# CCXLVI. THE EFFECTS OF ANAESTHETICS AND OF CONVULSANTS ON THE LACTIC ACID CONTENT OF THE BRAIN

By WILLIAM ELLIS STONE

*From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis*

THAT disturbances in the intermediary carbohydrate metabolism of the brain may be involved in certain "functional" disorders of the central nervous system is indirectly suggested by many observations. The beneficial effect of the "insulin shock" therapy introduced by Sakel [1937] for the treatment of schizophrenia is a particularly striking example, since one of the effects of hypoglycaemia is to diminish the supply to the brain of glucose, the normal substrate for oxidative catabolism in this organ [Dameshek & Myerson, 1935].

In epilepsy, also, the brain metabolism may be abnormal. Some form of anoxaemia has long been considered a possible etiological factor in convulsive disorders [Cobb, 1936]. The seeming antagonism between epilepsy and schizophrenia led Meduna [1937] to introduce the metrazol convulsion therapy for schizophrenia.

An older theory of narcosis, recently given a new aspect by the work of Quastel and his coworkers [Quastel & Wheatley, 1932; 1934; Jowett, 1938], suggests that an interference in brain oxidations may be involved in narcosis. Such an interference may be important in the prolonged narcosis therapy for schizophrenia [Palmer, 1937]. The work of Quastel & Wheatley [1933] also suggests a toxic effect of amines on brain metabolism as of possible importance in mental disorder.

The observations of Loevenhart *et al.* [1929] on the temporary effects of cyanide injection and of inhalation of high carbon dioxide-air mixtures on schizophrenic patients are of interest also.

The mechanism of glucose catabolism in the brain is a very controversial subject at present. Whether lactic acid is a direct intermediate, or whether it arises from pyruvic acid as a side reaction, is not known with certainty. But in either case, a disturbance at any point in the reaction chain or chains involved in glucose catabolism might be expected to cause an increase or decrease in the concentration of lactic acid in the brain. The object of this investigation was to discover whether or not such an increase or decrease is caused by anaesthetics or by convulsant drugs.

With the exception of the work of Kerr and coworkers [1936; 1937; Avery *et al.* 1935], the brain lactic acid values given in the literature are not dependable, because of post-mortem glycolysis. Kerr and coworkers froze the brain *in situ* in the living animal, but their technique required the use of anaesthetics, which decrease the brain lactic acid, as will be shown.

## EXPERIMENTAL

Mice were used in this investigation. These animals are small enough to be plunged into liquid air and frozen, without the use of anaesthetics or surgical procedures. The mouse is frozen solid in 2–3 sec., and reaches the temperature

of the liquid air in 60–90 sec. The brain is then removed, using chilled instruments and working on a surface consisting of a strip of cardboard chilled in liquid air. The tissue, instruments, and cardboard are chilled at intervals during the operation, so that the brain does not soften at any time. The whole brain is removed, and stored in liquid air, ready for crushing.

The crusher (Fig. 1) is similar to that described by Graeser et al. [1934], but much smaller, and simplified. The crushing surfaces are of tool steel; the ring and base of iron. The crusher is chilled by submerging for a few sec. in liquid air, the frozen tissue then placed on the anvil, with the ring in place, and the tissue crushed by several blows of a hammer on the steel plunger. The crushed tissue forms a solid disk which sticks in the iron ring, and is poked out with a glass rod into the $ZnSO_4$-$H_2SO_4$ contained in a tared centrifuge tube (with rubber stopper). In order to avoid loss of tissue during the transfer, a glass funnel with the stem cut off is used. The tube is restoppered, shaken well, and weighed.
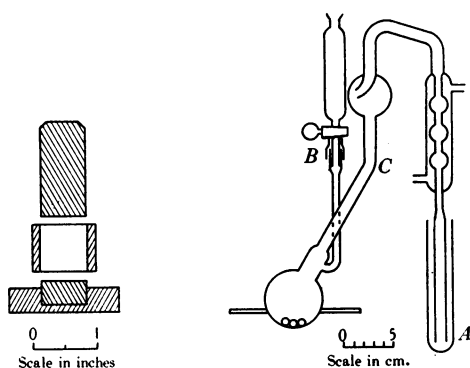


Fig. 1. Tissue crusher.    Fig. 2. Distillation apparatus.

### Determination of lactic acid

Lactic acid was determined by a modification of the method of Friedemann & Graeser [1933]. Since a mouse brain weighs only 0·3–0·4 g., it was necessary to use much smaller quantities than those employed by Friedemann & Graeser.

The distillation was carried out in the pyrex glass apparatus shown in Fig. 2. All rubber connexions have been eliminated except that at $B$, which does not become heated, and here the rubber does not come into contact with the solution. The apparatus is easily rinsed by inverting and running water in at $A$ and out at $B$. Draining through $B$ is satisfactory unless the angle at $C$ has been made too acute; 150° is about right. The apparatus is easily constructed from a 100 ml. distilling flask and the micro-Kjeldahl distillation apparatus described by Cavett [1931].

Protein removal is accomplished by the $Zn(OH)_2$ method. The crushed tissue is added to 3 ml. solution containing per litre 35·5 g. $ZnSO_4$, $7H_2O$ and 355·5 ml. $N$ $H_2SO_4$ [Blatherwick et al., 1935]. An amount of 0·5 $N$ NaOH is added sufficient exactly to neutralize the acid solution (determined by previous titration, using phenolphthalein). Water is then added to make a total volume of 7·5 ml., counting the tissue 80 % water. The mixture is allowed to stand for 30 min. with occasional shaking, and centrifuged.

To 5 ml. supernatant fluid are added 0·6 ml. $CuSO_4$ (containing 200 g. $CuSO_4$, $5H_2O$ per l.), and 0·6 ml. $Ca(OH)_2$ suspension (prepared by slaking 200 g.

fresh lime and diluting to 1 l.). Water is added to the mixture to make a total volume of 15 ml. This is allowed to stand for 30 min. with occasional shaking, centrifuged and decanted.

A 13 ml. aliquot of the solution is introduced into the distillation apparatus (at $B$), followed by 2 ml. of $H_3PO_4$-$MnSO_4$ mixture (100 g. $MnSO_4$, $4H_2O$ and 25 ml. 85 % $H_3PO_4$ per l.), and 5 ml. water. The funnel is then connected at $B$, with the stopcock closed, and filled to the 15 ml. mark with a colloidal suspension of $MnO_2$ (freshly prepared by mixing equal quantities of 0·013 % $KMnO_4$ and 0·03 % $MnSO_4$, $4H_2O$ solutions. Rapid flocculation does not occur unless $MnSO_4$ is slightly in excess). The receiver contains 2 ml. 0·1 $M$ $NaHSO_3$.

The solution is brought to boiling, and small portions of $MnO_2$ are run in at intervals, the whole 15 ml. being added during a 10 min. period. The total distillation period is 15 min., the rate being such as to give a final vol. of 20 ml. in the receiver. At the last, the receiver is lowered and the distillation continued for about 1 min. to rinse the outlet. Bumping is prevented by the presence of three glass beads in the flask. After the distillation, the receiver is cooled in ice water.

The excess $NaHSO_3$ is removed by 0·3 $N$ $I_2$, and the end-point adjusted with 0·005 $N$ $Na_2S_2O_3$ and 0·0025 $N$ $I_2$, using 1 ml. 1 % starch indicator. The bound $NaHSO_3$ is then released by adding 5 ml. saturated $Na_2HPO_4$, and titrated with standard 0·0025 $N$ $I_2$ (containing 10 g. KI per l.). A 2 ml. micro-burette is used, and an air-driven stirrer facilitates the titration.

1 ml. 0·0025 $N$ $I_2$ is equiv. 0·1125 mg. lactic acid.

Purification of the reagents used is necessary. Blank determinations usually required about 0·1 ml. of 0·0025 $N$ $I_2$.

Tests on known lactic acid solutions (prepared from Zn lactate) in amounts covering the range involved in these experiments indicated that the results were accurate to within $\pm 3$ mg. per 100 g. of brain tissue. Since this is much smaller than the differences between individual normal mice, the method is sufficiently accurate for the present purpose.

Blank tests on phenobarbital, ether, picrotoxin, metrazol, nicotine and NaCN in amounts likely to be present in experiments reported here, showed that these drugs do not form appreciable amounts of $NaHSO_3$-binding substances.

### Brain lactic acid in normal mice

Table I gives the data obtained on normal mice. Brain lactic acid values in mice not exercised before killing ranged from 12 to 25 mg. per 100 g. brain tissue (wet wt.), with a mean of 18·9 mg. per 100 g. Neither body wt. nor sex influenced the results significantly. Most of the mice were not subjected to a period of food deprivation before the experiment; such a period was found to have no influence on the brain lactic acid.

It was found that a period of strenuous exercise increased the brain lactic acid significantly. The mouse was placed in a covered beaker, which was then continuously tilted, shaken and rotated, forcing the mouse to exercise in attempting to right itself and maintain equilibrium. The brain lactic acid in these mice was 25–28 mg. per 100 g., with a mean of 26·8, an average increase of 7·9 mg. per 100 g. due to the exercise.

Since in strenuous exercise lactic acid accumulates in the blood, it was desirable to determine whether the increased brain lactic acid was due to diffusion from the blood into the brain. Three mice were injected with 4–8 mg. lactic acid (as Na salt) in 0·56 $M$ solution, by tail vein, and killed 5 min. later. The brain lactic acid values agreed with those of normal resting mice; hence it is

concluded that the extra brain lactic acid arising during exercise has its origin in the brain itself, and is due to increased activity of this organ. These observations are in harmony with those of Dameshek & Myerson [1935], who found no absorption by the brain of lactate from the blood in patients in insulin hypoglycaemia (during which brain lactic acid is low). Contrary observations have been made by others, however [McGinty, 1929; Himwich & Nahum, 1932].

Two experiments showed that adrenaline does not increase the brain lactic acid (Table I). These mice each received 0·06 ml. 1/10,000 adrenaline (0·2 mg. per kg.) subcutaneously. In one, this was divided into two equal doses, separated by a 3 min. interval, and the mouse was frozen 3 min. after the second injection. In the other, the adrenaline was given in one dose, producing marked signs of stimulation, and the mouse was frozen 3 min. after injection. These experiments confirm the findings of Kerr et al. [1937].

In the tables, the experiments are numbered in the order in which they were carried out.

Table I. *Brain lactic acid in normal mice*

| Exp. no. | Wt. of mouse g. | Sex | Brain lactic acid mg. per 100 g. | Remarks |
|---|---|---|---|---|
| | | | Without exercise | |
| 1 | 25 | F | 23 | — |
| 2 | 24 | F. | 25 | — |
| 10 | 24 | M | 16 | — |
| 15 | 20·5 | F | 23 | — |
| 18 | 28 | F | 16 | — |
| 23 | 21·5 | M | 19 | — |
| 30 | 25·5 | M | 16 | 14·5 hr. food deprivation |
| 44 | 30·5 | M | 19 | 4 hr. food deprivation |
| 61 | 20 | M | 12 | 3 hr. food deprivation |
| 67 | 19 | M | 20 | — |
| 79 | 19·5 | F | 18 | — |
| 87 | 19·5 | F | 20 | — |
| | | Mean | 18·9 | |
| | | | After exercise | |
| 5 | 21 | M | 25 | Exercised 3 min. |
| 9 | 21 | M | 27 | Exercised 5 min. |
| 14 | 23·5 | F | 27 | Exercised 4 min. |
| 19 | 32·5 | F | 27 | Exercised 4 min. |
| 27 | 21 | M | 28 | Exercised 5 min. |
| | | Mean | 26·8 | |
| | | | After Na lactate injection | |
| 77 | 22 | M | 24 | Injected 4 mg. lactic acid |
| 78 | 26 | F | 14 | Injected 4 mg. lactic acid |
| 86 | 20·5 | F | 17 | Injected 8 mg. lactic acid |
| | | Mean | 18·3 | |
| | | | After adrenaline injection | |
| 90 | 31·5 | M | 17 | 2 injections, 3 min. apart |
| 91 | 26·5 | M | 16 | 1 injection |

*Effects of anaesthetics*

Table II gives data obtained on mice killed in a state of anaesthesia. The experiments are arranged in the order of increasing duration of anaesthesia before killing, this period being measured from the time of loss of righting reflexes.

Table II. *Brain lactic acid in anaesthetized mice*

| Exp. no. | Wt. of mouse g. | Sex | Induction period min. | Period of anaesthesia min. | Brain lactic acid mg. per 100 g. |
|---|---|---|---|---|---|
| | | Phenobarbital, excitement stage | | | |
| 51 | 23·5 | M | 7 | 0 | 17 |
| 58 | 21·5 | M | 17 | 0 | 8 |
| 56 | 22 | M | 18 | 0 | 8 |
| 35 | 27 | F | 21 | 0 | 12 |
| | | | | Mean | 11·2 |
| | | Phenobarbital anaesthesia | | | |
| 41 | 27 | M | 15 | 15 | 9 |
| 49 | 26·5 | M | 30 | 18 | 8 |
| 48 | 25·5 | M | 13 | 22 | 8 |
| 34 | 24 | F | 30 | 42 | 11 |
| 36 | 18·5 | M | 30 | 70 | 6 |
| 50 | 22·5 | M | 25 | 196 | 14 |
| 57 | 24 | M | 20 | 210 | 6 |
| | | | | Mean | 8·9 |
| | | Amytal anaesthesia | | | |
| 47 | 26 | M | 43 | 4* | 9 |
| 46 | 29 | M | 10 | 15 | 8 |
| 45 | 23·5 | F | 10 | 23 | 8 |
| 43 | 26·5 | F | 10 | 25 | 7 |
| | | | | Mean | 8·0 |
| | | Ether anaesthesia | | | |
| 55 | 24 | M | 1 | 0·5 | 16 |
| 81 | 23·5 | M | 1 | 1 | 18 |
| 76 | 24·5 | F | 3 | 5 | 8 |
| 80 | 20·5 | F | 1 | 10 | 12 |
| 71 | 23·5 | F | 3 | 15 | 13 |
| 72 | 25·5 | M | 0·5 | 15 | 11 |
| 75 | 23 | M | 3 | 20 | 9 |

\* After a second injection.

The barbiturates were administered intravenously (tail vein), or in some cases subcutaneously, when the needle missed the vein. The manner of administration seemed to make little difference. Phenobarbital dosages were 0·04–0·06 ml. 10 % phenobarbital as the Na salt per mouse. Amytal dosages were 0·1–0·15 ml. 5 % Na amytal per mouse.

All three anaesthetics decreased the brain lactic acid significantly. With phenobarbital, it was shown that the decrease begins during the excitement stage. With ether, which induces anaesthesia much more quickly, the lactic acid decrease lags behind the onset of anaesthesia.

The lactic acid values obtained are lower than those reported by Kerr and coworkers for dog, cat and rabbit brains. However, it must be remembered that they analysed grey matter only, while the whole brain was used in the experiments reported here.

### Effect of insulin

Table III gives the data obtained on mice killed in "insulin shock". The mice were deprived of food for periods of 3–16 hr. before the experiments. No correlation seemed to exist between the length of this period and the severity of symptoms or the brain lactic acid. The insulin used was 40 units per ml., Eli Lilly Company. Injections were into the tail veins, or occasionally subcutaneous.

In all mice killed during insulin convulsions, and in some killed while in a flaccid condition, the brain lactic acid was much lower than normal. This

Table III. *Brain lactic acid during "insulin shock" in mice*

| Exp. no. | Wt. of mouse g. | Sex | Insulin injected units | Period of insulin action min. | Brain lactic acid mg. per 100 g. |
|---|---|---|---|---|---|
| | | Killed while in flaccid condition | | | |
| 25 | 25·5 | M | 1, 1, 2* | 268 | 14 |
| 26 | 22 | M | 1 | 107 | 16 |
| 28 | 23·5 | M | 1, 1, 1* | 240 | 2 |
| 68 | 20·5 | M | 2 | 35 | 6 |
| | | Killed during convulsions | | | |
| 29 | 21·5 | M | 1 | 63 | 6 |
| 31 | 28·5 | M | 3 | 80 | 5 |
| 37 | 24·5 | M | 3 | 230 | 5 |
| 38 | 25 | F | 3 | 53 | 9 |
| 42 | 29 | M | 3 | 88 | 9 |
| | | | | Mean | 6·8 |

* At intervals of 1–2 hr. Unsuccessful attempt to produce convulsions.

confirms the findings of Kerr *et al.* [1937], although earlier Kerr & Ghantus [1936] reported normal brain lactic acid values after insulin.

A low level of brain lactic acid during hypoglycaemia is in harmony with the view that the decreased glucose supply to the brain is an important factor in hypoglycaemic shock.

### Effects of picrotoxin

Picrotoxin in large enough doses causes severe epileptiform convulsions in mice. In these experiments, the dosage used was 0·04–0·08 ml. per mouse, of a solution containing 3 mg. picrotoxin per ml. (in 9 % alcohol), Eli Lilly Company. The injections were made into the tail veins, or occasionally subcutaneously.

Table IV. *Mouse brain lactic acid in picrotoxin convulsions*

| Exp. no. | Wt. of mouse g. | Sex | Duration of convulsions min. | Brain lactic acid mg. per 100 g. | Remarks |
|---|---|---|---|---|---|
| | | Killed at beginning of convulsions | | | |
| 6 | 21·5 | M | — | 26 | — |
| 7 | 23 | M | — | 19 | — |
| 12 | 22 | M | — | 24 | — |
| 20 | 27 | F | — | 13 | — |
| 21 | 23 | F | — | 15 | — |
| 24 | 25·5 | M | — | 27 | — |
| 32 | 30 | M | — | 18 | — |
| 33 | 25 | M | — | 12 | — |
| 39 | 20 | F | — | 18 | — |
| 40 | 24 | F | — | 13 | — |
| | | | Mean | 18·5 | |
| | | Killed during convulsions | | | |
| 3 | 31·6 | F | 3 | 49 | Very severe convulsion |
| 4 | 18 | F | 8 | 36 | Series of light convulsions |
| 8 | 21 | M | 6 | 39 | Last convulsion severe |
| 11 | 32 | M | 2 | 27 | All of brain except cerebellum |
| | | | | 30 | Cerebellum only |
| 13 | 22 | F | 7 | 30 | Severe convulsion |
| 16 | 20 | M | 5 | 34 | — |
| 17 | 33 | F | 10 | 37 | Series of convulsions |
| 22 | 21 | M | 6 | 42 | Series of light convulsions |
| | | | Mean | 36·9 | |

Convulsions invariably began in 5 to 8 min., being ushered in by a characteristic shaky appearance, hunching of the back and fighting movements of the front feet. In one mouse the convulsions terminated fatally after being allowed to continue for about 15 min.

In one group of experiments, the mice were frozen when the convulsion was just beginning, as judged by these characteristic signs, or immediately after it began. The brain lactic acid values were normal in this group, with a mean of 18·5 mg. per 100 g. (Table IV).

In a second group, the convulsions were allowed to continue for 2–10 min., and the mouse was then frozen during a convulsion. A significant increase in brain lactic acid occurred in this group, the mean value being 36·9 mg. per 100 g.

Kerr & Antaki [1937] reported experiments with picrotoxin, in which, however, the convulsion was terminated with an anaesthetic before the brain was frozen *in situ*. Using ether, they found a higher than normal brain lactic acid after picrotoxin, but in the case of amytal, which requires longer for induction of anaesthesia, and is pharmacologically antagonistic to picrotoxin, normal values were obtained.

### Effect of metrazol

Metrazol convulsions were induced by injection of 0·05–0·07 ml. per mouse, of an aqueous solution containing 2 % metrazol (cardiazol) and 0·02 % $Na_2HPO_4$. The convulsant action was quite irregular. Sometimes a violent convulsion began within 2 sec., while in other cases it was delayed as much as 7 min., and frequently two or more injections were required. If the injection was subcutaneous, the onset was slower than if intravenous. The convulsions were always very brief, lasting only a few seconds. They sometimes ended fatally. Occasionally recovery occurred before the mouse could be frozen; in these cases a second convulsion sometimes occurred.

The brain lactic acid values obtained on mice frozen during metrazol convulsions varied over a wide range, from normal to very high values, with a mean of 26·1 mg. per 100 g. (Table V). The occurrence of some very high values is of importance, considering the very brief nature of these convulsions.

Table V. *Mouse brain lactic acid in metrazol convulsions*

| Exp. no. | Wt. of mouse g. | Sex | Brain lactic acid mg. per 100 g. |
|---|---|---|---|
| 52 | 26·5 | M | 33 |
| 53 | 22·5 | M | 25 |
| 54 | 26 | F | 29 |
| 59 | 21 | M | 20 |
| 60 | 20·5 | M | 20 |
| 62 | 25 | M | 27 |
| 63 | 26 | M | 21 |
| 64 | 26 | M | 39 |
| 65 | 22 | M | 29 |
| 66 | 19·5 | M | 18 |
| | | Mean | 26·1 |

Kerr & Antaki [1937] found high brain lactic acid values after metrazol convulsions, even though amytal or ether was administered before freezing the brain *in situ*.

### Effect of nicotine

Nicotine convulsions were produced by subcutaneous injections of 5–11 mg. nicotine per kg., in 1·5, 2·5, or 5 % solution. As with metrazol, the response was quite variable. In some cases repeated injections were made. When a convulsion

occurred, it began with co-ordinated running movements, 45 sec.–2 min. after the injection. These merged into the convulsion, which was usually very brief. Occasionally a series of light convulsions occurred. Recovery or death followed usually within 3 min., if the convulsion was allowed to continue.

The brain lactic acid values found on mice killed during nicotine convulsions ranged from 14 to 27 mg. per 100 g., with a mean of 23·2 mg. (Table VI). These results are not significantly different from those obtained on normal mice.

Table VI. *Mouse brain lactic acid in nicotine convulsions*

| Exp. no. | Wt. of mouse g. | Sex | Brain lactic acid mg. per 100 g. |
|---|---|---|---|
| 82 | 18·5 | M | 27 |
| 83 | 19·5 | F | 24 |
| 84 | 19·5 | M | 26 |
| 85 | 27·5 | M | 23 |
| 88 | 19 | F | 25 |
| 89 | 28 | F | 14 |
|  |  | Mean | 23·2 |

## Effect of NaCN

Cyanide convulsions were found to be difficult to produce. The response was quite variable, and frequently the mouse died without having a true convulsion. However, 4 mice were frozen during convulsions, after injection of 7–10 mg. NaCN per kg., by tail vein, in 0·2, 0·3 or 0·4 % solution. In some, repeated injections were given, some of which may have been subcutaneous rather than intravenous. Probably all of these mice would have died very quickly if they had not been frozen.

The brain lactic acid values were very high, the mean being 62·8 mg. per 100 g. (Table VII).

Table VII. *Mouse brain lactic acid in cyanide convulsions*

| Exp. no. | Wt. of mouse g. | Sex | Brain lactic acid mg. per 100 g. |
|---|---|---|---|
| 69 | 22 | F | 82 |
| 70 | 24·5 | F | 56 |
| 73 | 21 | M | 51 |
| 74 | 22·5 | F | 62 |
|  |  | Mean | 62·8 |

The data are in harmony with those of McGinty [1929], who found that impairment of brain oxidations by cyanide causes lactate to be given out to the blood by the brain.

### DISCUSSION

The low brain lactic acid values found in anaesthetized mice are in accord with the view that the cerebral metabolism is depressed during anaesthesia. Dameshek et al. [1934] found that during amytal or ether anaesthesia a decrease occurs in the amounts of glucose and of $O_2$ removed from the blood by the brain. Quastel and his coworkers, as well as others, have observed that the $O_2$ consumption of brain tissue in vitro in the presence of certain substrates is depressed by anaesthetics [Quastel & Wheatley, 1932; 1934; Jowett, 1938].

However, the results reported here apparently do not support the hypothesis of Quastel, which attributes the anaesthetic action of the drugs to a specific inhibition of some part of an enzyme system involved in the oxidation of lactate or pyruvate. If this hypothesis were correct, one would expect to find an

accumulation of lactate in the brain during anaesthesia. It seems necessary to postulate some other effect of the anaesthetic on the brain tissue, besides the specific inhibition of oxidations observed by Quastel and coworkers. As to whether lactate formation from glucose is specifically inhibited by these anaesthetics, a search of the literature has revealed no data, although Loebel [1925] found that certain urethanes and higher alcohols inhibit respiration of brain tissue more than anaerobic glycolysis, and increase aerobic glycolysis. Inhibition of glycolysis would not explain all the observations of Quastel and coworkers.

The most obvious hypothesis to account for the variations observed in brain lactic acid content would be to suppose that the brain lactic acid content is dependent on the activity of the tissue, lagging somewhat behind the tissue activity, as in muscle. Thus, the brain lactic acid is low during anaesthesia, higher in the normal condition, still higher during exercise, which involves motor activity and highest during convulsions. McClendon [1912] has shown that tissue activity involves an increase in cell membrane permeability, and since intracellular metabolism and permeability are interrelated in some way as yet not understood, it is possible that certain metabolic changes are secondary to changes in membrane permeability. Such a hypothesis might also explain the observations of Quastel and coworkers. Spiegel & Spiegel-Adolph [1936] have shown that anaesthetics decrease the brain cell membrane permeability *in vivo*, confirming inferences drawn from observations on fish eggs by McClendon [1915] and on algae by Osterhout [1916].

The low lactic acid values occurring during insulin convulsions and during the excitement stage of phenobarbital anaesthesia do not accord well with the view that the brain lactic acid content is dependent on the tissue activity. The low values with insulin may be attributed to lack of the substrate, glucose, and in the case of phenobarbital, a "release" theory might be adopted, which would suppose that the higher centres are first anaesthetized, leaving the lower centres active during the excitement stage. The results with nicotine are also out of harmony with this first hypothesis, since the values are generally lower than those observed with metrazol, and none are out of the normal range.

A second hypothesis to account for the high values which occur during picrotoxin, and occasionally during metrazol convulsions, would be to suppose that these substances inhibit some part of an enzyme system involved in the brain oxidations, the accumulation of a measurable amount of extra lactate lagging somewhat behind the beginning of convulsions. In the case of cyanide convulsions, the symptoms are generally attributed to an inhibition of the cytochrome-indophenol oxidase system. Since the lactate accumulation was much greater with cyanide than with picrotoxin or metrazol, while the convulsions occurred with less regularity, it would be necessary to suppose that the inhibition of oxidation occurred at a different point in the oxidative mechanism. This is easily possible in a system so complex. The results with insulin are in harmony with this second hypothesis, since in hypoglycaemia the normal substrate for oxidation is lacking, and consequently the supply of oxidative energy required for the maintainance of homeostasis is deficient. The results with nicotine do not seem to be in accord with this hypothesis. Nicotine is of special interest in that it inhibits lactic dehydrogenase, increases anaerobic glycolysis, and decreases succinate oxidation by brain tissue *in vitro* [Himwich & Fazekas, 1935; Quastel & Wheatley, 1937]. Despite these reports, nicotine did not cause a significant increase of brain lactic acid *in vivo*.

That the extra lactic acid found in the brain during picrotoxin and metrazol convulsions does not come from the blood is shown by the normal or subnormal

values observed in conditions in which high blood lactic acid occurs, namely after lactate or adrenaline injection and during ether anaesthesia or insulin convulsions.

Finally, it is possible that the high brain lactic acid values occurring during picrotoxin or metrazol convulsions may be due to circulatory disturbances, either cerebral or systemic, resulting in cerebral anoxaemia. Cerebral vascular spasm is a possible cause of convulsions in some cases [Cobb, 1936]. In metrazol convulsions, Corrin [1938] observed temporary cardiac arrest in 10–15 % of cases.

### SUMMARY

1. A technique is described for determining the lactic acid content of the brain of the mouse, using liquid air and avoiding both post-mortem changes and the use of anaesthetics.

2. The lactic acid content of the brain of the normal mouse was found to vary from 12 to 25 mg. per 100 g. tissue, with a mean of 18·9 mg. per 100 g. This is increased to 26·8 mg. per 100 g. by strenuous exercise.

3. Anaesthetics (phenobarbital, amytal, ether) decrease the brain lactic acid content significantly. With phenobarbital the decrease begins during the excitement stage of anaesthesia; with ether it lags behind the induction of deep anaesthesia.

4. During insulin convulsions, the brain lactic acid is significantly decreased. During "insulin shock" without convulsions, a decrease is sometimes observed. This finding is attributed to the decrease in glucose supplied to the brain by the blood.

5. During picrotoxin convulsions which have progressed for 2–10 min., the brain lactic acid is significantly increased and is above the level observed in normal mice after strenuous exercise. At the beginning of the convulsion, no increase occurs above the level observed in normal resting mice.

6. During metrazol convulsions, the brain lactic acid varies from normal to significantly increased values.

7. During nicotine convulsions, no significant increase in brain lactic acid content was observed.

8. During cyanide convulsions, the brain lactic acid shows a large increase.

9. High brain lactic acid values cannot be attributed to diffusion of lactate from the blood into the brain.

10. Possible interpretations of the data, and their significance in relation to theories of anaesthesia and to the causation of convulsions are discussed.

### REFERENCES

Avery, Kerr & Ghantus (1935). *J. biol. Chem.* **110**, 637.
Blatherwick, Bradshaw, Ewing, Larson & Sawyer (1935). *J. biol. Chem.* **111**, 537.
Cavett (1931). *J. Lab. clin. Med.* **17**, 79.
Cobb (1936). *Med. Clin. N. Amer.* **19**, 1583.
Corrin (1938). *Arch. Neurol. Psychiat.* **39**, 864.
Dameshek & Myerson (1935). *Arch. Neurol. Psychiat.* **33**, 1.
—— —— & Loman (1934). *Amer. J. Psychiat.* **91**, 113.
Friedemann & Graeser (1933). *J. biol. Chem.* **100**, 291.
Graeser, Ginsberg & Friedemann (1934). *J. biol. Chem.* **104**, 149.

Himwich & Fazikas (1935). *Amer. J. Physiol.* **113**, 63.
—— & Nahum (1932). *Amer. J. Physiol.* **101**, 446.
Jowett (1938). *J. Physiol.* **92**, 322.
Kerr & Antaki (1937). *J. biol. Chem.* **122**, 49.
—— & Ghantus (1936). *J. biol. Chem.* **116**, 9.
—— Hampel & Ghantus (1937). *J. biol. Chem.* **119**, 405.
Loebel (1925). *Biochem. Z.* **161**, 219.
Loevenhart, Lorenz & Waters (1929). *J. Amer. med. Ass.* **92**, 880.
McClendon (1912). *Amer. J. Physiol.* **29**, 302.
—— (1915). *Amer. J. Physiol.* **38**, 173.
McGinty (1929). *Amer. J. Physiol.* **88**, 312.
Meduna (1937). Die Konvulsiontherapie der Schizophrenia. (Halle a.S.)
Osterhout (1916). *Bot. Gaz.* **61**, 148.
Palmer (1937). *J. ment. Sci.* **83**, 636.
Quastel & Wheatley (1932). *Proc. roy. Soc.* B, **112**, 60.
—— —— (1933). *Biochem. J.* **27**, 1609.
—— —— (1934). *Biochem. J.* **28**, 1521.
—— —— (1937). *J. biol. Chem.* **119**, 80P.
Sakel (1937). *Amer. J. Psychiat.* **94**, 111.
Spiegel & Spiegel-Adolph (1936). *Proc. Soc. exp. Biol., N.Y.,* **34**, 799.