moist cerebral cortex was increased by electrical pulses and the increase opposed by certain added agents.

6. Formation of lactic acid by cerebral tissues in minimal aqueous fluid was closer to its normal *in vivo* value than was its formation with the conventional excess of saline.

7. A considerable level of phosphocreatine could be maintained in moist cerebral tissues for at least 75 min., while they respired at 37° in oxygen.

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APPENDIX

Manometric Determination of the Solubility of Oxygen in Liquid Paraffin, Olive Oil and Silicone Fluids

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For the calculation of manometric vessel-constants for experiments conducted in non-aqueous fluids, the solubility of oxygen in the medium at the temperature of the thermostat was required.

The manometric apparatus of Van Slyke & Neill (1924) is not convenient for measurements at temperatures above that of the laboratory; the present method employs a conventional thermostat and shaking apparatus and involves measuring the entry of oxygen into the de-gassed oil; by this technique information can also be obtained about the rate at which the gas dissolves in the oil. A method somewhat similar in principle but for larger volumes especially of solid fats which have lower vapour pressures and in which gases are less soluble than the present oils, has recently been described by Davidson, Eggleton & Foggie (1952).

EXPERIMENTAL

The essential part of the apparatus is illustrated in Fig. 1. The oil reservoir *A* contained, besides the oil, anhydrous Na_2SO_4 and was mounted in the thermostat. Before a determination, O_2 (dried by passing over P_2O_5) was bubbled through the reservoir and out into the atmosphere via the three-way stop-cock *B*. Semi-pressure tubing (ext. diam. 8 mm.; int. diam. 3 mm.) connected the manometer *C* (Rodnight & McIlwain, 1954) via the T-piece *D* to a high-vacuum pump and also to *B*. The special manometer vessel *E*, of approx. 30 ml. total capacity, was fitted with a stop-cock (5 mm. tap bore and greased with high-vacuum rubber grease) leading to a side chamber; the latter, together with the tap bore, had a capacity of 1·5 ml. 2 ml. of oil were pipetted into the vessel, which was placed on the manometer cone (greased with silicone high-vacuum grease) and immersed in the thermostat. With the manometer stop-cock in the position illustrated in Fig. 1, the pump was switched on

and evacuation to remove gas dissolved in the oil continued for 15–20 min. (shorter times were used for silicone fluid of viscosity 1 cS.). At the end of this time the manometer and vessel were tipped so as to fill the side chamber with de-gassed oil and the stop-cock was closed. The pump was then isolated by closing the stop-cock F and warm O_2 carrying oil-vapour from A admitted to the vessel by turning stop-cock B first to position $B1$ and immediately afterwards to position $B2$. The manometer stop-cock was next turned to connect the vessel to the manometer limbs, shaking at 110–120 oscillations/min. commenced and manometer readings taken every 2 or 3 min. Pressure changes were recorded until the oil remaining in the main part of the vessel was saturated with O_2 and had begun to establish a vapour pressure at a steady rate (A–B, Fig. 2). Then the stop-cock F was opened and the de-gassed oil flowed into contact with the gas phase. Readings were continued every min. until a steady rate was again established (B–C, Fig. 2). Changes in atmospheric pressure during the experiment were corrected by a thermobarometer containing air only. In some experiments the flask was weighed before and after the estimation to determine whether any loss of oil had occurred during evacuation; no losses were found with liquid paraffin, olive oil or with silicone fluids of viscosity 5 cS. or greater; losses occurred with the silicone fluid viscosity 1 cS. and this was allowed for in the calculation.

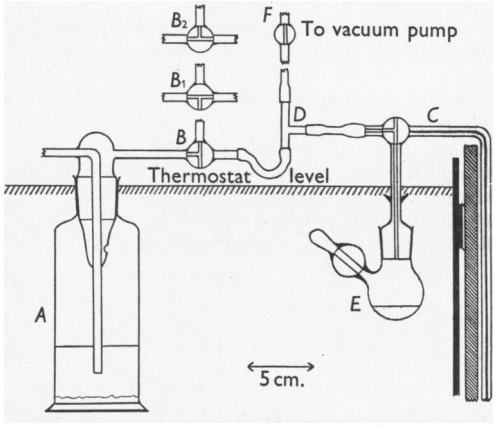

Fig. 1. Manometric apparatus used for the determination of the solubility of O_2 in non-aqueous fluids. For description see text.

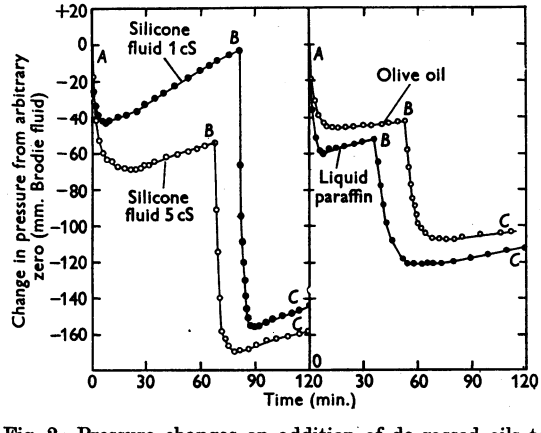

Fig. 2. Pressure changes on addition of de-gassed oils to oxygen atmospheres. At zero time A the de-gassed oil is isolated in the side chamber of the vessel and the remainder of the vessel filled with O_2. After pressure changes become regular the de-gassed oil is allowed to run into the vessel at B by turning a stop-cock and the resulting fall in pressure measured.

Typical curves illustrating the above procedure for the four oils examined are given in Fig. 2. The total change in pressure on adding the de-gassed oil was calculated by extrapolation of the rate at which the oil was evolving its vapour. Extrapolation could be avoided by waiting several hours for steady values and this procedure was found to give the same results.

The solubility of O_2 in the oil was calculated from the following considerations: Let V_g = vol. of vessel not occupied by oil; V_1 = total vol. of oil added to vessel; $V_2 = V_1$ less vol. of oil in side chamber; T = temp. of thermostat (° K); P_0 = standard pressure of 760 mm. Hg; P = pressure in vessel immediately before addition of de-gassed oil, being atm. pressure plus the difference in pressure registered in the manometer; h = change in pressure on addition of de-gassed oil (corrected); α (Bunsen coefficient) = ml. of gas (at s.t.p.) dissolved by 1 ml. of oil at temp. T and under a partial pressure of 760 mm. Hg.

Initially the O_2 in the vessel comprises that in the gas phase, $V_g\ 273P/TP_0$, and that in the oil phase, $V_2\alpha P/P_0$. At the end of the experiment these values are:

$$V_g\ 273(P-h)/TP_0 \quad \text{and} \quad V_1\alpha(P-h)/P_0.$$

As the total amount of gas remains unchanged it follows that

$$V_g\ 273\ P/TP_0 + V_2\alpha P/P_0 = V_g\ 273(P-h)/TP_0 + V_1\alpha(P-h)/P_0$$

and

$$\alpha = hV_g\ 273/T[P(V_1 - V_2) - hV_1].$$

RESULTS AND DISCUSSION

Values for the solubility of oxygen in liquid paraffin, olive oil and silicone fluids of viscosities 1 and 5 cS., as determined by this method are given in Table 1. The figures for silicone fluids are surprisingly high.

Table 1. *The solubility of oxygen in liquid paraffin, olive oil and silicone fluid*

Temperature, 38°; αO_2 (38°) = ml. of O_2 (at s.t.p.) dissolved by 1 ml. of oil at 38° and under a partial pressure of 760 mm. Hg. Where sufficient data is available mean values are quoted followed by the standard deviation and the number of determinations in parentheses.

Oil	αO_2 (38°)
Liquid paraffin (sp.gr. 0·835)	0·098 ± 0·0017 (5)
Olive oil, B.P. (1948)	0·102 ± 0·0061 (6)
Silicone fluid (viscosity 5 cS.)	0·251, 0·250, 0·210
Silicone fluid (viscosity 1 cS.)	0·304, 0·297

The scale of the method limits its accuracy to ± 5 % in a single determination, which is adequate for determinations of solubilities required for calculating manometer-vessel constants. Greater precision can be obtained by repeated determinations (see Table 1) and by increasing the dimensions of the apparatus. The special advantage of the present method compared with that of Davidson *et al.* (1952), lies in the fact that the initial pressure in the vessel is accurately measured, rather than estimated by extrapolation. In addition, temperature equilibration is ensured, as preliminary separation of the oil allows time for it to regain the temperature of the thermostat after cooling which occurs during evacuation.

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Electrical Pulses and the Metabolism of Cell-free Cerebral Preparations

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Metabolic response of sliced or chopped cerebral tissues to electrical pulses has shown several characteristics relating it to electrophysiological properties of the central nervous system (McIlwain, 1951*a*; 1954). Many of these properties can be interpreted as dependent on movements of ions to and from cells (see, for example, data collected by Eccles, 1953).

Certain changes in respiration and phosphorylation in cell-free preparations with applied electrical pulses (Abood, Gerard & Ochs, 1952) have been considered similar to those previously found in cell-containing systems. Responses in the two types of preparations have now been examined in parallel, and considerable differences found between them.

EXPERIMENTAL AND RESULTS

Particulate preparations

Glycolytic and respiratory responses to electrical pulses were less when cerebral tissues were very finely chopped and were doubtful or absent in 'homogenized' tissue, examined under conditions otherwise similar to those of the experiments with slices (Table 1*A*).

Media suitable for metabolism of sliced tissue are not optimum with cell-free preparations. In particular, in view of the connexion between changes in respiration, glycolysis, and phosphate derivatives in sliced cerebral tissue (McIlwain, 1952) it appeared desirable to examine preparations capable of oxidative phosphorylation. Two sets of conditions were chosen, using suspensions of the whole tissue, and a mitochondrial preparation.

Suspensions. In early experiments using electrodes of electrodeposited gold, partial but erratic inhibition of both respiration and phosphorylation was observed. Artifacts have previously been traced to the metal of the electrodes (McIlwain, 1951*a*) and molybdenum is free from many such effects (Ayres & McIlwain, 1953). Potential loss at molybdenum–saline interfaces is somewhat greater than at gold–or silver–saline interfaces, but full metabolic response in slices is readily obtained (Table 1*A*). Vessels with molybdenum electrodes were therefore used for examining effects of applied electrical pulses on suspensions under conditions in which phosphorylation was actively proceeding at some 200–300 μatoms P esterified/g. tissue/hr., with P/O ratios (atom/atom) of 1·2–1·8. Under these circumstances, pulses which with slices increased respiration twofold and glycolysis threefold, with suspensions increased oxygen uptake slightly (mean, 14 %) and phosphate esterification to nearly the same degree (10 %). The P/O ratio thus appeared to fall very slightly. This effect was extremely small in comparison with that which can be brought about by an agent such as 2:4-dinitrophenol (Table 1*B*) in the suspensions or by comparable pulses on the phosphates of sliced cerebral tissues (McIlwain & Gore, 1951).

It was concluded that the metabolic effects of electrical pulses on suspensions even during phosphorylation were of a different order from those brought about by pulses of the same characteristics on sliced tissues.