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THE ACTION OF NARCOTICS ON BRAIN RESPIRATION[1]

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Much work has formerly been done on the effects of narcotics on enzyme activity, with the general result [cf. Clark, 1937] that many enzyme actions are inhibited by sufficiently high concentrations of narcotics. Work done in this laboratory has made it clear that specificity of action of narcotics takes place in the brain, the oxidation of glucose and of lactic and pyruvic acids being affected more than that of other metabolites; and it has also been found that among hypnotics of the same chemical type those with greater hypnotic activity have the greater inhibitive action [Quastel & Wheatley, 1932]. These facts acquired a greater biological significance when it was found that the inhibitory action of certain narcotics was reversible [Quastel & Wheatley, 1934].

To establish that the biological action of narcotics is due to an inhibitory action of narcotics on oxidations, it is necessary to show that narcotics inhibit oxidations at concentrations which bring about narcosis in the intact organism. Preliminary experiments have shown [Jowett & Quastel, 1937*a*] that three narcotics investigated do in fact inhibit measurably the respiration of brain slices at concentrations of the correct order of magnitude.

In the present work a more extensive investigation on similar lines has been made. The effect of narcotics on the respiration of slices of the grey matter of the cerebral cortex has been examined in the presence of glucose, which is the main fuel of the brain. The glucose-oxidizing system of brain is very labile, and the most significant results will be obtained under conditions which damage nerve cells as little as possible. For

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experiments *in vitro* the tissue should therefore be employed in the form of slices.

The technical aspects of the work are of importance, as is shown for instance by the fact that respiration under the present conditions is more sensitive to ethylurethane than it was found to be by Bülow & Holmes [1932], who used minced brain.

Methods

The methods used are essentially those of Jowett & Quastel [1937*a*]. The manometric technique of Warburg is applied to slices (about 0·3–0·4 mm. thick, and of total dry weight 8–15 mg. in each vessel) of the grey matter of the cerebral cortex, shaken in 3 c.c. of a suitable medium. Young rats (80–150 g.) have been mainly used.

The medium is isotonic with 0·16 *M* NaCl, and contains 0·0128 *M* K^+, 0·001 *M* Ca^{++}, 0·0008 *M* Mg^{++}, and 0·02 *M* phosphate of *p*H 7·2, together with NaCl and 0·01 *M* glucose. Solutions of narcotics are made up in 0·16 *M* NaCl, and are added to the medium so as not to alter its salt or glucose concentration. Few of the narcotics are used at concentrations high enough to make the medium essentially hypertonic. Alcohol (in 50 p.c. solution) and chloretone are added to the medium from a side tube of the manometric vessel a few minutes after the vessels are placed in the thermostat at 39°. Other narcotics, when used, are already present in the medium in which the tissue slices are inserted about 10 min. before the vessels are placed in the thermostat. The atmosphere in the vessels is oxygen, and caustic soda (0·2 c.c. of 2 *N* solution) absorbs carbon dioxide. Readings are begun after 12–15 min. shaking at 39°, and are continued over a period of 2 hr. The respiration in presence of a narcotic is always stated with reference to the respiration of a "control" measured simultaneously and taken equal to 100. The respiration of brain under the conditions used, and in the absence of narcotics, is fairly constant over the experimental period of 2 hr.

Results

Six narcotics which have a generally similar action on respiration will first be considered. Alcohol behaves differently, and will be considered afterwards.

The main features of the action of the six narcotics in question are shown in Tables I and II and Figs. 1–7. All the narcotics exert an inhibitory action on respiration, which increases with their concentration.

TABLE I. The effect of luminal on respiration of rat brain. Average effect for 2 hr. period. Probable errors of the mean values are given where data are sufficient

Luminal conc. g./100 c.c.	Percentage effect	Mean	Inhibition calc.
0·01	−16·6, − 5·9, − 7·6, −19·5	−12·4±2·3	8·3
0·02	−28·7, −13·9, − 3·9, −13·9, −10·2	−14·1±2·8	15·3
0·03	−16·2, −32·7, −23·2, −23·2	−23·8±2·3	21·3
0·04	−22·3, −28·7, −27·7, −19·7, −16·9	−23·1±1·5	26·5
0·06	−42, −34	−38	35
0·08	−49, −45, −42, −49, −43, −49	−46±1	42
0·11	−73·5, −72·5	−73	50
0·15	−81·5, −84·5	−83	57

TABLE II. The effect of narcotics on respiration of brain. Average effect for 2 hr. period

Narcotic	Conc. g./100 c.c.	Animal	Percentage effect	Mean	Calc. inhibition
Chloral hydrate	0·015	Rat	− 9, −14	− 6	7
	0·03		−12, − 4, −15	−10	13
	0·06		−33, −20, −25	−26	24
	0·09		−44	−44	32
Ethylurethane	0·25	Rat	−10, − 2½	− 6	7
	0·5		−21, −15, −10	−15	13
	1·0		−28, −23, −18	−23	24
	2·0		−64	−64	38
Avertin*	0·01	Rat	−13	−13	13
	0·02		−19, −20	−20	23
	0·04		−38, −43	−40	38
	0·08		−75	−75	55
Magnesium ion†	0·0048	Rat	−12, − 1	− 6	6
	0·0096		−18, −14, 0	−11	11
	0·024		−28, −26, −34	−29	23
	0·048		−41, −26, −26	−31	37·5
	0·096		−48, −34	−41	55
	0·0178	Rabbit	−14, −25	−19	17
	0·0298		−14, −35	−24	26
	0·0593		−29, −36	−32	41
Chloretone	0·012	Rat	− 7, −18	−12	15
	0·024		−25, −26	−25	26
	0·0333		−35, −28, −34	−32	32
	0·0367		−43, −37	−40	35
	0·007	Guinea-pig	−17, − 6	−12	16
	0·014		−28, −20	−24	27
	0·021		−38, −41	−39	36
	0·0267		−56, −49	−52	41

* Avertin consists of 2 parts by wt. of tribromoethyl alcohol and 1 part of amylene hydrate. The concentration given in the table is that of tribromoethyl alcohol.

† The "control" medium contains 0·0019 g./100 c.c. of magnesium ion. The concentrations given in the table are those added to the control medium.

Tables I and II give the mean inhibitions exerted by the narcotics over a period of 2 hr. at 39°. The data obtained, as long as the

inhibitions do not exceed 35–40 p.c., can be represented by equations of the form

$$I/1-I=Kc,$$

I being the fractional inhibition of respiration, c the concentration of the narcotic, and K a constant dependent on the particular narcotic and varying also perhaps from one animal species to another. The equation can be derived from the assumption that reversible chemical combination occurs between the narcotic and some component of the respiratory system to which respiration is proportional. That the data

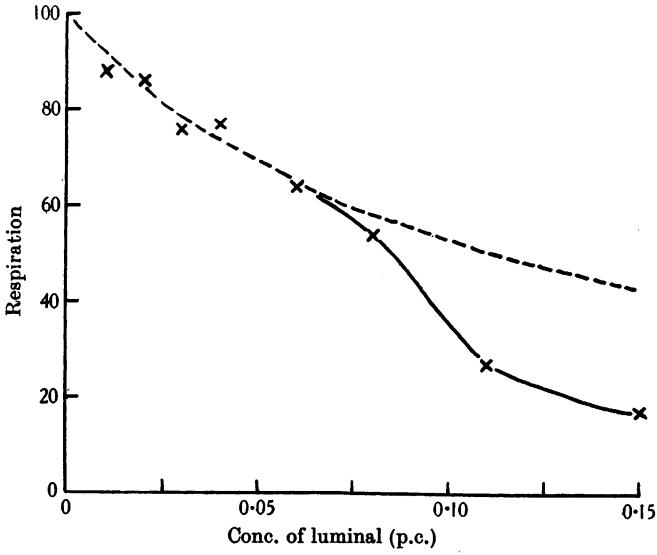

Fig. 1. The effect of luminal on the respiration of rat brain in presence of glucose (mean effect for 2 hr.). The dotted curve is a mass-action curve.

fit the equation does not of course prove the validity of the assumption; the equation however at least serves to represent the data within the experimental error. This may be seen by examining the data in Tables I and II, and also Figs. 1 and 6.[1]

The smaller inhibitions do not vary very greatly with time over a 2 hr. period (Figs. 2, 4, 5, 7). The initially lower values usually observed may be due to the time required for the narcotic to diffuse into the tissue slices. After some time the smaller inhibitions are usually approximately

[1] The data obtained by Jowett & Quastel [1937*a*] with luminal did not appear to fit a mass-action curve. This was due to there being insufficient data for the smaller inhibitions, where alone the relation holds.

steady. The steadiness supports the view that an approximate equilibrium is set up between the narcotic and the respiratory system of the tissue.

When the narcotics are present in concentrations which produce inhibitions exceeding about 40 p.c., definite divergence from the mass-action equation takes place, and in the sense that the observed inhibitions become greater than those calculated. (This is true for the organic narcotics; the inorganic narcotic, magnesium ion, shows if anything a

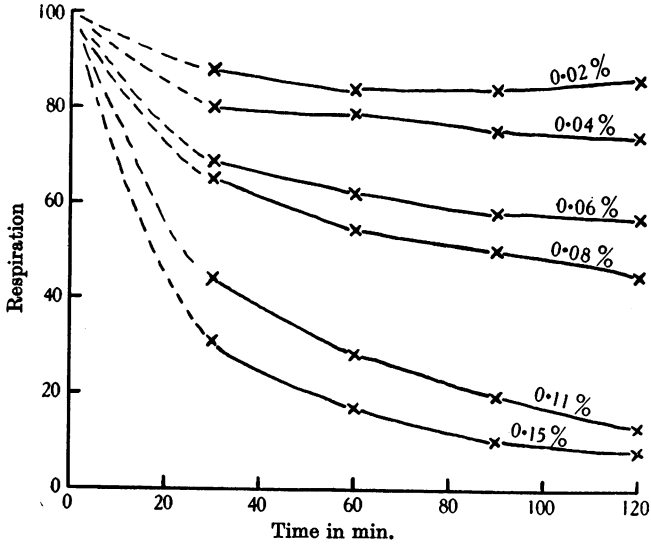

Fig. 2. The effect of luminal on the respiration of rat brain in presence of glucose, as a function of time. The curves are mean values from several experiments.

divergence in the opposite direction.) A clue to the nature of the divergence is found on examining the large inhibitions as a function of time (e.g. Figs. 2, 4, 5), when it is seen that the inhibitions increase considerably as time passes. The nature of the effect is best seen when values of K are calculated as a function of time and of concentration. The results of such a calculation for luminal have been plotted in Fig. 3, which shows that while there is a rough constancy of K for the lower concentrations of luminal, above a concentration of about 0·07 p.c. there is an increase of K progressive with time and concentration. The curves suggest that for a short time after exposure of the tissue to luminal the values of K may be constant even at high concentrations of luminal. The results may be interpreted on the view that a reversible inhibition occurs

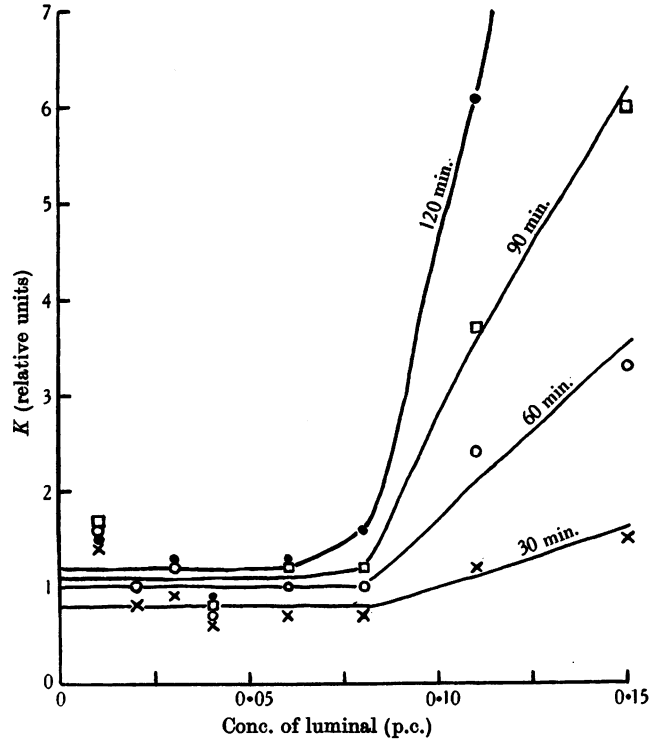

Fig. 3. The mass-action "constants" for the action of luminal on the respiration of rat brain in presence of glucose, as functions of time and luminal concentration.

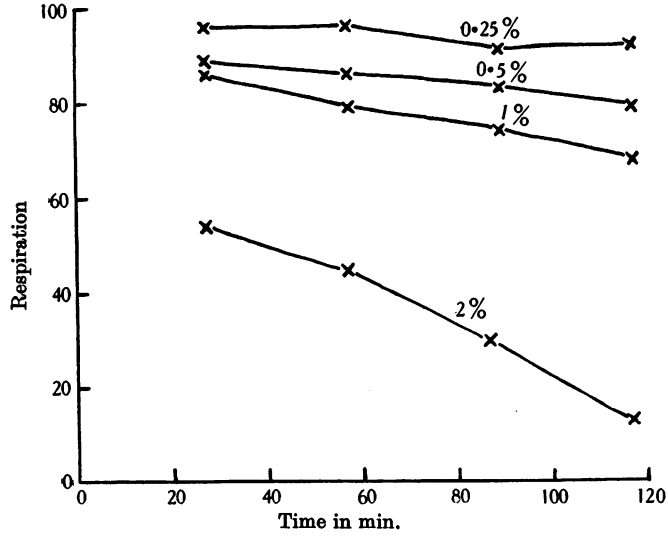

Fig. 4. The effect of ethylurethane on the respiration of rat brain in the presence of glucose.

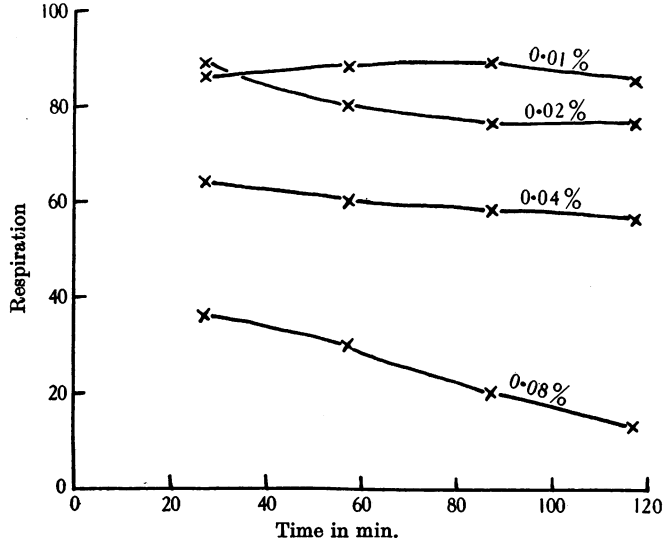

Fig. 5. The effect of avertin on the respiration of rat brain in the presence of glucose.

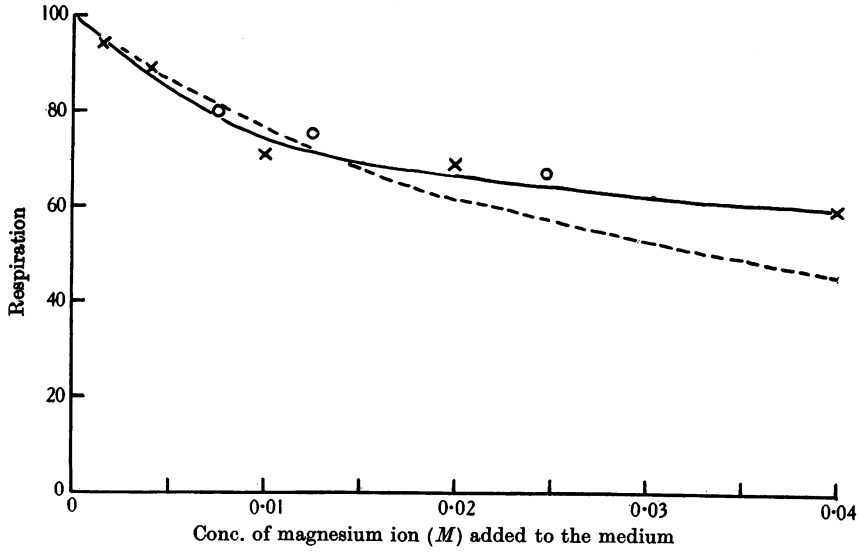

Fig. 6. The effect of magnesium on the respiration of brain in presence of glucose (rat brain ×, rabbit brain o). Mean effect for 2 hr. The dotted curve is a mass-action curve.

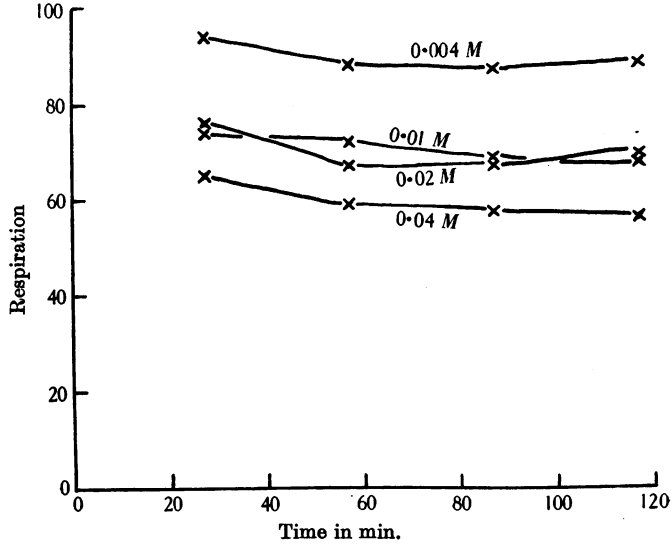

Fig. 7. The effect of magnesium ion on the respiration of rat brain in the presence of glucose.

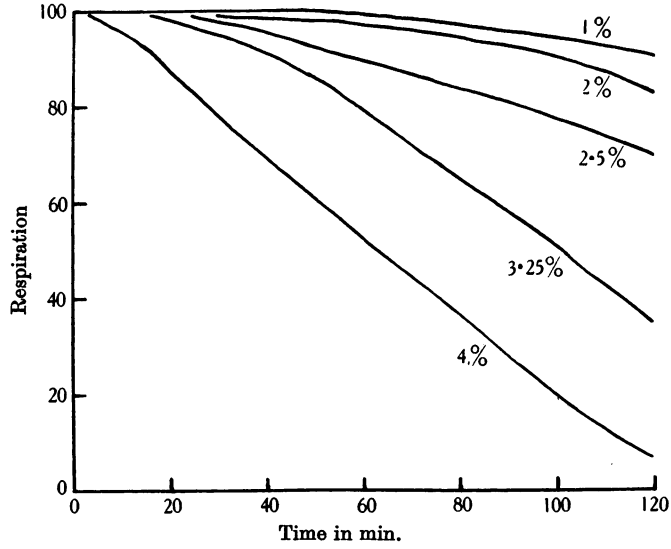

Fig. 8. The effect of ethyl alcohol on the respiration of rat brain in the presence of glucose (at 39°). The curves are smoothed from the means of several experiments.

initially, and that when the inhibition exceeds about 40 p.c. the respiration decreases further in an irreversible manner. Perhaps the equilibrium between narcotic and respiratory system is rather unstable, or possibly the cell is unable for long to maintain the full integrity of its respiratory function if its supply of energy is cut down too far. No definite explanation of the phenomenon is suggested at present.

The six narcotics discussed may probably be described as reversible in action, provided only moderate inhibitions are considered. Another type of action on respiration has already been found to occur in the case

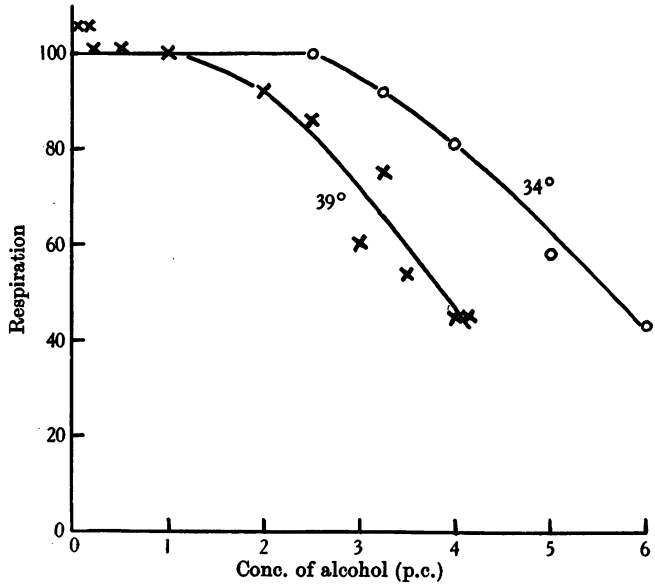

Fig. 9. The effect of alcohol (at 34° and 39°) on the respiration of rat brain in presence of glucose. Mean effect for 2 hr.

of ethyl ether [Jowett & Quastel, 1937*b*]. The action of ether on the respiration of brain in the presence of glucose is to cause progressive inhibitions, which never become steady. Ethyl alcohol has now been found to affect respiration in the same manner as ether. The effect of alcohol, as a function of time and concentration, is shown in Fig. 8. The inhibition, extrapolated back to the time of addition of alcohol, is nil. The inhibitions increase indefinitely as a function of time and concentration. Possibly at the lower concentrations there is a time-lag before the inhibition begins to show itself.

The rate at which the inhibitions develop depends on the temperature. This is shown in Fig. 9, where the mean inhibitions over a 2 hr. period

after addition of alcohol are shown for various concentrations at 39° and 34°. The inhibitions at the lower temperature are much smaller. Ether shows the same phenomenon. In the case of ether the inhibitions have been shown to be irreversible. This is probably true for alcohol also, and so the results are interpreted as meaning that the rate of irreversible inactivation of respiration has a high temperature coefficient.

The effect of narcotics on respiration at concentrations which narcotize the organism

The data on inhibition of respiration of brain slices by the reversible narcotics can be represented by means of the constants K, which have already been used to obtain the "calculated" values for inhibitions in Tables I and II. The values of K are given in Table III, the concentrations of the narcotics for this purpose being taken in mol./l., since the K values in this system of units give a truer measure of the inhibitory power of the narcotic. It will be seen that the values of K vary over a range of nearly two hundred times. The values of K are probably in error on the average by not more than 20 p.c.

TABLE III. Inhibitory activity of narcotics

Narcotic	Mol. wt.	Animal	K	Conc. of narcotic (M) producing 15 p.c. inhibition
Ethylurethane	89	Rat	2·8	0·063
Magnesium ion	24	Rabbit	28·5	0·0062*
,,	24	Rat	30	0·0059*
Chloral hydrate	165	,,	86	0·0021
Luminal	254	,,	230	0·00077
Chloretone	177	,,	253	0·00070
Evipan	260	Guinea-pig	325†	0·00054
Avertin	283	Rat	430	0·00041
Chloretone	177	Guinea-pig	470	0·00038

* Excess above the normal concentration.
† This value has been roughly estimated from data of Jowett & Quastel [1937*a*].

On the other hand no more than a very rough estimate can be made of the concentrations of the narcotics which are in equilibrium with the brain in any definite narcotic state in the intact organism. Estimates have been made by taking data for the doses which produce some degree of narcosis, and assuming that the narcotics are evenly distributed throughout the body. The dose in g./kg. has been assumed to equal the concentration in g./l. Three different errors may make the result inaccurate: (*a*) the depths of narcosis may be variable, (*b*) the narcotic dose may vary from one species to another, (*c*) the assumption of even distribution may be incorrect.

The third source of error is probably the most important. The assumption may give values which are too low, for the narcotic may be distributed only in the water of the body, or may be concentrated at the relevant time in the most vascular tissues. The assumption may on the other hand lead to values which are too high, since absorption may be incomplete at the time considered, or appreciable excretion or destruction of the narcotic may have occurred. What is really required for our purpose is a knowledge (not at present available) of the concentration of the narcotic in the blood—or, more exactly, in the ultra-filtrate of the serum—supplying the brain at the time when the more vascular parts of the brain are in equilibrium with the blood.

The data on narcotic doses taken for the present purpose are given in Table IV. Comment is required with regard to magnesium and evipan.

TABLE IV. Narcotic doses

Narcotic	Dose g./kg.	Administration	Species	Narcosis	Reference
Ethylurethane	2	(Subcutaneous?)	Rabbit	?	Kane & O'Connor [1937]
Magnesium	0·1–0·2	?	?	?	Schmidt & Greenberg [1935]
Chloral hydrate	0·2–0·25	Intraperitoneal	Sheep	Deep, 30–60 min.	Kucera [1933]
Luminal	0·2	?	Monkey	Light surgical	Keller & Fulton [1931]
Chloretone	0·18	Intraperitoneal	Guinea-pig	—	Jowett & Quastel [1937*a*]
Chloretone	0·2	Intraperitoneal	Cat, dog	Satisfactory	Haag & Bond [1927]
Evipan	0·0125	Intravenous	Rabbit	Very light, 10 min.	Jowett & Quastel [1937*a*]
Evipan	0·16–0·45	Intravenous	Rabbit	Good surgical, 70–195 min.	Maloney & Hertz [1935]
Avertin	0·08–0·10 0·3	Per rectum	Man Rabbit	Basal	Bäyer Bacq & Brown [1937]

In the case of magnesium some information is available regarding the concentration in the blood during narcosis; Schmidt & Greenberg [1935] state that the concentration is 7–21 mg./100 c.c. in the rabbit. Taking the mean as 14 mg. and subtracting the normal blood magnesium, a value of about 0·005 *M* is derived. The same value, it may be noted, would be derived from considering the dosage in this case. This value may not represent very accurately the magnesium ion concentration in the serum, for the available information on the state and distribution of magnesium in blood is suggestive rather than decisive.

As regards evipan, the fact that this drug is given intravenously makes estimation of its narcotizing concentration difficult. Two ways of esti-

mating it, however, agree fairly well. The first assumes it to be given by reckoning a dose producing light anæsthesia for 10 min. to be distributed throughout the blood only. The second assumes that a number of divided doses producing good surgical anæsthesia for some 70 min. are distributed evenly throughout the body.

In Table V are calculated the narcotizing concentrations of the narcotics, and these are compared with the concentrations (calculated from the K values) which produce 15 p.c. inhibition of the respiration

TABLE V. Narcotizing concentrations and comparison of narcotizing and inhibitory concentrations

Narcotic	Animal	Estimated narcotic dose g./kg.	Narcotizing conc. (M)	15 p.c. inhibitory conc. / narcotizing conc.	Inhibition p.c. due to narcotizing conc.
Ethylurethane	Rat	2	0·022	2·9	6
Magnesium ion	Rabbit	0·15	0·005*	1·2	12
Magnesium ion	Rat	0·15	0·005*	1·2	13
Chloral hydrate	,,	0·22	0·0013	1·6	10
Luminal	,,	0·2	0·00079	1·0	15
Chloretone	,,	0·18	0·0010	0·7	20
Evipan	Guinea-pig	0·16	0·00062	0·9	17
Avertin	Rat	0·3	0·00106	0·4	31
Chloretone	Guinea-pig	0·18	0·0010	0·4	32

* Excess above the normal concentration.

of brain slices in presence of glucose. This 15 p.c. inhibition has been selected as making the two sets of concentrations (in Tables III and V) approximate to equality. The range of the ratios is considerable, but is hardly more than would be expected from the error in estimating the narcotizing concentration.

The result of the comparison is not inconsistent with the view that the seven narcotics considered produce equal inhibitions of some 15 p.c. in the respiration of brain slices when present at concentrations which in the organism would produce an equal fairly deep narcosis. The data do not of course establish such a view, but are sufficient to show that a definite inhibition of respiration is produced by concentrations of narcotics of the order of those producing deep narcosis. The inhibitions, as calculated in the last column of Table V, range from 6 to 32 p.c.

The inhibitory action of alcohol, which has already been shown to be of a different type, does not begin to be appreciable at the narcotizing concentration. Alcohol produces narcosis at a concentration of about 0·5–1 p.c., and reference to Fig. 8 shows that not until the alcohol concentration exceeds 2 p.c. does the inhibition after 2 hr. amount to 20 p.c.

Alcohol is again behaving like ether, for which it has already been found [Jowett & Quastel, 1937*b*] that the narcotizing concentration is a threshold value for inhibitory effects.

Discussion

The view is already held [Winton & Bayliss, 1935] that inhibition of oxidations in the brain probably accounts largely for narcotic action. This view is perhaps based on experiments showing diminished oxygen consumption in the brain in the intact organism. It must however be pointed out that no work *in vitro*, prior to the present work and that of Jowett & Quastel [1937*a*], had established that narcotics diminish the respiratory power of tissues at the concentrations at which they act *in vivo*. The use of brain slices has made it possible to show that the respiratory power of the cells themselves is diminished in a direct way by narcotics at the pharmacologically active concentrations.

The action of narcotics on the oxidation of all substrates by brain is not a general one. The oxidations of glucose, fructose, lactate and pyruvate are all about equally sensitive to narcotics, but the oxidations of other substrates, such as succinate and α-glycerophosphate, are much less sensitive.

The mode of action of narcotics on the respiratory systems of cells is as yet unknown. A suggestion as to a possible point of attack has been made [Jowett, 1937], which will be investigated.

The present work strengthens the view that narcotics act biologically by inhibiting cell oxidations. The behaviour of five different chemical types of narcotics is in agreement with the view. On the other hand, alcohol and ether do not inhibit appreciably the oxidation of glucose at their narcotizing concentrations; at higher concentrations they inhibit the oxidation of glucose irreversibly. Alcohol and ether are less polar molecules than the other narcotics investigated, and it may be that this fact is related to their behaviour.

Summary

The effect of narcotics on the respiration of slices of cerebral cortex (mainly of the rat) has been investigated in presence of glucose. The behaviour of narcotics can be divided into two classes.

In the first class, and when the inhibitions do not exceed 40 p.c., the inhibitions of respiration caused by narcotics vary little with time, and are consonant with the view that the narcotic reaches a mass-action equilibrium with some component of the respiratory system, so that the

inhibition is given by a simple mass-action equation. With high concentrations of narcotics (excepting magnesium ion), the large inhibitions observed increase with time, and probably become irreversible.

Narcotics in this class include urethane, magnesium, chloral, luminal, evipan, chloretone and avertin. The inhibitions they produce are measurable (the figures ranging from 6 to 32 p.c.) at concentrations of the same order as those which produce general narcosis in the intact animal. Inhibition of glucose oxidation may therefore be the cause of the narcosis.

In the second class, alcohol and ether (the latter previously investigated) are found. These substances inhibit respiration progressively and probably irreversibly. The anæsthetic concentrations are only threshold values for the production of inhibition of respiration.

I am much indebted to Dr J. H. Quastel for his interest in this work.

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