enzyme at pH 2·0 and more slowly at pH values above 4·0.

3. The zymogen has one *N*-terminal residue and a molecular weight close to that of the enzyme.

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Effect of Ethanol on Respiration of Rat-Brain-Cortex Slices

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Potassium ions and some other agents enhance the respiration and glycolysis of nervous tissue *in vitro* (Ashford & Dixon, 1935; Dickens & Greville, 1935), as do applied electrical pulses McIlwain, 1951*a*). The respiration of stimulated tissue is affected by narcotics at pharmacologically active concentrations (Bronk & Brink, 1951; McIlwain, 1953*a*; Buchel, 1953), whereas that of unstimulated tissue is not.

Heavy ethanol intoxication in human subjects is associated with diminished cerebral oxygen consumption (Hine, Shick, Margolis, Burbridge & Simon, 1952; Battey, Heyman & Patterson, 1953). Recent work with potassium-stimulated brain-cortex tissue indicates that its oxygen consumption is slightly but significantly depressed at 'physiological' ethanol concentrations (Ghosh & Quastel, 1954; Sutherland, Hine & Burbridge, 1956; Fischer, 1957; Beer & Quastel, 1958*b*), whereas glucose consumption and lactic acid accumulation appear to be enhanced (Sutherland *et al.* 1956). Ethanol in corresponding concentrations slightly elevates unstimulated respiration, at least in the initial phase of the experiment (Fuhrman & Field, 1948; Ghosh & Quastel, 1954; Sutherland *et al.* 1956; Beer & Quastel, 1958*b*).

Here we report studies *in vitro* on the oxygen and glucose consumption and lactic acid formation in stimulated and unstimulated slices of cerebral cortex from the rat. The first aim was to determine whether ethanol had any effect at all in our experimental conditions. A depression of the respiration was observed in tissues stimulated electrically or with potassium chloride. Since changes in glucose concentration did not influence the ethanol effect, it was concluded that there cannot be any competition between ethanol and glucose for access to the enzymes. The nature of the non-competitive inhibition could not be defined.

MATERIAL AND METHODS

Apparatus. All experiments were performed with conventional Warburg constant-volume respiratory manometers and vessels of 13–16 ml. volume. The flasks for electrical stimulation of tissue were modified from the type described by McIlwain (1951*b*) as 'conical electrode vessels E' (Fig. 1). Before experiments, silicone grease was applied to the base of the platinum wires. After each experiment, the electrodes were removed, washed with detergent, rinsed with water, dipped for 15 sec. into dichromate–H_2SO_4 solution, rinsed with water and dried. To facilitate the application and removal of the electrodes, the

vessels were made wide-necked and provided with ground-glass collars for fitting them to the manometers. Calibration was done with mercury: the electrodes were of pure silver and their volume was calculated from their weight.

The stimulators were similar in principle to that described by McIlwain (1951*a*). Condensers (4 μF) operated with direct current from the mains (125 v) were charged and discharged through the vessels by means of autoradio vibrators (Philips AP 6014). The current to the condensers was regulated with variable resistors. Vibrator frequency was 90–100 cyc./sec., as stated by the factory. Each vessel was connected with a separate stimulator. Switches were arranged so that voltage and current could be measured at any time during the course of an experiment in each of the stimulated vessels. Voltage was measured at the flasks by means of an electronic voltmeter and current with an ordinary ammeter. A voltage gradient between the electrodes of 1·6–1·8 v/mm. was maintained during stimulation. Equipment was not available for measurements of shape, frequency and time-constant of the pulses, and respiratory response remained the practical criterion of successful stimulation. For the stimulation by K^+ ions 0·1 M-KCl was used.

Experimental procedure. The experimental conditions were as follows: 2·5 ml. of phosphate–glucose medium (McIlwain, 1951*a*) with 6 mM-glucose was used if not otherwise stated; concentration of ethanol was 0·087 M (0·4 %), when used; gas phase was pure oxygen; absorption of carbon dioxide was by 0·1 ml. of KOH (20 %) on folded filter paper; temperature was 37·5°, except when otherwise stated; shaking was at 96 complete strokes/min.; initial gassing and equilibration were for 5 + 10 min. at experimental temperature; after transfer of slices to vessels, they were gassed for 3 min., the required ethanol or KCl solutions (0·2 ml.) were pipetted into side arms and equilibration for 10 min. was allowed; after this, ethanol was tipped into the main compartment and stimulation began; the manometer reading was taken every 10 min. for 1 hr., beginning 5 min. after commencement of stimulation.

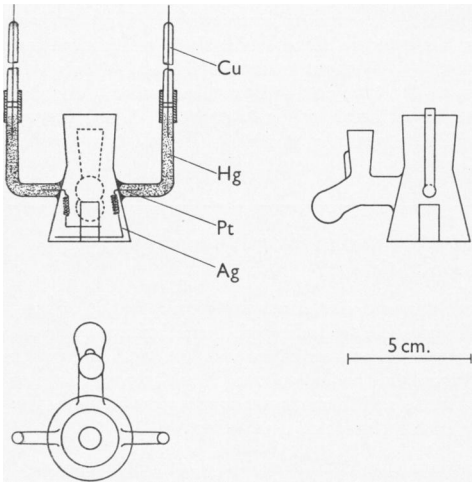

Fig. 1. Vessel employed for application of pulses to tissue slices. Connexions to stimulators are made with platinum lead-in wires (0·5 mm. diam.), side arms are filled with mercury and copper wire in glass tubing is attached to the side arms by means of rubber joints. The concentric electrodes are made of 0·5 mm. silver wire. The centre electrode ends in a helix pushed on the lead-in wire. The helical connexion of the larger electrode at the bottom is flexibly attached to the ring.

Electrical pulses passed at the chosen voltage through vessels containing experimental medium caused no measurable changes of pressure. Unstimulated tissue respired similarly in electrode flasks and in standard flasks. As a rule, experiments were set up with two standard vessels and two electrode vessels, one of each being used for controls and the other for ethanol exposure.

Adult rats from the Laboratory supply were used, with males and females in equal proportion in each experimental series. Slices were prepared in the cold room with cooled equipment and ice-cold experimental medium for cooling the brain on exposure. Preparation was as described by McIlwain (1951*a*), except that the cortex was not unfolded: instead the hemispheres were placed on the lateromedial surface in a guiding template, and slices (0·35 mm.) were cut directly, three slices being taken from each hemisphere. It was found essential to excise and cool the brain in at most 150 sec. to ensure full respiratory response to both potassium and electrical stimulation. Of the slices from one hemisphere, one (25–40 mg.) was used for determination of respiration of unstimulated tissue and two slices (50–70 mg.) for determination of respiration of stimulated tissue. To avoid systematic errors in the procedure, the following alternations were made: the first and second or the second and third slices were used for stimulation; tissue from the left or the right hemisphere was used as control, that from the other was exposed to ethanol; the first or the second pair of vessels contained control medium, the other ethanol–medium. The dry weight of twelve slices prepared in the same way as those used for respiration experiments was determined by drying for 4 hr. at 110° and was 20·7 % (S.D. 0·37 %).

Usually 10–15 min. elapsed from decapitation of the animal until the vessels were in the thermostat. After the last manometer reading had been taken, samples (1 ml.) were rapidly withdrawn for determination of glucose (Somogyi & Nelson, 1945) and for lactic acid determination (Barker & Summerson, 1941). In our hands, these analytical methods both gave S.D. 4 %.

Comparison was made of respiration during the first hour after equilibration of tissue slices stimulated during the whole period with that of unstimulated slices during the same period. This was because a continuous fall in response to stimulation was found in our experiments, as in those of Sutherland *et al.* (1956). Calculations of the results were based on unit fresh weight. No corrections were made for lactic acid present or glucose consumed at the beginning of the reading of the manometers, since such correction would not reduce the variation.

The statistical significance of the ethanol effect was calculated by the *t* test for non-independent means, since in every experiment the tissue in the control medium and in the ethanol-containing medium was from the same brain. The effect of other experimental variables was evaluated by Student's *t* test.

RESULTS

Unstimulated tissue

Oxygen consumption. During the experimental period, there was no indication of change in respiratory rate except that maximum uptake of oxygen was apparently not attained at the commencement of readings (Fig. 2). Changing glucose concentration did not influence oxygen consumption (see also Table 2). Ethanol caused an increase in respiration. This was statistically significant in the series with 6 mM-glucose as well as for the combined results for the experiments with all three glucose concentrations.

Glucose consumption. Glucose consumption was approximately the same at 3 and 6 mM concentration in the medium (Table 1). There was a significant increase when the initial glucose concentration was 10 mM (Table 2). Ethanol caused a significant augmentation of glucose consumption, both for the series with 6 mM-glucose and for the combined results for all three glucose concentrations. The change induced by ethanol was of the same order of magnitude and in the same direction as that in oxygen consumption.

Lactic acid formation. The formation of lactic acid (Table 1) was not significantly influenced by change in initial glucose concentration of the medium or by the presence of ethanol.

Glucose loss in terms of oxidation and glycolysis. Balance calculations were performed assuming that 6 molecules of oxygen are consumed for each molecule of glucose completely oxidized, or 2 molecules of lactic acid formed from each molecule of glucose in glycolysis. The figures given in the last column of Table 1 suggest that glucose was partly metabolized to compounds not measured in the present experiments. McIlwain, Anguiano & Cheshire (1951) performed similar balance calculations and found that all glucose that disappeared could be accounted for by respiration and glycolysis. Their experiments were, however, extended over at

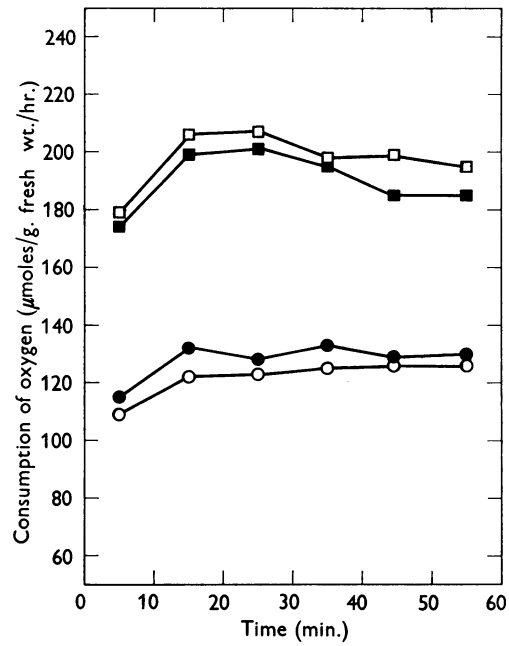

Fig. 2. Time course of respiration of rat-brain-cortex slices incubated *in vitro* in phosphate medium with 6 mM-glucose. ○, Without stimulation and without ethanol (mean of 41 expts.); ●, without stimulation in the presence of 0·087 M-ethanol (41 expts.); □, 0·1 M-KCl without ethanol (23 expts.); ■, 0·1 M-KCl in the presence of 0·087 M-ethanol (21 expts.).

Table 1. *Oxygen consumption, glucose consumption and lactic acid formation of unstimulated cerebral-cortex tissue*

The results are expressed as μmoles/g. of fresh tissue/hr. and standard deviations are given. In the column headed Experimental conditions, control series (C) and ethanol series (E) are indicated. Initial glucose concentration of the medium is given. *n*, Number of experiments. The ethanol concentration was 0·087 M. The level of significance of effects of ethanol is indicated in the footnotes. Figures in the last column show the percentage of glucose loss to be accounted for by the oxygen consumption and lactic acid formation, assuming that 6 molecules of O_2 are consumed or 2 molecules of lactic acid are formed for each molecule of glucose lost.

Experimental conditions	Concn. of glucose (mM)	*n*	Oxygen consumed (μmoles)	Glucose consumed (μmoles)	Lactic acid formed (μmoles)	Loss of glucose accounted for (%)
C	3	13	128±17	78±28	67±18	81·0
E	3	13	132±16	86±32	63±19	62·2
C	6	41	122±17	70±9	75±6	81·8
E	6	41	132±15*	86±10†	75±6	75·1
C	10	9	126±11	102±51	77±31	58·3
E	10	9	127±13	110±47	80±20	55·6

* $P < 0·01$ (combined ethanol effect at all concentrations of glucose $P < 0·01$).
† $P < 0·001$ (combined ethanol effect at all concentrations of glucose $P < 0·001$).

Table 2. *Evaluation of the effect of changing glucose concentration and temperature on respiration and metabolism of unstimulated cerebral-cortex tissue*

The control series with 6 mM-glucose at 37·5° serves as a standard of reference. Significance of differences between means has been obtained by t tests and is indicated as the P value. NS, Not significant.

Variable	3 mM	10 mM	30°	41·2°
Oxygen consumed	NS	NS	<0·001	<0·001
Glucose consumed	NS	<0·001	<0·05	<0·005
Lactic acid formed	NS	NS	NS	<0·025

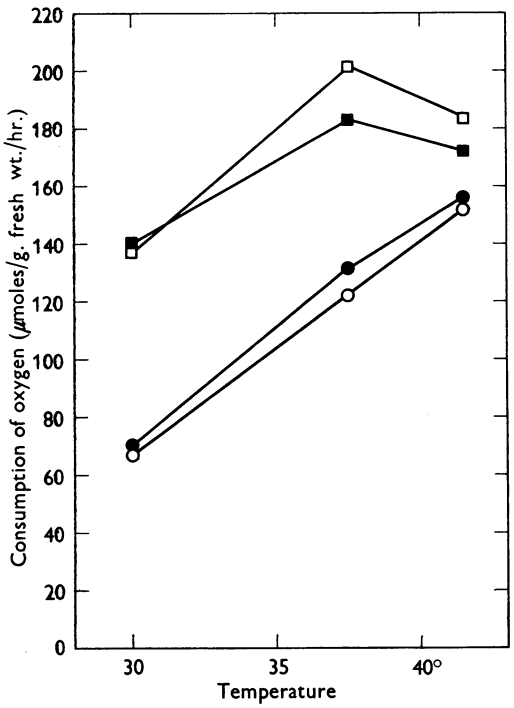

Fig. 3. Effect of temperature on the oxygen consumption of rat-brain-cortex slices incubated in phosphate medium with 6 mM-glucose. ○, Unstimulated, without ethanol (mean of 10 expts. at 30 and 41·5° and 41 expts. at 37·5°); ●, unstimulated in the presence of 0·087 M-ethanol (no. of expts. as without ethanol); □, stimulated without ethanol (10 expts. at 30 and 41·5°; 20 at 37·5°); ■, stimulated in the presence of 0·087 M-ethanol (no. of experiments as without ethanol).

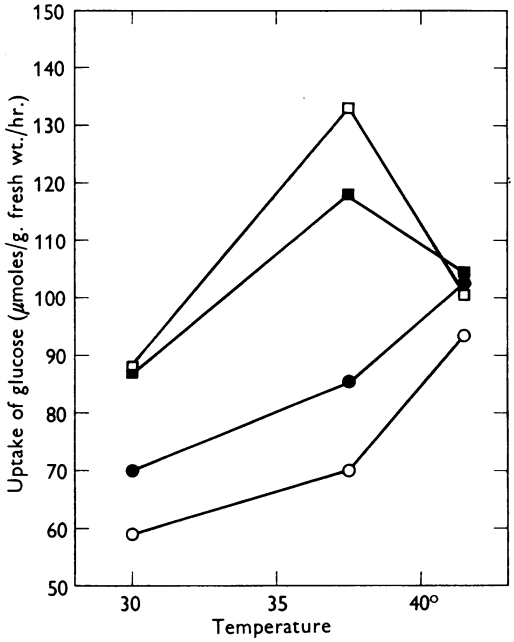

Fig. 4. Effect of temperature on the glucose uptake of rat-brain-cortex slices incubated in phosphate medium with 6 mM-glucose. ○, Unstimulated, without ethanol (mean of 10 expts. at 30° and 41·5° and 41 expts. at 37·5°); ●, unstimulated in the presence of 0·087 M-ethanol (no. of expts. as without ethanol); □, stimulated without ethanol (10 expts. at 30 and 41·5°; 20 at 37·5°); ■, stimulated in the presence of 0·087 M-ethanol (no. of expts. as without ethanol).

least 150 min. We found that in seven experiments of 2 hr. duration, glucose consumption was 51 ± 8 μmoles/g. of fresh tissue/hr. Lactic acid formation was 69 ± 15 μmoles/g. of fresh tissue/hr. Oxygen consumed and lactic acid formed corresponded to 106 % of the glucose loss.

Effect of temperature. Within the temperature range covered in our experiments, unstimulated respiration showed a linear relationship to temperature with Q_{10} 2·1, i.e. typical of biological phenomena dependent on enzymic reactions (Fig. 3). Glucose consumption was also significantly influenced by temperature (Fig. 4 and Table 2). A drop in temperature did not alter lactic acid production, whereas this was significantly increased by a rise in temperature to +41·5° (Fig. 5). Within the limits of experimental variation, the ethanol effect appeared to be unaltered by change of temperature except in the lactic acid production.

Stimulated tissue

Oxygen consumption. The values are given in Table 3. Evaluation of the results was based on the absolute values. This was because Fischer (1957) and Beer & Quastel (1958*b*) noticed that

with the same concentration of ethanol oxygen consumption of stimulated tissue could be depressed to lower values than that of unstimulated tissue. Potassium and electrical stimulation both increased the oxygen consumption to about 65% above the control unstimulated value at 37·5° with 6 mM-glucose. Variation in glucose concentration did not influence oxygen consumption significantly (Table 4). The respiration rate of electrically stimulated tissue plotted against time showed a progressive fall. The slopes were identical at all glucose concentrations (Fig. 6) and differed markedly from that of the respiration during potassium stimulation (Fig. 2).

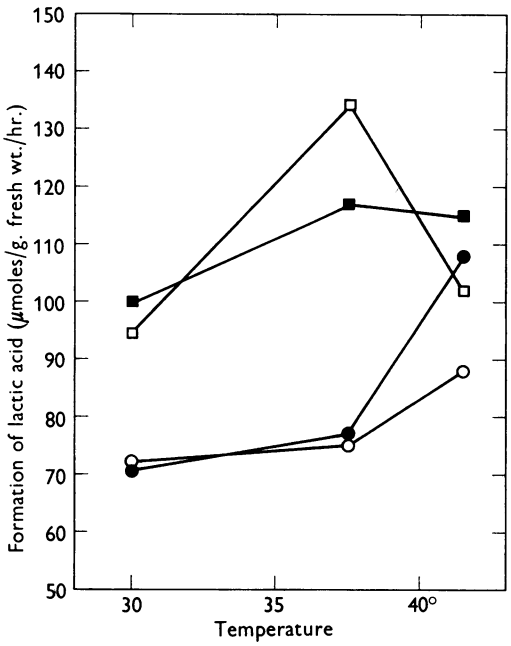

Fig. 5. Effect of temperature on the lactic acid formation of rat-brain-cortex slices incubated in phosphate medium with 6 mM-glucose. ○, Unstimulated without ethanol (mean of 10 expts. at 30° and 41·5° and 41 expts. at 37·5°); ●, unstimulated in the presence of 0·087 M-ethanol (no. of expts. as without ethanol); □, stimulated without ethanol (10 expts. at 30° and 41·5°, 20 at 37·5°); ■, stimulated in the presence of 0·087 M-ethanol (no. of expts. as without ethanol).

Ethanol had a statistically significant inhibitory effect. Potassium-stimulated tissue was apparently more resistant than electrically stimulated tissue. The inhibition caused by ethanol was the same at all glucose concentrations employed. This result was confirmed by statistical treatment and a Lineweaver–Burk plot based on the average oxygen consumption during the experimental hour. It was further corroborated by the fact that the effect of ethanol did not change with time and hence decreasing glucose concentration.

Glucose consumption. Both methods of stimulation increased the glucose consumption (Table 3). With potassium chloride, glucose consumption was significantly higher than with electrical excitation (Table 4). Increased glucose concentration was paralleled by augmented consumption (Tables 3 and 4). Ethanol decreased the glucose consumption in electrically stimulated tissue, a change in the same direction as in oxygen consumption, whereas there was an increase with potassium-stimulated tissue.

Lactic acid formation. As shown in Tables 3 and 4, potassium stimulation caused a substantially larger increase in lactic acid formation than electrical stimulation. When initial glucose concentration was decreased to 3 mM, lactic acid formation was diminished, whereas there was no effect of change from 6 to 10 mM (Table 3). The effect of ethanol was more irregular than on glucose con-

Table 3. *Oxygen consumption, glucose consumption and lactic acid formation of stimulated cerebral-cortex tissue*

Results are expressed as μmoles/g. of fresh tissue/hr. and standard deviations are given. *P* values for statistically significant differences between control and ethanol series are indicated. In the column headed Experimental conditions, K, potassium stimulation; S, electrical stimulation; C, control; E, ethanol (0·087 M). Initial glucose concentration is given. *n*, Number of experiments. NS, not significant.

Experimental conditions	Concn. of glucose (mM)	*n*	Oxygen consumed (μmoles)	*P* <	Glucose consumed (μmoles)	*P* <	Lactic acid formed (μmoles)	*P* <	Loss of glucose accounted for (%)
K, C	6	23	204±13	0·025	171±38	0·025	190±30	NS	75·4
K, E	6	21	196±13		189±45		195±30		68·9
S, C	3	13	192±19	0·025	91±21	NS	63±15	NS	69·8
S, E	3	13	175±17		81±17		69±19		78·6
S, C	6	20	202±13	0·001	133±27	0·001	134±21	0·001	75·7
S, E	6	20	183±15		118±26		117±26		75·4
S, C	10	9	200±11	0·025	168±39	NS	128±25	NS	57·9
S, E	10	9	184±18		145±47		125±39		64·3

Table 4. *Evaluation of the effect of changing experimental conditions on respiration and metabolism of stimulated cerebral-cortex tissue*

The electrically stimulated control series with 6 mM-glucose at 37·5° serves as standard of reference. Significance of difference between means has been obtained by t tests and is indicated as the P value. NS, Not significant.

Variable	0·1 M-KCl	3 mM	10 mM	30°	41·5°
Oxygen consumed	NS	NS	NS	<0·001	NS
Glucose consumed	<0·001	<0·001	<0·025	<0·001	<0·001
Lactic acid formed	<0·001	<0·001	NS	<0·01	<0·001

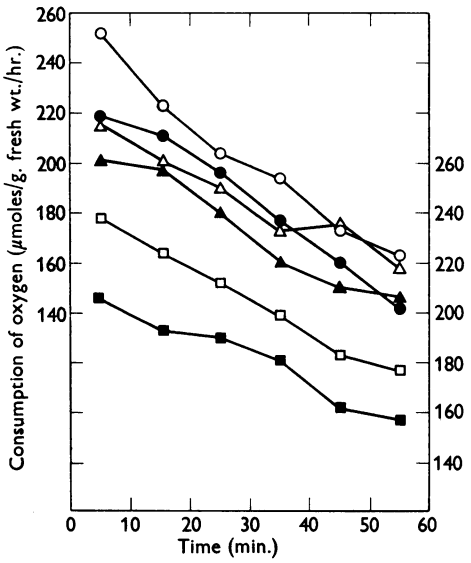

Fig. 6. Time course of respiration of electrically stimulated rat-brain-cortex slices incubated at 37·5° in phosphate medium with different initial glucose concentration. △, 3 mM-Glucose, no ethanol (mean of 13 expts.); ▲, 3 mM-glucose, 0·087 M-ethanol (13 expts.); □, 6 mM-glucose, no ethanol (20 expts.); ■, 6 mM-glucose, 0·087 M-ethanol, (20 expts.); ○, 10 mM-glucose, no ethanol (9 expts.); ●, 10 mM-glucose, 0·087 M-ethanol (9 expts.). Left ordinate, rate of oxygen uptake with 3 and 10 mM-glucose; right ordinate, rate with 6 mM.

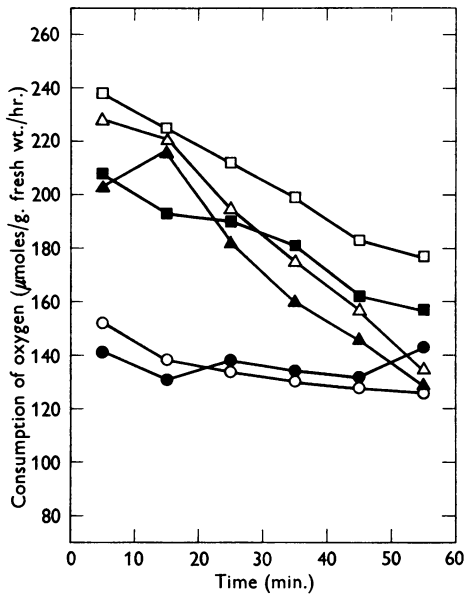

Fig. 7. Time course of respiration of electrically stimulated rat-brain-cortex slices incubated in phosphate medium with 6 mM-glucose at different temperatures. ○, At 30°, no ethanol (mean of 10 expts.); ●, at 30°, 0·087 M-ethanol (10 expts.); □, at 37·5°, no ethanol (20 expts.); ■, at 37·5°, 0·087 M-ethanol (20 expts.); △, at 41·5°, no ethanol (10 expts.); ▲, at 41·5°, 0·087 M-ethanol (10 expts.).

sumption. In the largest series with electrical excitation, however, there was a clear drop in lactic acid formation. This was also statistically significant ($P < 0·005$) when calculated for the combined results from the experiments with all three glucose concentrations at 37·5°.

Glucose loss in terms of oxidation and glycolysis. Results of balance calculations performed as for unstimulated tissue are shown in Table 3 (last column). The same remarks apply as for the unstimulated tissue. In seven experiments of 2 hr. duration, glucose consumption was 64 ± 7 μmoles/g. of fresh tissue/hr. Lactic acid formation was 79 ± 15 μmoles/g. of fresh tissue/hr. Oxygen consumed and lactic acid formed corresponded to 99·5 % of the glucose loss.

Effect of temperature. Both at 30° and 41·5° oxygen consumption of the electrically excited tissue was lower than at 37·5° (Fig. 3). The time course was strongly influenced by temperature (Fig. 7). The slopes of the curves representing rate of oxygen consumption as a function of time become steeper with increase in temperature. The average stimulation during the experimental hour was 104 % above the control at 30° (falling from 128 % during the first 10 min. to 90 % during the last period), 65 % at 37·5° (falling from 95 to 45 %), and 21 % at 41·5° (falling from 50 to −12 %). Because of this, the Q_{10} was calculated for the first 10-min. period of manometer reading on the basis of the results obtained at 30° and 37·5°. It was 1·75, i.e. considerably lower than for the

unstimulated tissue. Change in temperature caused highly significant changes in glucose consumption (Fig. 4 and Table 4). The inhibitory effect of ethanol on oxygen consumption had completely disappeared at 30° (Figs. 3 and 7). At 41·5° it was approximately the same as at 37·5°.

DISCUSSION

Stimulation of brain tissue *in vitro* appears to be possible only with preparations in which the cell structure is largely intact (Kimura, 1937). Under certain conditions applied electrical pulses alter the metabolism of homogenates and mitochondrial preparations (Abood, Gerard & Ochs, 1952), but these alterations differ qualitatively and quantitatively from those obtained with slices (Narayanaswami & McIlwain, 1954). Induced electrical activity of the type obtainable in undercut isolated slabs of cortical tissue (Burns, 1958) has not been observed in brain-cortex slices as used in manometric experiments (McIlwain & Ochs, 1952). However, there are other important metabolic and physiologic parallels between electrically stimulated brain slices and the brain *in vivo* (cf. Forda & McIlwain, 1953; McIlwain, 1954), whereas unstimulated slices represent conditions more remote from those prevailing in living, intact brain. Because of this, our results with stimulated and unstimulated tissue will be discussed separately.

Unstimulated tissue

Effect of ethanol. Increase in oxygen consumption when ethanol is added in relatively low concentration to media containing glucose as substrate has been observed by other workers (Fuhrman & Field, 1948; Ghosh & Quastel, 1954; Sutherland *et al.* 1956; Beer & Quastel, 1958*b*). The mechanism by which this occurs is obscure. It has been assumed that the elevated respiration is due to oxidation of the ethanol. Several investigators have been unable to show metabolism of ethanol in brain tissue (Bartlett & Barnet, 1949; Masoro, Abramovitch & Birchard, 1953; Beer & Quastel, 1958*a*). On the other hand, Burbridge, Sutherland, Hine & Simon (1959) have reported an appreciable consumption of ethanol in rat-brain slices. In our experimental conditions, oxidation of ethanol by rat-brain slices could not be demonstrated, whereas the ethanol was normally metabolized by liver slices. Ethanol has not been observed to act as an uncoupling agent of the oxidative phosphorylation (Truitt, Bell & Krantz, 1956). Our results demonstrate that the increase in respiration is accompanied by increased glucose consumption. Thus glucose probably furnishes the substrate for the enhanced oxidation. Low concentrations of ethanol slightly depolarize the neuronal membrane and increase neuronal excitability (Wright, 1947; Gallego, 1948; Larrabee & Posternak, 1952), and thus functional activation may be the underlying cause of the shift of respiration to a higher level.

Glucose concentration. The change in concentration of glucose employed did not influence the rate of respiration. This is in agreement with McIlwain's (1953*b*) observation that unstimulated tissue will be affected only when the glucose concentration drops to the range 0·5–1·5 mM.

Temperature. In a study on the respiration of unstimulated rat-brain tissue in the temperature range 0·2–47·5°, Field, Fuhrman & Martin (1944) demonstrated a non-linear relationship between Q_{O_2} and temperature, Q_{10} 2·128 over the range 10·0–37·5° and a progressive decrease in respiration with time when the temperature was over 40°. Field *et al.* made a larger number of observations than we did in the temperature range 30·0–41·5°, and their experiments were extended over periods of at least 90 min. This may explain why our results suggest a simple linear relationship of oxygen consumption to temperature, which is probably not a correct picture of the necessarily complicated responses. Our short experimental period makes it understandable why there is no significant drop in the rate of oxygen uptake towards the end of our experiments at 41·5°. Glucose uptake and lactic acid formation are higher at 41·5° than a linear dependence would imply.

Stimulated tissue

Effect of ethanol. The respiration and consumption of glucose were clearly decreased when ethanol was present. The opposite effects of ethanol on stimulated and on unstimulated tissue is an apparent paradox. However, if our suggestion is correct, that the depolarizing action of ethanol is related to its effect on respiration of unstimulated tissue, then these seemingly contradictory phenomena may both be related to the function of excitable neuronal structures. The ethanol concentration in our experiments represents approximately half the lethal blood concentration for rats. The magnitude of the depression of respiration obtained with potassium-stimulated tissue corresponds well to that observed by other investigators (Ghosh & Quastel, 1954; Sutherland *et al.* 1956; Beer & Quastel, 1958*b*). When condenser pulses were used for stimulation, more pronounced inhibition was obtained.

Comparison of ethanol and other depressants. In the presence of depressants, potassium stimulation and electrical stimulation have a different effect on glucose consumption and lactic acid accumulation. Sutherland *et al.* (1956) observed that ethanol (0·056 M) induced a significant increase in glucose uptake and lactic acid formation of tissue stimu-

lated with potassium. In our experiments with potassium stimulation, both were slightly larger with ethanol (0·087 M) than without. McIlwain (1953*a*) found that depressants caused a fall in respiratory rate, glucose consumption and lactic acid accumulation in stimulated isolated brain tissue, but that lactic acid formation was much less affected in tissue stimulated with potassium (0·033 M) than in electrically excited tissue. Thus our results with electrically stimulated tissue in the presence of ethanol show agreement with those obtained by McIlwain using other depressants. They also indicate that ethanol acts without delay and, during a 1 hr. period, independently of time. The same conclusion has been drawn with respect to ethanol by Beer & Quastel (1958*b*) and with respect to other depressants by McIlwain (1953*a*).

Effect of temperature. As is demonstrated by the result obtained at 41·5°, the respiratory and metabolic response to rising temperature will be an increase up to some critical temperature, above which a drop will occur. Progressive warming above body temperature also depresses the functional activity of the spinal cord (Brooks, Koizumi & Malcolm, 1955). It should further be noted that the slope of the graphs expressing rate of respiration as a function of time becomes steeper with increasing temperature (Fig. 7). This may in part be attributable to progressive inactivation of enzyme systems or cellular elements, which tends to be more rapid at higher temperatures. The relative increase in respiration when pulses are applied is higher at 30° than at 37·5°. This may be related to the known fact that whereas at moderately lowered temperature the excitation threshold of neuronal elements in the spinal cord is increased, the amplitude and duration of action potentials is also markedly larger (for review and experimental evidence, cf. Brooks *et al.* 1955).

Mechanism of inhibitory effect of ethanol. The mechanism of ethanol action is not known. The respiratory response of brain tissue to stimulation appears to be dependent on preservation of cell structure, on the operation of the citric acid cycle and on the presence of glucose or pyruvate as substrate (Kimura, 1937; McIlwain & Gore, 1953; Kratzing, 1953; McIlwain, 1953*b*, *c*; Beer & Quastel 1958*b*). Because pharmacologically active concentrations of ethanol depress the respiration of stimulated tissue only, it seems evident that the effect of ethanol is directed towards either the functional response to stimulation or the metabolic system supporting this response.

Himwich (1956) has suggested that ethanol may interfere with the metabolism of energy-rich phosphates. As mentioned in the preceding section, ethanol does not appear to act by uncoupling of oxidative phosphorylation. The hypothesis that conversion of ethanol into acetaldehyde contributes to its action in brain slices has been tentatively supported (Sutherland *et al.* 1956) but seems untenable in view of the results reported by Beer & Quastel (1958*a*, *b*).

Ethanol does not affect mitochondrial respiration in pharmacologically active concentration (Beer & Quastel, 1958*b*). This observation seems very important in view of the fact that the tricarboxylic acid cycle, fatty acid oxidation, oxidative phosphorylation and terminal-electron transport are all connected with the functioning of structurally organized enzyme systems within the mitochondria (for review, see Green, 1956–57). Results obtained by one of us (Wallgren, 1960) have shown that ethanol and malonate have no additive effect. Thus ethanol does not seem to interfere with any enzyme involved in the dehydrogenation of succinic acid.

Sutherland *et al.* (1956), as well as Ghosh & Quastel (1954), interpret their results as indicating an effect of ethanol on a potassium-sensitive phase in nerve respiration which is concerned with glucose or pyruvate metabolism. Our demonstration that ethanol acts independently of glucose concentration in the medium suggests that its depression of respiration is not due to competition with glucose. Quastel (1952) has shown that some other narcotics non-competitively inhibit pyruvate- or lactate-supported respiration of brain-cortex slices. The available evidence is not at variance with the conclusion of Beer & Quastel (1958*b*) that the membrane of excitable cells is the most likely site for the pharmacological action of ethanol. It also makes it probable that the depression of respiration is a secondary effect due to disturbance of an energy-demanding functional process.

SUMMARY

1. The effect of ethanol on the respiration of unstimulated and stimulated rat-brain-cortex slices was investigated. The consumption of glucose and the formation of lactic acid were also measured.

2. In unstimulated slices at 37·5° the addition of ethanol (0·087 M) increased the consumption of both oxygen (+8 %, $P < 0·01$) and glucose (+23 %, $P < 0·001$), but did not affect the formation of lactic acid.

3. In electrically stimulated tissue at 37·5° ethanol caused a decrease in the respiration (−9·5 %, $P < 0·001$), glucose consumption (−11 %, $P < 0·001$) and lactic acid accumulation (−12·5 %, $P < 0·001$). These changes were unaffected by varying the initial glucose concentration in the medium from 3 to 10 mM. In potassium-stimulated slices the effect of ethanol on the respiration was

less marked and the glucose uptake and lactic acid accumulation increased.

4. In unstimulated slices respiration, glucose consumption and lactic acid formation increased with rise of temperature (30·0–41·5°). In electrically stimulated preparations the functions mentioned above increased from 30° to 37·5° but decreased at 41·5°. Ethanol had no effect at 30°.

5. The relationship of these results to those obtained in earlier investigations with ethanol and other depressants is discussed. It is concluded that the action of ethanol is non-competitive and directed towards some process linked with the physiological functioning of nerve tissue.

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Purification of the Two Components of Leucocidin from *Staphylococcus aureus*

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Staphylococcal leucocidin inhibits the respiration (Neisser & Wechsberg, 1901) and produces characteristic morphological changes (Gladstone & van Heyningen, 1957) in the white blood cells of humans and rabbits. It has been shown that this activity results from the synergistic action of at least two substances, since the activity is lost when leucocidin preparations are separated on carboxy-

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