The Effect of Depressants on the Metabolism of Stimulated Cerebral Tissues

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Depression of the activity of the central nervous system by agents such as barbiturates* is accompanied by metabolic changes in the brain itself. The changes include a fall in respiration, lactic acid, and inorganic phosphate and a rise in phosphocreatine. In investigating the action of depressants, various attempts have been made to reproduce these changes under conditions simpler than those of the whole animal. The metabolic characteristics named can all be measured in cerebral tissues *in vitro*. However, none of the changes brought about in them by depressants *in vivo* has yet been unequivocally reproduced in cerebral tissues *in vitro* by concentrations of the depressants used therapeutically.

Thus, when change occurs in the aerobic formation of lactic acid, it is in opposite senses under the two conditions. It is unchanged or increased *in vitro* by a barbiturate (Hutchinson & Stotz, 1941), by chloral and butobarbitone (Buchel & McIlwain, 1950*a*, *b*; Rosenberg, Buchel, Etling & Levy, 1950) and by pentobarbitone, amylobarbitone, chloretone, tribromoethanol and phenobarbitone (Webb & Elliott, 1951). It is decreased *in vivo* by phenobarbitone, pentobarbitone, amylobarbitone and ether (Stone, 1938; Richter & Dawson, 1948). Phosphocreatine in the tissue also changes in opposite senses *in vivo* and *in vitro*. It is raised *in vivo* during the action of pentobarbitone (LePage, 1946; Dawson & Richter, 1950), but falls *in vitro* during the action of butobarbitone, allobarbitone, and chloral (Buchel & McIlwain, 1950*a*, *b*). Similarly opposed changes take place in the inorganic phosphate of the tissue (Buchel & McIlwain, 1950*a*, *b*).

Observations on respiration come closest to showing parallelism *in vivo* and *in vitro*. They have constituted a large proportion of biochemical work on narcotic action since the demonstration by Warburg (1910) (see also Minami, 1923) that such substances can inhibit respiration. Concentrations of depressants higher than those used therapeutically lower the respiration of cerebral cortical preparations *in vitro* (Loebel, 1925; Quastel & Wheatley, 1933; Quastel, 1951; for an assessment, see Butler, 1950; Himwich, 1951). This, however, is true also of certain barbiturates (Fuhrman, Martin & Dille, 1941) and other agents (Hutchinson & Stotz, 1941) which are not depressant but convulsant. Concentrations comparable to those which are depressive *in vivo* may slightly stimulate (Westfall, 1949, phenobarbitone), depress (Schueler & Gross, 1950, pentobarbitone; Wilkins, Featherstone, Schwidde & Brotman, 1950, phenobarbitone) or be without action on (Zorn, Muntwyler & Barlow, 1939, phenobarbitone) the respiration of cerebral cortex *in vitro*.

In considering possible reasons for the divergence between biochemical findings *in vivo* and *in vitro*, the state of activity of the cerebral tissue appears to be an extremely relevant matter which has not hitherto been explored. The action *in vivo* of depressants such as the barbiturates is accompanied by changes in the electrical activity of the cerebral cortex (see, for example, Brazier & Finesinger, 1945, and the review of Wikler, 1950). Slices of mammalian cerebral cortex *in vitro* do not appear to show electrical signs of activity (McIlwain & Ochs, 1952). Metabolic changes corresponding to those found *in vivo* in association with increased cerebral activity can, however, be induced in such slices by applying suitable electrical impulses to them (McIlwain, 1951*a*, *b*). Metabolic changes, in many ways comparable, can be induced also by increased concentrations of potassium salts (Ashford & Dixon, 1935; Dickens & Greville, 1935; McIlwain, 1952*b*; Gore & McIlwain, 1952), and by 2:4-dinitrophenol (McIlwain & Gore, 1951; see Dodds & Greville, 1933). It was, therefore, decided to examine the effects of some depressants of the central nervous system upon separated cerebral tissues while they were being subjected to the effects of these agents.

EXPERIMENTAL

Guinea pigs or rats were stunned by a blow on the neck, the brain removed and slices or chopped tissue were prepared from the cortex of the cerebral hemispheres as described by McIlwain (1951*a*, *b*).

The phosphate-glucose and bicarbonate-glucose salines, electrode vessels and tissue-holding electrodes were those

* The following trivial names are used: butobarbitone for 5-butyl-5-ethylbarbituric acid (Soneryl); pentobarbitone for 5-ethyl-5-(1-methylbutyl) barbituric acid; amylobarbitone for 5-ethyl-5-(3-methylbutyl) barbituric acid (Amytal); phenobarbitone for 5-ethyl-5-phenylbarbituric acid; allobarbitone for 5:5-diallylbarbituric acid (Dial).

described by McIlwain, 1951*a*, *b*). Stimulation was by condenser pulses (McIlwain, 1951*a*), which in all cases were alternating and at the frequency of 100/sec., and had the other characteristics described in the individual experiments below. Respiration was measured manometrically and lactic acid by determination according to Barker & Summerson (1941).

RESULTS

Results are given below with respect to three barbiturates and chloral. As magnesium salts in narcotic concentrations have already been studied by the present methods (Gore & McIlwain, 1952), depressants of three very different types have now been examined. The present agents were chosen to include some of practical utility and some on which considerable biochemical data were already available. Phenobarbitone, butobarbitone and allobarbitone represent aromatic, aliphatic and unsaturated barbiturates with some variations in their speed and type of action *in vivo*. Concentrations of chloral and butobarbitone were chosen which, in the observations of Buchel & McIlwain (1950*a*, *b*), McIlwain & Buchel (1951), Buchel-Olszycka (1947) and Olszycka (1938), had little or no action on the unstimulated respiration of guinea pig and rat cerebral cortex *in vitro*. Similar data with respect to phenobarbitone were drawn from Butler & Bush (1939) and Westfall (1949). Chloral, in these as in other experiments, presumably acts partly after its conversion to trichloroethanol (Butler, 1948, 1949).

Effects on electrically stimulated metabolism

Respiration. The course of respiration by normal and stimulated cerebral cortex is shown in Fig. 1 and many such experiments are summarized in Tables 1 and 2. In these, two intensities of stimulation have been used, one submaximal and the other somewhat above the minimum usually required for maximal stimulation.

Fig. 1 shows immediately that phenobarbitone has a markedly greater effect on the stimulated than on the unstimulated respiration of the tissue. The concentration of phenobarbitone used had only a negligible effect in the absence of stimulation. It considerably reduced the effect of the applied impulses at both of the voltages examined.

In these experiments the effects of the stimuli in presence and absence of the drug were examined in different vessels which in initial experiments were also attached to different, individual, stimulating units. Experiments at this stage therefore run in pairs, so that vessels and stimulators which in one experiment were used for following the effect in absence of drugs, were in the next used with the drug. The experiments of Table 1 and of Fig. 1 with phenobarbitone form such a pair. The difference is clearly due to the drug and not to the apparatus. Table 1 also shows that butobarbitone and chloral cause greater inhibition of respiration of stimulated than of unstimulated tissues. The effect with butobarbitone was shown in a glycylglycine- as well as in the usual phosphate-buffered saline, and was found in basal ganglion tissue as well as in cerebral cortex.

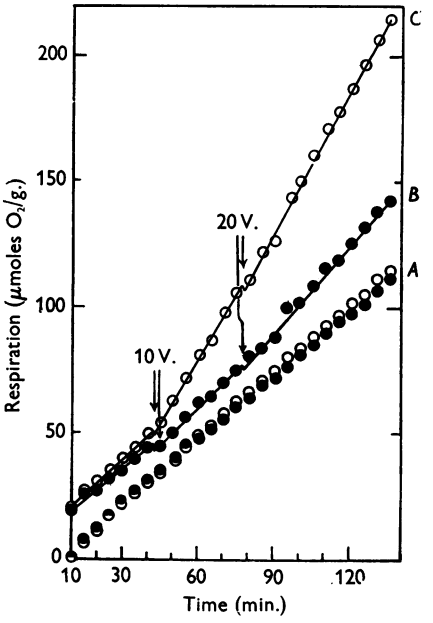

Fig. 1. Effect of phenobarbitone on respiration of stimulated and unstimulated fragments of slices of guinea pig cerebral cortex in salines in vessels *E*. O—O, glucose-phosphate saline only; ●—●, with $6{\cdot}8 \times 10^{-4}$ M-phenobarbitone. Unstimulated *A*; stimulated *B* and *C*. Stimulation by condenser pulses, time-constant 0·3 msec., as indicated by arrows. Ordinates for the experiments with stimulated material (*B* and *C*) increased by 20 μmoles/g. for clarity. Abscissae give times after placing the vessels at 37°.

The action of some depressants on the respiration of slices of cerebral cortex is shown, in papers quoted above (see also Jowett & Quastel, 1937), to change to a small degree with time, though this was not marked in the present experiments (see Fig. 1). In the experiments of Fig. 1 and Table 1 periods of stimulation always followed an unstimulated period. However, parallel experiments were run in every case in which similar tissues, with and without the drug, were unstimulated throughout. In the calculation of Table 1 the actions of the drugs have been compared during the same experimental period.

Results of adding depressants during respiration are quoted in Table 2. This arrangement enabled the effect of a stimulus from the same source and on the

Table 1. *Action of depressants on respiration and glycolysis of electrically stimulated cerebral preparations*

(Experiments were under the following conditions except when indicated otherwise in the first column. Slices of guinea pig cerebral cortex of 60–70 mg. fresh wt. and cut to about twenty pieces were suspended in 3·5 ml. of phosphate-glucose saline in vessels *E*. In one experiment rat slices were used. The manometric experiments began 23–30 min. after death of the animal and comprised successive periods (indicated) in which the tissues were without impulses, then with impulses at peak potentials of 10 V., then of 20 V.; readings were taken each 5 min. Half the vessels were without impulses throughout the experiments; half contained the drug, which was present in the appropriate media before the tissues were added.)

		Metabolism in vessels without stimulation				Metabolism in vessels with stimulation				Change (%) caused by drug in				
		Respiration (μmoles/g./hr.)				Respiration (μmoles/g./hr.)				Respiration			Lactic acid	
											Stimulated			
Drug (M × 10^{-4})		0–30 min.	30–60 min.	60–120 min.	Lactic acid (μmoles/ g./exp.)	0–30 min. (unstimu- lated)	30–60 min. (at 10 V.)	60–120 min. (at 20 V.)	Lactic acid (μmoles/ g./exp.)	Unstimu- lated	10 V.	20 V.	Unstimu- lated	Stimu- lated
Phenobarbitone	0	61	56	56	46	59	104	112	145	—	—	—	—	—
	6·8	60	56	55	56	58	81	92	122	−1	−22	−18	+22	−16
	17	55	54	54	95	55	62	68	144	−9	−40	−30	+104	−1
Butobarbitone	0	73	73	73	66	75	90	109	136	—	—	—	—	—
	6·4	68	68	66	98	70	76	81	164	−7	−15	−26	+48	+20
Butobarbitone	0	75	75	75	105	76	112	112	139	—	—	—	—	—
(in glycylglycine)	6·4	70	70	70	134	69	97	92	149	−8	−14	−18	+28	+7
Butobarbitone	0	24	24	21	32	23·5	32·5	46·5	56	—	—	—	—	—
(basal ganglia chopped)	6·4	26	23	20	46	22·5	33	37·5	74	0	0	−20	+44	+32
Chloral (rat)	0	74	74	74	51	70	118	154	139	—	—	—	—	—
	8·6	74	74	74	73	70	109	123	127	0	−8	−20	+42	−9
Chloral	0	60	60	59	34	59	95	101	129	—	—	—	—	—
	17	54	54	54	56	54	73	80	103	−10	−23	−21	+64	−20

Table 2. *Addition of depressants to respiring cerebral cortex*

(Drugs were not in contact with the tissue until after the first period of stimulation, when they, or saline, were tipped from a side arm. Experiments comprised successive 30 min. periods (*a*) without impulses; (*b*) with impulses at 20 V.; (*c*) with impulses at 20 V. in presence of drug; (*d*) without impulses but in presence of drug. Other conditions were as in the experiments of Table 1. Initial, unstimulated rates were of 62–68 μmoles/g./hr. Values marked with an asterisk (*) are derived from experiments in which no impulses were applied, and periods at similar intervals after commencing metabolism were compared in two different vessels. Other values were derived from rates at different intervals after commencement, but in the same vessel; period *a*/period *d*, or period *b*/period *c*.)

Solution tipped	Concn. in medium after tipping ($M \times 10^{-4}$)	Change in respiratory rate (%) following addition of drug	
		Periods without impulses	Periods with impulses
Saline	—	0, −5, 0, −1	−4, −8, −2, −2
Chloral	4·3	+4	−18
Chloral	43	−23	−52
Butobarbitone	6·8	0, 0*	−18
Butobarbitone	10	−4, +12, −17, +5 +10*, −12*, +3*, −9*	−34, −11 −28, −23
Butobarbitone	20	−14, −30, −17*	−38, −34
Allobarbitone	4·3	+9, +5	−20, −27
Allobarbitone	43	−21, −23	−40, −44

same sample of tissue to be observed in the same vessel in the presence and in the absence of the drug. Again, greater inhibition of stimulated than of unstimulated respiration was found. In a given experiment the periods with drugs necessarily

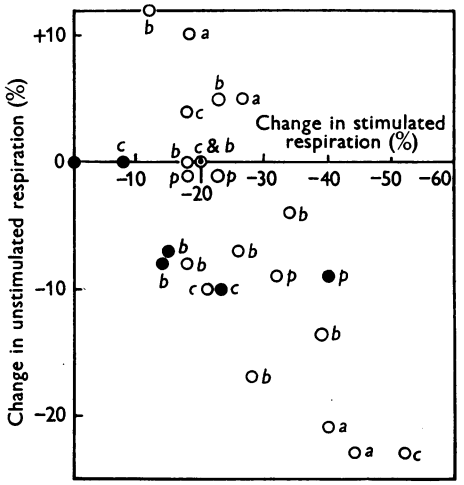

Fig. 2. Comparison of the change brought about by depressants in stimulated and unstimulated respiration of guinea pig cerebral cortex. Stimulation by condenser pulses at 10 V. (●) and 20 V. (○). *a*, allobarbitone; *b*, butobarbitone; *c*, chloral; *p*, phenobarbitone.

followed those without. To control possible differences arising from this, the effect was determined of adding, in place of the drugs, the same volume of saline. The second stimulated and unstimulated periods then showed slightly lower rates than the first, but the changes were very much smaller than those following addition of the drugs.

Direct comparison between the effect of the various agents on stimulated and unstimulated metabolism is made in Fig. 2 by plotting points with these values as co-ordinates. This suggests a relationship between the two effects, and emphasizes that a mean inhibition of some 20 % in stimulated respiration is attained without any mean inhibition of unstimulated respiration. The data available do not show effects specific to the different agents. Averaging all values of Fig. 2 shows that a mean inhibition of 5 % in unstimulated respiration was associated with a mean inhibition of 18 or 22 % (10 or 20 V. impulses) in stimulated respiration.

Corresponding values for Table 2 were −5 and −29 %, while saline alone caused changes of −2 and −3·5 %.

Lactic acid formation. During the experiments described above, lactic acid accumulated in reaction mixtures with unstimulated tissue at a mean rate of about 20–30 μmoles/g./hr. The rate was increased by the depressants, and greatly increased by electrical stimulation.

The impulses, however, always had a lesser effect in the presence of depressants. This is shown in the values given in Table 1. Thus, in the first series of experiments with phenobarbitone, the increase following stimulation in the absence of drug was 99 μmoles/g./experiment, while with the two concentrations of the drug the increases were 66 and 50 μmoles. This might have been due, in part, to the existence of an upper limit to the rate of lactic acid formation by the tissue, and to this being already approached by the stimulus in the absence of the depressant. However, in four of the experiments of Table 1, the lactic acid formed with depressant and stimulus is less in total quantity than that formed with the stimulus alone. This is true also of the

experiment of Fig. 3 in a different medium. This is in spite of the opposite effect of the depressant on the accumulation of lactic acid in the absence of applied impulses.

An impression of the course of formation of lactic acid during such experiments is given by Fig. 3. The ordinates here give the change in pressure in vessels with tissue stimulated and unstimulated in a bicarbonate-containing glucose-saline. The increase of pressure in such experiments with cerebral cortex is almost entirely due to the formation of lactic acid (see McIlwain & Grinyer, 1950; McIlwain, Anguiano & Cheshire, 1951). Fig. 3 shows well, in the upper two curves, how the effect of the phenobarbitone on unstimulated glycolysis is reversed during stimulation.

Promptness and reversibility of action. Depressants were added during the measurement of respiration in the experiments of Table 2. The manometric readings from which respiratory rates were derived were taken each 5 min. before and after addition. The lower rate recorded in the presence of the depressant appeared to be established without delay. The accuracy of the measurements was such that this implied the depressant to become fully effective within 3 min. after its addition.

The experiments of Table 3 were carried out to see whether the effect of phenobarbitone on the tissue on which it had been acting would be lost when most of the drug was removed. Tissues were held in the vessel in a grid electrode in salines with and without the drug. After initial periods of metabolism without and with impulses, vessels were removed from manometers and their salines replaced by fresh salines without phenobarbitone. This was done in all vessels, for it was found that replacement of saline lowered metabolic rates in subsequent periods even when the nature of the saline was not changed. When, however, salines containing the depressant were replaced by ones without, stimulated respiration rose. This effect of the drugs is therefore, like their actions *in vivo*, reversible.

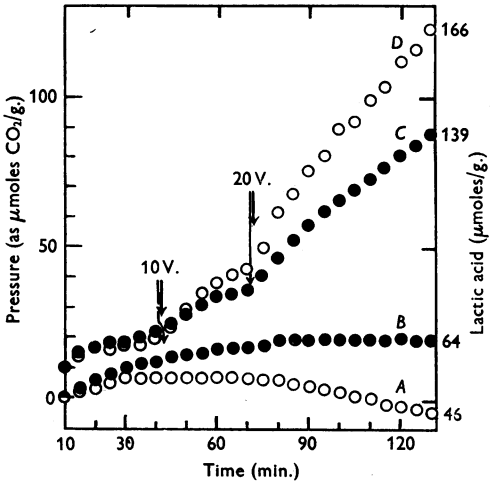

Fig. 3. Effect of phenobarbitone on glycolysis of stimulated and unstimulated fragments of slices of guinea pig cerebral cortex in salines in vessels *E*. ○, glucose-bicarbonate saline only; ●, with $1{\cdot}71 \times 10^{-3}$ M-phenobarbitone. Unstimulated *A*, *B*; stimulated *C*, *D*. Ordinates give the pressure changes which were found manometrically, calculated as μmoles CO_2/g. tissue; those for *C* and *D* have been increased by 10 μmoles/g. for clarity. Abscissae give times after placing vessels at 37°. The lactic acid found chemically at the end of the experiment is given by the values to the right of the figure.

Table 3. *Reversal of the action of phenobarbitone on electrically stimulated respiration*

(Respiration in each vessel was measured during four periods of 30 min. each, given successively in the table. During the first two of these the vessels were unstimulated, then stimulated; then followed 10 min. without impulses, during which respiration was not measured. The media of all of the vessels were then replaced by fresh salines without phenobarbitone, returned to the thermostat for 10 min., and respiration measured during the final 30 min. periods (without and with impulses). Impulses throughout were at 18 V., applied to slices in the electrodes in vessels *A* (McIlwain, 1951*a*.)

Initial two periods			Final two periods			Increase in respiration due to stimulation in second period/increase in first (%) (100*y*/*x*)
Respiration (μmoles/g./hr.)		Increase in respiration due to stimulation (%) (*x* = 100(*b* − *a*)/*a*)	Respiration (μmoles/g./hr.)		Increase in respiration due to stimulation (%) (*y* = 100(*d* − *c*)/*c*)	
Unstimulated *a*	Stimulated *b*		Unstimulated *c*	Stimulated *d*		
Without phenobarbitone throughout						
48	111	131	40	78	97	74
56	114	103	51	96	88	85
62	116	64	60	87	45	71
With phenobarbitone ($1{\cdot}5 \times 10^{-3}$ M) in initial two periods only						
56	87	55	45	90	88	160
60	96	57	66	105	59	104
57	66	15	63	84	32	212

Effect on tissues stimulated with 2:4-dinitrophenol and potassium salts

Other substances which inhibit the electrically stimulated metabolism of cerebral tissues have been shown to be without action on tissues in which the respiration is stimulated to a comparable degree by 2:4-dinitrophenol or by potassium salts (McIlwain, 1951*c*). The depressants now being studied showed, on the contrary, a relatively uniform action independently of the mode of stimulation of the tissue.

2:4-Dinitrophenol was used in the present experiments at 5×10^{-5} M, a concentration found (McIlwain & Gore, 1951) to cause maximal rise in respiration. It caused, during the present experiments (Table 4), an approximate doubling in respiratory rate, the stimulated values being close to those obtainable electrically (Table 1). Both phenobarbitone and chloral lowered these rates by about 25%, when present at concentrations having little effect on unstimulated metabolism.

Accumulation of lactic acid was also increased by 2:4-dinitrophenol, but the effect of depressants on the accumulation was less than that observed during electrically stimulated metabolism. The lactic acid formed during the experiments of Table 4 was throughout less than that formed in those of Table 1; contributing to this were the shorter experimental period, the use of intact slices, and the absence of additional handling of the slices involved in the use of tissue-holding electrodes.

Potassium chloride was used in most experiments in a concentration (0·033 M) giving a large but not maximal increase in respiration, and a small change only in lactic acid accumulation. One set of experiments was also carried out with 0·1 M-potassium salts, which gave maximal effect on respiration and also markedly increased glycolysis. Respiration stimulated with either concentration of potassium salts was markedly inhibited by both phenobarbitone and chloral. The effect on accumulation of lactic acid was relatively small.

Considering all the experiments of Table 4 with both 2:4-dinitrophenol and potassium chloride as stimulants, the mean inhibition of unstimulated respiration by the depressants was 5%, while they inhibited stimulated respiration by 25%. This is very similar to the effects of the depressants on electrically stimulated tissue. The mean changes caused by depressants in lactic acid formation during chemical stimulation were much smaller than during electrical stimulation.

DISCUSSION

Present studies and previous work suggest for depressants two action types, one on the metabolic behaviour of brain, the other on electrical phenomena. These will be first discussed and then compared.

Metabolic changes. New findings are (1) that stimulation of cerebral tissues electrically or otherwise increases the sensitivity of their respiration to depressants; (2) that lactic acid formation in electrically stimulated sections is lowered by depressants. Both findings show that stimulated sections simulate events *in vivo* more closely than do unstimulated ones. Quantitative comparison is as follows. By arterio-venous difference and blood flow in man, Himwich, Homburger, Maresca & Himwich (1947) found cerebral respiration to be little affected by pentothal during the early stages when consciousness was clouded, but to be inhibited some 30% after loss of contact and during surgical anaesthesia. Deep pentothal narcosis in the monkey was observed to be associated with inhibition of respiration by 35% (Schmidt, Kety & Pennes, 1945). In these cases, the concentration of drug in the blood stream or in cerebral tissues was not determined, but Grenell & Davies (1950) investigating perfused cat brain, found 5×10^{-4} M-pentobarbitone to inhibit cerebral respiration by 20–25%. This order of concentration and inhibition has been reproduced during the present experiments with stimulated tissues (Tables 1 and 2). As has been indicated throughout this paper, comparisons between the present results and those of other workers frequently involve related, rather than identical, drugs and species.

Preferential action on the more active central nervous system is of obvious advantage in a depressant drug. Such drugs have been selected specifically in order to depress partially and reversibly; not to cause complete loss of function, but to convert a greater to a lesser level of activity. While many types of mechanism other than biochemical are likely to be involved in this, the present findings show that contributions to such selectivity can be made by reason of the different levels of respiratory activity in normal and excited tissue. Depression of the metabolism of electrically stimulated slices was lost when the drug was removed, as is the depression *in vivo*.

Selective inhibition of the stimulated respiration suggests that the factors limiting respiration of slices when their oxygen uptake was at the level of some 120 μmoles/g./hr. were different from those limiting respiration at the level of 60 μmoles/g./hr. It is not known whether the factor in either case corresponds to that stage in respiration of brain homogenates concluded by Greig (1946) to be most sensitive to depressants. It is probable, however, that the higher rate corresponds most closely to the rate of metabolism by cerebral cortex *in vivo*. A mean value for the normal oxygen consumption of the brain of monkeys *in vivo* given by Schmidt *et al.* (1945)

Table 4. *Effect of depressants on respiration and glycolysis of cerebral cortex stimulated with 2:4-dinitrophenol and potassium salts*

(For each of the first four groups of experiments, weighed intact slices from the cerebral hemispheres of a guinea pig were distributed between two sets of vessels, one set containing ordinary glucose-phosphate saline and the other set with saline containing the stimulating agent. Respiration was measured for a period of 45 min., the depressants then added from side arms, and respiration measured for a further 45 min. The depressants added were dissolved in the type of saline (with or without the stimulating agent) present in the main compartment of the vessels. The side arms of the control vessels contained only the corresponding saline, and the lactic acid in these vessels was compared at the end of the experiment with that found in vessels to which drugs had been added. In the last two sets of experiments, marked with asterisks (*), vessels with and without chloral or KCl from the commencement of the experiment were compared; duration 90 min.)

Added drug	Final concentration ($M \times 10^{-4}$)	Metabolism in media without stimulant: Respiration (μmoles O_2/g./hr.) Before addition	Metabolism in media without stimulant: Respiration (μmoles O_2/g./hr.) After addition	Metabolism in media without stimulant: Lactic acid (μmoles/g./exp.)	Metabolism in media with stimulant: Respiration (μmoles/g./hr.) Before addition	Metabolism in media with stimulant: Respiration (μmoles/g./hr.) After addition	Metabolism in media with stimulant: Lactic acid (μmoles/g./exp.)	Change (%) caused by drug in mean values for: Respiration Unstimulated	Change (%) caused by drug in mean values for: Respiration Stimulated	Change (%) caused by drug in mean values for: Lactic acid Unstimulated	Change (%) caused by drug in mean values for: Lactic acid Stimulated
					5×10^{-5} M-2:4-Dinitrophenol						
Phenobarbitone	0	57	54	18	120	120	52	—	—	—	—
Phenobarbitone	11	57, 60	57, 57	17, 21	117, 132	102, 90	47, 45	−2	−25	+6	−10
Chloral	0	54	54	20	129	126	52	—	—	—	—
Chloral	11	58	55	25, 19	120, 114	90, 96	59, 65	−5	−26	+10	+19
					0·033 M-K^+						
Phenobarbitone	0	60, 63	54, 66	26, 28	96, 96	84, 99	30, 35	—	—	—	—
Phenobarbitone	6	60, 57	66, 57	28, 29	84, 96	66, 78	27, 21	+5	−20	+5	−27
Phenobarbitone	12	60, 62	57, 48	24, 26	102, 102	78, 72	31, 35	−13	−36	−7	0
Chloral	0	66	66	24	81	78	26	—	—	—	—
Chloral	12	63, 72	57, 66	26, 20	90, 90	63, 63	24, 27	−9	−30	−4	+2
Chloral*	0	57	—	32	84, 87	—	38, 35	—	—	—	—
Chloral*	12	54	—	30	75, 72	—	27, 29	−5	−14	−6	−23
					0·10 M-K^+						
Chloral*	0	66	—	40	111, 102	—	59, 59	—	—	—	—
Chloral*	12	60	—	45	84, 90	—	65, 61	−9	−18	+13	+7

corresponds to 79 μmoles/g./hr. Grey matter from the cerebral cortex respires *in vitro* at twice the rate of cerebral white matter.

Varied effects of depressants on respiration of cerebral preparations were quoted at the beginning of this paper. It is possible that certain such variations may be related to the level of respiration attained under the experimental conditions used. Jowett & Quastel (1937) found phenobarbitone to have a greater effect in the absence than in the presence of calcium and magnesium salts. Westfall (1951) again reported phenobarbitone to inhibit or stimulate according to the content of magnesium and calcium salts in the surrounding medium, and several of these changes were in the sense that a high rate of respiration was depressed, and a low one increased. Schueler & Gross (1950) found inhibition *in vitro* with pentobarbitone at anaesthetic concentrations in whole blood. Rates are not recorded in known units, but are likely to be higher in such a medium than in simple salines (cf. Krebs, 1950). Moreover, in a study published after the present results had been obtained, Webb & Elliott (1951) compared the effects of depressants on the respiration of cerebral cortex slices in presence and absence of added potassium salts. The potassium salts had relatively little action on respiration; no explanation of this divergence from their normal effect could be given. But, associated with the small stimulation of respiration, there was an absence of increased sensitivity to depressants.

Electrical phenomena. Of the means used in the present investigation for increasing the activity of cerebral tissue, electrical stimulation is most relevant to circumstances *in vivo*, when the brain shows continuous electrical activity. The inhibition by depressants of electrically induced activity in nervous tissues has frequently been observed, although electrical rather than metabolic means of observing such activity have been more frequently employed.

Thus the threshold to stimulation may be raised or axonal conduction depressed in various elements of the central nervous system, or its spontaneous electrical activity changed in the cerebral cortex (Derbyshire, Rempel, Forbes & Lambert, 1936; Forbes & Morison, 1939; Hoagland, Himwich, Campbell, Fazekas & Hadidian, 1939), the spinal cord (Heinbecker & Bartley, 1940; Bremer & Bonnet, 1948) or in a sympathetic ganglion (Posternack & Larrabee, 1948, 1950). This can occur at anaesthetic concentrations, and particular characteristics measured by electrical methods are affected somewhat differently by different depressants (Swank & Watson, 1949; Finesinger & Brazier, 1944; and some of the preceding references). Moreover, the responses to stimulation as measured by electrical techniques may be prevented at concentrations of depressants lower than those necessary for effects on the resting respiratory activities of the tissue. This was found to be the case in the stellate ganglion of the cat (Larrabee, Ramos & Bulbring, 1950), where pentobarbitone or chloretone depressed synaptic transmission at anaesthetic concentrations which had no effect on the oxygen uptake of the tissue, in absence of stimulation. Depression of these various aspects of functional activity may well be mediated by metabolic effects of the drugs such as those on acetylcholine described by McLennan & Elliott (1951).

Cerebral tissues can respond to a fall in energy-consuming processes by a fall in energy-yielding processes. Schmidt *et al.* (1945), among others, showed that the rate of metabolism of the brain *in vivo* depended on its level of activity; comparable phenomena *in vitro*, and the mechanisms available to establish such connexions have been surveyed elsewhere (McIlwain, 1950, 1952*a*). Thus depression of electrical activity under the circumstances described above would be expected to be associated with a fall in respiration and glycolysis in the excited tissues.

Possible balance of inhibitions in vivo. The conclusions arrived at in the preceding two sections are different and complementary. Metabolic findings indicate that respiration, however stimulated, becomes more susceptible to concentrations of depressants active *in vivo*; inhibition of respiration may thus be responsible for the depression of function *in vivo*. The second section indicates that depression of function can lead secondarily to lowered respiration without the depressants acting on any respiratory step.

The question of which, if either, of these processes preponderates *in vivo* can probably be answered from data already available (McIlwain & Buchel, 1951). Energy-rich compounds are observed to accumulate during narcosis *in vivo* and this makes it likely that in the brain as a whole, inhibition of energy-consuming activity is greater than that of energy-yielding processes.

However, inhibition of both processes may be necessary in a successful depressant. Disproportionate inhibition of energy-yielding mechanisms would be expected, as in hypoxia or hypoglycaemia, to be destructive to cerebral tissues. Disproportionate inhibition of energy-consuming mechanisms may also lead to instability. Thus, atropine at 5×10^{-4} M has no effect on the respiration of cerebral cortex *in vitro* either in an unstimulated condition or when stimulated by 2:4-dinitrophenol and by potassium salts, but it prevents the response of such tissues to electrical stimulation (McIlwain, 1950). The central effects of atropine (excitement, hallucination, and delirium) are not those of a depressant. A greater or lesser imbalance in inhibition of

energy-yielding and energy-consuming reactions may be responsible for certain barbiturates being convulsant, and for the transitory excitory phase in the action of many depressants.

SUMMARY

1. Phenobarbitone, butobarbitone, allobarbitone and chloral inhibited the respiration of electrically stimulated sections of rat and guinea pig cerebral tissues. They did so at concentrations below 10^{-3} M which had little or no effect on respiration of the same preparations in the absence of stimulation.

2. Stimulation of the respiration of cerebral cortex by potassium salts and by 2:4-dinitrophenol also rendered it more sensitive to depressants. In this, the depressants acted differently from a substance such as atropine which had a selective effect on electrically stimulated tissues.

3. The depressants decreased the lactic acid formed from glucose by electrically stimulated cerebral tissues. This change occurs also during narcosis *in vivo* and is in the opposite sense to that induced by the depressants in unstimulated tissues *in vitro*.

4. Inhibition of the respiration of electrically stimulated tissue was prompt and could be reversed when the drug was removed.

5. It is concluded that a successful depressant acts *in vivo* by inhibiting both energy-yielding and energy-consuming processes in the central nervous system.

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Techniques in Tissue Metabolism. 1. A Mechanical Chopper

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The apparatus now described was devised to chop small and irregular fragments of animal tissues for metabolic experiments, with as little disruption as possible of cell structure. It will also cut larger pieces of tissue. Small and irregular specimens were being examined because they are often the only ones available at biopsy or when small organs, or parts of them, are being investigated. Cell structure was required to be relatively intact in order to disturb as little as possible the many metabolic characteristics on which such structure exerts a controlling influence. These include levels of metabolites and metabolic rates, concentration or exclusion of added substances, and response to inhibiting and stimulating agents. Several instances of the importance of such structural factors in preparations from the central nervous system have been encountered recently (McIlwain & Grinyer, 1950; McIlwain, Buchel & Cheshire, 1951; McIlwain, 1951*b*). In some of these studies (see also McIlwain, Ayres & Forda, 1952) specimens had been reduced to a size small enough for diffusion of metabolites to the tissue, by a method consisting of 'chopping' free-hand, by repeatedly passing a blade vertically into the tissues. The machine now described was built to do this in a regular and reproducible fashion.

EXPERIMENTAL

Preliminary observations

At least three different types of treatment have been used to fragment tissues.

Cutting. A blade with a relatively small cutting angle is moved both across and into the specimen simultaneously. Its motion is in the plane of the blade and has components both parallel and perpendicular to its cutting edge. Efficacy in cutting does not markedly vary with the speed of motion of the blade.

Shearing with scissors. Opposed cutting edges with large cutting angles are made to approach the specimen in directions perpendicular to their cutting edges.

Chopping. This motion, as of an axe or of a knife in chopping vegetables, has been applied little in dissection. It is, however, applied to hardened or supported tissues in microtomes. A sharp blade with relatively small cutting angle is used and approaches the tissue directly without necessarily any motion parallel to the cutting edge. The result, when applied to soft tissues, depends greatly on the speed of approach of the blade.

It appeared that the static rigidity of embedded tissues might be simulated by the resilience and inertia which they show, while still fresh, to a sudden blow. If a scalpel about the size and weight of a pocket knife is held by the extremity of its handle and its blade swung against a piece of liver lying on a pad of filter paper, the tissue can easily be cut (chopped) cleanly and suffers little depression at the point of entry of the blade. On the other hand, if the blade is laid on the tissue and pressed directly into it, appreciable force is needed for the cut and the tissue is distorted. (If a cutting motion is given to the blade, then again, much less force is required.)

There appeared to be relatively little advantage, when 'chopping' with a scalpel, in giving the blade an additional motion parallel to the cutting edge, as in ordinary cutting. A simple chopping motion was therefore made the basis of the machine described.

Description of the chopper

Fig. 1, Pl. 1, is a view of the chopper from the position of a person using it, and Fig. 2, Pl. 1, a view from a position at his left-hand side. Fig. 3 gives constructional details and omits the motor and rheostat which are at the back of Fig. 1, Pl. 1.

Motion of the blade. Chopping is by the blade *B*, held in arm *A*. This is lifted by the trigger cam *C*1 and then released, when it is drawn by its own weight and by the spring *Sp*1 into the specimen on the cutting table *T*. This is the only motion of the blade; it is lifted and released at each revolution of the shaft *Sh*1.

While the machine is cutting, shaft *Sh*1 is rotated by an electric motor, being continuous with the shaft of a helical