pantothenic acid (McIlwain & Hawking, 1943). The failure of this substance as a drug may be attributed to the presence of pantothenic acid in the body of the host in a concentration sufficient to nullify the action of the drug. There is reason to hope that these difficulties would not arise with drugs designed to simulate some feature of the mycobactin structure. Such drugs might prove of importance in the chemotherapy of tuberculosis or leprosy.

SUMMARY

1. Methods for large-scale production of *Mycobacterium phlei* are described: the organism contained large amounts of growth factor for *Myco. johnei* when grown on media made from beef, but not on other media.

2. A method for the assay of the growth factor is described.

3. The factor has been isolated as a crystalline aluminium complex and also in an amorphous metal-free form. The name 'mycobactin' is suggested for the compound.

4. Mycobactin is a hitherto undescribed compound. Evidence is given in support of the empirical formula $C_{47}H_{76}O_{10}N_8$. Details are given of its general properties and of a number of derivatives.

5. The concentration of mycobactin in living *Myco. phlei* grown on beef infusion medium is estimated to be about 3 mg./g. Optimum growth of *Myco. johnei* is promoted by the presence of 40–80 µg. mycobactin/ml. of medium.

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Techniques in Tissue Metabolism

2. APPLICATION OF ELECTRICAL IMPULSES TO SEPARATED TISSUES IN AQUEOUS MEDIA

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Fluctuating electrical-potential gradients exist in the greater part of the organs of the animal body. Characteristics of the gradients produced in nervous and muscular elements are used to indicate the functional state of organs *in vivo*; applied impulses greatly change the activity of such organs and the metabolic activities of their constituent tissues. The application of electrical impulses *in vitro* to separated tissues under conditions which allow active metabolism has been described in previous papers from these laboratories (McIlwain, 1951a; McIlwain, Anguiano & Cheshire,

1951; McIlwain & Gore, 1951; Kratzing, 1951). Large changes in the metabolic behaviour of certain tissues have been caused, while others were unaffected.

The present paper describes: (1) apparatus chosen and devised to furnish and measure the electrical impulses applied in such experiments; (2) arrangements of electrodes for leading the impulses to the tissue; and (3) measurement of the effects of the impulses on (a) the electrical fields set up by the impulses around the tissue, and (b) the metabolic response of the tissue.

EXPERIMENTAL AND RESULTS

Source of electrical impulses

Sine-wave alternating currents. Current at 50 cyc./sec. was obtained from mains through a transformer and potentiometer. Voltage and current were measured with an Avometer no. 7, a switch being arranged to connect it in parallel or series with the experimental vessels (see below). In this way, up to six vessels could be supplied, their individual currents determined at the beginning and end of the experiment and the total current observed throughout. Other frequencies were obtained from a beat frequency oscillator

The circuit is shown in Fig. 1. A 45 v supply was obtained by full-wave rectification through a selenium rectifier, and the resistance R_{14} was used to control the output voltage of the instrument. Condensers C_{11} and C_{12} were found necessary in order to reduce the effective impedance of the supply to a value that did not lower the voltage produced on charge below that of the discharge. The relay used was a Carpenter type 3E10. This is a polarized relay with both sides stable, high contact pressure and short transit time. A bias magnet can be adjusted so that the change-over when driven from a 50-cycle sine wave occurs at the same voltage point on each half cycle. The charge and discharge pulses are therefore evenly spaced at 10 msec. intervals. In practice it

Fig. 1. Circuit diagram of condenser pulse generator and monitor. Resistors had the following values: R_1 , 100 k Ω ; R_2 , 470 k Ω ; R_3 , 100 k Ω ; R_4 , 33 k Ω ; R_5 , 10 k Ω ; R_6 , 33 k Ω ; R_7 , 100 k Ω ; R_8 , 10 k Ω ; R_9 , 330 k Ω ; R_{10} , 120 k Ω ; R_{11} , 1 M Ω ; R_{12} , 10 k Ω ; R_{13} , 15 k Ω ; R_{14} , 1 k Ω ; R_{15} , 500 Ω ; R_{16-23} , 1 M Ω ; $R_{24,25}$, 500 k Ω ; R_{26} , 200 k Ω ; R_{27} , 470 k Ω ; R_{28} , 10 k Ω ; R_{29} , 100 k Ω ; R_{30} , 50 k Ω . Condensers were as follows: C_1 , 0.1 μ F; C_2 , 0.01 μ F; $C_{4,5}$, 0.1 μ F; C_{6-10} , 4 μ F; $C_{11,12}$, 100 μ F electrolytic working at 50 v. S , 2-pole 4-way wafer-switch, Yaxley type. Meter M , 0-2M-amp.; 2 in. scale marked 0-36 v. B , 45-v battery; relay, see text.

type L.O. 800 B of Birmingham Sound Reproducers. This gave an output adequate (according to frequency) for from two to four vessels of resistance 30-50 Ω ; its output was set to match a 15 Ω load. When in use the waveform delivered was continuously observed with an oscilloscope and very little distortion was seen at frequencies between 10 cyc./sec. and 10 kcy./sec.

Condenser pulse generator and monitor. The instrument was designed for the present work and gives pulses of exponential time-voltage relationship at a rate of 100/sec. The duration of these pulses can be varied and is measured with a cathode-ray tube. The duration is dependent on the load connected to the instrument, but means are provided for adjusting the duration independently of this. The peak voltage of the pulses can be varied and is measured by balancing it against a bias voltage the value of which is determined by a meter.

was found that a capacitance of 4 μ F in series with the electrodes used gave pulses with time constants of 0.35-0.5 msec. In order that up to four vessels could be supplied in parallel, five 4 μ F condensers were arranged so that from one to four could be connected in series with the vessels. A variable series resistance R_{15} allowed fine adjustment between each step of the capacitor switch. As adjustment of R_{15} results in a fall of voltage across the preparation, it is necessary to compensate this by increasing the charging voltage, but as the maximum voltage required has not been above 24 v there is ample voltage available to correct any fall in voltage on operating this series resistance.

The type of circuit used for the time base is that of a Miller transitron oscillator. This has the advantage of requiring only one valve, and the frequency range required is covered by one control, R_1 . The saw-tooth waveform of the Miller transitron is linear over the part used for time measurement.

Synchronization is achieved by applying a 50 cyc./sec. signal to the suppressor grid of the oscillator at the junction of R_5 and R_6 , the signal being obtained from one side of the relay energizing supply. No trouble was found in locking the time base at 50 and 100 cyc./sec. The duration of the pulses is expressed as the time constant of the output circuit and measured as the time taken for loss of 63% of the peak voltage. To do this, the time base was set to give two pulses (10 msec. interval) visible through a graticule in front of the screen. The graticule was ruled horizontally with lines 10 and 3.7 mm. above the base-line. The peak of the discharge was adjusted to the upper of these, and the intercept of the discharge on the lower one was measured.

Fig. 2. A-D, grid electrodes; F, G, electrode vessels. The grid electrodes are in elevation in A, sectional end elevation in B, and plan in C (the upper electrode) and D (the lower). Hatched areas are of enamelled silver wire; the wire is bared at points P, not hatched. Black areas represent stainless steel wire. Helical connexions are shown only in the plan. E: electrode arm suitable for manometric vessel E (McIlwain, 1951 b), carrying tungsten electrode a soldered at b to a tinned copper wire c which reaches beyond the glass tube; a rubber washer at d and cement at e close the glass tube, and electrical connexion is made by a clip at the upper end of the wire. F: electrode vessel F; G: dipping electrodes G in a test tube.

With the range of voltage used in this instrument one stage of amplification was ample to operate the 3 in. cathode-ray tube used (VCR 138). The method of voltage measurement used was chosen because it does not necessitate stabilized shift supplies and calibrated shift controls. Gain is controlled by negative feedback across R_8 , the slider of R_7 being normally at earth. The signal is applied to the grid through C_6 and a known bias can be applied by operation of R_{12} , the value being indicated by the meter M. Voltage measurement is made by biasing the valve with resistance R_9 until only the positive-going signal is observed on the screen; then this signal is cut off completely by operation of R_{12} . The voltage required to do this is then equal to the peak value of the signal.

Cathode-ray tube type VCR 138 was used with 2 kv on the final anode. The ganged X controls R_{16-17} and Y controls R_{18-19} keep the mean potential of the plates close to that of the final anode. It was found that there was coupling between the plates resulting in a distortion of the sharp rise for each pulse, but this was not severe enough to affect measurement made on the screen.

Electrodes

Numbering and lettering of vessel and electrode types is consecutive with that of earlier papers (McIlwain, 1951 a, b) which are those referred to when no other specific reference is made.

Metals employed. In addition to descriptions given previously, considerable use has been made of tungsten and molybdenum in electrodes. Neither of these metals was found to interfere with ordinary manometric measurements and they were thus suitable when platinum was not. Also, they yielded gas-tight joints with Pyrex glass more satisfactorily than did platinum. Tungsten was found very

suitable for making gas-tight electrical connexions through the walls of manometric flasks. It could not, however, be worked sufficiently easily for shaped electrodes to be made from it. It has thus been used in vessels A and A¹ in the fashion shown in Fig. 2. After sealing, it was cleaned from its adherent surface film either with emery paper or chemically. Chemical cleaning was necessary in inaccessible places such as the side arm b in Fig. 2E and was done by heating with solid NaNO_2 . Mechanical cleaning was found most suitable in position a and was necessary after every few experiments.

Molybdenum wire is sufficiently flexible, and not too elastic, to be bent to shapes needed for vessels E. It was found easy to make an initial seal through Pyrex glass with molybdenum wire, but rapid working was necessary before secondary changes took place. These produced a bloom on the glass and bubbles by the sealed wire. Molybdenum electrodes in vessels E have, however, been employed extensively because of their freedom from the danger of causing gas changes and producing inhibitory substances. Gold-plated platinum electrodes require testing during each

group of experiments for freedom from these effects (McIlwain, 1951*a*).

Lead-in wires to electrodes. In place of the temporary arrangements previously described which involved contacts made with mercury, soldered or welded connexions have now been devised. At *b*, Fig. 2*E*, soldered connexion is made, in a glass side arm, between an electrode and a tinned copper wire. The lead from a stimulator is clipped to the top of *c*. The glass side arm rises above the level of water in the thermostat, or of acid in a cleaning bath, and is sealed by litharge-glycerol cement resting on a rubber gasket.

At *f*, Fig. 2*F*, a welded connexion between platinum and tungsten or nichrome has been made and completely sealed in glass. The tungsten wire leads to the top of the side arm, which is of capillary tubing, and there another weld at *h* connects it with a nickel stub to which the stimulator lead is clipped. This connexion is also sealed in the glass. This arrangement was devised by Mr T. W. Wingent. In some cases a spiral has been made in the wire at *g* to relieve tension developed on cooling.

Electrode arrangements

Concentric vessels E. These were previously made by inserting lengths of wire, already fused to electrode arms, to the shaped conical vessel and later bending the wires to shape. It was difficult in this way to obtain a group of vessels with electrodes sufficiently uniform. Recently a short length of fine capillary has been sealed into the vessel first, and then the electrodes, already bent to shape, put in the vessel and their lead-out wires threaded through the capillary. The final sealing has been carried out with the vessel full of hydrogen. This prevents oxidation of the cleaned wire and is especially useful in producing vacuum-tight molybdenum-to-glass seals.

Concentric vessels F. Principles applied in designing vessels *E* were found applicable to small vessels of volume about 4 ml., illustrated in Fig. 2*F*. These were designed for examining small tissue specimens and were used with 10–20 mg. fresh weight of tissue in 0.8–1.2 ml. of fluid. The effects of applying electrical impulses to cerebral tissue in these vessels, quoted in Table 3, show a scatter of values not very much greater than that observed in vessels *E*.

Grid electrodes H. Results with an earlier type of these electrodes have already been reported (McIlwain, Ayres & Forda, 1952). Dr C. C. Kratzing and Mr H. L. Buddle helped in their design. They were made to fit vessels *A* and are illustrated in Fig. 2*A–D*. Enamelled silver wire was wound to a plastic frame and the silver exposed at a series of places which formed a grid between which the tissue lay. In use, the lower grid *D* is immersed in the saline, a tissue slice floated above it and lifted out with the grid, held by its helical connecting wire. The point of the upper grid *C* is slipped under the stainless-steel frame at the right-hand end of *D* and the left-hand ends of *C* and *D* pressed together, when they are held by the stainless-steel clip at that end. The grids with tissue are placed in vessels *A* already containing saline, and the helices of the grid pushed home on the electrodes of the vessel.

Measurement in saline of potentials resulting from the application of electrical impulses

Tissue floating freely in saline between electrodes is affected metabolically by applied impulses; these can affect the tissue only through potentials set up

in the fluid. Measurements were made to determine such potentials in the glucose-saline between the electrodes described above. Potentials in such systems depend greatly on properties of metal-fluid junctions; these have been investigated in relatively simple solutions (for reviews see Bowden & Agar, 1938; Agar, 1947; Kortum & Bockris, 1951; Butler, 1951) but not, apparently, in physiological glucose-salines at neutral pH with the range of impulse types with which the present experiments were concerned.

The saline contained 134 mM-NaCl; 5.4 mM-KCl; 1.34 mM-KH₂PO₄, MgSO₄, and CaCl₂; 13 mM-glucose; and 10.4 mM-Na₂HPO₄ brought to pH 7.4 by HCl.

Apparatus for measurement. The wires to which potentials were applied, of types described in individual experiments below, were clamped so that they were immersed in fixed positions in the saline in a shallow dish. This imitated a manometric vessel of types *A* or *E* with respect to its diameter (35–40 mm.) and the depth of the saline (5–6 mm.). The dish was fixed horizontally on the stage of a James Swift travelling microscope. Probe wires were fixed to the objective of the microscope so that their points were in focus at about the centre of its field. This did not hinder the use of the microscope, with its eyepiece graticule, for measuring linear distances and enabled the probe wire to be adjusted to varying depths in the liquid by the focusing rack of the microscope. The separations of the wires were measured by a mm. scale and micrometer on the microscope body, except when short distances of some 0.01 mm. were concerned, when the eyepiece graticule was used.

The probe wires were made by brazing silver wire 0.0032 in. in diameter to longer wires as leads, and completely insulating them with a plastic (Araldite, Aero Research Co.). They were then cut to expose the tips only. As will be seen from the results given below the apparatus enabled readings of potential to be taken at each 0.05 mm. between wires 1 mm. apart.

The dish and the wires used in these experiments were cleaned by immersion in conc. HNO₃ immediately before use. Immersion was of some hours duration except with silver wire, when it was brief, and molybdenum wire, when brief immersion in HNO₃ containing about 1% of conc. HCl was used. Mechanical cleaning of silver and gold wires by scraping them with a clean knife gave the same results.

Potentials resulting from application of sine-wave a.c. to parallel wires

Wires, exposed for a length of 1 cm., were placed a few mm. apart in the saline. This arrangement imitated that of electrode *A* (McIlwain, 1951*a*, Fig. 9) or part of the grid of electrode *E*. The result of applying sine-wave a.c. was examined at first qualitatively by placing two probes between the wires. The potential difference between these probes was taken to a Cossor double-beam oscilloscope (model 1049) and compared with the input sine wave. The results indicated an approximately uniform potential gradient, but as shown in Fig. 3 alteration in the voltage-time relationships of the

current occurred in the saline. These alterations were greatest close to the wires and least at points about equidistant from them.

To measure the variation in peak potential with distance, the circuit of Fig. 4A was first used with moderate success. Here a single probe was employed and its potential balanced against a measured

Fig. 3. Voltage-time relationships between silver wires 0.71 mm. diam. and 2.0 mm. apart in glucose-phosphate saline, determined by silver probe wires 0.0128 mm. diam., 0.2 mm. apart and in a line perpendicular to the electrodes. To the electrodes was applied a sine-wave a.c. of peak potential 2.85 v. A and B: with the probe nearest to an electrode, 0.01 mm. from it; C and D: with probes equidistant from the corresponding electrode. On each trace is superposed one derived from the signal applied to the electrodes, through an attenuator, adjusted to give a peak-peak potential of 0.2 v. A and C: applied signal of 50 cyc./sec.; B and D of 2000 cyc./sec.

Fig. 4. Circuits used in measuring potential gradients. A, voltage applied was measured at V_1 . R_1 (50 Ω) was of resistance about equal to the electrode system E_1E_2 (40–100 Ω). In a given position of the probe electrode P, a position on R_1 was found such that current at A was minimal. V_2 then gave the potential difference between the probe and electrode E_2 . V_1 is a voltmeter giving virtual values. Voltage and current at V_2 and A was measured on a double-beam oscilloscope. Cossor model 1049. B, in use, resistance R_2 was adjusted to give a zero reading on the galvanometer G, with no signal across T_1 . Only signals in phase or 180° out of phase with the signal in T_2 give appreciable deflection of galvanometer (G). A null point is found on R_1 as in the instrument of Fig. 4A.

fraction of the potential applied. A true balance at a null point was not always obtained owing to the alteration in voltage-time relationships undergone in solution. For this reason, fluctuations out of phase with the input sine wave were filtered out by the apparatus of Fig. 4B. Both these are null-point instruments and depend on finding the potential which must be applied to a probe wire to prevent flow of current between it and the solution; its potential can then be taken as equal to that of the point in solution at which it is placed.

Fig. 5. Potentials in glucose-phosphate saline at 20° between two 1 cm. lengths of silver wire (0.46 mm. diam.) 8 mm. apart, to which sine waves of peak voltage 6.5 v were applied; O, at 50 cyc./sec. and ●, at 2000 cyc./sec. The points give measurements which were made with the circuit of Fig. 4B. The out-of-phase components, not measured by this circuit, gave a complex residual waveform of which the maximum amplitude at the midpoint between the wires was 50 mv peak-peak. The readings plotted as 0 and as 8 mm. were taken with the probe as close as possible to the electrodes, without making metallic contact. Its distance from the wire was then less than 0.01 mm. The line is not drawn through the points but gives the theoretical relationship explained in the text.

The maximum gradient established by alternating currents between two parallel silver wires as electrodes is shown by Fig. 5. In obtaining these values, the tip of the probe wire was adjusted to be in the plane of the electrodes at several chosen points at different distances from them. An abrupt change in potential was found between each electrode and the solution. As the probe wire was

moved towards an electrode, the potential measured in the solution perhaps 0.01 mm. from the wire changed sharply as metallic contact was made with it. In the solution, the potential change with distance along the line joining the centre of the two wires was sigmoid. The relationship between potential (V) and distance (d) between two such wires, radius R and distance D from centre to centre, would be expected on theoretical grounds (Attwood, 1949) to be

$$V = k \log \{ [2\sqrt{[(\frac{1}{2}D)^2 - R^2]}/d] - 1 \}.$$

In Fig. 5, the sigmoid line gives this relationship for wires of the size and distance employed, and for a value of k chosen so that the total voltage fall in solution has the observed value of 5.5 v. The agreement with the experimental points is good.

Although in Fig. 5 the potential gradient varies with distance from the electrodes it has between them defined upper and lower limits. The line of Fig. 5 is not asymptotic with the ordinates. It becomes straighter as the wires become closer. For studying the effect of a number of variables on such gradients, the higher and lower gradients have been read from graphs such as that of Fig. 5. In this way the dependence of the gradients on the following factors were determined.

Nature of probe wire. Most of the electrodes to which potentials were applied were of noble metals and electrodes of the same nature were generally used for the probe to minimize the introduction of metal-metal potentials. When stainless-steel wire was used as probe with the apparatus of Fig. 4B, curves similar to Fig. 5, but displaced vertically were obtained. Displaced curves were in some cases obtained between silver wires and a silver probe.

Relationships between applied voltage and potential gradient and current in solution. With given electrodes a fixed distance apart, increase in voltage of applied impulses led to change in all the characteristics of voltage-distance curves such as Fig. 5. This has been studied in detail with 50 cyc./sec. a.c. between 0.5 and 4 v, as many metabolic experiments have been carried out with currents of these characteristics. The abrupt potential change between metal and solution increased with increasing voltage, but not linearly (Fig. 6A). Nevertheless, the potential gradients between the electrodes, when expressed as described above, did increase in nearly direct proportion to the applied voltage. Current in solution was found to be directly proportional to applied voltages except when these were below 0.2 v or so.

Potentials established at varying frequencies. Peak voltage-distance curves for sine-wave a.c. were often closely similar over a considerable range of frequencies, as can be seen in the comparisons of Table 1 and Fig. 5 between 50 and 2000 cyc./sec.

Above 2000 or 3000 cyc./sec., however, the abrupt voltage change between the metal and solution increased until at 10000 cyc./sec. and 6.5 v it was twice that of Fig. 5.

Nature of electrode wires. Table 1 shows that the behaviour of molybdenum and stainless steel as electrodes was markedly different from that of silver or electro-deposited gold. With molybdenum and steel the potential change at the metal-solution interface was large and the potential gradient established in the solution was low. Gold as a drawn wire showed a larger fall in potential at the interface, and a smaller potential gradient in solution, than did electro-deposited gold.

Fig. 6. Potential between parallel silver wires established by sine-wave alternating currents of different voltages. Values were derived as described in Table 1 from experimental results plotted in Fig. 5. Wires: parallel, silver, as in Fig. 5, 1 mm. apart; a.c., 50 cyc./sec. A, the abrupt change in potential between wire and solution (sum of the changes at the two wires). B the larger and C the smaller potential gradient established in solution.

Potentials in grid electrodes. The grid electrodes of Fig. 2D have been used in many investigations, including those in which voltage-response curves have been determined. Accordingly, the potential distribution in them has been measured.

Potential-distance curves were in all cases more complex than between two wires (Fig. 7). The interval between two wires in the holder always included a short plateau of little or no voltage change. The maximum gradient was again adjoining the wires and was of about two-thirds the value obtained by dividing peak applied voltage by mean distance between the wires (in Fig. 8, $3.15/2.1 = 1.5$ v/mm.). It is not practicable to make and maintain exactly uniform the position of all the wires in a group of

holders *E*, and measurements have accordingly been made at several intervals in more than one holder under the conditions of Fig. 7. The results in Table 2 show variations to be expected, but the following uniformities: (a) In no case were points of unusually high potential gradient found, and the highest gradient, of some 2 v/mm., occupied some 10–30 % of the total distance. (b) 50–80 % of the

distance was at a voltage gradient of half the maximal. Potentials found in the grid electrodes, while a slice of cerebral cortex was clamped between them were similar to those without tissue, but the potential fall across the tissue was greater than across the same distance in saline. This presumably reflects the greater impedance of the tissue and appears likely to be important in its stimulation.

Table 1. Potentials established by a.c. impulses between parallel wires in glucose-saline solutions

(Two 1 cm. lengths of the wire described below, 0.46 mm. in diameter, were placed parallel and 2 mm. apart in phosphate-buffered glucose saline. Wires were of the drawn metals, except in the instance noted, when gold was electro-deposited on platinum as described by McIlwain (1951*a*). Sine-wave a.c. at peak potential of 2.8 v was applied to the wires. Peak potential between one wire and a probe of the same metal (except with molybdenum wire, when a silver probe was used) were measured with the instrument of Fig. 4*B*, giving curves of the sort illustrated in Fig. 5, from which are derived the values of this table. Where two gradients are given, section *B* of the solution comprises the parts nearest to the wires and of greater potential gradient, section *A* is the central part.)

Metal	Impulse applied									
	100 cyc./sec.					2000 cyc./sec.				
	Sum of the two abrupt potential changes at inter-faces (v)	Section				Sum of the two abrupt potential changes at inter-faces (v)	Section			
		A		B			A		B	
		Distance (mm.)	Gradient (v/mm.)	Distance (mm.)	Gradient (v/mm.)		Distance (mm.)	Gradient (v/mm.)	Distance (mm.)	Gradient (v/mm.)
Silver	0.7	1.2	0.9	0.8	1.6	0.55	0.9	0.9	1.1	1.6
Gold (electro-deposited)	1.15	1.4	0.85	0.6	1.15	0.15	1.6	1.15	0.4	6.0
Gold (drawn)	2.1	1.4	0.4	0.6	0.5	0.8	1.5	1.1	0.5	1.5
Molybdenum	2.7	2.0	0.19	—	—	2.8	2.0	0.16	—	—
Stainless steel	2.6	2.0	0.2	—	—	2.2	2.0	0.4	—	—

Fig. 7. Potential distribution in glucose-phosphate saline between the silver wires of part of a grid electrode of Fig. 2. The centres of the wires are at *A*, *B* and *C*. Sine-wave a.c. of 3.15 v peak potential was applied and the measurements are of peak voltages at the points of maximum voltage found by moving the probe electrode vertically during determinations. These points of maximum voltage were found to be close to lines joining the centres of the wires. The probe electrode was of silver, as described on p. 610, except that its point was bent to negotiate the wires of the grid; measurements were made using the circuit of Fig. 4*B*.

*Potentials resulting from application
of condenser pulses*

The apparatus of Fig. 4A was found suitable for following the potentials established in solution on applying pulses of exponential time-voltage relationship. These were derived from the instrument of Fig. 1. The voltmeter V , in Fig. 4A was replaced by an oscilloscope in order to measure the peak voltage and duration of the signal applied to the electrodes. Balancing was not difficult since the signal, seen on the oscilloscope, was closely similar in its time-voltage relationship to the applied pulse. Between parallel wires the change of peak potential with distance was similar to that found when sine-wave currents were applied (p. 611).

such vessels, with electrodes of electro-deposited gold and of molybdenum, has accordingly been measured.

Examples of the potentials established are shown in Fig. 8 and Table 2. Loss of voltage between the electrodes and solution is similar to that found with sine-wave currents. The potential gradient is not uniform, but is greatest at points closest to the centre electrode. This is the situation between coaxial cylinders, and the radial gradient between the wires as they lay in a shallow layer of saline was reasonably close to the gradient expected (Attwood, 1949) between such cylinders. The line of Fig. 8B gives the change of potential in solution (V) with distance (d) from the centre ring of outer radius r calculated from $V = k \log(d/r + 1)$. The gradient is

Fig. 8. Potentials established by condenser pulses between concentric electrodes. *A*, dimensions of electrodes (gold-plated wire, 0.46 mm. diam.) in the vessel examined below; r_1 , r_2 , r_3 , radii at which voltage was observed. *B*, points give the potentials observed in glucose-phosphate saline along the three radii of *A* when pulses of peak potential 18 v and time constant 0.4 msec. were applied; the line is not drawn through the points but from theoretical considerations given in the text. *C*, abrupt potential change between wires and solution (sum at the two wires) when condenser pulses of varying peak voltage and 0.4 msec. time constant were applied at 100/sec. to concentric electrodes. *D*, gradients established in solution between the wires at varying voltage; ●, near the centre wire; ○, near the outer wire. Values for *C* and *D* were derived from a number of measurements of the type shown in *B*, arbitrarily dividing the curve as indicated by the dotted line in *B* to obtain the two values for the gradient.

Potentials at concentric electrodes. Concentric electrodes in conical manometric flasks were designed (McIlwain, 1951b) so that the bulk of the fluid of the flask lay between the wires, and tissue floating freely in the fluid would be subject to the applied impulses. Wires have usually been about 1 cm. apart and used with condenser pulses, because at the voltage required for stimulation, alternating currents expended too much energy in the fluid. The gradient established by condenser pulses in several

seen in Fig. 8D to increase with increasing applied voltage, but not in direct proportion to the voltage.

Potentials established between electrodes of molybdenum wire were found to be similar to those between the gold wires of Fig. 8. In this respect the brief pulses of higher voltage gave results different from the sine-wave a.c. of Table 1. A greater fall in potential at molybdenum-saline than at gold-saline interfaces is, however, reflected in the values of Table 2.

Table 2. *Potential gradients with grid and concentric electrodes*

(Grid electrodes were of silver wire as shown in Fig. 2*C*. Concentric electrodes were in vessels *E*; 1 to 3 were of electro-deposited gold on platinum and 4 was of molybdenum. Electrodes were immersed in glucose-phosphate saline at pH 7.4, 6 mm. in depth in a dish 35 mm. in diameter. Potentials (peak) were determined as described in Fig. 4 and gave curves, similar to those shown in Figs. 7 and 8, from which were read the gradients listed below.)

Electrode	Applied current; peak potential (v)	Distance over which gradients extended (% of distance between wires)		
		0-0.6 (v/mm.)	0.7-1.2 (v/mm.)	1.3-2.3 (v/mm.)
Grid 1	Sine wave, 3.15	8	79	13
Grid 2	Sine wave, 3.15	17	73	10
Grid 3	Sine wave, 3.15	23	58	19
Grid 4	Sine wave, 3.15	13	58	29
Grid 5	Sine wave, 3.15	17	53	31
Grid 6	Sine wave, 3.15	20	50	30
		Distance over which gradients extended (% of distance between wires)		
		0.45-0.7 (v/mm.)	0.8-1.1 (v/mm.)	1.2-2.5 (v/mm.)
Concentric, vessel 1				
Radius 1	Condenser pulse, 18	23	55	22
Radius 2	Condenser pulse, 18	18	62	20
Radius 3	Condenser pulse, 18	0	77	23
Concentric, vessel 2				
Radius 1	Condenser pulse, 18	18	62	20
Radius 2	Condenser pulse, 18	22	64	14
Concentric, vessel 3				
Radius 1	Condenser pulse, 18	24	67	9
Concentric, vessel 4				
Radius 1	Condenser pulse, 18	38	39	23

Metabolic response of tissues in different vessels

Variation in response with grid and concentric electrodes. This assessment was carried out in order to see how similar were the responses using series of vessels and electrodes prepared in the manner described above.

Four concentric electrode vessels made according to Fig. *E* (McIlwain, 1951*a*) were examined, two with electrodes of molybdenum wire and two of gold-plated platinum. These were employed in ten experiments for the determination of the respiratory rate of guinea-pig cerebral cortex. In each experiment tissue from one guinea pig in one 'molybdenum' and one 'gold' vessel was subjected to successive periods without electrical impulses, with impulses at 12 v and with impulses at 24 v. In the series of experiments the order in which the vessels were handled was randomized. The responses are recorded in Table 3 and were expressed as percentage of the initial rates because this reduced variations resulting from the source of tissue, its weighing and handling. The mean responses in the different vessels proved to be very similar. Variance ratios for the mean square deviation of results in each vessel, to the error variation, corresponded to a probability

of >0.2. Also the results at 12 v were examined separately because the effect of a given impulse might be expected to show greatest dependence on electrode arrangements when the response is not maximal. Here the ratio of variance in vessels/error variance was 1.56, again corresponding to a probability of >0.2 ($N_1 = 3, N_2 = 16$). Thus variation between the vessels was not sufficiently great to be differentiated from the variation among results in a given vessel by the ten experiments described. Any difference in response due to the greater potential loss at molybdenum than at gold surfaces was not great enough to be shown.

Variation in respiratory response was also examined in the smaller ring-electrode vessels *F* of Fig. 2, and found to be of the same order of magnitude (Table 3). Smaller variation was observed in the grid electrodes. This is probably due to the tissue being more rigidly fixed in relation to the wires.

Electrodes G dipping into test tubes. In a previous study of the effects of impulses on cerebral tissues (McIlwain, 1951*a*) it was remarked that very simple apparatus with two wires in a tube should be sufficient to cause changes in the metabolism of susceptible tissues. This has now been found to be

Table 3. *Consistency of values for respiratory response of cerebral tissues with different electrodes*

(Guinea pig cerebral cortex was used in glucose-phosphate saline. Group *A* gives results of five experiments with each of four vessels (differently numbered) of type *E*; each standard deviation reflects the behaviour of one vessel on five occasions. Condenser pulses of time constant 0.3 msec. were applied at the voltages quoted. The tissue was a chopped suspension of particles approx. $0.3 \times 0.3 \times 2$ mm. Group *B* employed similar impulses and tissue preparations. Group *C* employed sine-wave a.c. at the peak voltages quoted and tissue slices. Groups *B* and *C* are with six different vessels of each type, examined in one (group *B*) or two (group *C*) experiments.)

Vessels	Electrode	No. of expts. in group	Respiration (Rate as % of unstimulated rate, ± standard deviation)	
			Group <i>A</i>	
			At 12 v	At 24 v
<i>E</i> , 1	Mo rings	5	164 ± 12.3	206 ± 14
<i>E</i> , 20	Mo rings	5	172 ± 11.9	198 ± 16.7
<i>E</i> , 21	Au rings	5	178 ± 12.0	205 ± 17
<i>E</i> , 22	Au rings	5	172 ± 8.0	217 ± 17.6
			Group <i>B</i>	
			At 2.5 v	At 7.5 v
<i>F</i>	Au rings	6	120 ± 12	182 ± 23
			Group <i>C</i>	
			At 0.85 v	At 1.13 v
<i>A</i>	Ag grid	6	117 ± 5	138 ± 11.2

Table 4. *Effect of impulses from dipping electrodes G on lactic acid formation by fragments of guinea pig cerebral cortex in test tubes*

(Cortex slices cut to about twenty fragments were transferred to 2 ml. of glucose-phosphate saline with Ca salts, in 15×1.6 cm. test tubes *a*, *b*, *c*, and *d* fitted with dipping electrodes *G*. These were shaken in a bath at 37° in air for 45 min. Specimens of 0.5 ml. of the liquid were then taken for determination of lactic acid. Impulses: *C*, condenser pulses, 100/sec., of which the peak voltage and time constant are quoted; a.c., sine wave of 50 cyc./sec. and of the virtual voltage and amperage quoted.)

Expt. and tube	Tissue (o, outer; i, inner slices) (mg.)	Impulses	Lactic acid (μmoles/g. tissue)
1 <i>a</i>	o, 63	—	32.8
<i>b</i>	i, 62	—	30.8
<i>c</i>	i, 69	—	27.4
<i>d</i>	i, 64	—	30.9
2 <i>a</i>	o, 63	—	31.0
<i>b</i>	i, 61	<i>C</i> ; 21 v, 0.3 msec.	88.4
<i>c</i>	i, 62	—	29.5
<i>d</i>	i, 59	<i>C</i> ; 20 v, 0.45 msec.	80.3
3 <i>a</i>	o, 64	—	31.5
<i>b</i>	i, 67	a.c.; 3 v, 18 ma	50.8
<i>c</i>	i, 60	—	29.4
<i>d</i>	i, 71	a.c.; 3 v, 24 ma	68.6

the case. On the basis of results with vessels *E* and *F*, concentric electrodes of molybdenum or silver wire were attached to a glass rod suitable for dipping into test tubes (Fig. 2*G*).

The tubes with suspended tissue were shaken at 2 oscillations/sec. in a thermostat at 37°. Table 4 shows that application of impulses to the electrodes

in such experiments increased the formation of lactic acid by fragments of cerebral cortex suspended in a glucose saline. The effect is similar to that observed previously in manometric apparatus (McIlwain *et al.* 1951). In the present experiment, unstimulated formation of lactic acid was higher than in conical manometric vessels. Conditions of oxygenation of the tissue in the tubes were not optimum as air and not oxygen was employed, and the area of fluid in contact with the gas phase was smaller in the test tubes.

DISCUSSION

Use of apparatus. The apparatus described above has been in use for periods between 1 and 4 years and has been found dependable. Choice among different stimulating and electrode systems may be made as follows. The relationship between tissue and electrodes is most definite and reproducible with grid electrodes *G* between which the tissue is held. Such electrodes carrying the tissue are also convenient when the tissue is to be removed completely at the end of an experiment, e.g. for analysis. The grid electrodes *D* described earlier (McIlwain, 1951*a*) permit analysis of larger pieces of tissue but the field between them is not so regular and reproducible as in electrodes *G*, and has not been studied in detail.

Vessels with concentric electrodes between which the tissue floats freely were designed for use with small fragments of tissue and during experiments these are distributed between the electrodes in a random fashion. Larger pieces of tissue become entangled in the electrodes to such an extent that their aeration is in doubt. The arrangement is not

suitable for analysis of constituents of the tissue itself, which is difficult to collect after metabolism. Concentric dipping electrodes have, however, been employed for determination of tissue constituents by making the electrodes to fit a homogenizer tube; after passage of impulses, the electrodes were removed, a deproteinizing agent added and the homogenizer pestle inserted in the course of 2 sec. These electrodes are simple to prepare for class experiments and together with a transformer or a simple relay to give condenser pulses are inexpensive.

Potentials between electrodes and response threshold. The abrupt change of potential at the electrode-solution interface appears to be akin to a resistance overpotential (Bowden & Agar, 1938). It was satisfactorily low with silver and electro-deposited gold (0.3–0.6 v at one electrode with the potential applied). With silver, a film of silver chloride, later blackening, could be seen, but this was not the case with gold. The abrupt potential change was in all cases less with sine-wave currents at 2000 than at 100 cyc./sec.

The distribution of potentials in the saline was not as simple as in, for example, the experiments of Rashbass & Rushton (1949), mainly because of the relatively large volume of fluid surrounding the electrodes. Nevertheless, the characteristics which were examined followed theoretical expectations. The volume of saline in the present experiments conditioned also the electrical characteristic which was measured for the purpose of correlation with metabolic response. Applied potential has been measured rather than current, since only a fraction of the current passes through the tissue. Moreover, the current passing in the vessel as a whole varies during the shaking which is necessary to oxygenate the saline.

The gradients measured give only a partial

picture of the field to which the tissue is exposed. The part of the field which was measured was chosen to include the points of maximal potential gradient, for this is of greatest importance in judging thresholds to metabolic response. The parts of the field more distant from the wires and not measured are also the parts most liable to variation when the vessels are shaken. From the values given in the tables, the threshold of cerebral tissues to both sine-wave alternating currents and condenser pulses of suitable duration and frequency can be seen to be at a peak voltage gradient of about 0.2 v/mm. Characteristics required in electrical pulses for metabolic response in cerebral tissues will be described in more detail elsewhere.

SUMMARY

1. An instrument is described which works from alternating current mains and produces condenser pulses suitable for applying to separated tissues. The pulses are alternating. Their potential and duration can be varied and measured, and cover the range of characteristics required for altering the metabolism of excitable tissues.

2. Electrodes and vessels are described which enable currents of various characteristics to be applied to separated tissues while their metabolism is measured by conventional procedures. They include a simple and inexpensive type.

3. The potentials established in nutrient saline solutions, between such electrodes, have been measured and assessed.

4. The uniformity of potential gradient and of metabolic response in a number of electrode systems has been determined.

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