# LXXIX. THE EFFECTS OF NARCOTICS ON TISSUE OXIDATIONS

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IN view of the hypothesis that narcotics exert their characteristic effects on the organism through effects on oxidations of the brain [Quastel & Wheatley, 1932, 1], a more extended study of the effects of narcotics on tissue oxidations appeared desirable.

It has already been found [Quastel & Wheatley, 1932, 1] that narcotics inhibit the oxidations of glucose, lactate and pyruvate by the brain. In the present work this finding has been confirmed. An extension of the investigation to other tissues has shown that inhibition of oxidations is not restricted to the brain, or to carbohydrates and their breakdown products. A beginning has been made with a study of the mechanism of action of narcotics on oxidations, and also with a comparison between the concentrations at which narcotics bring about narcosis and those at which they inhibit oxidations.

### *Experimental details*

The manometric methods of Warburg have been used with tissue slices. The portion of the brain used has always been the grey matter of the cerebral cortex. Media used have been isotonic, and made up on the lines followed by Jowett & Quastel [1935]. Unless otherwise stated, the medium contained 0·001 $M$ $Ca^{++}$, 0·0008 $M$ $Mg^{++}$, and 0·02 $M$ phosphate of $p$H 7·2, in addition to NaCl and various named constituents; $p$H 7·2 has been preferred to the more usual 7·4 so as to improve the buffering in the alkaline direction when lactate and pyruvate are being oxidized. Measurements have been made at 39° unless the contrary is stated, since this temperature is considered a better approximation than the more usual 37–38° to the temperature *in vivo* of the internal organs. In most experiments the volume of medium has been 3 ml., and 8–15 mg. dry weight of tissue have been present in each vessel. Oxygen has been the gas present in all respiration experiments.

## THE EFFECT OF NARCOTICS ON OXIDATIONS IN THE CEREBRAL CORTEX

In Table I are summarized the results of experiments on the effects of 0·08 % luminal (sodium phenylethylbarbiturate) on the respiration of brain in the presence of various substrates. It is evident that the inhibition of respiration depends on the substrate that is being burnt. The inhibition is greatest for glucose, followed by lactate and pyruvate. Glutamate shows a smaller inhibition, and the oxidation of succinate is not inhibited.

It has already been found by Quastel & Wheatley [1932, 1], using minced whole brain, that narcotics inhibit the oxidations of glucose, lactate and pyruvate, and to a lesser extent that of glutamate, while the oxidations of

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Table I. *The effect of 0·08 % luminal on brain respiration*

K+ 0·002 *M*. Mean figures for a 2-hour period
The average deviations of single experiments from the mean are given

| Substrate | No. of exps. | $Q_{O_2}$ without narcotic | % effect of narcotic on $Q_{O_2}$ |
|---|---|---|---|
| | A. Rat brain | | |
| Nil | 5 | − 2·89 ± 0·18 | − 5 ± 6 |
| Glucose 0·04 *M* | 4 | − 10·77 ± 0·70 | − 57 ± 5½ |
| Glucose 0·01 *M* | 4 | − 12·20 ± 0·33 | − 55 ± 2½ |
| *d*-Lactate 0·02 *M* | 2 | − 13·55 ± 0·63 | − 35 ± 1½ |
| Pyruvate 0·02 *M* | 2 | − 11·10 ± 0·65 | − 27 ± 3 |
| Glutamate 0·02 *M* | 3 | − 8·03 ± 1·05 | − 15 ± 9 |
| Succinate 0·02 *M* | 2 | − 9·53 ± 0·10 | + 7 ± 4 |
| | B. Guinea-pig brain | | |
| Glucose 0·04 *M* | 1 | − 11·8 | − 44 |
| Glucose 0·01 *M* | 1 | − 11·0 | − 40 |

succinate and *p*-phenylenediamine are unaffected. The present experiments were made with slices of the grey matter of the cerebral cortex, under conditions considerably different from the earlier ones, and it is therefore of value that the former results are confirmed.

When no oxidizable substrate is added, the small oxidation of the brain slices is not appreciably affected by 0·08 % luminal. This finding shows that the respiration in absence of added substrates is not due to the oxidation of traces of glucose, lactate or pyruvate present in the tissue. It is unlikely that these substances would be present in appreciable quantities, for in the preparation of the slices some will be lost; also in the medium, before measurements are made, further loss will occur, partly by oxidation and partly by diffusion. It is now, however, definitely shown that the residual respiration of brain is due largely to substances other than glucose, lactate or pyruvate. The only clue to the nature of the substances oxidized is provided by the finding of Dickens & Greville [1933] that the R.Q. of the oxidation is unity.

If the concentration of luminal is doubled, the respiration in absence of added substrates is then considerably inhibited (Table II). The experiments in Table II show that the increase in respiration due to succinate varies little with

Table II. *The effect of luminal on succinate oxidation by rat brain*

K+ 0·002 *M*

| Exp. no. ... | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|
| Conc. of luminal % | 0 | 0·08 | 0 | 0·08 | 0 | 0·15 |
| $Q_{O_2}$ in absence of substrate: | | | | | | |
| 1st hour | 4·52 | 4·33 | 4·90 | 4·52 | 4·25 | 1·66 |
| 2nd hour | 1·23 | 1·45 | 1·34 | 1·26 | 1·10 | 0·47 |
| $Q_{O_2}$ in presence of 0·02 *M* succinate: | | | | | | |
| 1st hour | 11·22 | 12·57 | 11·10 | 11·41 | 11·42 | 10·81 |
| 2nd hour | 8·02 | 8·75 | 7·76 | 7·97 | 7·70 | 8·27 |
| Increase in $Q_{O_2}$ due to succinate: | | | | | | |
| 1st hour | 6·70 | 8·24 | 6·20 | 6·89 | 7·17 | 9·15 |
| 2nd hour | 6·79 | 7·30 | 6·42 | 6·71 | 6·60 | 7·80 |

time and is not inhibited by luminal. The increase is actually raised by the presence of the narcotic, which shows that there is some competition for oxidation between succinate or its oxidation products and the residual substrates of

the brain. Competition between succinate and lactate in the brain has already been demonstrated by Quastel & Wheatley [1932, 2]. It may be noted that the constancy with time of the increase in respiration due to succinate in the absence of narcotics coexists with competition, and such constancy therefore cannot be used as an argument for additivity of respiration.

In the experiments reported in Table I, the inhibition of respiration by the narcotic increases considerably with time when glucose and lactate are the substrates. No definite increase has been found with pyruvate and glutamate.

### *The effect of $K^+$ concentration*

The increase with time of the inhibition of respiration by narcotics is related to the composition of the medium, and especially to the $[K^+]$.

In Table III is shown the effect of varying the $[K^+]$ of the medium on the inhibitory effect of narcotics on brain respiration. Considering the experiments

Table III. *The effect of narcotics on the respiration of brain at high and low $[K^+]$*

$K^+$ 0·002 or 0·0128 *M*. When chloretone is present, it is added from a side-tube 5 min. before readings are begun. Experimental period 2 hours.

| Fig. | Narcotic | Animal | Substrate | % effect of narcotic (a) low $[K^+]$ | % effect of narcotic (b) high $[K^+]$ |
|---|---|---|---|---|---|
| | | A. Temperature 39° | | | |
| 1 | Luminal 0·08 % | Rat | Glucose 0·01 *M* | −54 | −49 |
| | ,, 0·08 % | ,, | ,, | −57 | −47* |
| | ,, 0·08 % | Guinea-pig | ,, | −39 | −43 |
| | ,, 0·08 % | ,, | Pyruvate 0·01 *M* | — | −28 |
| | ,, 0·08 % | ,, | *d*-Lactate 0·02 *M* | — | −31 |
| 2 | Chloretone 0·033 % | Rat | Glucose 0·01 *M* | −52 | −35 |
| 3 | ,, 0·033 % | ,, | ,, | −41 | −28 |
| | ,, 0·033 % | ,, | ,, | — | −34 |
| | ,, 0·027 % | Guinea-pig | ,, | −62 | −56 |
| 4 | ,, 0·027 % | ,, | ,, | −60 | −49 |
| | ,, 0·037 % | Rat | *dl*-Lactate 0·02 *M* | −45 | −45 |
| 5 | ,, 0·037 % | ,, | ,, | −47 | −47 |
| | ,, 0·037 % | ,, | Pyruvate 0·01 *M* | — | −39 |
| | ,, 0·037 % | ,, | ,, | — | −40 |
| | | B. Temperature 29° | | | |
| 6 | Chloretone 0·033 % | Rat | Glucose 0·01 *M* | −34 | −36 |
| | ,, 0·033 % | ,, | ,, | −21 | −33 |
| | ,, 0·033 % | ,, | ,, | −22 | −21 |

* The $[K^+]$ in this case was 0·006 *M*.

at 39°, we see that the mean inhibition is usually (in 7 experiments out of 10) lower when the $[K^+]$ is raised. The K concentrations are of the same order of magnitude as that in serum (about 0·006 *M*).

To observe the real nature of the effect it is necessary to examine the inhibitions as a function of time, which is best done graphically.

The graphs (Figs. 1–5) show that, when the $[K^+]$ is low (0·002 *M*), the inhibitions increase with time, often considerably. The effect of the higher $[K^+]$ (0·0128 *M*) is to stabilize the inhibition. The stabilization is complete in some instances (e.g. the action of chloretone on rat brain with glucose as substrate, Figs. 2 and 3), in other instances less complete (e.g. Figs. 1 and 5), but in every

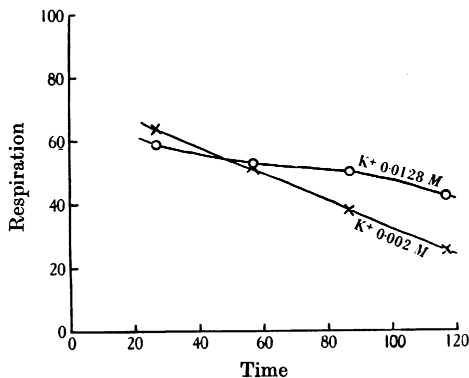


Fig. 1.

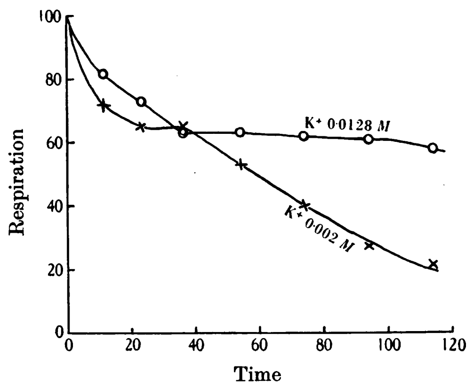


Fig. 2.

Fig. 1. Effect of 0·08 % luminal on respiration of rat brain in presence of glucose.

Fig. 2. Effect of 0·033 % chloretone on respiration of rat brain in presence of glucose.

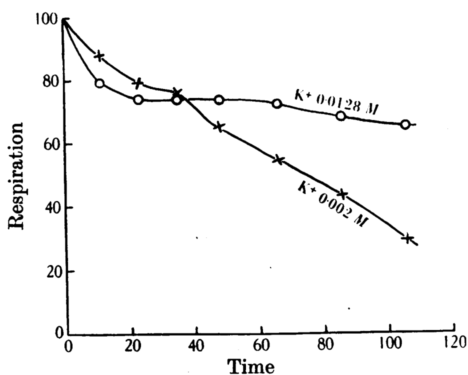


Fig. 3.

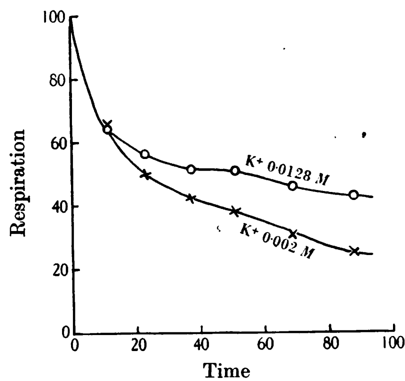


Fig. 4.

Fig. 3. As Fig. 2.

Fig. 4. Effect of 0·027 % chloretone on respiration of guinea-pig brain in presence of glucose.

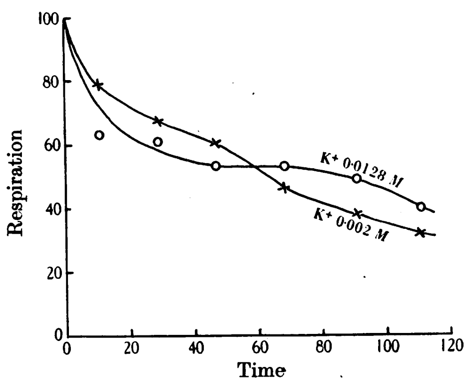


Fig. 5.

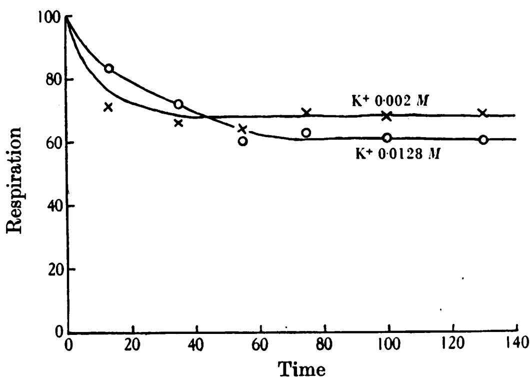


Fig. 6.

Fig. 5. Effect of 0·037 % chloretone on respiration of guinea-pig brain in presence of lactate.

Fig. 6. Temperature 29°. Effect of 0·033 % chloretone on respiration of rat brain in presence of glucose.

experiment the inhibition changes less with time when the [$K^+$] is raised from the low level of 0·002 $M$. A tendency is sometimes seen for a temporary steady inhibition to show itself even at this low [$K^+$]. It may be noted that Quastel & Wheatley [1934], who found steady inhibitions with narcotics in their experiments on the reversibility of the inhibitions, used a [$K^+$] of 0·0128 $M$, although this was not stated in their paper.

When the temperature is lowered 10° to 29°, the inhibitory effect of chloretone no longer varies appreciably with the [$K^+$] (e.g. Fig. 6). A steady inhibition is found whether the [$K^+$] is high or low. The increasing inhibition at 39° is probably due to loss of K from the cell at the low external concentration, due to irreversible changes in the cell, which proceed much more slowly when the temperature is lowered. The mechanism of action of K is perhaps related to the fact, which will be discussed in a later communication, that respiration is higher and steadier in media containing the higher [$K^+$]. Perhaps higher respiration maintains the nerve cell for a longer time in its normal state, better able to resist irreversible changes.

*The rate of development of inhibition*

Chloretone is a narcotic sufficiently soluble to be added to the medium from a side-tube in the dissolved state, and in the experiments with chloretone it is therefore possible to study the rate of development of its inhibitory action almost from the time of applying it to the tissue slices. The manometric method used is not very suitable for measuring quantitatively rapid changes in rate, and the results show considerable variation in the observed time-course of development of inhibition (cf. e.g. Figs. 2, 3, 5, 6). Determined graphically, the time required at 39° for the inhibition to reach half its steady or approximately steady value in the high-potassium medium varies from 3 to 11 min. after the time of addition of the narcotic. The average time required is 7 min.; the exact value is uncertain, but the order of magnitude should be correct. The time required for complete development of a steady inhibition is naturally a less definite quantity, but it may be estimated at 25–45 min. At 29° the time required for the development of the inhibition is perhaps longer. The limiting factor in the rate of development of the inhibition may well be the rate of diffusion of the narcotic into the tissue slices. The rate of diffusion is at least probably of the same order of magnitude as the rate of development of inhibition.

*The temperature coefficient of the inhibition*

A comparison of the magnitudes of the steady inhibitions exerted by chloretone on the respiration of rat brain in presence of glucose at the two temperatures 39 and 29° (Table III) shows that, in the high-K medium, temperature does not affect these inhibitions appreciably. The absence of a temperature coefficient is in contrast with the behaviour of ether, which will be considered in a later communication.

*The effect of narcotic concentration*

In Figs. 7 and 8 are shown the results of experiments in which the narcotic concentration was varied. The inhibition of respiration increases with the concentration of narcotic, but the curves are of a sigmoid character. It is however likely on theoretical grounds that the inhibition should follow the law of mass action, which when simply applied gives a very different type of curve. It occurred to us that the apparent deviation from the law of mass action

might be due to the uptake by the tissue at low concentrations of an appreciable proportion of the narcotic in the medium, so that the concentrations as plotted are, at low values, higher than the equilibrium concentrations. The correction to be applied should on theoretical grounds be greater the lower the narcotic concentration if we are dealing with an adsorption of the narcotic by certain tissue elements.

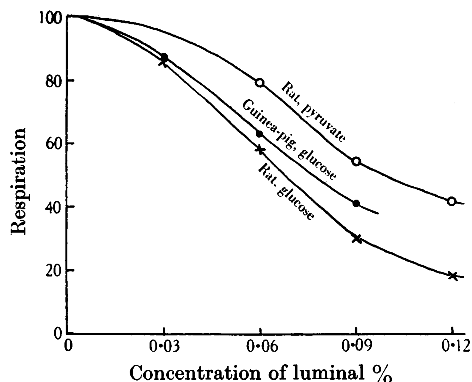


Fig. 7.

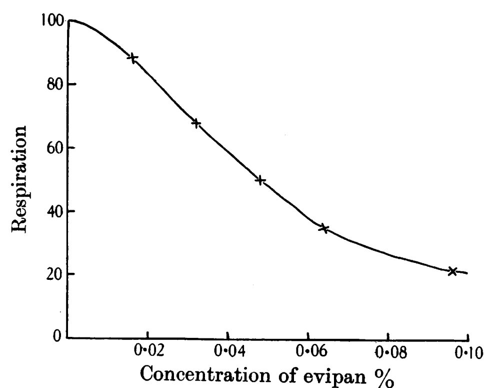


Fig. 8.

Fig. 7. Effect of varying concentrations of luminal on brain respiration. K+ 0·002 *M*.

Fig. 8. Effect of varying concentrations of evipan on respiration of guinea-pig brain in presence of glucose. K+ 0·006 *M*.

Experiments were therefore made in which the amount of brain tissue per unit volume of medium was varied, and the inhibition of respiration caused by the narcotic was examined in relation to this factor. A similar method of demonstrating adsorption has been used by Jowett & Brooks [1928] in another connexion.

The data in Table IV show that at tissue concentrations of about 10 mg. dry weight per ml. of medium the inhibition exerted by the narcotic is less than at

Table IV. *The effect of tissue concentration on narcotic inhibition*

K+ 0·0128 *M*. Glucose 0·01 *M*

| Exp. | Animal | Narcotic | mg. dry wt. per ml. medium | Effect of narcotic on respiration % |
|---|---|---|---|---|
| 1 | Rat | Luminal 0·08 % | 1·5 | −63 |
| | ,, | ,, 0·08 % | 3·7 | −63 |
| | ,, | ,, 0·08 % | 5·3 | −65 |
| 2 | ,, | ,, 0·035 % | 4·3 | −23 |
| | ,, | ,, 0·035 % | 9·7 | −16 |
| 3 | Guinea-pig | ,, 0·035 % | 2·7 | −26 |
| | ,, | ,, 0·035 % | 4·8 | −26 |
| | ,, | ,, 0·035 % | 11·1 | −19 |
| 4 | ,, | ,, 0·035 % | 2·6 | −33 |
| | ,, | ,, 0·035 % | 9·5 | −17 |

low tissue concentrations. This shows that at high concentrations the tissue, as suggested, takes up an appreciable proportion of the narcotic.

The observed effect is in the right direction to explain the deviation of the curves of Fig. 7 from the law of mass action. In the experiments of Fig. 7, however, the tissue concentration is only 3–5 mg. dry weight per ml., and it does not appear likely from Table IV that at such tissue concentrations the adsorption of narcotic is sufficient to alter the inhibition seriously.

Hence the suggested explanation probably does not suffice to explain the sigmoid curves. A further possibility is that the narcotic inhibits according to the law of mass action some step or steps in oxidation that do not ordinarily limit the rate of the consecutive chain of reactions that makes up the total oxidation, so that the mechanisms of those steps can be inhibited considerably before the rate of the whole chain is slowed down very appreciably. A test of this conception must await a detailed investigation of individual steps in oxidation processes; if the conception is correct some step or steps will be found which are more sensitive to narcotics than is the sum-total of oxidations.

*The effect of $Ca^{++}$ and phosphate on inhibitions*

The $[K^+]$ in the medium has already been shown to influence the inhibitory action of narcotics on brain oxidations.

It has been found that other ions have also some effect on their action. In the experiments shown in Table V the inhibitions were measured over four successive half-hour intervals, the figures given in the table being the first and the last of the four values obtained.

Table V. *The effect of phosphate and $Ca^{++}$ on the inhibition of brain respiration by 0·08 % luminal*

Glucose 0·01 *M*

The effect of phosphate concentration

| Exp. | Species | % inhibition in presence of (a) 0·02 *M* P | (b) 0·06 *M* P | |
|---|---|---|---|---|
| 1 | Guinea-pig | — | 42–49 | $K^+$ 0·0128 *M*, $Ca^{++}$ 0 |
| 2 | ,, | 45–61 | 43–50 | |
| 3 | ,, | 42–61 | — | |

The effect of Ca and Mg ($Ca^{++}$ 0·001 *M*, $Mg^{++}$ 0·0008 *M*)

| Exp. | Species | % inhibition (a) $Ca^{++}$, $Mg^{++}$ present | (b) absent | |
|---|---|---|---|---|
| 4 | Guinea-pig | 34–48 | 42–61 | $K^+$ 0·0128 *M*, 0·02 *M* P |
| 5 | Rat | 34–60 | — | $K^+$ 0·006 *M*, 0·02 *M* P |
| 6 | ,, | 33–60 | 53–74 | $K^+$ 0·006 *M*, 0·02 *M* P |
| 7 | ,, | — | 49–76 | $K^+$ 0·006 *M*, 0·02 *M* P |

The data show that at a higher phosphate concentration than the usual 0·02 *M* the inhibitions are to some extent stabilized. The effect may be due to stabilization of the *p*H through the increase of the buffering power of the medium.

When $Ca^{++}$ and $Mg^{++}$ are present the inhibition caused by luminal is less, at least with rat brain, than when these ions are absent. As $Mg^{++}$, in a single experiment, has no effect, the lessening of the inhibition is probably due to the $Ca^{++}$. The effect may be related to the supposed influence of $Ca^{++}$ in lessening permeability or reactivity of cells.

*The effect of varying substrate concentrations*

It was found by Davies & Quastel [1932] under anaerobic conditions that the reduction of methylene blue by minced brain in presence of carbohydrates, lactate and pyruvate was inhibited by narcotics, and that with lactate as substrate the inhibition varied with the substrate concentration. Apparently there was competition between substrate and narcotic for the dehydrogenases.

Under our aerobic conditions we have found no evidence that the inhibitions exerted by narcotics on respiration of brain slices depend on the substrate concentration. Data in Table I show that varying the glucose concentration does not affect the luminal inhibition, and data given in Table VI show that varying the concentration of lactate or pyruvate does not affect the inhibition of respiration by luminal or chloretone.

The apparent discrepancy between the two results is probably due to the very different conditions of the two sets of experiments, which involve different media, different states of the tissue, and aerobic as against anaerobic conditions. It may be noted that Davies & Quastel had to use ten times as high a chloretone concentration to secure inhibitions of the order obtained in our experiments.

We are inclined at present to believe that the difference between the results is due to the use of anaerobic conditions by Davies & Quastel, which, it is suggested, results in a lowered sensitivity to narcotics. The action of narcotics under anaerobic conditions is at present being investigated.

Table VI. *The effect of substrate concentration on inhibitions*

$K^+$ 0·0128 *M*. Experimental period 2 hours

| Exp. | Species | Substrate | Narcotic | Inhibition of $Q_{O_2}$ % |
|---|---|---|---|---|
| 1 | Rat | Pyruvate 0·01 *M* | Chloretone 0·037 % | 37 |
| | | ,, 0·08 *M* | ,, | 37 |
| 2 | Guinea-pig | ,, 0·06 *M* | Luminal 0·08 % | 27 |
| | | ,, 0·01 *M* | ,, | 28 |
| 3 | Guinea-pig | *d*-Lactate 0·06 *M* | ,, | 39 |
| | | ,, 0·01 *M* | ,, | 31 |

*The destruction of pyruvate*

It has been found (Table VII) that, when the respiration of brain in presence of pyruvate is inhibited by luminal, the disappearance of pyruvate from the medium is inhibited to an approximately equal extent. Pyruvate has been estimated colorimetrically [Jowett & Quastel, 1937].

Table VII. *The effect of 0·12 % luminal on the oxidation of pyruvate by rat brain*

$K^+$ 0·006 *M*, pyruvate 0·01 *M*. 2 hours
$Q_P$ = rate of formation of pyruvate in the same units as $Q_{O_2}$

| Exp. | Metabolic quotient | Value of quotient: (*a*) Alone | (*b*) With luminal | Effect of luminal % |
|---|---|---|---|---|
| 1 | $Q_{O_2}$ | −13·1 | −5·6 | −57 |
| | $Q_P$ | − 7·6 | −4·4 | −42 |
| 2 | $Q_{O_2}$ | −15·2 | −6·4 | −58 |
| | $Q_P$ | − 8·4 | −4·1 | −51 |

Such an inhibition is consistent with an action of the narcotic on the first stage of the oxidation of pyruvate, but does not prove that the action is on the first stage. The narcotic might act on a later stage, and the inhibition of this later stage might in turn inhibit the first stage, for accumulation of intermediary metabolites might bring about inhibition of an earlier stage.

It may be observed that the ratio of pyruvate disappearance to oxygen consumption is greater than the theoretical value for complete combustion (0·40). This shows that brain slices, like kidney slices (as can be seen from data given by Krebs [1933]), do not burn pyruvate completely.

*Production of pyruvate from lactate*

Solutions of lactate in which brain slices have been respiring give colours, when tested by the colorimetric method already referred to, which are probably to be attributed to pyruvate. It would of course be expected that some pyruvate should be found in the medium, since a definite concentration of pyruvate must be present in the tissue slices in which pyruvate is being formed and destroyed. The concentration of pyruvate in the medium may well be (after 2 hours' respiration) in dynamic equilibrium with that in the tissue slices; it is therefore likely that the pyruvate concentration in brain slices burning lactate is of the order $2 \times 10^{-4}\ M$, which is the approximate concentration found in the medium. The figures given in Table VIII are only to be taken as approximate. They

Table VIII. *The production of pyruvate from lactate by brain slices, in absence or presence of 0·08 % luminal*

| Exp. | Substrate | Species | [K+] | Pyruvate conc. ($M \times 10^4$) (a) Alone | (b) In presence of luminal |
|---|---|---|---|---|---|
| 1 | *d*-Lactate 0·02 *M* | Rat | 0·0128 *M* | 2·2 | 1·6 |
| | ,, 0·02 *M* | ,, | 0·002 *M* | 2·0 | 0·9 |
| 2 | ,, 0·06 *M* | Guinea-pig | 0·0128 *M* | 2·9 | 2·6 |
| | ,, 0·01 *M* | ,, | 0·0128 *M* | 1·6 | 1·1 |

show however that in the presence of luminal the pyruvate concentration is lower than in its absence. Similarly it has been found that chloretone lowers the pyruvate concentration.

It may be concluded that narcotics have some inhibitory action on the oxidation of lactate to pyruvate. If this were not so, narcotics should raise the pyruvate concentration, for, as already shown, they inhibit the disappearance of pyruvate.

The nett production of pyruvate is small in relation to the other metabolic quotients, figures for $Q_P$ being of the order 0·3–0·8.

## THE EFFECTS OF NARCOTICS ON THE METABOLISM OF LIVER, KIDNEY AND DIAPHRAGM

With brain, which in absence of added substrates has a low and rapidly falling respiration, it probably involves little error to regard the total respiration, in the presence of a substrate which raises the respiration greatly, as due entirely to oxidation of that substrate. An inhibition of the total respiration of the order of not more than, say, 50 % may be considered as an inhibition of the oxidation of the added substrate.

With most other tissues the respiration is seldom raised by added substrates to such an extent that the "control" respiration can be disregarded. It is impossible from respiration measurements alone to deduce quantitatively the effect of an inhibitor on the oxidation of an added substrate. The "control" respiration and the oxidation of an added substrate cannot be assumed to proceed additively, nor can it be assumed that the "control" respiration is replaced entirely by the oxidation of the added substrate. The truth probably lies between these two assumptions, and the effect of an inhibitor on the oxidation of an added substrate probably lies between (*a*) the inhibition of the total respiration and (*b*) the inhibition of the "extra" respiration due to the substrate.

In the present experiments (Table IX) on respiration, both these figures have been calculated, and fortunately they usually allow us to form a semi-quantitative idea of the effect of the narcotic. Experiments must usually be made with

Table IX. *The effect of 0·08 % luminal on the respiration of rat tissues*

$K^+$ 0·002 *M*. Experimental period 2 hours

| Exp. | Tissue | Substrate | $Q_{O_2}$ without narcotic: (*a*) Alone | (*b*) With substrate | % effect of narcotic on: (*a*) $Q_{O_2}$ alone | (*b*) Total $Q_{O_2}$ with substrate | (*c*) Extra $Q_{O_2}$ due to substrate |
|---|---|---|---|---|---|---|---|
| 1 | Liver | — | 7·48 | — | −39 | — | — |
| 2 | ,, | — | 5·60 | — | −17 | — | — |
| 3 | ,, | — | 8·13 | — | −33 | — | — |
| 4 | ,, | *d*-Lactate 0·02 *M* | 7·76 | 10·51 | −44 | −45 | −48 |
| 5 | ,, | ,, | 6·95 | 9·80 | −29 | −45 | −85 |
| 6* | ,, | ,, | 7·36 | 10·67 | −32 | −40 | −58 |
| 7* | ,, | ,, | 8·23 | 11·57 | −38 | −39 | −43 |
| 8 | ,, | Butyrate 0·02 *M* | 6·47 | 7·86 | −34 | −38 | −55 |
| 9 | ,, | ,, | 7·37 | 8·45 | −36 | −40 | −67 |
| 10 | ,, | Succinate 0·02 *M* | 6·76 | 26·04 | −34 | −15 | − 8 |
| 11 | Kidney | *d*-Lactate 0·02 *M* | 15·75 | 32·5 | −18 | −11 | − 5 |
| 12 | ,, | ,, | 15·2 | 34·9 | −15 | − 6 | 0 |
| 13 | ,, | Pyruvate 0·02 *M* | 14·2 | 26·0 | −14 | −42 | −75 |
| 14 | ,, | ,, | 13·7 | 26·7 | −29 | −48 | −68 |
| 15 | ,, | Glucose 0·04 *M* | 15·9 | 21·7 | −14 | −17 | −26 |
| 16 | ,, | ,, | 19·8 | 24·4 | −32 | −37 | −57 |
| 17 | ,, | Butyrate 0·02 *M* | 15·3 | 21·2 | −30 | −10 | +44 |
| 18 | ,, | ,, | 16·1 | 25·1 | −11 | − 8 | − 3 |
| 19 | ,, | *dl*-Alanine 0·02 *M* | 14·8 | 38·4 | −32 | −31 | −31 |
| 20 | Diaphragm | Pyruvate 0·02 *M* | 6·5 | 9·4 | −24 | −40 | −79 |
| 21 | ,, | ,, | 6·6 | 9·5 | −24 | −32 | −48 |
| 22 | ,, | *d*-Lactate 0·02 *M* | 7·0 | 9·3 | −39 | −32 | −10 |
| 23 | ,, | ,, | 5·2 | 9·5 | −17 | −22 | −28 |

* Rat previously starved for 3 days. Fed in other experiments.

great care to obtain the required accuracy; we have rejected a number of experiments which presented internal evidence of inconsistent behaviour of one or more of the four sets of tissue slices used.

In these experiments the same concentration of luminal has been used as in Table I, and the experiments on liver, kidney and diaphragm are therefore comparable with those on brain. The results show that oxidations in brain and in other tissues show much the same sensitivity to luminal. A typical experiment showing the time-course of the respiration in presence and absence of luminal is illustrated in Fig. 9.

The mean inhibitions of the respirations of liver, kidney and diaphragm without added substrates are respectively 34, 22 and 26%. The inhibitions vary appreciably, as might be expected from the probably variable make-up of the respiration. The inhibitions are less than that shown by brain in presence of glucose under similar conditions (Table I). In the presence of 0·033% evipan[1] (Table X) there appears to be a greater difference between the sensitivity of brain respiration and that of other tissues.

The oxidation of glucose by kidney is probably inhibited (Table IX). Pyruvate oxidation by kidney is considerably inhibited and that by diaphragm is also inhibited. The oxidation of lactate by liver is inhibited, as is probably that by diaphragm; the oxidation of lactate by kidney seems unaffected. Roughly, it can be said that the oxidations of glucose, lactate and pyruvate are affected by luminal to about the same extent as in brain. Succinate oxidation by liver is unaffected by luminal, as is also the case with brain.

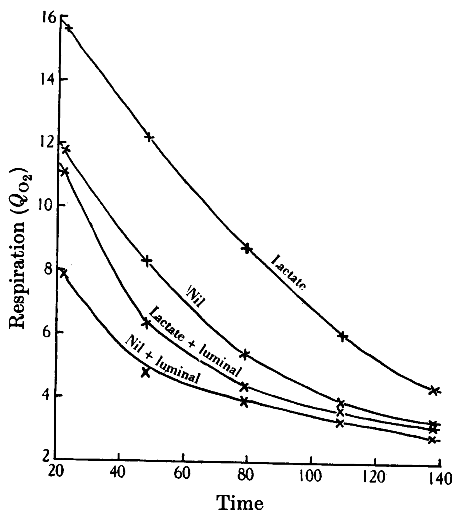


Fig. 9. Respiration of rat liver in presence and absence of lactate and of 0·08 % luminal. $K^+$ 0·002 *M*.

Kidney and liver are able to oxidize many substances that brain does not, and with these organs it is possible to investigate, therefore, the sensitivity to narcotics of many other oxidations than those of carbohydrates and allied substances.

Table X. *The effect of 0·033 % evipan on the respiration of guinea-pig tissues in presence of glucose*

$K^+$ 0·006 *M*, glucose 0·01 *M*, *p*H 7·4

| Tissue | $Q_{O_2}$ alone | $Q_{O_2}$ in presence of evipan | Effect of narcotic % |
|---|---|---|---|
| Kidney | 15·2 | 15·95 | + 5 |
| Liver | 4·25 | 4·15 | − 2 |
| Testis | 8·65 | 7·25 | −16 |
| Spleen | 7·7 | 6·4 | −17 |
| Brain | 14·2 | 9·5 | −33 |

As a representative of fatty acids, butyric acid was studied. The effect on respiration makes it probable that the oxidation of butyrate by liver is inhibited by luminal, and the conclusion is strengthened by the finding that luminal inhibits the nett acetoacetic acid formation from butyrate (Table XI). The oxidation of butyrate by kidney seems little affected by luminal. If butyrate oxidation is unaffected, it might be expected that oxidation of acetoacetate would also be unaffected, since butyrate is probably oxidized through acetoacetate as intermediary. The destruction of acetoacetate by kidney is in fact (Table XI) little retarded by 0·08 % luminal.

[1] Evipan is sodium *N*-methyl*cyclo*hexenylmethylbarbiturate.

Table XI. *The effect of 0·08 % luminal on acetoacetic acid formation and breakdown in rat tissues.**

A. Liver. Formation from butyrate ($K^+ = 0{\cdot}002\,M$)

| Exp. | Butyrate conc. | $Q_{Ac}$ (alone) | $Q_{Ac}$ (with narcotic) | Effect of narcotic % |
|---|---|---|---|---|
| 1 | 0 | +0·23 | −0·04 | −100 |
| | 0·02 *M* | +1·07 | +0·19 | −82 |
| 2 | 0 | +0·43 | +0·23 | −46 |
| | 0·02 *M* | +1·58 | +0·80 | −50 |

B. Kidney. Breakdown of acetoacetate ($K^+ = 0{\cdot}002\,M$)

| Exp. | Acetoacetate conc. | $Q_{Ac}$ (alone) | $Q_{Ac}$ (with narcotic) | |
|---|---|---|---|---|
| 1 | 0·008*M* | −5·4 | −4·6 | −14 |
| 2 | 0·008*M* | −4·8 | −4·2 | −12 |

* Cf. Jowett & Quastel [1935] for details of the determinations.

As a readily oxidized amino-acid, alanine has been examined. From respiration measurements it appears that the oxidation of alanine by kidney is inhibited by luminal. The rate of ammonia production is likewise inhibited (Table XII).[1]

Table XII. *The effect of 0·08 % luminal on the oxidative ammonia production from alanine by rat kidney*

$K^+$ 0·002 *M*. 1½–2 hours. 0·02 *M dl*-alanine
No $NH_3$ was found in absence of alanine

| Exp. | $Q_{NH_3}$ (alone) | $Q_{NH_3}$ (with narcotic) | Effect of narcotic % |
|---|---|---|---|
| 1 | +7·2 | +3·3 | −54 |
| 2 | +6·9 | +3·8 | −45 |
| 3 | +5·7 | +3·1 | −45 |

We will now consider whether luminal inhibits the oxidative deamination of alanine, or only the subsequent oxidation of the pyruvic acid formed. If the first stage is not itself inhibited, the inhibition of ammonia formation must be exerted indirectly, and probably through a competitive inhibition of the deaminase by accumulation of pyruvate. However, Krebs [1933] has found that in presence of arsenite accumulation of pyruvate occurs without inhibition of the deaminase. There is therefore in all probability a direct action of luminal on the deaminase.

These experiments show that the inhibitory action of narcotics is not restricted to the oxidation of glucose and its breakdown products by the brain. Oxidations of these substances in other tissues are inhibited to about the same extent by luminal, and oxidations of fatty acids and amino-acids may be inhibited. Much more investigation is desirable on the inhibiting effects of narcotics on different types of oxidations.

*The effects of narcotics under vital conditions*

The view was advanced by Quastel & Wheatley [1932, 1] that narcosis is due to a depression of oxidations in the nervous system, the oxidations concerned being those of glucose, lactate and pyruvate. Parallelism between

[1] We are indebted to Mr A. H. M. Wheatley for his assistance with the experiments of Table XII, and also with the evipan experiments (Fig. 8, Table X).

hypnotic activity and inhibitory action on brain oxidations was found among narcotics of the same chemical type.

To establish the view it will be necessary to prove that narcotics inhibit oxidations in the nervous system at concentrations which bring about narcosis without resulting in death.

The concentrations of narcotics that we have used in most of our present experiments cause considerable inhibitions of carbohydrate oxidation in the brain, and such concentrations would probably be lethal. Before turning to the effects of lower concentrations, we will consider some of the evidence already available that the metabolism of the organism is affected in narcosis.

Work on the intact organism has in fact shown that narcotics affect carbohydrate metabolism [Peters & Van Slyke, 1931]. During anaesthesia the glucose and lactic acid concentrations in the blood are raised, and an acidosis appears parallel with the lactic acid accumulation. During fairly short periods of anaesthesia, there is no great alteration in the ketone bodies of the blood. In prolonged narcosis, used as a treatment for certain types of mental disorder, ketosis often develops, which disappears on administration of glucose and insulin [Quastel & Ström-Olsen, 1933; Ström-Olsen, 1933]. It may be concluded that an inhibition of some stage or stages of carbohydrate utilization is probably caused by narcotics, in which liver or muscles or both are concerned. It is as yet uncertain whether the effects on carbohydrate metabolism in these organs are exerted through a direct action on the tissues concerned, or indirectly through nervous, hormonal or other circulatory influences. If the action should be direct, it is likely that carbohydrate metabolism in the brain will also be affected, for our experiments have shown that *in vitro* oxidations in brain tissue are at least as sensitive to narcotics as those of other tissues.

We return to the problem of showing that oxidations in brain or other tissues are inhibited *in vitro* by concentrations of narcotics that cause narcosis *in vivo*. At present we can offer no more than a comparison of the order of magnitude of the concentrations of narcotics (*a*) which affect measurably brain oxidations *in vitro* and (*b*) which exist in the blood in contact with the brain during narcosis. The latter concentrations are the more uncertain.

With evipan *in vitro* a concentration of 0·016 % causes an inhibition of about 10 % in the respiration of guinea-pig brain in presence of glucose (Fig. 8). When 12·5 mg./kg. of evipan are injected intravenously into a rabbit, an immediate anaesthesia, without disappearance of the optic reflex, is produced, which lasts only 10 min. If we assume that the evipan is distributed at first only throughout the blood, and take the blood as 8 % of the body weight, it will be concluded that the narcotic concentration to which the rabbit brain is at first subjected is 0·016 %.

With chloretone, we have no figures for the effect at low concentrations, but estimate that 0·01 % chloretone would inhibit the respiration of guinea-pig brain in presence of glucose by 10–15 %. It was found that 180 mg./kg. of chloretone, injected intraperitoneally into a guinea-pig, caused narcosis. Assuming the chloretone to be distributed uniformly throughout the animal, its concentration during narcosis would be 0·018 %.

With luminal, a concentration of about 0·025 % inhibits the respiration of rat or guinea-pig brain in presence of glucose by 10 % (Fig. 7). According to Keller & Fulton [1931], injection of 200 mg./kg. into monkeys abolishes response of the motor cortex, but is barely sufficient for surgical operations. Assuming the luminal to be uniformly distributed throughout the body, its concentration (in monkeys) during narcosis is 0·02 %.

These approximate data and assumptions are sufficient to show that the narcotic concentrations which produce narcosis *in vivo* are of the same order of magnitude as the narcotic concentrations which *in vitro* inhibit measurably the respiration of the cerebral cortex. A more exact conclusion would demand much more exact information.

## Summary

1. An investigation has been made of the effects of narcotics (luminal, chloretone and evipan) on the respiration of tissue slices.

2. The following results have been obtained with brain.

(*a*) The respiration is more sensitive to luminal in the presence of glucose, lactate and pyruvate than in the presence of other substrates examined.

(*b*) The inhibition of respiration by narcotics tends to be independent of time when the potassium ion concentration of the medium is sufficiently high, while at low potassium ion concentrations the inhibition increases with time.

(*c*) Calcium ion influences the inhibitions.

(*d*) The inhibition due to chloretone develops rapidly after addition of the narcotic and has no temperature coefficient.

(*e*) The substrate concentration has not been found to influence inhibitions of respiration by narcotics.

(*f*) The variation with narcotic concentration of the inhibition of respiration by narcotics follows a sigmoid curve.

3. Narcotics inhibit oxidations by slices of liver, kidney and diaphragm, both when no substrates are added and in presence of some substrates, such as pyruvate. The respiration without added substrates is less sensitive to narcotics than is the respiration of brain in the presence of glucose. Narcotics inhibit the oxidation of butyrate by liver and that of alanine by kidney.

4. The following individual stages in oxidation appear to be inhibited by narcotics: (*a*) the oxidation of lactate to pyruvate by brain, (*b*) the oxidative deamination of alanine by kidney.

5. Narcotic concentrations which produce narcosis *in vivo* are of the same order of magnitude as those which inhibit measurably the respiration of the cerebral cortex *in vitro*.

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