in the spinal cord.

A wide range of techniques was devised to ensure: a) that an administered drug affects the spinal and not some other cell which, in turn, influences the spinal reflexes, and b) that what is recorded is the drug's modification of the spinal and not of the peripheral mechanisms.

Some experimenters drew conclusions as to the effects of drugs on spinal reflexes from experiments in which the drug was administered intravenously, while others deemed it necessary and worthwhile to remove the spinal cord from the vertebral column and keep it alive in an artificial medium for

the exclusion of at least some of the many complicating variables.

Therefore, first will be discussed some of the difficulties involved in those pharmacological experiments in which the drug was administered in the "whole animal".

Then experiments on the spinal and decerebrate animals will be mentioned. Drugs were still administered through the vascular system. It is to be noted that in these cases, at least, the possibility of a direct or indirect action of drugs on reflexes through upper centres was excluded.

A discussion of experiments is then in order in which, in addition, attempts were made to administer the drugs in such a way that they could only reach the spinal cord.

Finally, those experiments will have to be described in which the spinal cord was given an artificial medium, other than blood, in which to survive. The few experiments of this kind are most important in this connection, since our method belongs to this category.

1. Experiments on the "Whole Animal".

The most convenient but least reliable and conclusive way of testing the possible effects of drugs on spinal reflexes is their introduction into the intact vascular system. This can be done only when preliminary experiments have been done to preclude the possibility of drug action:

a) on the peripheral aspects of the reflex arc, i.e., by modifying sensory reception, peripheral nerve conduction or neuromuscular transmission;

b) on neurologically higher centres, which, in turn, may affect spinal reflexes, or

c) on physiological or homeostatic mechanisms (e.g., vasomotor) which may have secondary effects on the spinal cord (e.g., by anoxia).

However, even if a drug is known to affect one or more of the mechanisms just outlined, it may affect the spinal cord in a different order of concentration. For instance, strychnin may affect nerve conduction in concentrations of 10^{-4} but may alter the spinal reflexes in concentrations of 10^{-7}. Once it is known that a drug, in certain concentrations, does not affect the higher centres and peripheral mechanisms, its intravascular administration to test its effect on the spinal cord may be legitimate:

a) in statistical analyses of, e.g., a spinal convulsant or depressant, or

b) when no other method of administration is possible, as in humans.

One of the earliest experiments on the action of ACh and anti-cholinesterases on the motor responses of the C.N.S. was done on a virtually whole animal by McKail et al., (1941). They recorded the responses of the cat's tibialis to stimulation of the motor cortex, pyramidal tract, or the homolateral popliteal nerve (to elicit the spinal flexor reflex). Drugs were injected intra-arterially or intravenously.

Just as an illustration, some of the variables in their procedure which complicate any interpretation of their results will be enumerated:

a) the cats were anaesthetized with urethane or dial. It is conceivable - in any event, the possibility cannot be excluded - that the response of the anterior horn cell to ACh and to other drugs they tried, is altered in the presence of an anaesthetic.

b) Intravascular injections cause vascular and respiratory changes which, through anoxia and excess CO_2, may by themselves alter the response of the spinal cord.

c) Even though the drugs injected had no visible effect on the musculature, they may have altered nerve conduction and the mechanisms of neuro-muscular transmission. (It is to be recalled that they recorded from the tibialis.)

Their experimental evidence does not permit drawing conclusions as to the actual site of action of drugs.

2. Experiments on the "Spinal Animal".

The first prerequisite of a method for testing the effects of drugs on the spinal cord is a "spinal animal". The chances are that most drugs to be tested will have an effect on upper centres which may respond even so as to counteract the drug's action on the spinal cord.

The frog is easily made spinal by pithing through the foramen magnum. But in a mammal, e.g., the cat, precautions have to be taken not to interfere with the blood supply of the cord if it is to stay alive, and also to ensure maintained respiration. Various methods, other than cutting or crushing (e.g., anodizing current or binding around the cord),

were designed to spinalize animals without severance of its blood supply. This author has started to decerebrate the frog by dipping its head into liquid nitrogen (-195°C), but this technique was not repeated a sufficient number of times to permit comment on its efficacy.

It is to be noted that at this stage of complexity of the method, the drugs are injected intravascularly, and thus, there still exists a possibility of peripheral effects of drugs. Nevertheless, this limitation was disregarded in a very large number of pharmacological experiments.

Some physiological studies of the effects of drugs on the spinal cord were also done on the spinal animal with an intact vascular system. Bonnet and Bremer (1937) studied the effects of drugs (ACh, eserine, and nicotine) on the after-discharge of spinal reflexes in the spinal frog. They recorded the reflex response of the semi tendinosus to stimulation of the central end of the homolateral sciatic nerve. Drugs were injected into one of the aortic arches.

Bradley and Schlapp (1950) studied the effects of strychnin (and CO_2) on the response of the ventral root L_7 or S_1 to peripheral nerve stimulation. Note that if reflex activity is recorded, as they did, from a ventral root, drugs can be conveniently used despite their effect on the neuromuscular mechanism.

3. Experiments on the Pharmacologically Isolated Spinal Cord.

In these experiments an attempt was made to administer the drugs in such a way that it reached the spinal cell

directly and quickly in high enough concentrations to be locally active, but not in sufficient concentrations to act on the periphery once absorbed in the circulatory system. Most of these methods pertain to the mammal since in the Amphibia the circulation to the muscle can be cut out and reflexes can still be elicited from the muscle without its blood supply.

These experiments can be classified according to the method of the administration of drugs:

a) Microinjection.

Kennard (1951) injected substances (e.g., ACh) into the anterior horn cell region of the cat's spinal cord by a glass micro-pipette (40 μ outside diameter) which was doubly bored (one bore for the drug, the other for the control solution). The minimal volume of injection was 10^{-4}mm. To locate the site of injection, he deposited a carbon particle (4μ) after the injection, and sectioned the cord after the experiment. Contractions of antagonistic muscles were recorded mechanically.

This method of testing the effects of drugs on the spinal cord is potentially a very good one.

b) Local application of the drug on the surface of the cord.

A host of experiments was done by this method, e.g.,

Lefebvre and Minz (1936)

Bonvallet and Minz (1938)

Bernhard and Skoglund (1947).

c) Close arterial injection.

Feldberg et al. (1952, 1953), for the first time, described a technique of close arterial injection into the

spinal cord of the chloralosed cat. Drugs, e.g., 0.2 ml of 10^{-4} ACh, were injected into a cannula in the basilar artery. They stimulated the trunk of C_2 and recorded from C_1.

If this technique could be simplified, it would certainly become invaluable for demonstrating effects of drugs which are rapidly destroyed in the blood on the spinal cord. It is to be noted that close arterial injection was one of the methods whereby the ACh transmission at the neuromuscular junction was conclusively proven. It has so far been difficult to adapt it to the spinal cord because of the anatomical relationships of the vertebrae and the arterial supply of the spinal cord.

d) Intrathecal injection.

Intrathecal injection is another way of obtaining high concentrations of the drug within the spinal cord. This method was used in testing the effects of eserine on the spinal cord by Calma and Wright (1947) on the cat, and by Kremer (1942) in man.

e) Perfusion of the subarachnoid space.

Merlis and Lawson (1939) perfused the lumbar subarachnoid space with Ringer. The spinal dura was exposed by laminectomy and two No. 20 gauge spinal needles were inserted into the subarachnoid space, one at the 11th thoracic segment (inflow), and the other at the first sacral segment (outflow). The perfusion pressure was kept well below the systemic pressure in order to obviate asphyxiation of the spinal cord. After a simultaneous control record of the knee jerk (as an example

of the cross-extensor reflex) and of the response of the tibialis muscle (a flexor reflex) to stimulation of the posterior tibial nerve, they could switch from Ringer perfusion to one with a low concentration of the drug to be tested. The main criticism against this technique is the possibility of absorption of the drug intended for the spinal cord into the circulatory system.

f) The "Ischemic limb" technique.

A very extensive analysis of the effects of drugs on the knee jerk was done by Schweitzer and Wright (1937/38) (for reference to all previous papers, see Schweitzer et al., 1939). They injected their drugs intravenously. To ensure that these did not reach the limb from which recording was made, they used the "Ischemic limb technique", i.e., they occluded the blood supply to a limb for five minutes and the control record was taken. Then they allowed the preparation to recover and again disrupted the limb circulation while the injection of the drug was made.

g) Double perfusion method.

Bülbring and Burn (1941, 1948) devised a most ingenious technique for the perfusion of the spinal cord and the collection of venous effluents to study the possibility of chemical transmission in the spinal cord.

To restrict the action of drugs to the spinal cord, they devised a system in which there were two circulations of blood perfusion, one supplying the spinal cord, and the other the muscles. They used three dogs for each experiment. Two

pump lung circuits (with two dogs) perfused separately the spinal cord (via external iliac) and the lower limb from which recording was made. They elicited the flexor reflex and the knee jerk through stimulation of the tibial nerve and recorded from the quadriceps and tibial muscles, respectively. The drugs were injected into the spinal blood circuit.

This experimental design of double perfusion of the mammalian spinal cord isolated from upper centres and the muscles from which the reflexes are recorded, though very complicated, would be an ideal one if only instead of blood from another dog, oxygenated Ringer could be substituted in the spinal circuit. The demonstration of the chemical nature of synaptic transmission should then be a relatively easier task through the Ringer effluent which perfused the active spinal cord.

4. Experiments on the Spinal Cord in an Artificial (Ringer) Medium.

In all the foregoing experiments, the spinal cord was dependent on its blood supply for oxygen, nutritive materials and removal of metabolites. A number of experimenters, however, succeeded in maintaining, mainly in Amphibia, the spinal cord in an oxygenated Ringer environment, a category of experiments to which our own preparation belongs. If the chemical products of synaptic activity are to be collected in a perfusate, the advantages of a perfusion fluid whose exact composition is known, over blood, are obvious.

The author has been unable to find reference to experiments in which the mammalian spinal cord, without natural blood supply, was perfused.

Three experiments, all on the frog, belong under this heading. Ine one, the whole spinal cord was removed from the vertebral column. In the second, it was perfused with Ringer through the vascular system; and in the third, the spinal cord was maintained through intravascular Ringer perfusion.

a) Barron and Matthews (1938) while investigating the potential changes in the spinal cord of the frog, were able to isolate a 10 mm segment of the spinal cord with its roots attached and maintain it in a functional state for several hours by continually irrigating with glucose-gum-Ringer's solution from a flask under a pressure of half an atmosphere of pure oxygen.

The method was further perfected by Eccles (1946) who used the ninth and tenth spinal roots for eliciting and recording reflex potentials, and studied pharmacological action on the potentials.

He removed the spinal cord with attached roots from the decerebrate frog, and immediately placed it in Ringer in an airtight chamber through which moist oxygen was passed. During experimental periods the Ringer was sucked out so that the cord lay on the paraffin floor of the chambers in an atmosphere of moist oxygen. Pharmacological action was studied by immersing the spinal cord in a solution of the drug in

Ringer for thirty minutes during which the drugs he investigated exerted their maximal effect. In later experiments, the cord was divided by a medial longitudinal incision, and the contralateral side removed to provide better access of the drugs to the grey matter in a shorter time. After thirty minutes, the drug solution was drawn off and the experiment continued.

Provided that the pure oxygen content of the chambers was maintained with one to two minutes interruption for manipulation, the spinal cord exhibited a uniform level of reflex activity for many hours.

b) Torda (1940) perfused the toad with Ringer solution through the thoracic aorta at a pressure of 50 mm Hg. The test substances were added to the perfusate. All the connections of the gastrocnemius from which recording was made with the remainder of the animal, except the sciatic nerve, were cut, including the vessels leading to the muscle, so that perfused substances could only have access to the spinal cord with no peripheral effects.

The contralateral extensor reflex was elicited by stimulating the central end of the contralateral sciatic nerve and was inhibited by stimulating the central end of two intra-abdominal branches of the homolateral sciatic nerve.

Torda does not mention if oxygenation of the Ringer was necessary for the maintenance of the cord. However, it is possible that in the particular season in which she conducted her experiments, the atmospheric oxygen absorbed in

the Ringer solution may have been sufficient to keep the spinal cord alive.

Amongst all the methods enumerated so far, perhaps the most convenient one for the administration of drugs and collection of spinal effluents is that of Häusler and Sterz (1952) and Sterz (1953). It was developed contemporaneously and independently of our preparation which it resembles in many details.

Their preparation consisted of two frogs. One for the collection of spinal effluents, and the other for demonstrating active substances therein and for testing the effects of drugs on the spinal cord.

Preparation 1.

For the collection of effluents, the frog was pithed and through the hole a cannula was inserted, pointing towards the vertebral column. The frog was completely eviscerated (circulatory system presumably completely destroyed) and the lower limbs were removed, with the exception of the sciatic nerves on which stimulating electrodes were arranged. The whole preparation was suspended in a vertical position. The vertebral column was transected just below the exit of the sciatic nerves so that the Ringer, perfused through the cannula from a Mariotte's bottle above the preparation, could drip into a test tube through this hole. They perfused the spinal cord at a very slow rate (1 - 2 drops per minute) and collected effluent samples of 1 - 2 cc. (each 15 minutes):

a) immediately after the preparation was set up;

b) during several control periods of 15 minutes each, and c) during 15 minutes of strong stimulation of both sciatic nerves following one hour's rest. The effluents were then tested on preparation No. 2 (and apropos, assayed for histamine on the guinea pig ileum).

Preparation 2.

Preparation No. 2 was set up in such a way that drugs or effluents (collected from the first preparation) could be tested on reflexes.

The spinal cord was again arranged for perfusion as in preparation No. 1; however, this time the extremities were left in the preparation and were perfused with Ringer through a cannula in the aorta pointing towards the feet. Thus, the legs and the spinal cord had separate perfusion systems (analogous with Bülbring's method - 1941). The whole preparation again was suspended vertically. The left leg was stimulated five times for one second at 30 second intervals, and was then given ten minutes rest to allow the reflexes to recuperate from "fatigue" phenomena before the cycle of stimulation could be recommenced. (Note that this slow rate of stimulation may have obviated the necessity of supplying extra oxygen for the spinal cord in their case.) The right leg was connected to a writing lever and the movements of the leg, due to excitation of the contralateral leg, were registered on a kymograph.

This preparation differs from ours in two important respects:

a) they recorded the response of a whole leg, while in our

experiment we were specifically concerned with testing the excitatory and inhibitory mechanisms of the spinal cord when the flexor reflex and its reflex inhibition were recorded alternately, and

b) in our experiment attempts were made to supply the spinal cord with an extra quota of oxygen to circumvent the continuous complicating effects of anoxia on the spinal cord.

V. THE METHOD

Preview

The method was designed to meet, as closely as possible, the requirements of an ideal preparation (outlined in the Introduction) for testing drugs and spinal effluents on spinal reflexes.

To achieve neurological isolation, frogs were at first pithed and later the spinal cord was transected in the brachial region. Thus, only the remaining approximately 10 mm segment of the spinal cord with attached dorsal and ventral roots and the sciatic nerves remained functional.

To achieve physiological isolation of the spinal cord from the rest of the body, the normal circulation of the frog was disrupted. The oxygen requirement of the cord was supplied by the perfusion of the circulatory system with gaseous oxygen (97% O_2 and 3% CO_2 in most experiments) through the truncus arteriosus. The systemic arches were tied and the vena cava cut, so that the gaseous oxygen, after its entry into the truncus, circulated through the arteries which originate in the upper aorta (amongst which are the vertebral and possibly other small arteries supplying the cord), and left the system either through broken vessels or through the opened inferior vena cava.

The spinal cord was perfused with Frog-Ringer, containing glucose, through a cannula inserted into the vertebral column at the end of the urostyle. The perfusate could be collected into a test tube through suction. Since the Ringer circulated only through the cord, it served as a convenient route of administration of drugs for testing their effects on reflexes. Also a very slow perfusion rate was hoped to permit the chemical modification of the fluid as it bathed the reflexly active spinal cord.

Reflex contractions of the right biceps femoris (a flexor muscle) served as an indication of spinal cord activity. The stimulating apparatus was so designed that it delivered alternately excitatory (E) and inhibitory (EI) stimuli of the desired relative strength and duration and at desired but fixed intervals. The E stimulus was a shock to the right leg alone, while the EI stimulus consisted of the same E stimulus (of one second duration) during a longer shock (of two seconds duration) to the left leg which was thus inhibitory to the contralateral biceps.

A continuous (control) record of the flexor and inhibited flexor reflexes was recorded on a slowly revolving kymograph. Experimental variables were introduced when a sufficiently long and stable control record was obtained.

A detailed description of all aspects of the method is now in order.

A. OPERATION

1. Decerebration

The frog (Rana Temporaria) was chosen for the preparation. Since it is a small and cold-blooded animal, maintenance of its spinal cord without a natural blood supply promised to be more successful than an attempt to perfuse the spinal cord of a warm-blooded, large animal. Not only was artificial temperature regulation thus obviated (since one could work at room temperature), but since oxidative metabolism proceeds at a much lower rate in the frog than in the mammal, less oxygen and glucose per gram of active spinal cord per minute was expected to be required. Moreover, the muscle from which the recording was made, could survive without its blood supply on anaerobic metabolism.

The frogs were pithed through the foramen magnum with a blunt needle, and bleeding from the occipital arteries was stopped with cotton wool. To effect complete decerebration and to permit experiments on reflexes of the hind leg, uncomplicated of brachial reflexes, the spinal cord was later transected in the brachial region.

2. Muscle Dissection.

After a lot of trial, the biceps femoris (a flexor muscle) was found to qualify best for the recording of spinal

cord activity, for the following reasons:

a) It is a small muscle on the dorso-medial aspect of the leg, lying between the M. semi-membranosus and the vastus externus of the triceps and is, thus, convenient to record from, when the preparation is placed on the frog board on its ventral aspect.

b) It is easily accessible via a small slit through the overlying skin.

c) It responds with ample contractions to the flexor reflex elicited by stimulation of the homolateral toe, and

d) Its flexor contraction is readily inhibited by the cross-extensor reflex due to stimulation of the contralateral toe.

Since there is no circulating O_2 and glucose in the hind legs, one would expect the muscle to fatigue rapidly. However, the muscle seemed to afford the required activity (i.e., in a six hour preparation, 500 tetani of one second duration at 30 to 40 second intervals) without signs of fatigue, presumably with the amount of oxygen that reached the muscle fibres by diffusion from the atmosphere, or through anaerobic metabolism.

In dissecting the biceps, precautions had to be taken as follows:

a) It was very easy to damage the main vessels of the thigh and, more important, to injure the sciatic nerve, all running beneath the muscle (see Fig. 3). Furthermore, the sciatic nerve bifurcates below the biceps to form the N. tibialis, and N. peroneous which crosses over the surface of the knee

just at the point of insertion of the biceps and is, therefore, easily injured when the tendon is cut. This nerve is, of course, important for the delivery of afferent impulses from the stimulated homolateral toe to the spinal cord.
b) Once the muscle is dissected out, it is very easy to pull on it and tear its motor nerve. It was best, therefore, not to detach the tendon from the knee bones until the preparation was ready for recording.

3. Abdominal Operations for Vascular Oxygen Perfusion.

To expose the abdominal and thoracic cavities, the frog was placed on its back and the skin over the ventral abdominal wall was slit. The abdominal muscles were removed up to, and including, the sternum.

Arrangements were then made to perfuse the vascular system with gaseous oxygen. The lungs were tied to prevent short-circuiting of the gas. Since in the course of experiments certain variables were imposed on the preparation through its vascular system, e.g., in the administration of gaseous N_2, large per cent of CO_2, or a gaseous anaesthetic, it was, of course, essential to prevent them from reaching the biceps from which recording was made, or else the peripheral effect of the variables would have obscured the central (spinal) effects. The aorta, therefore, had to be tied at some point. Originally, the aorta was tied where the systemic arches unite and redivide to form the dorsal aorta and

mesenteric artery. However, since the aorta may give off collaterals to the spinal cord beyond this region, in later experiments, the dorsal aorta was tied just before it divides to form the iliac arteries.

A cannula was then inserted through the heart and strongly tied into the truncus arteriosus. The vena cava was cut, and gaseous oxygen saturated with water vapour administered to the truncus from a cylinder at an initial pressure of 100 mm Hg. As the peripheral resistance to the flow of gas decreased by clearing the circulatory system of blood, a greater flow per minute was necessary to maintain the required pressure. The gas at this pressure was expected to reach the spinal cord either through broken larger vessels, or possibly through the arteriolar-capillary system. The gas left the preparation either through broken vessels or through the venous system by the cut vena cava.

After checking that no major hole on the functional aorta permitted the escape of gas, the frog was turned onto its ventral side again and the legs were pinned down on the supporting board by the toe, the knee bone, the forearms and the head (see Figs. 2 and 3).

4. Vertebral Perfusion.

Arrangements were now made for perfusing the spinal cord. The skin was reflected over the vertebral column, and the muscles and bone were carefully dissected away over

the cervical region of the spinal cord. The spinal cord was transected with blunt forceps to destroy brachial reflexes, and the spinal and medullary tissue, central to the cut, was cleared away so as to allow the escape of fluid which perfused the vertebral column through a cannula easily inserted at the point where the urostyle ends. The perfusion fluid reached the cord at the desired pressure (at adjustable rates), perfused the cord, and was then collected in the brachial region by suction.

5. Getting Ready for Recording.

The "excitatory" (see 32 in Figs. 1, 2, and 3) and "inhibitory" (31) electrodes were now placed on the right and left toes respectively. (The electrodes were wound of silver wire - see Fig. 3 - insulated with nail-polish and sealed in glass tubing). To ensure constant current flow and to prevent drying of the skin, the preparation was constantly sprayed with Ringer solution through an atomizer (22 in Figs. 1 and 2). In order to keep the amount of spraying solution constant, a Mariotte's bottle (23) was kept at a desired pressure head. The solution was forced through the sprayer by compressed air.

The tendon of the biceps was now detached from the knee (if not done previously) and the thread (27) bound around the tendon (see Fig. 3) was led over a pulley to an isotonic lever (28) which recorded the contractions of the muscle on a slowly (2 mm/min.) revolving Palmer kymograph. The preparation was now ready for stimulation.

B. THE APPARATUS.

The apparatus consists of the following sections:

- A frog-board and apparatus for recording muscle contractions on a kymograph (already mentioned on p. 36).
- Apparatus for intravascular gaseous oxygen supply.
- Apparatus for intravertebral Ringer perfusion.
- Apparatus for spraying the preparation (already described on p. 36).
- Apparatus for collection of effluents.
- Stimulator and electrodes.

Throughout the detailed description of parts of the apparatus which is to follow, numbers in brackets will refer to the common Legend of Figs. 1 and 2.

Fig. 1 (next page) shows the entire apparatus photographed in action.

Fig. 2 was drawn to illustrate the details of the intravertebral Ringer perfusion, intravascular gaseous oxygen perfusion, spraying and perfusate collecting apparatus, which are not clear from Fig. 1 at first sight.

Fig. 3 is a close-up picture of the preparation as it is stimulated on the toes, perfused intravascularly and intravertebrally, and as the perfusate is collected.

LEGEND TO FIGURES 1 and 2

1 - Gas cylinder.
2 - Pressure reducing valve.
3 - Pipeline supplying gas to the bubbler (8).
4 - Erlenmeyer - half filled with Ringer for moistening the gas.
5 - Manometer No. 1 (0 - 300 mm Hg) for registering pressure at which gas is delivered to the frog.
6 - Manometer No. 2 (0 - 300 mm Hg) for registering pressure on the other side of a resistance between the frog and the bubbler. The difference in pressures (M2 - M1) is an indication of the flow rate of the gas.
7 - Capillary tubing delivering gas into truncus of the frog.
8 - Gas bubbler (No. 1 porosity - Sciex).
9 - Mariotte's bottle with Ringer solution for intravertebral perfusion.
10 - Drop counter No. 1 for normal perfusion of Ringer.
11 - Drop counter No. 2 for injection of drugs or effluents.
12 - Thick rubber tubing for the injection of drugs or effluents.
13 - A 25 cc. reservoir (pipette) from which Ringer passes back into (9) when injection is made at (12).
14 - Rubber tubing connecting Mariotte's bottle with drop counter No. 1.
15 - Rubber tubing connecting Mariotte's bottle with the pipette (13).
16 - Thin capillary tubing for delivery of perfusion fluid (or drug) into the vertebral canal.
17 - Three-way stop cork with which one can switch from Ringer to drug perfusion of the spinal cord.
a - Connection of the three-way stop cork (17) with drop counter No. 1 (10).
b - Outlet to the frog.
18 - Thin capillary tubing for the collection of perfusate in the brachial region of the spinal canal.
19 - Test tube into which perfusate is collected.

(cont'd. on p. 40)

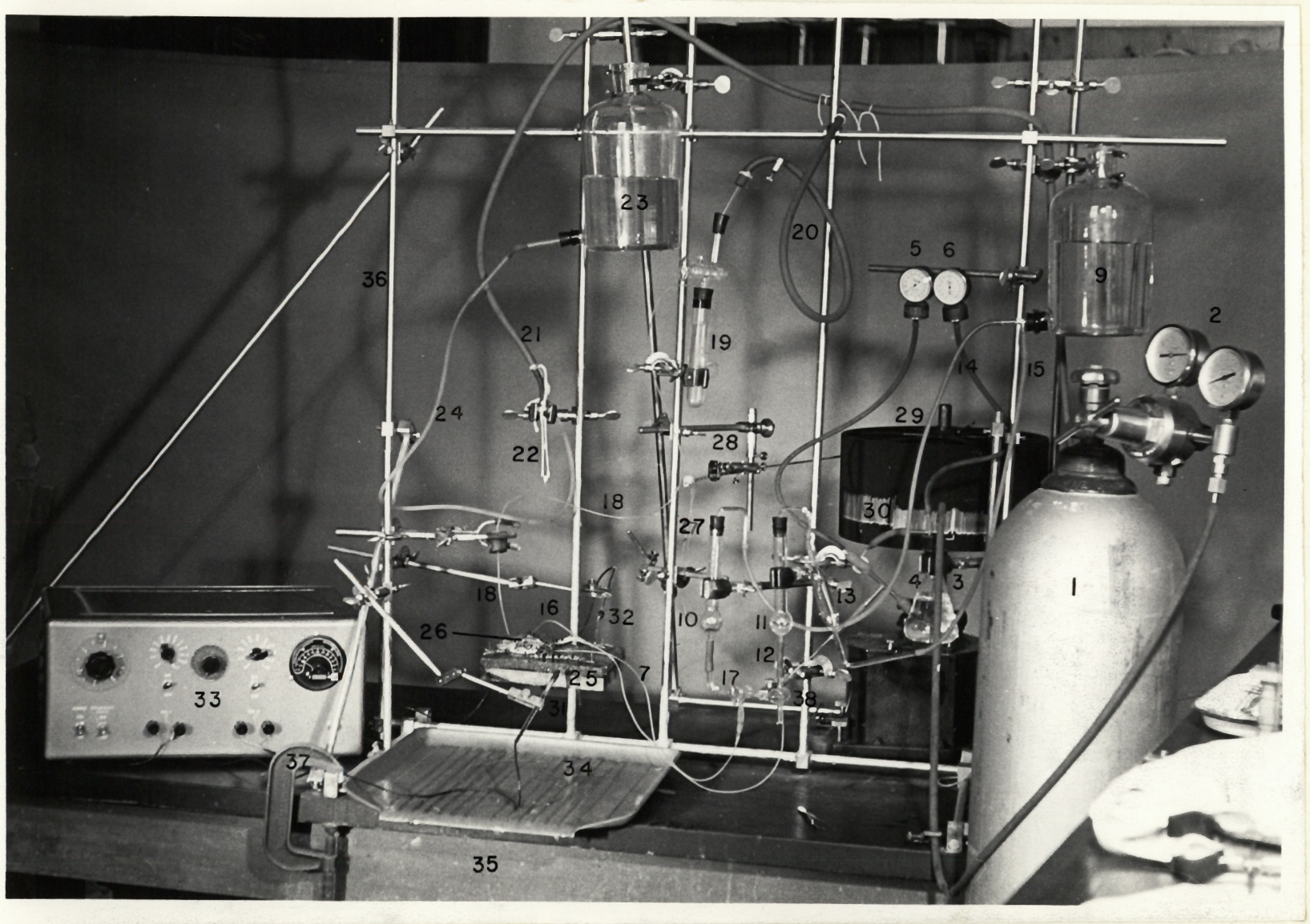

FIGURE 1. The Apparatus - photographed in actual operation.
(For Legend, see opposite page.)

LEGEND TO FIGURES 1 and 2 (cont'd.)

20 - Compressed-air line for the collection of perfusate.
21 - Compressed-air line for the sprayer.
22 - The sprayer (atomizer).
23 - Mariotte's bottle containing Ringer solution for spraying the preparation.
24 - Rubber tubing connecting spraying reservoir (23) with the sprayer.
25 - Frog board.
26 - Frog (for details see Fig. 3).
27 - Thread connecting the biceps muscle to the lever (following 28).
28 - Lever recording contractions of the biceps.
29 - Kymograph (C.F. Palmer), revolution rate variable from .01 - 580 mm/sec.
30 - A record of the reflex contractions of the biceps in a strychninized frog.
31 - The inhibitory (I) electrode applied to the left toe of the frog.
32 - The excitatory (E) electrode applied to the right toe of the frog.
33 - The stimulator (for details, see Fig. 6).
34 - A drain board for the spraying fluid.
35 - Table.
36 - Frame-work of the apparatus.
37 - Clamp fastening the whole framework to the table.
38 - Three-way stop cork for cleaning drop counter No. 2 (11) of previous drug injection.
d - (38) connected to thick rubber tubing (12) for injections.
c - Outlet.
39 - An injection needle (No. 23 gauge) to act as a resistance to gas flow.
40 - Pins for fastening the preparation to the frog board.
41 - Intravertebral cannula (No. 21 gauge).
42 - Glas tubing into which electrodes are sealed.

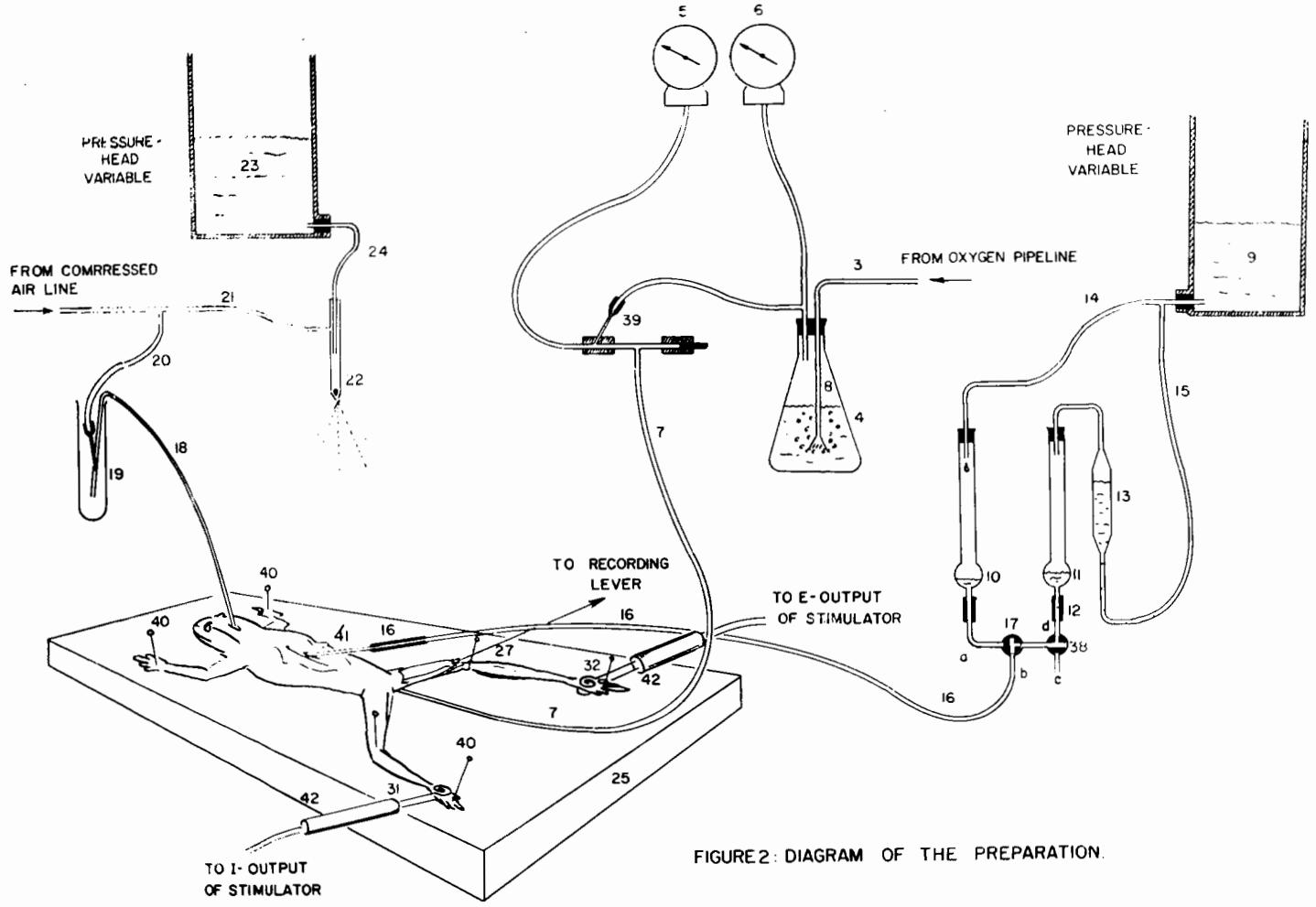

FIGURE 2: DIAGRAM OF THE PREPARATION.

FIGURE 2.

(See common Legend for Figs. 1 and 2)

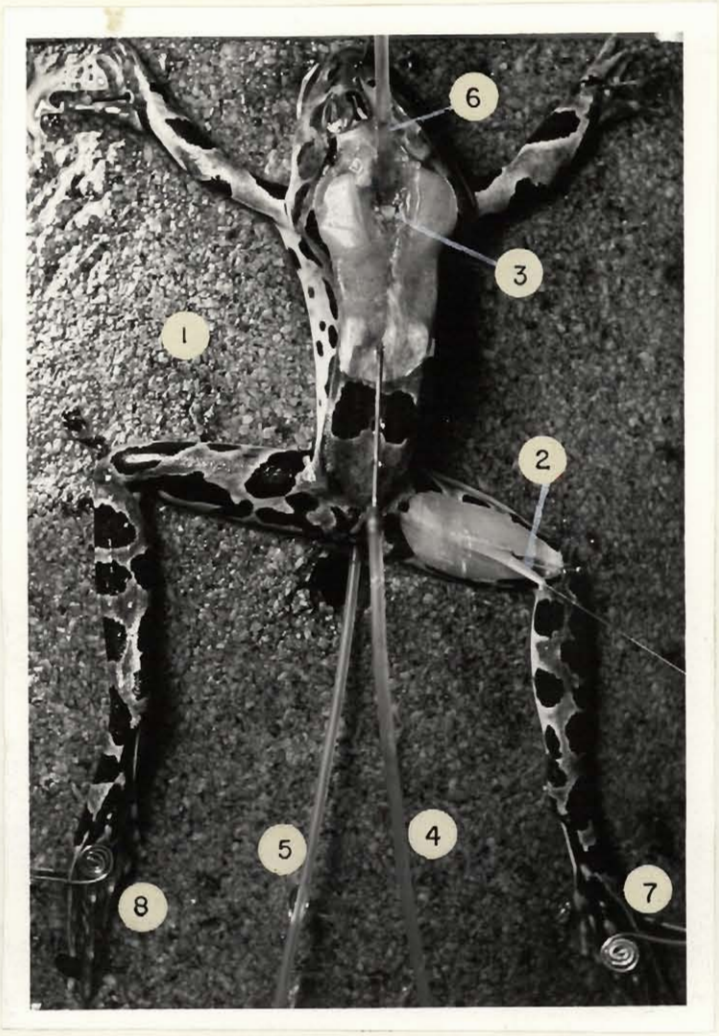

FIGURE 3. A Close-up View of the Frog.

1 - Cork board onto which frog was pinned.
2 - The biceps femoris muscle from which recording was made.
3 - The cut end of the spinal cord.
4 - The intravertebral Ringer perfusion line.
5 - The intravascular gaseous oxygen perfusion line.
6 - Capillary tubing for collection of spinal effluents.
7 - The excitatory (E) electrode.
8 - The inhibitory (I) electrode.

1. Apparatus for Oxygen Supply.

(Figs. 1 and 2.)

Oxygen was delivered from a commercial cylinder (1) with a pressure reducing valve (2). The usual mixture used was 97% O_2 and 3% CO_2. Of course, other mixtures, e.g., pure oxygen, pure nitrogen, 97% nitrogen and 3% CO_2, compressed air, or N_2O could be substituted. In order to saturate the gas with water vapour and to record the pressure and the rate of flow of the gas, the apparatus was arranged as follows; (see especially Fig. 2).

The pressure valve was connected by rubber tubing (3) to a No. 1 porosity gas bubbler (8) inserted into a tightly stoppered Erlenmeyer flask (4) half filled with Ringer. One end of a T-tube served as an outlet for the gas now saturated with water vapour from the Erlenmeyer - the second end was connected with a manometer M_2 (6) - the body of a sphygmomanometer was used for this purpose - and the third end of the T was connected to an injection needle, (No. 23 gauge) which served as a constriction (39), a small resistance to the flow of gas so that the difference of pressures recorded on its two sides is proportional to the rate of flow. The injection needle was now connected to another T-tube. The second arm of this T was connected to a second manometer M_1, and the third side of the T was connected, through capillary tubing (7), to the heart cannula (an injection needle No. 28 gauge, the end of which was cut down and grooved) which when

inserted in the truncus, delivered the gas to the preparation. It is to be noted that the pressure recorded by M_1 was the final pressure at which oxygen reached the frog's arteries. The optimum pressure was found to be 100 - 150 mm Hg. Furthermore, $M_2 - M_1$ is proportional to the rate of flow of gas, for which the optimum was found to be 130 - 160 mm Hg. At the beginning of any experiment the rate of flow for a given final pressure was, of course, very small and increased as the vascular system was cleared of blood and as more and more vessels were broken on the arterial side, thus decreasing the (frog) resistance to the flow of gas.

2. Apparatus for Intravertebral Ringer Perfusion.

(See Fig. 1 and especially Fig. 2)

The rate of intravertebral Ringer perfusion was regulated by the pressure head of the fluid in the Mariotte's bottle (9) above the preparation. This was so adjusted that the rate of perfusion was between four and twenty drops per minute, but slower or faster rates could be obtained by altering the height of the bottle, or by changing the resistance to flow in the thin tubing (16) which delivered the perfusion fluid into the vertebral column.

In many experiments the effects of drugs on spinal reflexes were tested. Therefore, it was essential to be able to switch from Ringer perfusion to a drug perfusion of definite

quantity or duration without changing the drop rate. To achieve this, the perfusion apparatus was supplemented as follows: (Fig. 2). The bottom outlet of the Mariotte's bottle (9) was tightly plugged with a stopper, into which one end of a T-tube had been inserted. The second end of the T-tube was connected by rubber tubing (14) to drop counter No. 1 (10). The third end of the T emerging from the Mariotte's bottle, was connected with rubber tubing (15) to a 25 mm pipette (13), conveniently bent, which led into drop counter No. 2 (11).

The two drop counters were connected by a double T-joint system (17 and 38).

Drop counter No. 1 was connected to the system at "a". Drop counter No. 2 was connected by thick rubber tubing (12) with "d". To "b" was joined a thin rubber tubing (16) which delivered the perfusion fluid to the cannula (41) inserted in the vertebral column just at the end of the urostyle. "c" was an outlet.

Before intravertebral perfusion was started, one had to make sure that Ringer filled both outlets (14 and 15) from the reservoir (9) and that it was actually dripping into both drop counters and out through "c" above. Then "c" was closed and (17) opened to either "a" or "d". The drop rate had to be equal from both outlets as could be measured by a stop watch.

When intravertebral perfusion with Ringer was started, (17) was opened to the frog and to drop counter No. 1, but was closed to drop counter No. 2.

If a drug perfusion (of, say, 5 cc.) was desired, the drug was injected through the thick rubber tubing (12) which connects drop counter No. 2 with the double T-joint arrangement. Since injection is made into a closed system, it displaces an equal volume (5 cc.) of Ringer from the pipette (13) back into the reservoir (9) where the pressure head is not changed appreciably. When a sufficiently long and stable control record is obtained, (17) is suddenly opened to drop counter No. 2 and closed to drop counter No. 1. Perfusion of the cord with the drug solution is now continued at the same rate as with Ringer before. (The diagram in Fig. 2 is drawn to show the arrangement of the T-joints, 17 and 32, while perfusing the preparation with a drug).

After the drug perfusion is over, one can either let Ringer perfusion follow the drug from the same drop counter No. 2 (11), or one can switch back by (17) to drop counter No.1. Drop counter No. 2 can then be washed out with Ringer through outlet "c".

3. Arrangements for Collection of Spinal Cord Effluents.

Once the perfusion fluid or the drug has traversed the spinal cord, it had to be removed efficiently for two reasons:

a) When the effects of a drug were examined, the drug had to be removed as fast as possible, since, once the circulation is destroyed, most of the deactivating mechanisms of the animal are

excluded. Thus, in order that the effects may be reversible, the drug had to be removed by physical means.

b) Since the ultimate aim of the experiments was to collect the perfusion fluid and to detect some chemical modification in it by assays, either on the same or on a second preparation, the effluent had to be carefully collected in small test tubes.

Effluent collection was achieved by suction (see Figs. 2 and 3). A very thin plastic tubing (18) was placed in the vertebral canal in the brachial region, now clear of spinal tissue from the previous transection. The other end of the tubing dipped into the collecting vessel (19). Just before its end, an injection needle (No. 23 gauge) was inserted, pointing towards the collecting tube so that compressed air (20), when applied through it, exerted a mild suction on the fluid in the vertebral canal.

When the collected fluid was to be immediately reperfused, the collecting tube dipped into a syringe placed so that the level of fluid in it was the same as obtained in the original Mariotte's bottle (9). The other end of the syringe was connected to that end of the double T-joint arrangement (d) which was previously attached to drop counter No. 2 for the perfusion of drugs. Thus, the reperfusion fluid went through at the same rate as the normal perfusion fluid in the control period.

Effluent samples could also be perfused through a second preparation or injected later through the drop counter No. 2 originally designed for drug perfusion.

4. The Stimulator.

A stimulator had to be so designed that it delivers electric stimuli, of variable but fixed duration and magnitude at adjustable but regular intervals, to the frog's toes in order to elicit spinal reflexes on which the effects of different experimental conditions can be tested. Since we were interested in the effects of drugs on the "inhibitory" as well as "excitatory" mechanisms of the spinal cord, it was thought best to elicit, e.g., the flexor reflex and its alternate inhibition by the contralateral cross-extensor reflex.

Thus, the stimulator was required to deliver
a) an E-stimulus (to the foot homolateral to the flexor muscle from which recording was made), and
b) an EI-stimulus which consisted of two stimuli, the E-stimulus being of the same duration and magnitude as before, and the I-stimulus which is of longer duration and applied to the contralateral foot, timed so that it both precedes and outlasts the E-stimulus.

A detailed description of the design and make-up of the apparatus is now in order.

LEGEND TO FIGURES 4, 5 and 6.

A - Self-maintaining switch which completes the 110 V circuit of M1.
B - Switch, which completes the 110 V circuit of M2.
C1&2 - Cams regulating duration of I-stimulus adjusted at two seconds.
C3&4 - Cams regulating duration of E-stimulus delivered during the I-stimulus (adjusted at 1 sec. so that I precedes and outlasts it by half a second).
C5&6 - Cams regulating duration of the E-stimulus alone, adjusted to be equal to C3 and C4 at 1 sec.
C7 - Cam operating switches A and B.
D - Delay scale (10-120 sec.).
E - Excitatory stimulus.
EI - Inhibited excitatory stimulus.
F - Fuse.
I - Inhibitory stimulus.
L - Lever slowly moved by M2 to close S5 on D.
M1 - Motor, turning C1 - C7 (revolves at 3 r.p.m., 180° at a time), and thus responsible for the operation of the arrangements for the delivery of stimuli of specific duration, at desired times, and also for stopping M2 and maintaining itself.
M2 - Motor responsible for the adjustable interval between stimulations.
P1&2 - Variable potentiometers (10 Ω 4w) for E and I stimuli.
S1&2 - On-off switches for transformers of E and I stimuli.
S3 - On-off switch for the entire apparatus (main).
S4 - On-off switch for the motors (M1 and M2).
T1&2 - Step down transformers (110/20 V) for E and I stimuli separately.
V - Variac.
Y1&2 - Two condensers, (.06u/600V) to prevent sparking across contacts A and B.

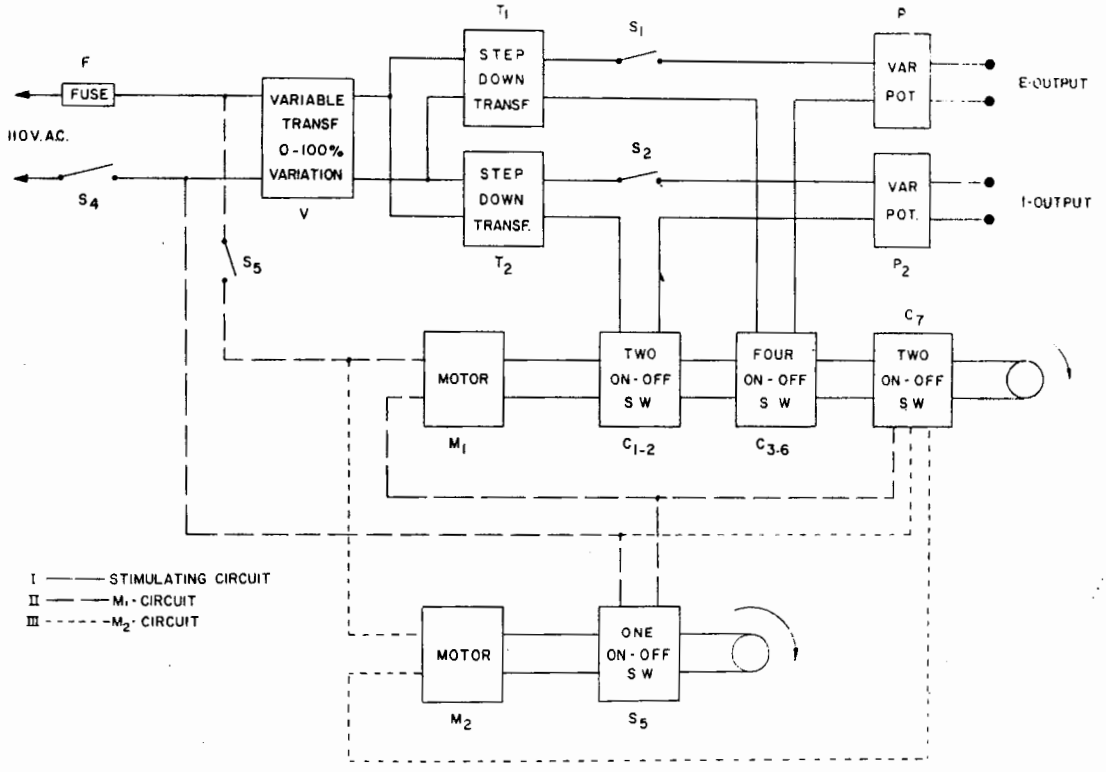

FIGURE 4 BLOCK DIAGRAM OF STIMULATOR

FIGURE 4.

(See Legend on opposite page)

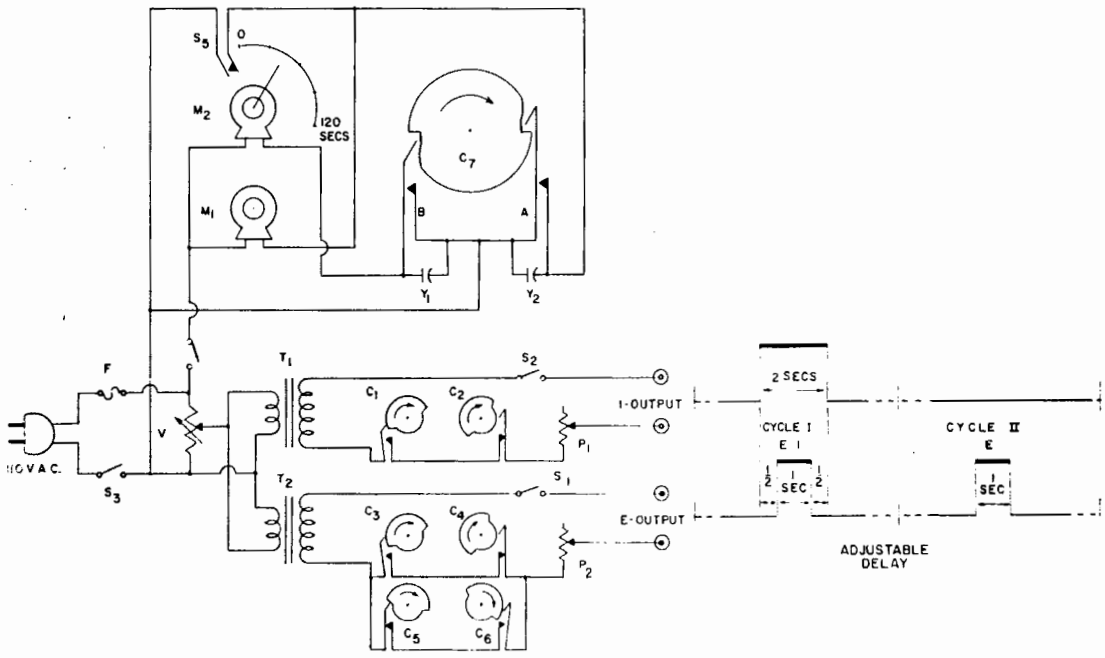

FIGURE 5 CIRCUIT DIAGRAM OF STIMULATOR

FIGURE 5.

(See Legend on page 49.)

The stimulator consists of three main circuits:

I. The actual stimulating circuit for the delivery of the E and EI-stimuli of desired strength (drawn in —— in Fig. 4).

II. The circuit which connects a motor (M_1) to the power line as well as to a system of switches designed to control the motors' starting and stopping action. It delivers the E and EI-stimuli of a predetermined relative duration (drawn in ---- in Fig. 4).

III. The circuit which connects a second motor (M_2) to the power line. The function of M_2 is to achieve a variable, but thenceforth regular, interval between the E and EI-stimuli (drawn in .... in Fig. 4).

i. The stimulating circuit (See Fig. 5 and —— in Fig. 4).

The stimulating circuit consists of a 110 V A.C. receptacle, feeding a "Variac" (V). The purpose of the Variac is to enable the operator to control the amplitude of both I and E-stimuli simultaneously.

The Variac fees two separate step-down transformers (T_1 and T_2), ratio 5:1. Their purpose is to isolate the E and I-outputs from each other, i.e., there is no physical connection of any sort between the two outputs, other than through the frog tissue.

One lead of each transformer goes through a convenient on-off switch (S_1 and S_2), to a variable potentiometer (P_1 and P_2). The purpose of each potentiometer is to enable the operator to control the amplitude of each output (E and I)

independently.

The other lead of each transformer goes to the other end of the potentiometer output through a separate arrangement of switches for the E and EI circuits. Each arrangement is designed to close and open its transformer lead continuously.

The I arrangement consists of two simple on-off switches in series, opened and closed by two cams (C_1 and C_2). The two cams are mounted on the shaft of motor M_1. The I circuit is completed only when both switches are closed. The duration of closure is variable from 0 - 10 seconds, by rotating the cams on their common shaft relative to each other. Once adjusted, the duration of each stimulus is no longer variable.

The E arrangement consists of four switches, two in series, then two more in series, but in parallel with the first two, i.e., series-parallel. These switches are driven by four cams (C_3 to C_6), also mounted on the shaft of M_1. The switches are arranged so that while the I-circuit can be closed only once in each revolution of M_1, the E-circuit is closed twice.

ii. <u>The M_1-Circuit</u>. (---- in Fig. 4).

M_1 is a sixty cycle synchronous motor, geared down to 3 r.p.m., or 180° in ten seconds. M_1 drives a shaft on which seven cams are rigidly mounted. The purpose of six of the cams (C_1 to C_6) is described above. The seventh cam (C_7) is circular (nowhere excentric), has two notches such that its two switches

(A and B) make and break twice during each revolution or once in 180°. The M_1 arrangement is catalogued as an "M_4 Timer", made by Kramer Timer Company, Centerbrook, Conn., U.S.A.

iii. The M_2-Circuit. (.... in Fig. 4).

The second motor (M_2) is a time delayed switch, i.e., a motor which turns a lever (L) slowly until it closes a switch (S_5). The delay before the lever closes S_5 is mechanically adjustable, from 0 - 120 sec. on the scale D.

From Fig. 5 the mechanical action of the cam (C_7) on switches A and B is apparent; B makes upon striking a notch, while A breaks when striking a notch. C_7 is cut so that B makes slightly before A breaks so that M_2 may start turning while M_1 is still revolving. Also A makes before B breaks, so that the circuit of M_1 may remain closed after B and S_5 open, (see sequence below).

iv. A typical sequence of operations.

Supposing one wants a fifty second interval between stimuli (L is set at 50 sec. on scale D - see Fig. 6).

Time in sec.

0 Switch power on by S_3, (S_4 is closed). Assume A and B are in notches. B completes the 110 V circuit of M_2 which, therefore, turns. After 40 sec. M_2 closes S_1.

Time
in sec.

40 S_1 closes the 110 V circuit. M_1 begins to turn at 3 r.p.m. for 180°.

41 A closes due to contour of cam C_7 (see Fig. 5).

42 B opens due to contour of cam C_7. When B opens, the circuit of M_2 is broken and the lever of M_2 flies back by spring action to its starting position. S_1, therefore, opens. M_1 continues to turn since A is now closed, i.e., it is a self-maintaining circuit.

49 B strikes the notch vacated by A seven seconds ago. B closes. M_2 starts to turn its lever arm.

50 A opens. M_1 stops turning and will not start until S_5 closes again.

90 S_5 closes. M_1 turns, A closes.

91 B opens, S_5 opens, M_2 flies back and waits.

99 B closes, M_2 starts.

100 A breaks, M_1 stops, etc.

In the ten seconds during which M_1 is making a half revolution, i.e., between 40 and 50, and between 90 and 100 sec. above, cams C_1 to C_6 are also turned through the half revolution. The action of the cams for the I-stimulus (C_1 and C_2) and of those for the E-stimuli (C_3 to C_6) will now be described separately.

C_1 and C_2 can be adjusted relative to each other as well as relative to C_7. Thus, C_1 and C_2 may be adjusted both to

close and open their switches anywhere between 40 and 50 seconds. For instance, C_1 switch was adjusted to be already closed at 40 seconds. C_2 switch closes at, say, 45 seconds. The I-stimulus now appears at the I-terminals for two seconds since both switches are closed. (As will be shown later, the switches on C_3 and C_4 are both closed at 45½ seconds and open at 46½ seconds and, thus, the E-stimulus appears at the E-terminals during that time.) C_1 opens at 47 seconds and the circuit is broken. M_1 completes the remainder of the 180° in the three seconds that follow.

After a further delay (from 50 - 90 seconds) obtained again through M_2, M_1 commences to turn once more to 180°. During this period, C_2 opens and C_1 closes but both are never closed together at any time during this half revolution. Therefore, no I-stimulus appears at the I-terminals.

In both the above half revolutions, the E-stimulus appeared. In the first half revolution, it appeared together with the I-stimulus. In the second half revolution, without I (see output in Fig. 5). Thus, in one revolution of M_1 in two cycles, one E and one EI stimulus were delivered to the terminals.

During the first half revolution, C_3 and C_4 operated as described for C_1 and C_2 above, only for a shorter duration, i.e., C_3 is adjusted to be already closed at 40 seconds. C_4 closes at 45½ seconds. The E-stimulus now appears at the E terminal for one second (it is to be recalled that the I-stimulus

started to appear at 45 seconds). At 46½ seconds, C_3 opens and the E-circuit is broken (recall that the I-stimulus continues to be on for half a second more). During this revolution, the C_5 and C_6 switches (responsible for the E-stimulus alone) were never closed together at the same time.

After the second delay of 40 seconds (at 90 seconds), obtained through M_2, M_1 commences to turn from 180° to 300°. During this revolution, C_3 and C_4 switches are never closed at the same time, but the switches of C_5 and C_6 are both closed for one second, i.e., at 90 seconds C_5 is closed; at 95½ seconds C_6 closes; at 96½ seconds C_5 opens. Note that in this revolution only the E-stimulus appears, due to the action of C_5 and C_6, whereas at least one of the switches of C_1 to C_4 (responsible for the EI-stimulus complex) is always open.

The E-stimuli operated through cams C_3 and C_4 and C_5 and C_6, respectively, had to be adjusted to be equal (one second approximately). Adjustment could be aided by an Electronic Scaler (Potter Instrument Company) by which the total number of A.C. impulses delivered during each cycle could be counted. The E-stimulus consisted of 64 impulses and the I consisted of 128 impulses.

The whole circuit was encased. On the outside of the case appeared the following (see Fig. 6):

1 - On-off switch (S_3) for the mains.

2 - On-off switch (S_4) for the stimulus cycle.

3 - On-off switch (S_3) for E-stimuli.

4 - On-off switch (S_1) for the I-stimulus.

5 - Variac scale (1 - 20 V) (V).

6 - Potentiometer scale (P_1) for I (from 0.1 - 1.0 x).

7 - Potentiometer scale (P_2) for E (from 0.1 - 1.0 x).

8 - Indicator of M_1 (to show the experimenter if the E, or EI, cycle is to follow).

9 - Scale of M_2 (D) in which the interval between stimulation be adjusted at 10 - 120 seconds, by a lever (L).

10 - A pilot lamp to indicate if mains are on.

FIGURE 6. The Stimulator.

Summary

1 - The absolute and relative durations of the E and EI-stimuli were adjusted when the apparatus was built so that both E's lasted one second, (64 A.C. impulses), and the I (which appeared only once in two cycles) lasted two seconds (128 A.C. impulses), preceding and outlasting one of the E-stimuli by half a second, (see time relationships of stimuli on Fig. 5).

2 - The strength of the stimuli could be varied during a day's experiment, as follows:

a) E-strength could be varied independently of I (by means of P_2).

b) I-strength could be varied independently of E (by means of P_1).

c) Both E and I could be changed simultaneously (by means of V).

3 - The interval between stimulations could be adjusted at anywhere between 120 seconds on D. Once adjusted, stimuli were delivered at that interval during the whole experiment, without further interference from the experimenter.

4 - If E or I-stimuli alone were desired, the other stimulus could be switched off by S_1 or S_2, respectively.

C. METHODS OF OXYGEN AND DRUG ADMINISTRATION.

1. Methods of Oxygen Supply.

If a preparation is put up without any special oxygen supply, but with the normal circulation destroyed, the reflexes usually decline within one half to one hour. This is in agreement with findings in the literature (Heubach, 1876; Ringer, et al., 1878; Bergman, 1897; Winterheim, 1907). However, the occasional frog survived five hours, provided intravertebral perfusion was maintained at a relatively fast rate, (Fig. 8). It seems that in these frogs, the amount of atmospheric oxygen, dissolved in the perfusion fluid, was just enough to keep the spinal cord alive.

However, in most preparations, this was not the case, and some additional method of oxygen supply was necessary.

a) Supply of oxygen in intravertebral perfusion fluid.

In addition to the atmospheric oxygen dissolved in the intravertebral perfusion fluid, the O_2-tension can be increased by bubbling pure O_2 through the fluid, before perfusion. This was tried in some of the initial experiments, with not much success, however. It seemed that, in order to supply the necessary quota of oxygen, the rate of intravertebral perfusion had to be significantly increased. This was disadvantageous for two reasons. Firstly, since the eventual purpose of the method is

to collect some possibly chemical products of spinal cord activity in the effluent, the slower the rate of perfusion, the better the chances are for the hypothetical substance to reach assayable concentrations.

Secondly, since the perfusion fluid serves as the immediate "milieu interieur" for the cells, too fast a circulation may have physically altered normal neuronal activity. This method of oxygen supply was thus abandoned.

b) Supply of oxygen dissolved in Ringer, intravascularly.

An alternative way of getting O_2 dissolved in fluid to the spinal cord was attempted. The frog was perfused through the truncus with Ringer, saturated with oxygen. However, this method was found to be inadequate, because the whole body became edematous - and if gum accasia was added, to bring it to the colloid osmotic pressure of whole blood - the solution became so viscous that its circulation could not be made fast enough to deliver the necessary amount of oxygen.

c) Intravertebral gaseous supply of oxygen.

One of the two best methods for supplying oxygen promised to be through bubbling the gas through the spinal cord directly. This was done by inserting the gas cannula (of very small gauge, No. 27) into the intravertebral cannula itself. This method proved to be a very reliable one, as shown by two facts:

1 - A preparation which collapsed through intravascular gaseous perfusion could be revived by intravertebral gaseous perfusion (see Fig. 25).

2 - A preparation which was running on intravertebral gaseous perfusion, when put on the intravascular gaseous method, showed the signs of anoxia (Fig. 24). In some frogs, this method of perfusion seemed to supply more oxygen than necessary and this, though there is no conclusive evidence for it, seemed to depress the reflexes. A 50% oxygen and 50% nitrogen mixture was best to be administered in these cases.

However, since this method of perfusion was not thoroughly reinvestigated till towards the end of this project, most of the results outlined in this paper, and elsewhere (Pedley, 1953), have been obtained with the intravascular gaseous perfusion method.

d) Intravascular gaseous perfusion of oxygen.

This most novel method of oxygenation was tried accidentally and appeared to be very promising. The gas, after its entrance into the aorta, must have reached the spinal cord somehow, since it appeared to maintain the reflexes for ten to twelve hours, in some cases. The question arose as to how the oxygen reached the spinal cell. Two possibilities suggested themselves:

i) That oxygen was actually delivered by the spinal cord capillaries. It is to be noted that this can only happen if there are collaterals from the aorta, supplying the spinal cord, since the vertebral artery was severed when the spinal cord was transected in the brachial region, to effect complete isolation from upper centres.

ii) The second possibility is that the extra quota of oxygen reaches the spinal cord from broken vessels larger than capillaries. That oxygen can diffuse through the large amount of nervous tissue in sufficient amounts to maintain the cord, is shown by the fact that intravertebral gaseous perfusion maintains the spinal cord, in fact, more reliably so than the intravascular gaseous perfusion.

No conclusive experiments could be carried out in the available time, to be able to decide between these two alternatives. When the mesentery, or a thin abdominal muscle, were observed under the microscope, as the truncus was being perfused with gaseous oxygen, the gas was seen to penetrate the arterioles and one or two capillaries in a field under low magnification (which is 5 - 10% of all the capillaries seen in the area) and also filled the veins. However, whether the oxygen got to the veins through true capillaries, or A.V. connections, could not be determined from the small number of preliminary experiments of this kind.

One little experiment is perhaps suggestive that some capillary circulation of the perfused gas is possible. A microscopic field of low magnification of the mesentery, e.g., is chosen so that artery, arterioles, some capillaries, and veins are conveniently seen full of oxygen after gaseous perfusion of the truncus. If now a 1% solution of Evan's blue in Ringer is perfused through the truncus, all vessels in view, even those previously not visibly filled with oxygen, are coloured blue, and the capillaries previously expanded

with oxygen do not contain it any more. Now, since the pressure head was not lowered when the Evan's blue was administered, the oxygen must have left the capillaries in the venous direction.

If further investigation proved that gaseous oxygen, introduced into a frog's artery, can circulate through the capillaries, it may then be worthwhile - as a method - to experiment on the possibility of maintaining a (mammalian) tissue by this method of oxygen supply.

2. Administration of Drugs.

a) Administration of gaseous drugs.

In order to test the effects of a gaseous drug on spinal reflexes, the gas could be conveniently administered, either intravertebrally - by bubbling through the spinal cord, or intravascularly.

If the gas is rapidly acting, the oxygen supply can be suspended for a short period of time and the gas perfusion substituted.

If the drug takes a longer time to act, it can be either administered together with the oxygen, under partial pressure, or it can be supplied by a different way, i.e., if the preparation gets its oxygen supply intravascularly, the gaseous drug can be administered through the intravertebral route, and vice versa. In this author's experience with N_2O, the intravertebral route of gaseous drug administration is

more effective, in that the action is more rapid and lower concentrations suffice (see Fig. 32). However, if the differential action of the drug on the inhibitory and excitatory mechanisms is investigated, the intravascular administration is advisable, since, with the action being much slower, one has a chance to see what happens to the reflexes while they collapse.

b) Administration of drugs dissolved in Ringer.

The most convenient method of administration of drugs is through the intravertebral perfusion line. The apparatus which permits the switching over from normal Ringer to drug perfusion, without changing the drop rate, has already been described. The chief disadvantages of intravertebral administration of drugs are:

i) That the spinal dura may act as a barrier to some (non-lipid soluble) drugs;

ii) The drug does not have a chance to come into as intimate a contact with the spinal cell through diffusion from a distance, as it would if the drug were administered through the vascular system, reaching the nerve cell through capillaries.

Attempts were made to perfuse a drug which had a long latency of action (e.g., Myanesin), intravertebrally, through the vascular route. The results were disappointing. This method appeared to be more inefficient than the intravertebral one, probably because the number of vessels still in communication with the spinal cord (after the vertebral arteries had been severed when transecting the spinal cord

in the brachial region) could not bring the drug to high enough concentration within the spinal cord to be active, except if drastically high concentrations were perfused intravascularly.

Thus, in all the experiments the drugs were administered intravertebrally.

D. DRUGS AND MATERIALS.

1. The Intravertebral Perfusion Fluid.

a) Purposes of intravertebral perfusion fluid.

The intravertebral perfusion fluid served the following purposes in most of the experiments:

i) It served as an artificial milieu interieur for spinal activities. Since we could work at room temperature, the perfusion fluid had only to be balanced with respect to osmotic pressure, relative concentration of different ions, and pH.

ii) It served to supply the necessary glucose for neuronal activity. If glucose was omitted from the medium, the preparation usually declined irreversibly, as indicated in Fig. 28, showing the effects of glucose lack.

iii) It served as a means for delivering at least some of the oxygen required for spinal cord activity, as shown by the sudden collapse of reflexes in a frog without any special oxygen supply, when the intravertebral perfusion was cut off.

Also it served as a medium for the delivery of the full quota of oxygen in those experiments in which oxygen was either bubbled through the perfusion fluid, or through the spinal cord directly.

iv) It served as a medium for the administration of drugs whose effects on spinal reflexes were to be tested.

v) And, finally, as a medium into which the possibly chemical products of spinal cord activity can diffuse to be collected and assayed. (In any event, the intravertebral perfusion was essential once the circulation was destroyed, since - when perfusion was cut off, as shown in Fig. 29 - the reflexes collapsed.)

b) Composition of Frog-Ringer.

The composition of the Frog-Ringer solution, used for spinal cord perfusion and for spraying, was:

NaCl	- 0.650 g %
KCl	- 0.014 g %
$CaCl_2$	- 0.012 g %
NaH_2PO_4	- 0.012 g %
Na_2HPO_4	- 0.059 g %
pH	- 7.4 ± 0.1
Glucose	- 0.20 g %

For intravertebral perfusion, chemicals were dissolved in glass-distilled water. For the spraying fluid, normally (metal) distilled water was used.

To raise the colloid osmotic pressure of the perfusion fluid, one gram % Gum Accasia was added (according to Kato's - 1950 - formula) to the intravertebral perfusion fluid in some experiments, with no perceptibly beneficial effects on reflexes.

c) Composition of stock solutions.

Stock solutions were prepared once a week, as follows:

Solution A (50 ml/l final solution)

NaCl - 130.0 g/l

KCl - 2.8 g/l

$CaCl_2$ - 2.4 g/l

Solution B (30 ml/l final solution)

0.15 M Mono- and dibasic Sodium Phosphate solutions were mixed in 1:4 ratio. Thus, 1000 cc. of Solution B consisted of:

200 cc. of NaH_2PO_4 - 20.7 g/l

800 cc. of Na_2HPO_4 - 21.3 g/l

Glucose (2 g/l) was added fresh to the final solution every morning.

2. Gases Used.

Oxygen, nitrogen and nitrous oxide were administered intravascularly or intravertebrally, through a pressure reducing valve as described above (p. 43).

The oxygen and nitrogen mixtures were obtained from

Dinsmore Company (Dominion Trade Mark) and the nitrous oxide from Ohio Chemical Company of Canada.

The following gases were used:

Pure O_2	97% O_2 - 2% CO_2
Pure N_2	97% N_2 - 3% CO_2
Pure Nitrous Oxide	95% O_2 - 5% CO_2
Compressed Air	95% O_2 - 5% CO_2
93% O_2 + 7% CO_2	50% O_2 - 47% N_2 - 3% CO_2.

3. Drugs Used.

All the drugs were first diluted with glass-distilled water to concentrations of 10^{-2} or 10^{-3} and were then diluted before use with Frog-Ringer to the desired concentrations.

The following drugs were used:

Drug	Company	Form
Ether	Merck	In 3.5 % alcohol
Histamine dihydrochloride	Burroughs Wellcome Co.	Powder
Myanesin	B.D.H. (courtesy of)	In 10^{-1} solution
Nicotine tartrate	Merck	Powder
Strychnine sulphate	Merck	Crystals

VI. R E S U L T S

Preview

A. STRENGTH RESPONSE CURVES.

Before one could introduce an experimental variable, the preparation had to be stabilized under the new conditions of stimulation and oxygen and Ringer perfusion of the spinal cord, so that a relatively even "control" record was obtained. Examples of an "ideal" and a "satisfactory" control records (Figs. 7 and 8) will first be presented to acquaint the reader with the kind of record obtained with the preparation.

Since this is a new method, it was first necessary to establish the "norms" of the preparation, i.e., to obtain an idea of the rate of change of the amplitude and duration of the E-response and per cent inhibition with change in the E or I-stimulus strength. Information of this nature was indispensable for any interpretation of a drug's effects on reflexes. Thus, records will be presented to show:

1) An illustrative example of ten experiments in which the height of excitatory responses was plotted against change in the E-stimulus (Figs. 9 and 10).

2) An example of five experiments in which the reflex conditions of the biceps were recorded on a fast moving kymograph to

obtain the relationship between the duration of the E-responses and E-stimulus strength (Figs. 11 and 12).

3) An illustration of five experiments in which the relative rates of change of the amplitude of the E-response and of its inhibition were plotted against decreasing E-stimulus strengths, while the inhibitory stimulus strength remained constant (Figs. 13 and 14).

4) An example of four experiments in which the E and EI responses were recorded on the fast moving kymograph, to see whether the amplitude and duration of the EI-responses vary with equal rates with change in E-strength (Figs. 15 and 16).

5) An illustration of experiments in which the E-stimulus was constant and the I-stimulus strength was varied (Figs. 17 and 18).

B. MALTREATMENT OF THE PREPARATION.

Under this heading will be presented results of those experiments which were carried out in an attempt to ascertain optimal methods of spinal cord oxygenation, frequency of stimulation and intravertebral Ringer perfusion.

1) Anoxia.

a) Records are presented to show that the response of the spinal cord to anoxia in our preparation is very similar to the effects of asphyxia on reflexes in a spinal frog with intact circulation (Figs. 19 and 20).

b) The effects of anoxia with different methods of oxygenation of the spinal cord will be shown, e.g., via Nitrogen dissolved in the intravertebral perfusion fluid, administr-

ation of gaseous Nitrogen, intravascularly or intravertebrally. (Figs. 21, 22 and 23).

c) The relative effectiveness of intravascular and intravertebral gaseous perfusion methods will be compared (Figs. 24 and 25).

2) Interval between Stimulations.

A record will be shown to illustrate six experiments in which the interval between stimulations was increased or decreased in stages (Figs. 26 and 27).

3) Collapse of Reflexes brought about by Modification of the Intravertebral Perfusion Fluid.

a) Effects of glucose lack (Fig. 28).

b) Effects of stopping intravertebral perfusion (Fig. 29).

c) Effects of reperfusion of effluents (Fig. 30).

C. THE EFFECTS OF SOME DRUGS AS ILLUSTRATIONS OF THE USE OF THE METHOD.

In order to illustrate how drugs can be tested on the flexor and inhibited flexor reflexes of this preparation, records of the effects of some of the drugs tried will be presented here, as follows:

1) Depressants: N_2O (Figs. 31, 32, and 33).
Ether (Fig. 34).
Myanesin (Fig. 35).

2) Excitants : Strychnine (Fig. 36).
Histamine (Fig. 37).
Nicotine (Fig. 38).

Full interpretation and discussion of the significance of these records will not be attempted in the present paper, since they constitute the subject matter of a separate Master's thesis (Pedley, 1953).

- RESULTS -

P A R T A.

STRENGTH-RESPONSE CURVES

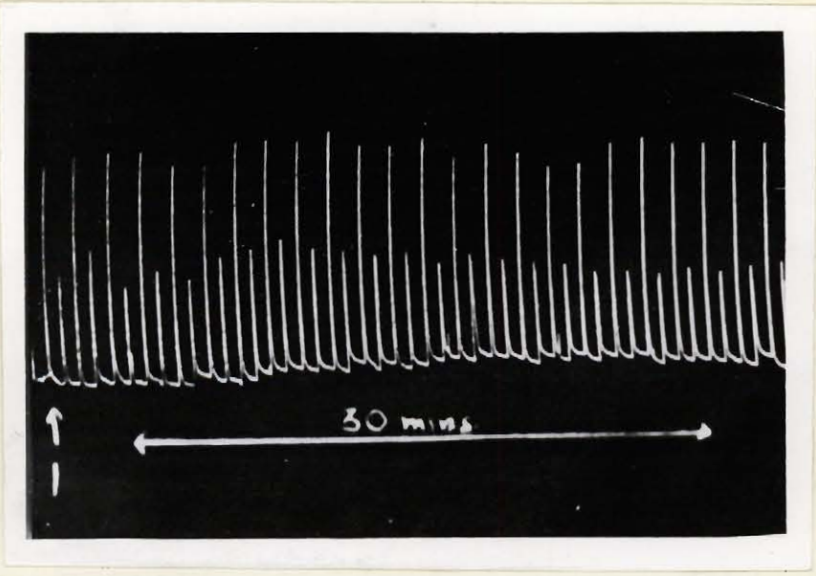

FIGURE 7. An ideal control record.

This is the kind of control record that was aimed at before the beginning of an experiment. The large (E)-strokes are the flexor reflex response of the biceps femoris to stimulation of the homolateral foot. Alternately interspersed are the (EI) flexor responses, inhibited by the contralateral cross-extensor reflex, brought about by simultaneous stimulation of both feet. Note that the excitatory (E) and inhibitory (EI) responses are surprisingly steady, considering the complexity of the reflex arcs involved.

In subsequent legends, experimental conditions will be specified in the following sequence and notation; (in brackets will be shown the usual range of values):
Method of perfusion: intravascular gaseous perfusion of 97% oxygen plus 3% CO_2 - unless otherwise specified.

PP - Perfusion pressure of the gas (80-150 mm Hg).
PR - Intravertebral Ringer perfusion rate (1 drop in 2 sec. to 1/15 sec.)
SR - Stimulation rate (1 stimulus in 25 sec. to 1/50 sec.).
E - Excitatory (homolateral) stimulus strength in Volts (0.5 - 2.5 V).
I - Inhibitory (contralateral) stimulus strength in Volts (0.5 - 2.5 V).

Legend to Fig. 7.

1 - Control record - frog on intravertebral perfusion of pure oxygen - PP - 80 mm Hg
PR - 1/5 sec.
SR - 1/40 sec.
E - 1 V
I - 1 V.

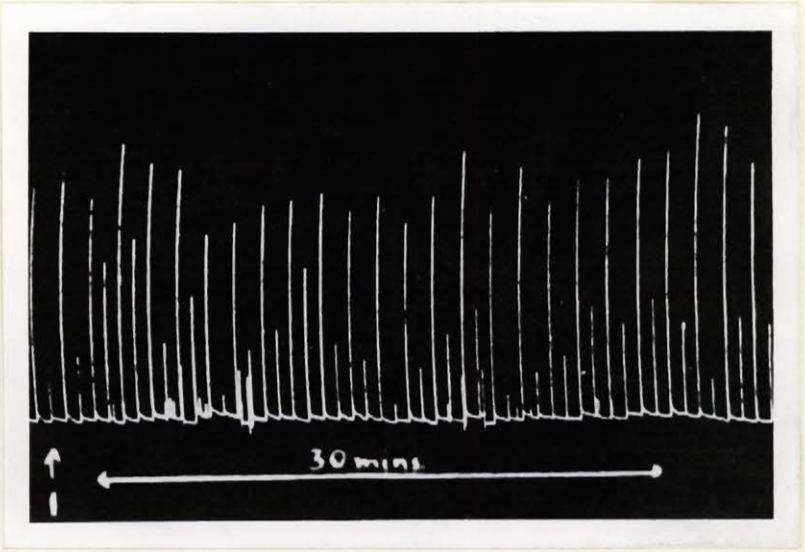

FIGURE 8. A satisfactory control record.

This figure shows a control record in which a completely even series of responses was sacrificed for the sake of operating on submaximal stimuli. Note that the E-response is much less stable than in Fig. 7, and that inhibition varies from 100 - 35%. This record was, however, still considered a satisfactory one since an experimental condition could produce a 100% change in excitation and a qualitatively significant change in inhibition.

1 - A control record on a frog without a special oxygen supply. Four hours before this record was taken, the heart was excised, and intravertebral Ringer perfusion started immediately.

PR - 1/6.5 sec.
SR - 1/40 sec.
E - 1.0 V
I - 1.1 V.

Only two out of five preparations did survive longer than three hours without special oxygen supply.

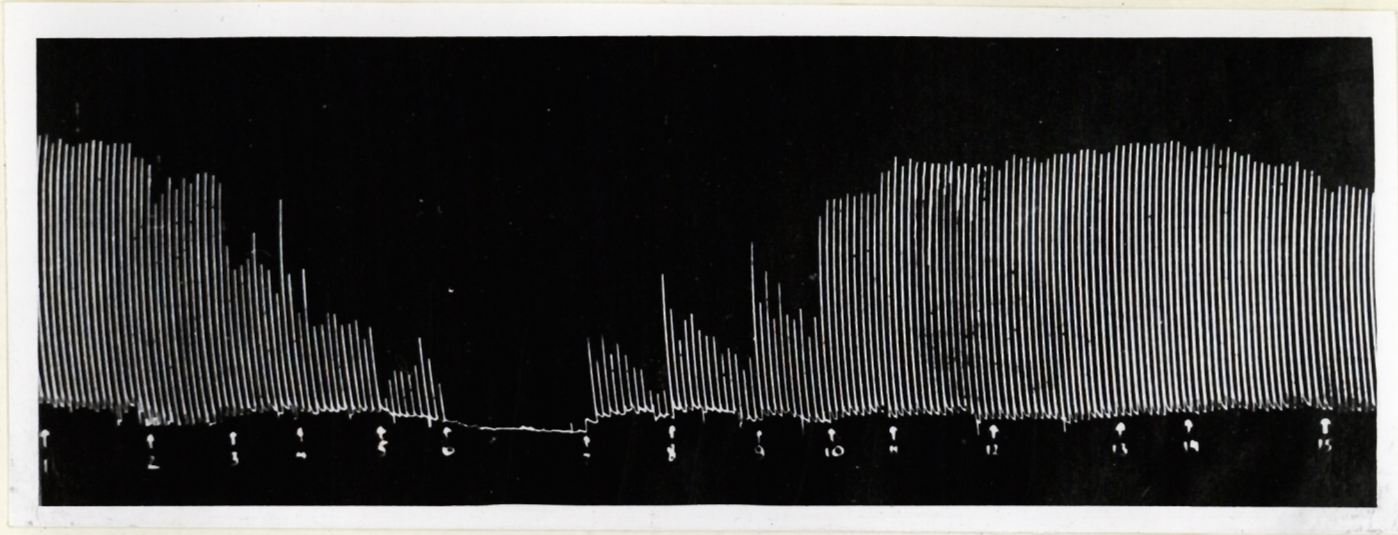

FIGURE 9. E-stimulus strength vs. E-response on the slow moving kymograph.

This figure shows the flexor responses of the biceps to progressively lower and to increasing strengths of excitatory (homolateral) stimuli. For a particular stimulus strength the E-response usually declines first until it reaches a stable level. Note that very strong stimuli result in diminishing responses due either to fatigue, or to activation of some homolateral inhibitory mechanism.

The frog was on intravascular perfusion (of 97% oxygen and 3% CO_2).
PP - 115 mm Hg.
PR - 1/2.5 sec.
SR - 1/30 sec.
I - off.

No.	Stim.strength Volts	Response mm	No.	Stim.strength Volts	Response mm	No.	Stim.strength Volts	Response mm
E1	1.50	43.3	E6	0.75	0.0	E11	1.50	41.0
E2	1.35	40.7	E7	0.90	8.3	E12	1.80	42.0
E3	1.20	26.2	E8	1.05	13.3	E13	3.00	45.0
E4	1.05	15.7	E9	1.20	20.2	E14	6.00	40.0
E5	0.90	7.1	E10	1.35	35.0	E15	10.00	36.5

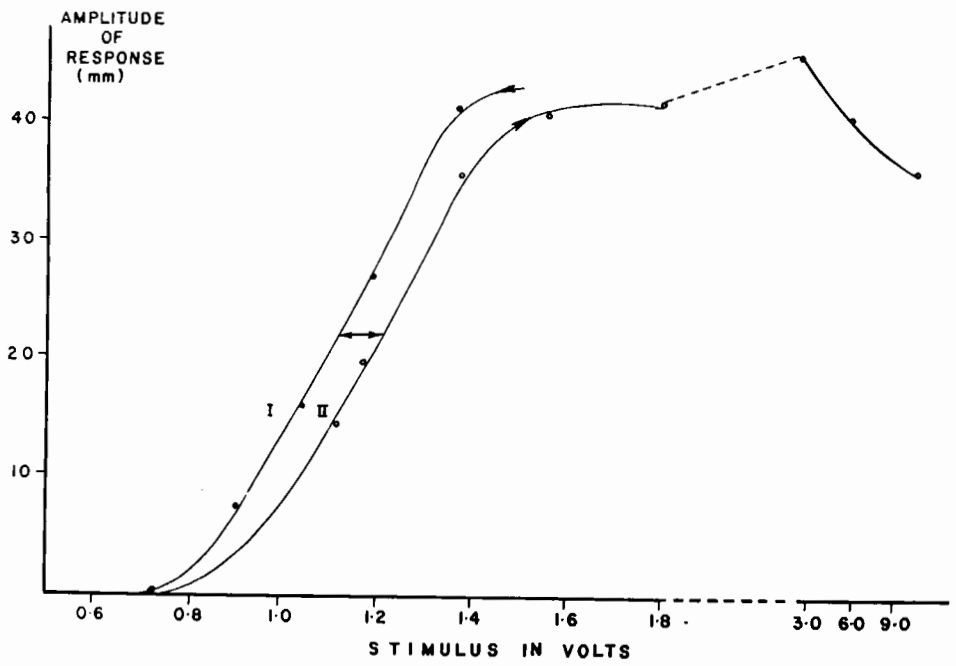

FIGURE 10. Strength response curves plotted from Fig. 9.

In this figure are plotted stimulus strengths of the excitatory (E) stimulus vs. amplitude of the E-response.

The horizontal line between the two curves indicates the voltage with which the stimulus strength had to be increased in order to obtain the particular response that could be elicited with a weaker stimulus 45 min. before. The preparation fatigued (at least at the beginning of the day) at the rate of 0.1 V/hr.

Note that the ratio E-stimulus strengths (in Volts) necessary to elicit 75% and 25% maximal E-responses in this preparation is 1.3. In ten experiments on six frogs, the mean value for this ratio was found to be 1.44, with a Standard Deviation of 0.24.

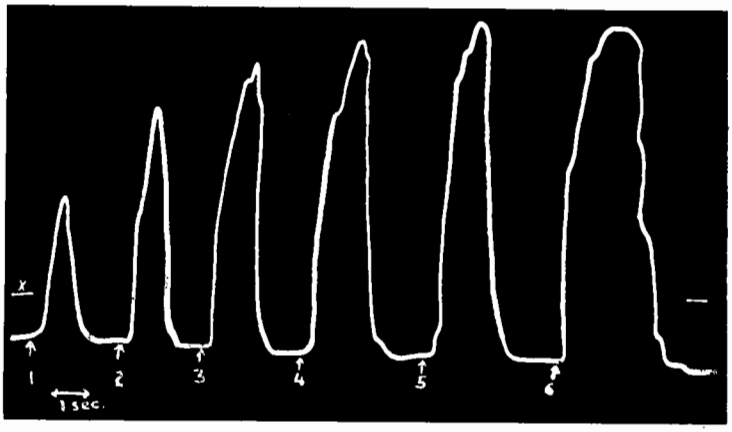

FIGURE 11. The effects of increasing E-stimuli on the height and duration of the flexor response, recorded on a fast moving kymograph.

This record shows the flexor response of the biceps to increasing strengths of E-stimuli, recorded on a kymograph revolving at a fast rate of 5 mm/sec. Note that the width (duration) of the flexor response increases with increasing strengths of stimuli, even after the height of the response reached a maximum. (Record retraced from the original).

Frog on intravascular perfusion of 97% O_2 + 3% CO_2.
PP - 100 mm Hg.
PR - 1/3.5 sec.
SR - 1/100 sec.
I - off.

No.	E-stim. strength Volts	Width of response at x mm
1	1.50	4.5
2	0.65	6.0
3	0.75	7.5
4	0.90	9.8
5	1.05	11.2
6	1.50	18.0

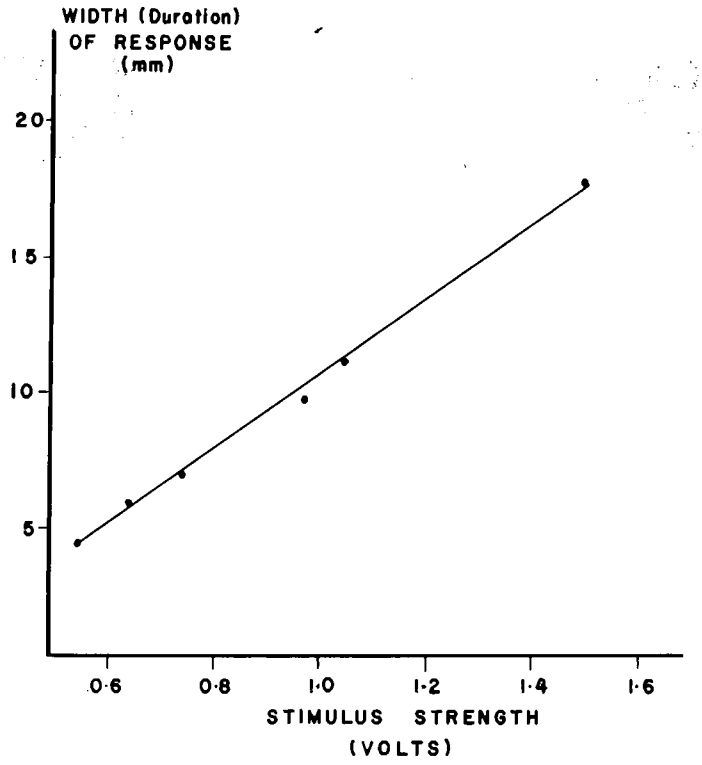

FIGURE 12. E-stimulus strength vs. width (duration) of response, plotted from Fig. 11.

Note that at least at these stimulus strengths, the duration of the tetanus (after discharge) is directly proportional to the stimulus strength (though the duration of the stimulus remains constant at one second).

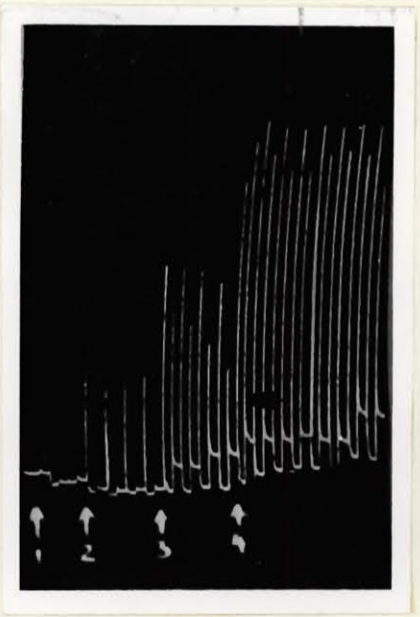

FIGURE 13. The effects of increasing E-stimulus strengths while the I-stimuli remain constant.

This figure shows an experiment in which the E-stimulus was increased in three stages, while the I-stimulus remained constant.

Note that the inhibition shown after 4 in the figure, is only apparent since the excitatory stroke starts from a tone level which was raised by the previous EI-stimulus with as much as the E-strokes are higher than the EI-strokes.

1 - Just sub-threshold record. Frog perfused intravascularly with 97% oxygen plus 3% CO_2.

PP - 110 mm Hg.
PR - 1/6 sec.
SR - 1/30 sec.
E - 0.30 V
I - 0.66 V

2 - E - 0.35 V

3 - E - 0.70 V

4 - E - 1.05 V.

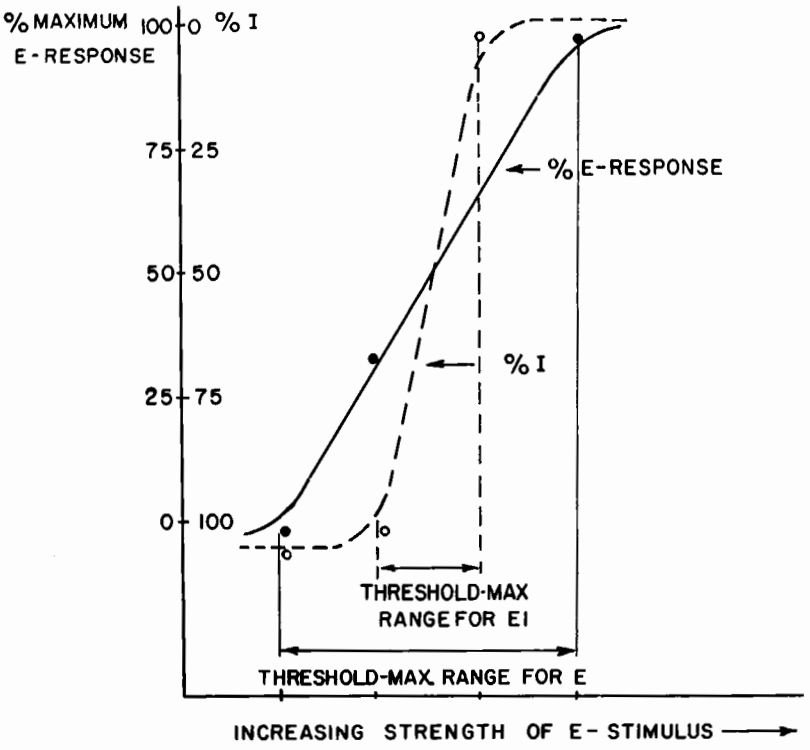

FIGURE 14. Increasing strength of E-stimulus (while I remains constant) vs. per cent maximum E-response and per cent I, drawn from Fig. 13.

This diagram illustrates the fact that the strength response curve for inhibition (when E-strength is increased and I-stimulus remains constant) is much steeper than that for the E-response. Note that the range between E-stimulus strength necessary to elicit the minimum and maximum E-responses is three times as large as the range between E-stimuli which allow 100% and 0% inhibition, respectively.

Thus, a relatively small change in the E-stimulus strength could make or break inhibition in some experiments. It is for this reason, that it was so difficult to obtain 50% inhibition at will, and to have conducted a sufficiently large number of successful experiments to report on a strength response curve on EI, analogous with Fig. 8.

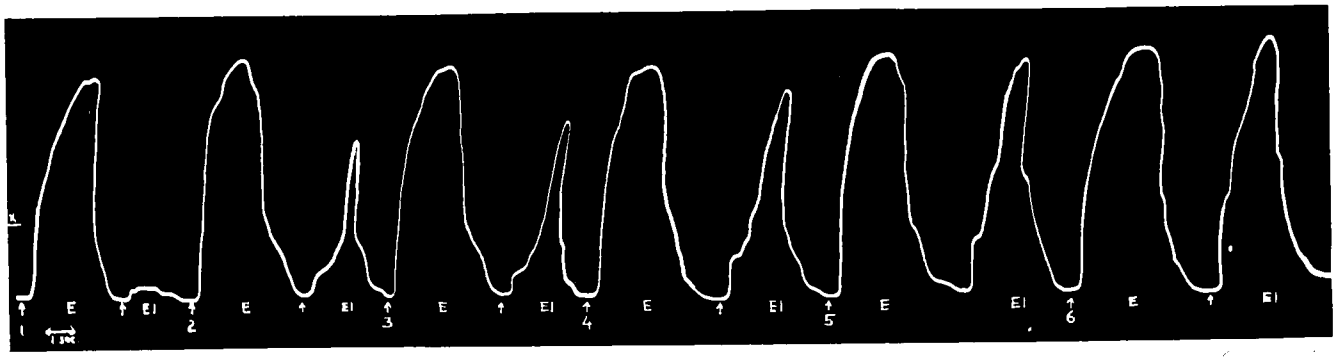

FIGURE 15. The effects of increasing E-stimuli (while the I-stimulus remains constant) on per cent inhibition of height and duration of the EI-response, recorded on the fast moving kymograph (Retraced from the original).

Note that the E-stimuli are maximal, and that the duration of the after-discharge remains inhibited long after the height of the response is not.

Frog on intravascular perfusion of 97% O_2 and 3% CO_2:

PP - 150 mm Hg.
PR - 1/4 sec.
SR - 1/110 sec.

No.	Stim. strength Volts E	I	Amplitude E mm	I mm	%	Width E mm	I mm	% I
1	2.50	2.6	64	4	94	16	-	100
2	2.70		68	45	33	18	2.5	86
3	3.00		66	50	24	19	5	74
4	3.15		66	59	10	19	9	48
5	3.30		68	68	0	19	14	26
6	3.95		70	72	-3	16	16	27

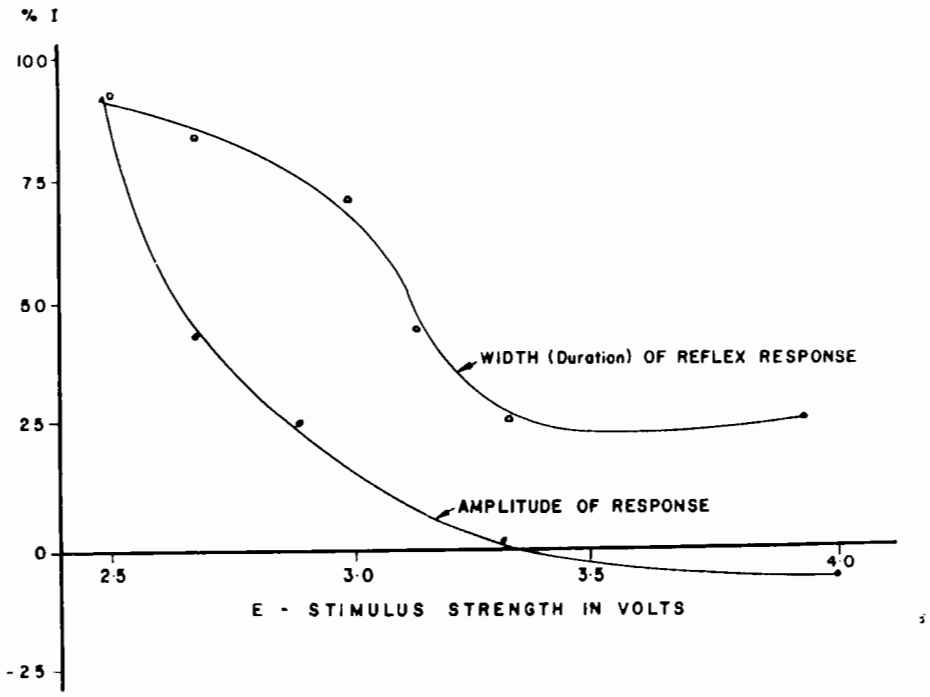

FIGURE 16. Per cent inhibition of amplitude and duration of the flexor response plotted against increasing strength of the E-stimulus (while the I-strength remains constant). (Plotted from Fig. 15).

This graph illustrates clearly that increasing the strength of the E-stimulus makes the (constant) I-stimulus ineffective in inhibiting the amplitude of the response much faster than its duration. In fact, the last EI-response (6) is already "reversed", i.e., larger than the E-response preceding it.

As a result, it is suggested that, in order to have a truer indication of spinal activity, it may be worth while to design a method whereby the total (integrated) "area" of the muscle contraction, rather than its amplitude, is recorded.

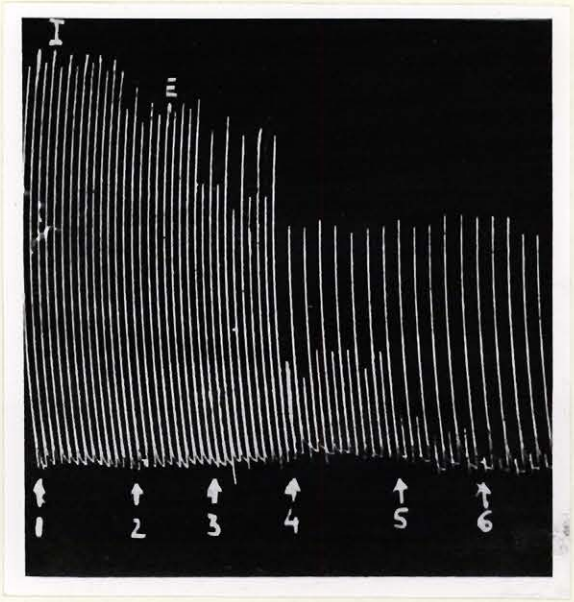

FIGURE 17. The effects of increasing I-strengths (while E remains constant) on per cent inhibition and amplitude of E-response.

Frog on 97% O_2 plus 3% CO_2 intravascularly.

PP - 150 mm Hg.
PR - 1/2 sec.
SR - 1/30 sec.

No.	Stim. strength Volts E	I	Response mm E	I	%I
1	3	.62	55	57	-4
2	3	.72	50	47	+6
3	3	.86	45	36	20
4	3	1.04	32	14	56
5	3	1.15	32	5	85
6	3	1.30	31	1	97

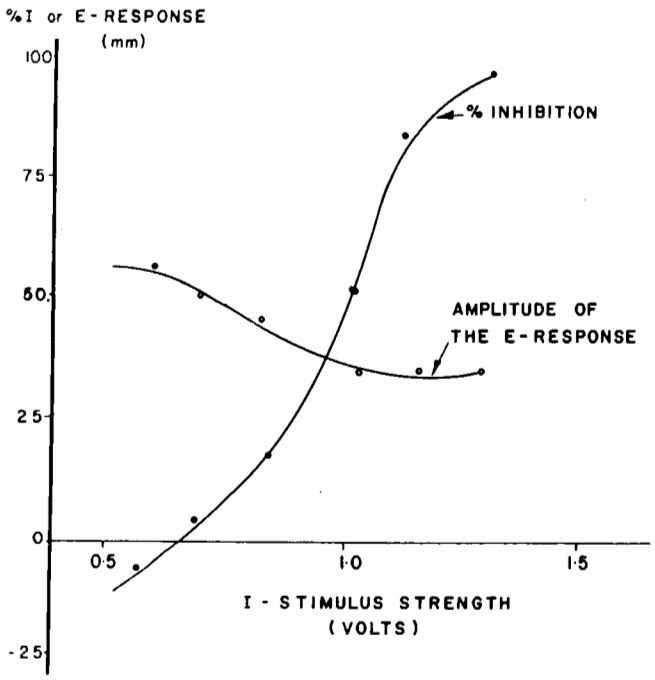

FIGURE 18. Graph, showing the effects of increase in the I-stimulus strength (while E remains constant) on per cent I, and amplitude of the E-response.

Note that the E-response to a constant stimulus decreases with increasing I-strengths.

- RESULTS -

P A R T B.

MALTREATMENT OF THE PREPARATION

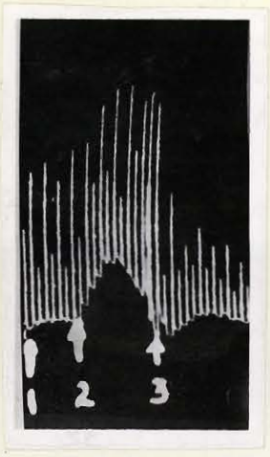

FIGURE 19.

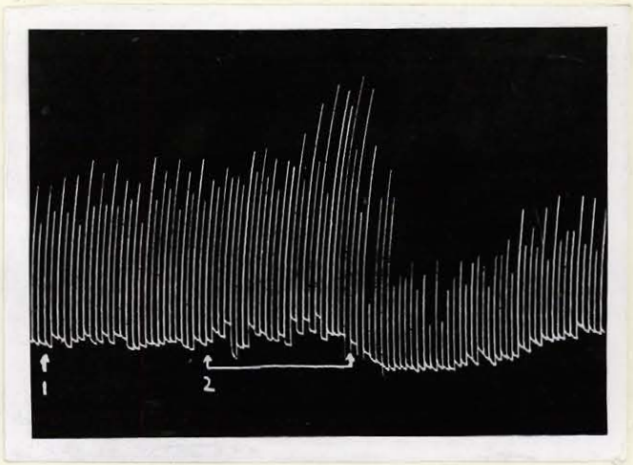

FIGURE 20.

Fig. 19 - The effects of asphyxia on the E and EI responses in a frog on its own circulation (with no intravertebral Ringer perfusion).
1 - Control record. SR - 1/30 sec.; E - 1-2 V; I - 1.5 V
2 - Truncus clumped.
3 - Truncus released.

Note a) the potentiation of E-responses during asphyxia;
b) Collapse of inhibition just before truncus released;
c) Collapse of reflexes to 50% of control, after normal circulation is reinstituted.

The rise in tone is partly genuine and probably partly due to anoxia rather than asphyxia, since CO_2 in concentration of 3-7% had no clearcut effects in other experiments.

- ... -

Fig. 20 - The effects of anoxia on the E and EI responses in a frog whose spinal cord depended on intravascular supply of gaseous oxygen for six hours before the experiment.
1 - Control record. Intravascular perfusion of O_2.
PP - 110 mm Hg
PR - 1/4.5 sec.
E - 1.4 V
I - 1.0 V
2 - Anoxia, produced through substitution of N_2 in the intravascular gaseous perfusion.

The surprising similarity between records of Figs.19 and 20 indicates that the substitution of the frog's normal blood supply with intravertebral perfusion of Ringer solution and intravascular perfusion of gaseous oxygen, has not substantially altered the physiological response of spinal reflexes to anoxia.

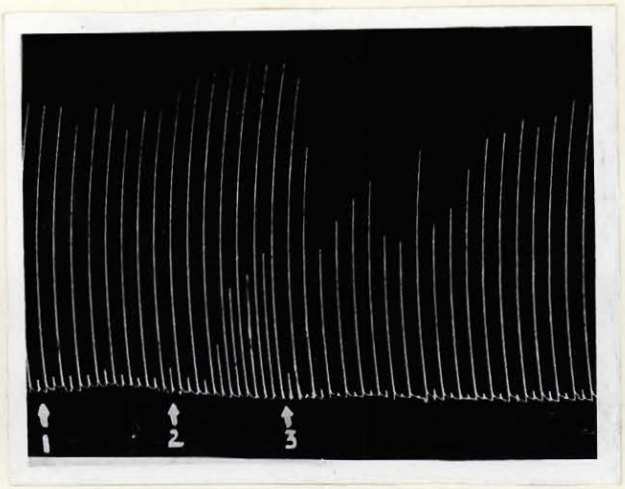

FIGURE 21. The effects of anoxia on reflexes in a frog without its own circulation. The spinal cord is kept alive with O_2 bubbled through the intravertebral perfusion fluid.

1 - Control record. Intravertebral perfusion fluid saturated with O_2.

PR - 1 drop/7sec.
SR - 1/50 sec.
E - 1 V
I - 1.3 V - Note that inhibition is complete.

2 - Anoxia record. Intravertebral perfusion fluid saturated with N_2.

3 - Post-anoxic record. Intravertebral perfusion fluid resaturated with O_2.

Note again, the remarkable similarity to Fig. 19 in that there is a) Potentiation of E-responses during anoxia together with
b) Progressive collapse of 100% inhibition to 50%, and
c) There is collapse of the E-responses after the readmission of O_2.

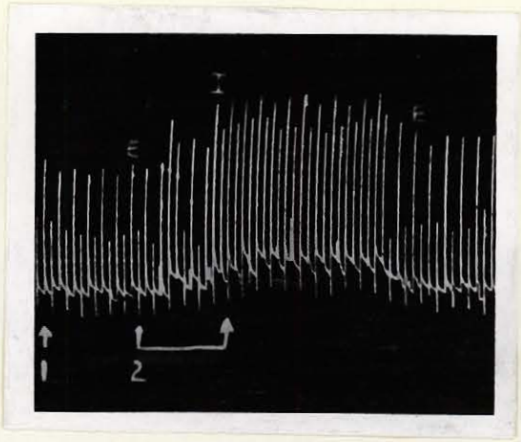

FIGURE 22. Effects of anoxia on reflexes. (Presented here, by courtesy of Dr. K. Terroux).

1 - Control record. Intravascular gaseous perfusion of 97% O_2 plus 3% CO_2.

PP - 110 mm Hg.
PR - 1/4 sec.
SR - 1/30 sec.
E - 0.4 V - submaximal
I - o.6 V.

2 - Anoxia. Intravascular perfusion of 97% N_2 plus 3% CO_2.

3 - Oxygen readmitted. Intravascular perfusion of 97% O_2 plus 3% CO_2.
Perfusion pressures were maintained at 110 mm Hg throughout.

This figure was presented here as an illustration of that fraction of experiments on anoxia, in which - though the control record was submaximal - the collapse of E-responses after the readmission of oxygen was not obtained.

Note that the effect of anoxia was collapse, in fact, complete reversal of the 50% inhibition in the control record. Thus, due to anoxia, the EI-responses became larger than the E-responses. There is some potentiation of the E-responses during the collapse of I.

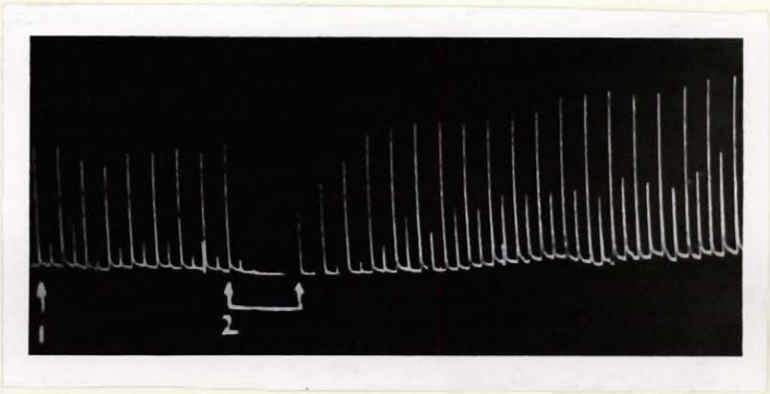

FIGURE 23. The effects of anoxia on reflexes when the spinal cord is kept alive by intravertebral gaseous oxygen perfusion.

1 - Control record. - Intravertebral gaseous perfusion of 97% O_2 plus 3% CO_2.

PP - 80 mm Hg.
PR - 1/5 sec.
SR - 1/50 sec.
E - 0.5 V
I - 0.6 V.

2 - Intravertebral perfusion of 97% nitrogen plus 3% CO_2 for the indicated period.

Note that removal of the oxygen supply results in instantaneous collapse of reflexes, i.e., the spinal cord is rendered completely dependent on the intravertebral gaseous perfusion method, for its oxygen supply.

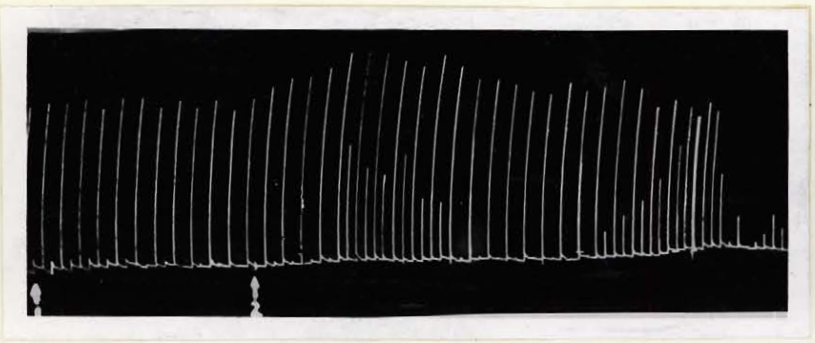

FIGURE 24.

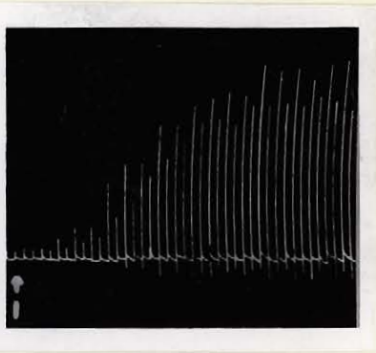

FIGURE 25.

Fig. 24. - Showing the relative effectiveness of intravascular and intravertebral methods of perfusion of gaseous oxygen in maintaining spinal reflexes.

1 - Control record. - Intravertebral perfusion of a mixture of 50% O_2, 47% N and 3% CO_2. (A mixture of gases with a low per cent oxygen was chosen intentionally for this experiment).

PP - 80 mm Hg.
PR - 1/5 sec.
SR - 1/60 sec.
E - 0.5 V
I - 0.5 V.

2 - Switched to intravascular perfusion of the same mixture of gases at 130 mm Hg. The drop rate of intravertebral Ringer perfusion was adjusted so that it remained the same as in the control period.

Note that the reflexes show the initial effects of anoxia (i.e., potentiation of E-responses and collapse of I) right after changing to the intravascular gaseous perfusion method, and collapsed completely within 45 minutes on the stimulus strength used. Increasing the stimulus strength brought back the reflexes temporarily.

- ... -

Fig. 25. - Shows the beneficial effects of intravertebral perfusion (at 1) after the reflexes collapsed in a frog, on the intravascular perfusion method.

The gradual recovery of reflexes, due to better oxygenation, is well demonstrated.

- ... -

Figs. 24 and 25 indicate that the intravertebral gaseous oxygen perfusion method may be a more reliable way of supplying oxygen to the spinal cord than the intravascular perfusion of O_2.

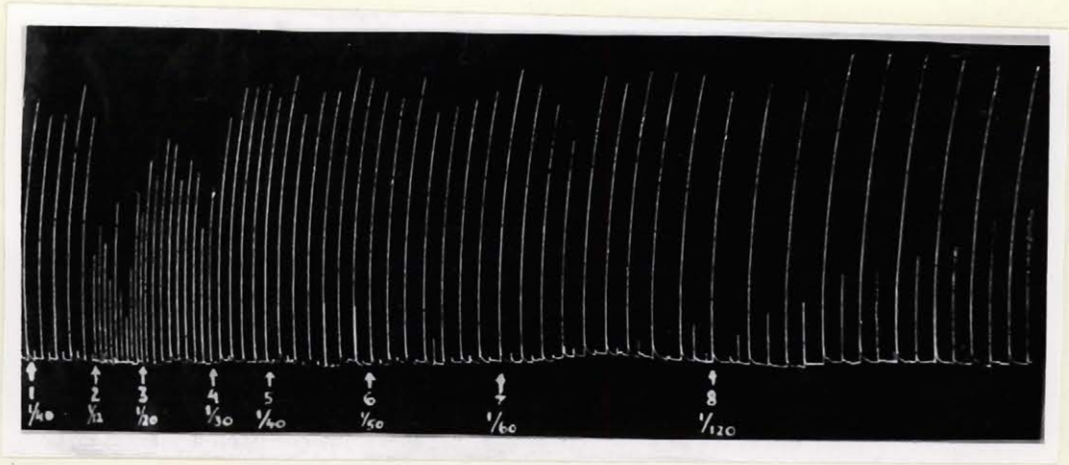

FIGURE 26. Shows the effects of increasing the interval between stimulation.

1 - Control record. - Frog on intravascular perfusion of 97% O_2 and 3% CO_2.

PP - 130 mm Hg.
PR - 1/3 sec.
SR - 1/40 sec.
E - 1.15 V
I - 1.2 V.

2 - The interval between stimulations was decreased from one stimulus in 40 seconds, to one in twelve seconds.

Note the immediately diminished E-responses. Inhibition is unable to break through the contralateral I-stimulus which is given only once in 24 seconds. Therefore, EI-responses are not seen at all.

3, 4, etc. - The interval between stimulations was increased, as indicated in the figure.

Note that: a) the responses become steadier;
b) the amplitude of the E-response becomes larger;
c) inhibition becomes less effective.

Inhibition is complete between 1 and 7, and then, due to greater effectiveness of the E-stimulus, the EI strokes gradually appear.

The site of fatigue at high frequency of stimulation has not been investigated in these experiments.

It is to be pointed out that the preparation responds, with almost maximal response, to stimulation at 1 in 30, or 1 in 40 seconds. These were the usual frequencies used in the experiments. However, responses at these frequencies of stimulation are not as even as they can be when the interval between stimulations is greater.

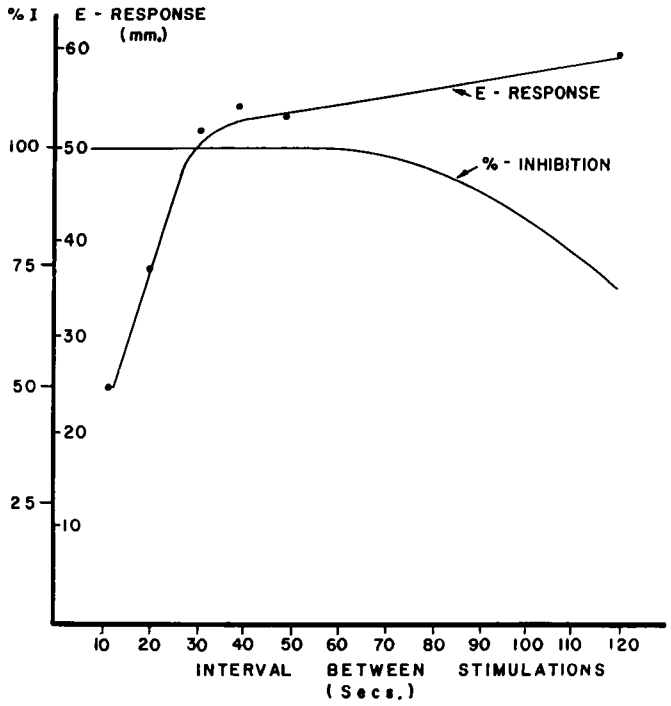

FIGURE 27. Amplitude of the E-response and per cent inhibition vs. interval between stimulations. (Drawn from Fig. 26).

Note that when the E-response is not very effective, because of the small interval between stimulations, inhibition is complete. When there is time for the reflex arc to rest between stimulations, the E-response is larger and the E-component of the EI-stimulus breaks through the inhibition due to the I-component.

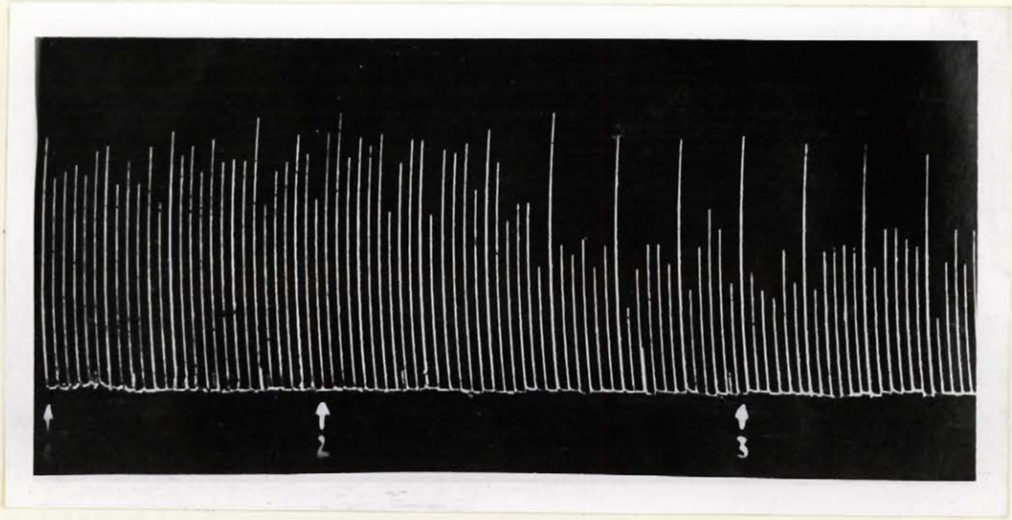

FIGURE 28. The effects of glucose lack.

This figure shows the effects of omitting glucose from the intravertebral perfusion fluid.

1 - Control record. - Intravascular gaseous perfusion.

PP - 100 mm Hg.
PR - 1/8.5 sec. - perfusion fluid contains the usual amount of 0.2% glucose
SR - 1/40 sec.
E1 - 2.8 V - electrode on toe (small responses)
E2 - 1.4 V - electrode more toward the ankle (larger test shocks).

2 - Glucose lack. - Produced by switching from normal Ringer perfusion to a solution of the same composition, except without glucose in it. The perfusion rate remains constant.

3 - Back to normal intravertebral Ringer perfusion.

Note that the effect of glucose lack is to lower the responses to the same stimulus with 50%. No recovery after such partial collapse was obtained in four experiments.

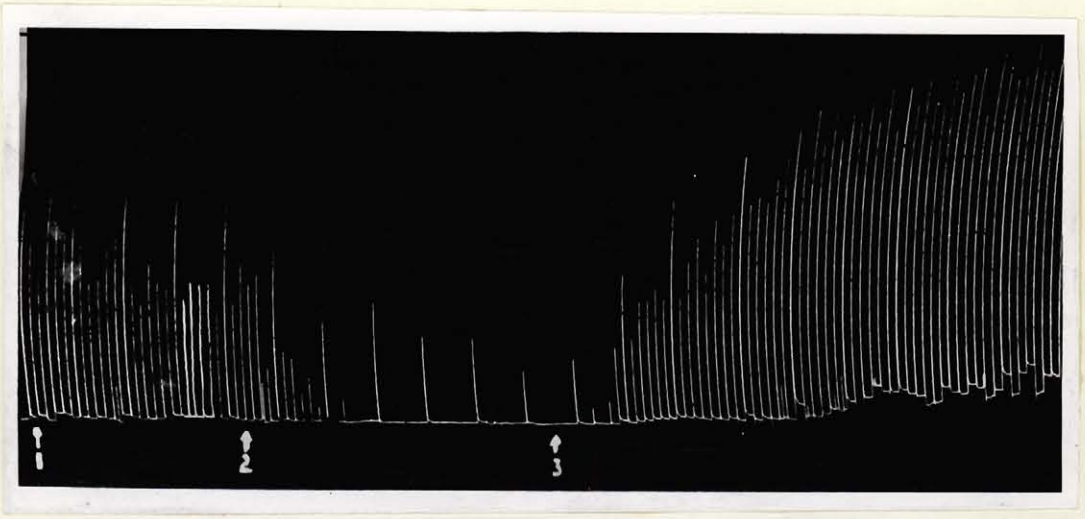

FIGURE 29. The effects of cutting off the intravertebral Ringer perfusion.

1 - Control record. - Intravascular gaseous perfusion.

PP - 105 mm Hg.
PR - 1/8.5 sec.
SR - 1/40 sec.
E1 - 2.8 V - on toe (small strokes)
E2 - 1.4 V - further towards ankle (larger test strokes at 240 sec. intervals).

2 - Cut off intravertebral perfusion of Ringer.

3 - Intravertebral perfusion started again. The perfusion rate was the same as during the control period.

Note that a) cutting off the intravertebral perfusion results in rapid collapse of the flexor reflex;
b) the collapse is proportional to the rate of stimulation since the response elicited six times less often takes approximately six times longer to collapse;
c) radmission of intravertebral perfusion of Ringer brings about rapid recovery with potentiation of the reflexes over the control level. This potentiation persisted for 30 minutes, which makes its interpretation very difficult.

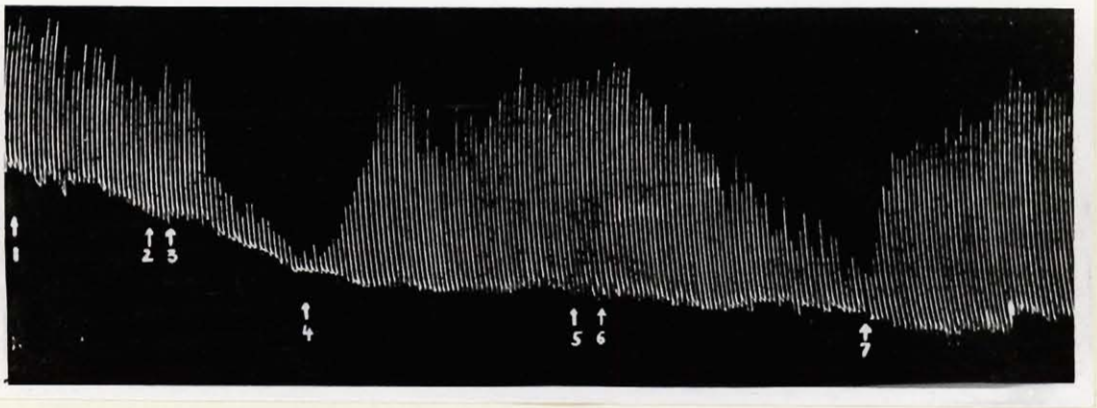

FIGURE 30. The effects of reperfusion of effluents.

In these two experiments, 4 cc. of perfusate which had traversed the spinal cord once, was reperfused for 15 min. the first time, and for 30 min. the second time (see method on p.47). Since it took 130 sec. to collect 4 cc. of effluent, and since after all reperfusion was ended, we were still left with 3½ cc., the fluid had a chance to recirculate seven times in the first experiment, and 15 times in the second.

1 - Control record. - Falling tone is the usual record obtained at the beginning of a day's experiment. Intravascular gaseous perfusion method: PP - 80 mm Hg.; PR - 1/3.2 sec.; SR - 1/30 sec.; E - 0.5 V; I - off.

2-3 - Collection of effluent No.1 (4cc. in 130 sec.)
3 - Start reperfusion of effluent No.1 for 15 min. PR-1/3.5 sec.
4 - Stop reperfusion and normal Ringer perfusion reinstituted.
5-6 - Collection of effluent No.2 (4 cc.).
6 - Continuous reperfusion of sample No.2 for 30 min.
7 - Stop reperfusion and back to Ringer perfusion.

The drastic collapse of reflexes, both (a) when the Ringer effluent is continually reperfused (Fig.30);and (b) when intravertebral Ringer perfusion is suspended, and only approx. ¼-½ cc. of solution remains in the vertebral canal (Fig.29), is perhaps indicative of some chemical modification of the fluid in contact with the spinal cord for longer periods.

This chemical modification, causing collapse of the reflexes, may be related to reflex activity, since the greater the number of stimulations per unit time, the more rapid the collapse (Fig.29).

Furthermore, the collapse is probably not wholly due to glucose lack, since Figs. 29 & 30 are very different from Fig.28 in that in the latter (a) reflexes do not collapse completely, and (b) once the reflexes have collapsed 50%, they do not recover.

However, no conclusive results were obtained so far with perfusion of a sample of continually reperfused effluent when time was taken to get a reliable control record between the collection of reperfusate and its reperfusion.

- RESULTS -

P A R T C.

THE EFFECTS OF DRUGS

AS ILLUSTRATIONS OF THE USE OF THE METHOD

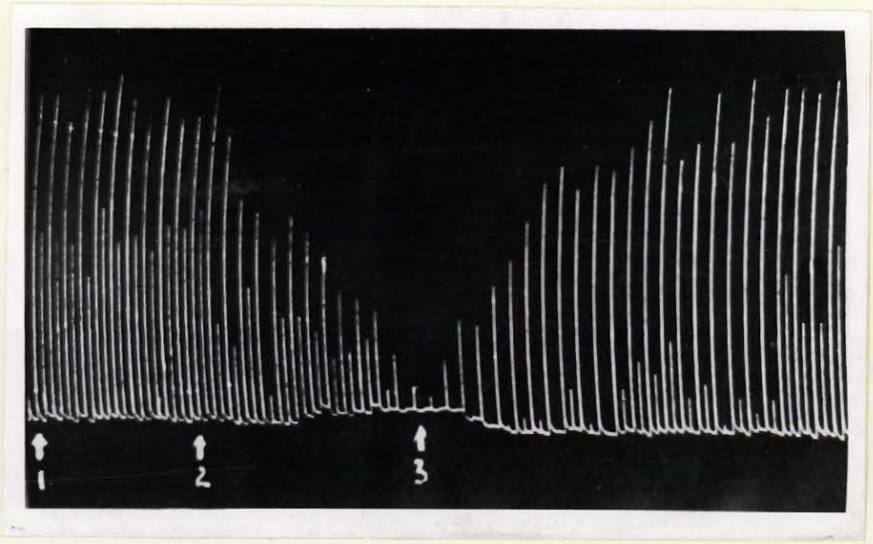

FIGURE 31. The effects of nitrous oxide (N_2O) on the E and EI-responses.

1 - Control record. - Intravascular gaseous perfusion of oxygen.

PP - 98 mm Hg.
PR - 1/8 sec.
SR - 1/30 sec.
E - 1.5 V - submaximal E-stimulus
I - 0.9 V.

2 - Intravertebral perfusion of N_2O at 100 mm Hg. was started, while the intravascular perfusion was continued as during 1.

3 - Intravertebral perfusion of N_2O discontinued.

Note that the effects of N_2O were:

a) collapse of the reflexes as expected from an anaesthetic;
b) potentiation of inhibition on recovery from the N_2O effect.

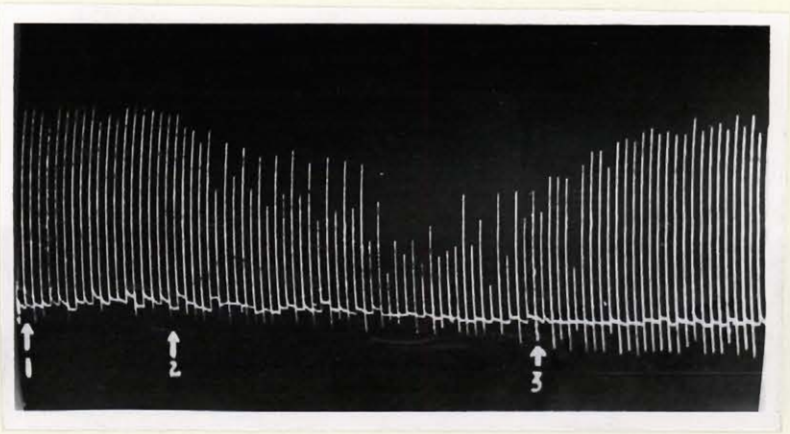

FIGURE 32. The effects of a smaller concentration of N_2O on spinal reflexes.

1 - Control record. - Intravascular gaseous perfusion.

PP - 120 mm Hg.
PR - 1/18 sec.
SR - 1/40 sec.
E - 2.5 V
I - 3.0 V - no effective inhibition is visible.

2 - Switch to intravascular perfusion of 70% N_2O plus 30% (97% O_2 plus 3% CO_2).

3 - Back to 97% oxygen plus 3% CO_2.

Note that the collapse of the E-response is only partial and persists as long as the N_2O is administered. The partial collapse is in contrast with the full collapse that is obtainable with pure N_2O (see Fig. 31).

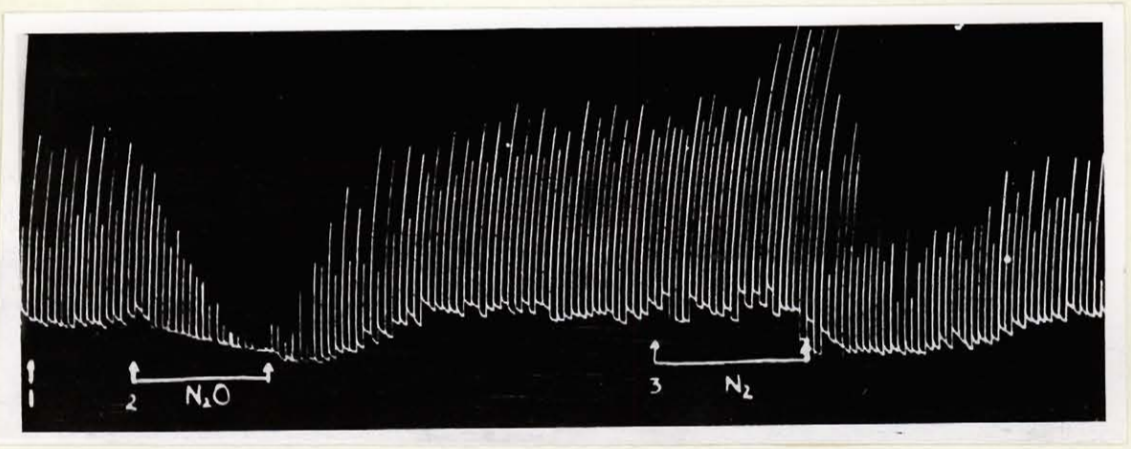

FIGURE 33. Comparison of the initial effects of nitrous oxide and anoxia on the reflexes.

1 - Control record. - Intravascular gaseous perfusion of oxygen.

PP - 110 mm Hg
PR - 1/4.5 sec.
SR - 1/40 sec.
E - 1.4 V
I - 1.0 V.

2 - Intravascular perfusion of oxygen stopped and N_2O perfused.

3 - Intravascular perfusion of N_2O stopped and O_2 perfused.

Note that N_2O collapses the reflexes immediately, while anoxia has an initial potentiating effect, and in this case, collapse of reflexes occurs only when oxygen is readmitted.

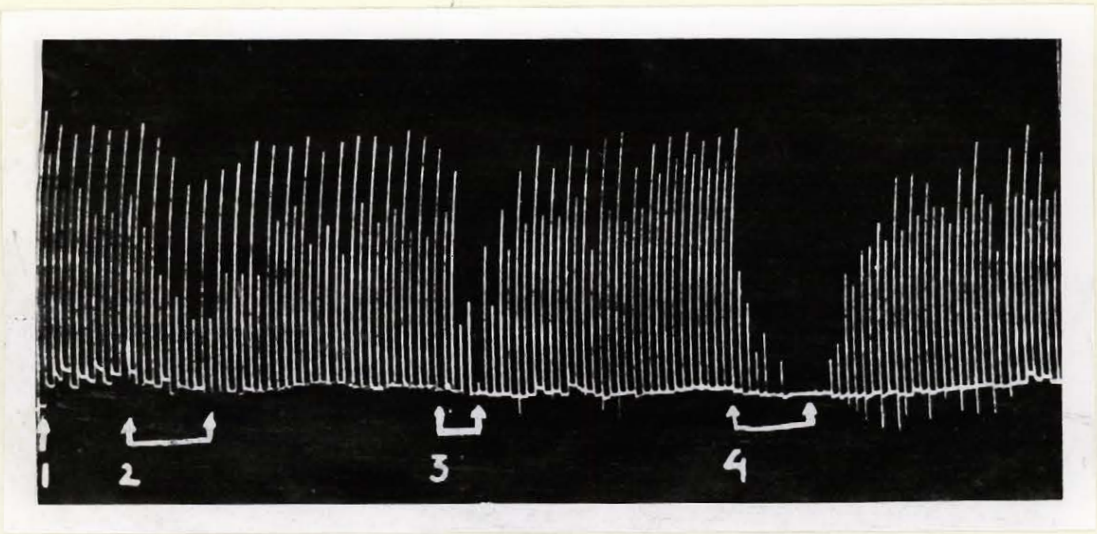

FIGURE 34. The effects of different concentrations of ether perfusion of the spinal cord, for different durations.

1 - Control record. - Intravascular gaseous perfusion.
PP - 110 mm Hg.
PR - 1/6 sec.
SR - 1/30 sec.
E - 0.3 V
I - 0.5 V.

2 - Perfusion of approx. 10^{-3} ether (10^{-2} of a saturated solution of ether) for two minutes.

3 - Perfusion of ether (2×10^{-3}) for two minutes.

4 - Perfusion of ether (2×10^{-3}) for five minutes.

Note that a) the preparation was able to differentiate between a solution of 1×10^{-2} and 2×10^{-2} (see contrasting record, following 2 and 4);

b) that the preparation could differentiate the duration of perfusion of the drug (see records following 3 and 4); and also

c) that ether had no differential effect on inhibition, though it may have affected the excitatory mechanism differentially. In agreement with Figs. 13 and 14, the increase in per cent I, following 2, can be accounted for by depression of excitation (analogous with lowering of the E-stimulus strength). In this respect, the effect of ether is different from that of N_2O since, as shown clearly in Fig. 31, N_2O potentiates inhibition after its collapsing effect is over.

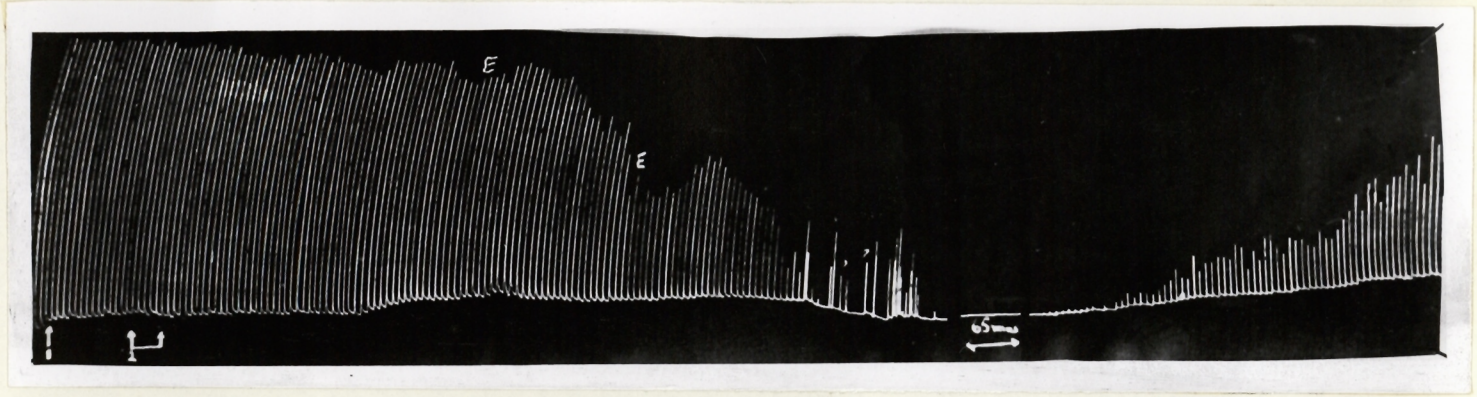

FIGURE 35. The effects of Myanesin.

1 - Control record. - Intravascular gaseous perfusion method.

PP - 108 mm Hg.
PR - 1/22 sec.
SR - 1/40 sec.
E - 1.2 V
I - 1.2 V.

2 - Perfuse 5 cc. of 10^{-5} Myanesin in Ringer.

Note the long latency of action (approx. 45 min.) which was confirmed in six experiments. It is perhaps attributable to the spinal dura acting as a barrier to diffusion of myanesin to the spinal cell.

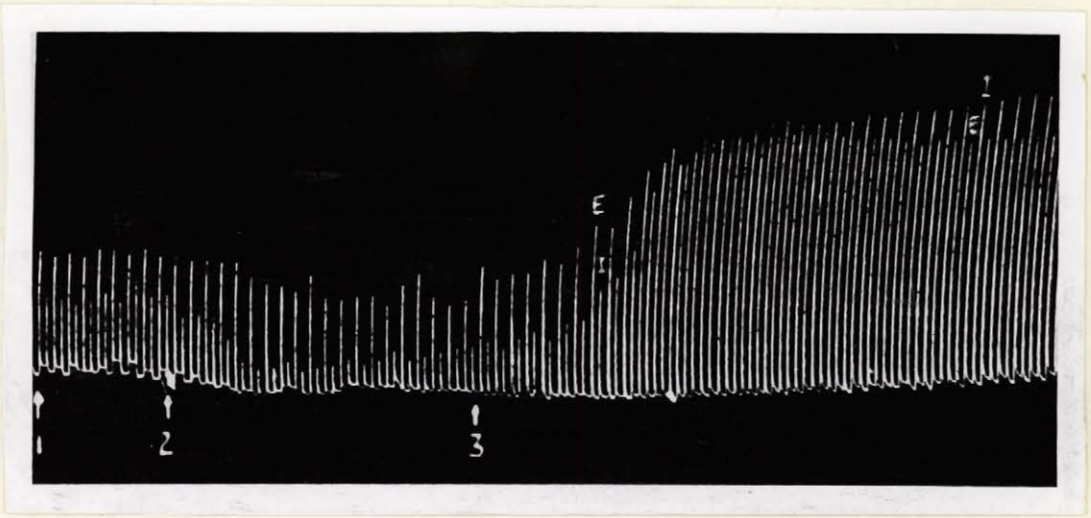

FIGURE 36. The effects of strychnine.

1 - Control record. - Intravascular gaseous perfusion method.

PP - 160 mm Hg.
PR - 1/40 sec.
SR - 1/30 sec.
E - 0.6 V
I - 0.6 V.

2-3 - Strychnine sulphate, perfused in a concentration of 5×10^{-5} for 24 min. through the intravertebral Ringer perfusion line.

Note that not only is the 50% inhibition, shown in the control record, completely abolished as a result of strychnine, but the EI-strokes sum and become larger than the E-strokes, when the strychnine has taken full effect.

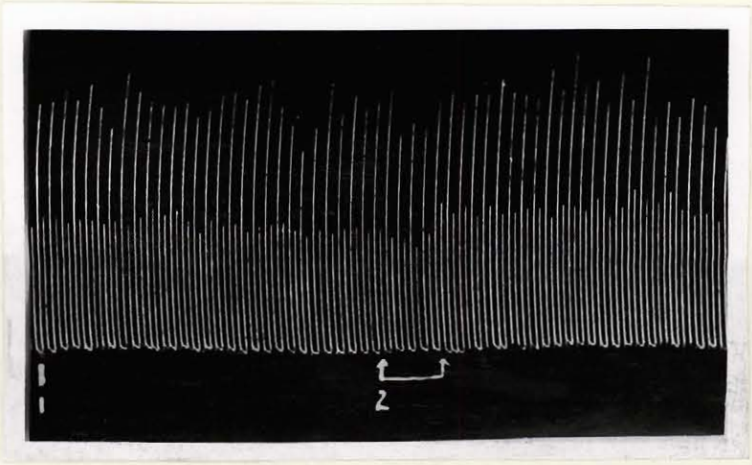

FIGURE 37. The effects of histamine perfusion.

1 - Control record. - Intravascular gaseous perfusion method.

PP - 115 mm Hg.
PR - 1/5 sec.
SR - 1/30 sec.
E - 1.2 V - maximal stimuli
I - 2.0 V.

2 - 0.15×10^{-5} histamine (in 5 cc. Ringer) perfusion.

Although the control record is satisfactory, and the decrease in inhibition at the point of injection could be interpreted as a result of an increase in the effectiveness of the excitatory stimulus (not shown in the record because the E-responses of the muscle are maximal), the change is certainly not significant enough to corroborate Häusler's (1952) finding that histamine potentiates flexor reflexes.

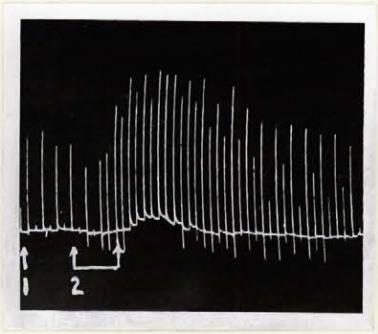

FIGURE 38. The effects of nicotine.

1 - Control record. Intravascular gaseous perfusion method.

PP - 135 mm Hg
PR - 1/20 sec.
SR - 1/40 sec.
E - 0.654 V
I - 0.55 V.

2 - Perfusion of 2 x 10^{-5} nicotine tartrate for six minutes.

Note increase in tone, potentiation of E, and (probably as a result) collapse of I.

VII. D I S C U S S I O N

The Control Record

The preparation had to be stabilized before any experimental variable could be introduced. An ideal control record was shown in Fig. 7, but a record comparable to Fig. 8 was usually considered satisfactory.

An ideal control record was one in which (like in Fig. 7):

a) the heights of the E and EI-responses are <u>even</u>, for at least 15 minutes, so that a change in the record can be attributed to the experimental variable without dispute;

b) the stimulus is <u>submaximal</u> so that slight changes in the conditions, or small doses of drugs, can have an effect;

c) the EI-response is approximately <u>50%</u> of the E-response so that the enhancing, or abolishing, effect of a variable on inhibition can show up equally well.

Of course, no experiment could be started without at least a stable, even record. This could be achieved on 80% of the frogs, within one half to one hour from the first stimulation of the preparation. During this time, the reflexes usually declined and gained a new tone level (see "1" in Fig. 30), in their adaptation to the new conditions of

oxygen and glucose supply and rates of stimulation.

Restabilization of the reflexes, after an experiment was completed, depended on the reversibility of the experimental effects. Most of the effects produced were easily reversible so that two to six experiments could be performed on the same preparation. However, some drugs, like strychnine and myanesin, or sometimes a long duration of anoxia, would result in an irreversible state of affairs so that no further experiments could be performed on the same preparation.

In most cases, the reflexes could not be considered stable for longer periods than one hour. However, some frogs remained reliable for two and a half hours, and others after giving a relatively stable record for one half hour, suddenly changed for no visible reason. Therefore, returning of the reflexes to the pre-experimental control levels was essential, before one could consider an experimental record to be valid.

During a day's experiment, the preparation usually fatigued so that the stimulus strength had to be increased. From Fig. 10, it is seen that the rate of fatigue, at least at the beginning of the day, was approximately 0.1 V per hour.

In most of the experiments it was possible to ensure that the stimuli were submaximal, usually, however, only at the expense of a record with a variation of the E, and EI-strokes, by about $\pm 10\%$ and $\pm 20\%$, about their respective means.

However, to keep the EI-strokes at 50% of the E-stroke height consistently, was found to be a most difficult task because the amount of inhibition, as recorded in this method, was not only a function of the effectiveness of the inhibitory stimulus, but also a function of the excitatory stimulus. Thus, the chances for variation of %-inhibition were doubled. Moreover, the strength response curves for inhibition appeared to be much steeper than those for excitation, as seen in Fig. 14, i.e., an increase in stimulus strength which would increase the E-response from 40 to 50% maximum, abolished the existing 100% inhibition completely. Therefore, the experimenter was satisfied with EI-strokes which varied from 0 - 50% of the E-stroke height. Alternately, an inhibitory record which was either just threshold, or just complete, was also considered satisfactory. If an experimental variable did not tend to abolish inhibition, in a record with complete inhibition, the next experiment with the same variable had to be started with a record with just no inhibition, and vice versa. However, one had to ensure that inhibition was not very supramaximal, or sub-threshold.

Advantages and Shortcomings of the Preparation

This new preparation for studying pharmacological action on spinal reflexes, described in detail in the Method, has certain advantages over other methods reported in the literature:

a) It is relatively simple and easy to set up (in about half an hour);

b) Experiments can be carried out at room temperature, without further temperature regulation of the preparation;

c) Since the isolated segment of the spinal cord is small (approx. 30 mm^3), its maintenance without a natural blood supply is a relatively easy task;

d) The effects of drugs on spinal reflexes can be conveniently tested without their obscuring effect on the muscle from which the reflex activity is recorded. However, while the drug traverses the vertebral canal, it comes in contact with the sciatic nerves (and dorsal and ventral roots), in addition to the spinal cord. Therefore, conclusions as to a drug's effects on the spinal cord can be drawn only in the case of those drugs which either do not act on peripheral nerve conduction, or else, affect the nerve in much stronger concentrations than they act on the spinal cord. (It is to be noted that no perfusion method can exclude the effects of drugs on dorsal and ventral roots. They are indispensable for eliciting and recording of the reflexes, and any drug which comes in contact with the spinal cord, through intravertebral perfusion, must come in contact with the roots too).

e) The muscle which records the flexor reflex and its inhibition by the cross-extensor reflex, does not seem to need a special oxygen or glucose supply.

f) The oxygen supply to the cord is independent of local vascular effects of drugs.

g) Our method of stimulation allowed recording of a continuous indication of the excitatory and inhibitory state of the cord, with respect to the flexor reflex. However, recording from the muscle could have been improved by registering the total area under the reflex contraction of the muscle, rather than its amplitude. (Fig. 15).

h) Since the spinal cord perfusion fluid enters the vertebral canal at one end and leaves it at the other, without coming in contact with significant amounts of active tissue, other than active spinal cord, interpretation of possible findings of active substances in the perfusate should be clear cut.

It must be pointed out, however, that the method has not yet been sufficiently improved to allow a reliable analysis of spinal cord effluents (see Discussion in Miss N. Pedley's Thesis), mainly because the reflexes cannot be considered stable for long enough periods.

Further experiments will have to be carried out to ascertain the site of fatigue which occurs during a day's experiment (Fig. 10), and during high frequency of stimulation (Fig. 26).

The possible site of fatigue along the reflex arcs used in the method are:

1) Receptors; 2) Afferent nerve conduction; 3) Fatigue in the spinal cord; 4) Motor nerve conduction; 5) Neuromuscular transmission; 6) Muscular fatigue.

It is, however, probable that there are only three possible "bottle-necks" involved:

i) Fatigue of the receptors;

ii) Fatigue in the spinal cord;

iii) Muscular fatigue.

If further experiments proved that fatigue of the preparation under the adopted conditions of stimulation was due to exhaustion of the receptors, precautions could then be taken to avoid this, e.g., by stimulating with a lower frequency of stimulus impulses, for shorter durations, or by a more diffuse method of stimulation.

Fatigue of the muscle from which reflexes are recorded, could be obviated by perfusing the aorta with oxygenated Ringer beyond the point where it was tied to prevent the oxygen (or other gas) intended for the spinal cord, from reaching the same muscle (e.g., according to Häusler's method).

If, however, the site of fatigue does prove to be within the spinal cord, perhaps (in addition to more moderate stimulations), a better method of oxygenation than through vascular gaseous oxygen perfusion will have to be adopted. (See Legend to Figs. 24 and 25).

VIII. S U M M A R Y

(A) - A new spinal cord perfusion method has been described, which permits:

a) Testing effects of drugs on spinal reflexes, and

b) Collection of spinal cord effluents.

A 10 mm segment of a frog's spinal cord "in situ" was kept alive, at room temperatures, by perfusing the vascular system through the truncus arteriosus, with a gas mixture of 97% oxygen and 3% CO_2, at pressures of 100 - 150 mm Hg. Tying the two systemic arches, just proximal to their abdominal junction, prevented circulation of the gas to the hind legs.

The cord was perfused with Ringer solution through a cannula, inserted in the caudal end of the vertebral canal, and the perfusate was collected in the cervical region, where the neural arches and spinal and medullary tissue were removed.

The flexor responses of the biceps femoris muscle to (E) stimulation of the skin of the homolateral foot for one second (60 cycle A.C.), and its alternate inhibition (EI) by the cross-extensor reflex, due to stimulation of the contralateral foot (for two seconds) were recorded on a smoked drum. The stimuli were repeated at regular intervals of 30 to 50 seconds. The preparation was kept moist by a spray

of Ringer perfusion.

Drugs, through the intravertebral perfusion line, and various gases, intravascularly, could thus reach the spinal cord without directly affecting the biceps muscle from which the reflexes were recorded.

(B) - In the results, the stimulus response characteristics of the preparation were presented to show the relationship between the E or I-stimulus strengths and the rate of change of the amplitude and/or duration of the E and EI-responses.

Further figures show the effects of maltreating the preparation. These were the outcome of experiments, conducted to ascertain optimal conditions of spinal cord oxygenation, frequency of stimulation, and intravertebral Ringer perfusion.

To illustrate the use of the preparation in testing the effects of drugs on spinal reflexes, records were presented to show the action of some spinal cord depressant and excitant drugs.

(C) - The merits and shortcomings of the preparation as compared to other methods reported in the literature, were discussed.

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