THE EFFECT OF BORATE ON THE OXYGEN UPTAKE OF BRAIN TISSUE IN KREBS PHOSPHATE RINGER SOLUTION

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Summary

The effect of borate on the respiration of guinea pig brain cells in Krebs phosphate Ringer solution in the absence of any added substrate and in the presence of glucose and fructose has been investigated.

In the absence of substrate borate rapidly depresses the oxygen uptake, causing finally a very low, apparently constant "borate-resistant" respiration.

In the presence of glucose in concentrations sufficient to maintain the respiration rate of the tissue in the absence of borate, the addition of borate in 50 to 100 times excess over the glucose has no early effect on the respiration.

To inhibit the effects of borate to the same degree, fructose has to be present in four to six times higher concentration than glucose.

In the absence of additional hexose, the R.Q. of the tissue respiring in the presence of borate falls rapidly below 0.9. In the presence of a sufficient concentration of the two sugars it remains unaltered, slightly lower than 1.0.

Addition of 0.03M mannitol to the medium has no effect on the action of borate.

Glucose in very low concentrations maintains the normal respiration rate of the tissue in the presence of borate for a period depending on its concentration. Increasing concentrations progressively delay the onset of the respiratory depression. Fructose in low concentrations fails to maintain the respiratory rate; increasing concentrations merely raise the oxygen uptake until it coincides with that of the borate-free control.

The observations are tentatively discussed; the investigation is being continued.

I. INTRODUCTION

Cases of borate poisoning due to accidental ingestion, to prolonged lavage, or merely to extensive use of boracic powders for epidermal lesions such as eczema, have been reported repeatedly, and fatal intoxications have occurred (Abramson 1949; Rivas 1950). The drug affects particularly the central nervous system, causing convulsions, stupor, and coma; in post-mortem examinations the concentration of borate in the brain was found to be considerably higher than in the body fluids and other organs (Pfeiffer, Hallman, and Gersh 1945). It appears, however, that the effects of borate on nervous tissue have not been investigated in detail. The present work deals with the effect of borate on the oxygen uptake of guinea pig brain cells in Krebs phosphate Ringer solution. It will be shown that the drug exercises a strong inhibitory effect on the oxygen uptake of the tissue, that this effect is counteracted by glucose and fructose, and that, in the presence of borate, there are significant differences in the utilization of low concentrations of glucose and fructose.

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II. Materials and Methods

Guinea pigs were killed by a blow on the neck. A brain mince was obtained by pressing the cerebrum through a 60-mesh copper wire sieve. The mince consisted of thin shreds, 2-3 mm long, which on microscopic examination showed mainly intact cells and very little debris. After centrifugation of the mince in phosphate Ringer solution containing glucose, the supernatant showed no oxygen uptake; whilst the respiration of the sedimented cells remained essentially unaltered. To shorten the interval between the death of the animal and the start of manometric observation, the crude mince was mostly used without previous washing. Slices were prepared by hand with the aid of a microscope slide. After insertion into the main compartment of the Warburg flask they were teased apart with pins to prevent curling and sticking. Mince was treated in the same way to ensure homogeneous suspension. No difference in oxygen uptake was observed between slices and mince. Since the preparation of slices is relatively slow, most experiments were performed with mince; all essential observations were duplicated with slices.

Borate solutions were made by dissolving borax in distilled water and bringing to pH 6.9-7.0 with concentrated hydrochloric acid. A 0.08M borax solution made in this way is approximately isotonic. Usually 0.5 ml of a more concentrated (0.2M) solution was added to 2.5 ml of the medium, thus making the final solution slightly hypertonic with a concentration of 0.13M with respect to boric acid. Control experiments with 0.5 ml of 0.4M NaCl showed that the small increase in tonicity had no effect on the respiration of the cell.

The pH of borate solutions made in this way is increased by dilution and decreased by the addition of substances forming acidic borate complexes; the pH of each ingredient was therefore adjusted to assure a final pH of the medium within the range 7.3-7.4. Control experiments showed that variations of up to 0.2 pH units made no significant difference in the oxygen uptake. (According to Elliott and Birmingham (1949) the respiration of rat brain suspensions in phosphate Ringer is optimal between pH 7.0 and 7.6.) The pH determinations were made with a Jones' pH meter (N. L. Jones, Melbourne).

For the determination of the borate content of the tissue, slices only were used. These were dried on filter paper, weighed, and after addition of a few drops of sodium hydroxide to avoid sublimation of boron trioxide, carefully ashed on platinum. The white residue was dissolved in distilled water and the boron determined by the colorimetric quinalizarine method of Berger and Truog (1939).

Glucose and fructose solutions were made by dissolving the sugar in phosphate Ringer solution. Mannitol was dissolved in distilled water.

All reagents used were of "Analar" quality; the fructose was Extra Pure (Hoffmann-La Roche, Basel).

All concentrations are given as final molar concentrations in the medium, borate concentrations being calculated as boric acid.

Oxygen uptakes were measured in standard Warburg flasks and are expressed as μl O_2 per 100 mg fresh tissue. Between 120 and 140 mg tissue were

used with flasks of 25-30 ml volume containing 3 ml fluid medium. Unless stated otherwise, the conditions were: initial pH 7.3-7.4; temperature 37.5°C; gas phase air; fluid medium Krebs phosphate Ringer solution (Krebs 1933); volume of added borate solution 0.5 ml of a molarity to give a final boric acid concentration of 0.13M; volume of added hexose or mannitol 0.5 ml; time between death of animal and start of observation 40-60 min; duration of manometric observation 5 hr. Readings were taken at least every half hour. Each graph is based on at least three separate runs.

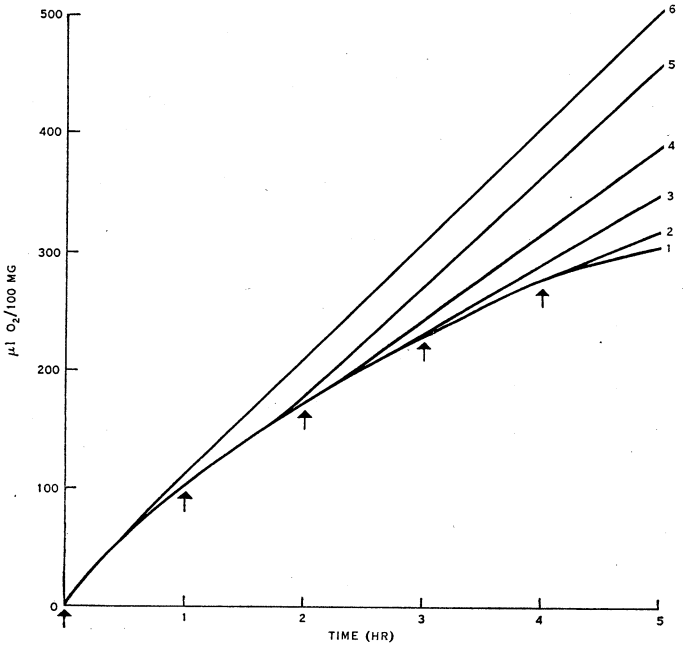

Fig. 1.—Oxygen uptake of guinea pig brain in Krebs phosphate Ringer, with glucose added after various time intervals; the ability of glucose to restore the original oxidation rate falls with increase in the time of addition. Final glucose concentration, 0.01M.

Curve 1: No glucose
Curve 2: Glucose added after 4 hr.
Curve 3: Glucose added after 3 hr.
Curve 4: Glucose added after 2 hr.
Curve 5: Glucose added after 1 hr.
Curve 6: Glucose added at start of measurement.

III. Results

(*a*) *Preliminary Observations*

(i) *Normal Respiration.*—Our preparations showed, in Krebs phosphate Ringer containing 0.01M glucose, oxygen uptakes of 90-110 µl O_2 per 100 mg fresh tissue per hour, and maintained this rate for at least 5 hr.

In the absence of glucose, the initial rate of respiration was the same or almost the same as in its presence, but very soon the oxygen uptake began to fall. There were great variations in the time of onset of this decrease, which varied from brain to brain, from 30 min to about 2 hr after start of observation. The rate of decrease reached its maximum after a further ½-1 hr; later the decrease was slower, though still steady (Figs. 1 and 4).

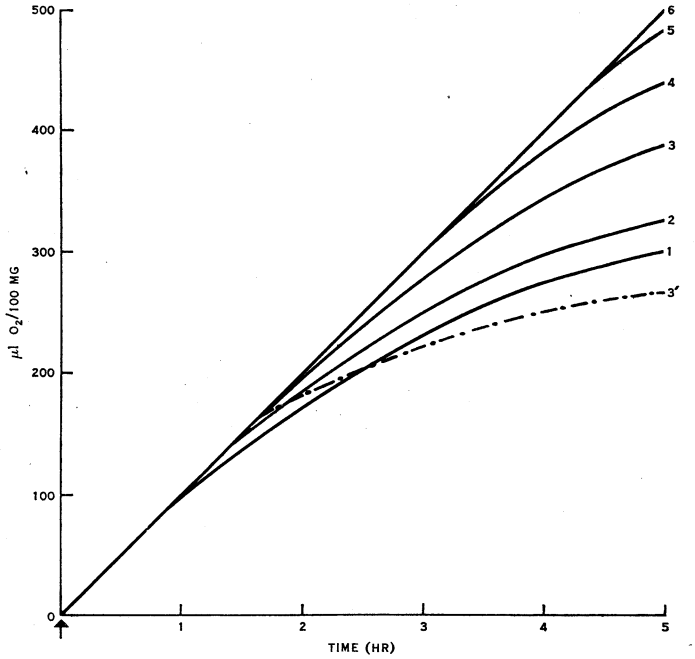

Fig. 2.—Effect of glucose at various concentrations. Glucose added at start of measurement.

Curve 1: No glucose.
Curve 2: 0.000125M glucose.
Curve 3: 0.00050M glucose.
Curve 4: 0.0010M glucose ("maintenance concentration").
Curve 5: 0.0020M glucose.
Curve 6: 0.010M glucose.
Curve 3′: 0.00050M glucose with 0.13M borate (same as Fig. 9, curve 3).

(ii) *Maintenance Concentration.*—The concentration of glucose commonly used in metabolic experiments is 0.01M; the minimum concentration capable of maintaining a constant respiration for at least 4 hr—referred to here as "maintenance concentration"—was found to be considerably lower, about 1/10 of the above concentration (Fig. 2). The maintaining effect of the glucose depended on its concentration rather than on the quantity available, since the respiration could not be maintained for a longer period by increasing the fluid volume while preserving the same low glucose concentration.

(iii) *Respiration in the Presence of Fructose.*—Under the conditions of our experiments there was no difference in the utilization of fructose and glucose. Equal concentrations of the two sugars maintained the respiration to the same degree and the maintenance concentration of both was the same.

(iv) *Late Addition of Hexose.*—When glucose or fructose (0.01M) was added to tissue which had been respiring for some time in Krebs phosphate Ringer alone, its effect depended on the degree of exhaustion of the tissue. Maintenance of the original rate of respiration was obtained only on addition during the period when the tissue still respired at its original rate or when this rate had only just begun to decline. When the hexose was added later, namely when the rate of respiration had already fallen for 20-30 min, its effect became noticeable only after a time lag (Fig. 1); the respiratory rate then increased slowly and became constant after 1-2 hr, though at a considerably lower level than that shown by the control with hexose present from the start. Very late addition of the sugars had little or no restoring effect, but it still prevented a further decline of the oxygen uptake.

(v) *Respiration in the Presence of Mannitol.*—Mannitol is one of many hydroxy compounds forming strong complexes with boric acid (cf. glycerol, α-hydroxy acids, salicylic acid). It is not toxic in the concentrations used. Its addition to brain cells in concentrations up to 0.03M was found to have no effect on the oxygen uptake of the tissue respiring either alone or in the presence of glucose or fructose.

(vi) *Relative Ability of Glucose and Fructose to Form Borate Complexes.*—It is known that "ketoses form much greater quantities of the boric acid complex than do aldoses" (Boeseken 1949); an attempt was made to estimate approximately the relative ability of glucose and fructose to form these complexes at concentrations similar to those of our experiments.

At room temperature, 20 ml of 0.1M borate solution of pH 7.4 showed, on addition of solid glucose to 0.01M concentration, a pH drop of 0.2 units and required 0.3 ml of 0.1N sodium hydroxide solution to restore the pH to 7.4. With fructose, the pH dropped by almost one unit and the solution required 1.4 ml sodium hydroxide to restore the pH, indicating that fructose forms—under these conditions—acidic complexes to an extent of about 70 per cent. of its amount, which is 4-5 times higher than with glucose. Under the actual experimental conditions (phosphate Ringer 0.13M borate, pH 7.4, 37.5°C), the extent of complex formation can be expected to be still higher.

Unfortunately it was not possible to estimate to what extent free sugars and their modifications may still be present under the conditions of our experiments. The physicochemical data available from the literature (Isbell *et al.* 1948; Boeseken 1949; Ross and Catotti 1949) shed little light on this question since they hold only under conditions vastly different from ours.

(vii) *Borate Content of the Tissue.*—Slices were incubated in borate-free and in borate-containing medium for periods between 20 and 120 min. They were then removed, rinsed with phosphate Ringer, dried, weighed, and analysed. In slices incubated in borate-free medium, no boron could be detected by the

colorimetric method used. The borate content of the slices incubated in borate-containing medium was found to be, weight for weight, identical with that of the medium. The presence or absence of glucose, fructose, or mannitol made no difference to the boron content of the slices. No selective absorption of boron could thus be detected.

(*b*) *Effect of Borate Alone*

(i) *Effect on Respiration.*—When brain slices or mince, directly after preparation, were suspended in a medium containing 0.05-0.15M boric acid, their respiration, compared with that of the controls, had decreased considerably by the time manometric measurement could be started. The degree of this depression depended on the concentration of borate rather than on its quantity.

Borate concentrations of the order of 0.001M and lower had no effect on the oxygen uptakes during the experimental time.

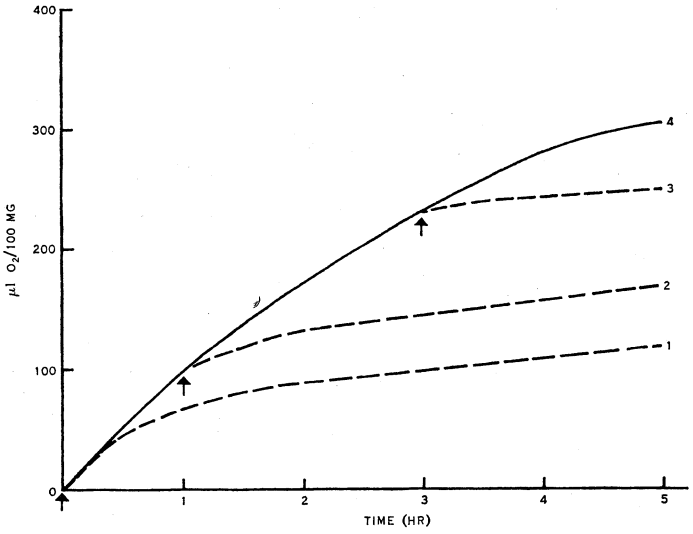

Fig. 3.—Borate added after various time intervals; no glucose. Time-lag on early addition. Borate concentration 0.13M (expressed as H_3BO_3).

Curve 1: Borate added at start of measurement.
Curve 2: Borate added after 1 hr.
Curve 3: Borate added after 3 hr.
Curve 4: No borate.

(ii) *Onset of the Borate Effect.*—When borate was added to the medium at the start of manometric observation, the onset of the respiratory depression became noticeable only after a delay (Fig. 3), which varied from brain to brain from 15 min to almost 1 hr. The effect appeared to be more delayed if the borate-free control was able to maintain a constant respiration rate for 1-2 hr, but to occur earlier if the tissue alone was unable to maintain its original respiration. Once borate had begun to show its effect, the rate of oxygen uptake decreased rapidly, a maximum rate of decrease being reached after about 30

min and maintained for a short time. Later the decrease became slower, and finally an almost constant very low rate of oxygen uptake was reached and maintained for the rest of the experimental period. This rate, 10-15 μl O_2/100 mg tissue per hour, was lower than the final rate reached by the borate-free controls within the same time, and it appeared to be almost equal with all brains examined. The nature of this "borate-resistant" oxidation was not further investigated.

When the borate was added at a time when the tissue showed already signs of exhaustion insofar as its respiration had begun to decline, the initial period of delay was no longer pronounced. The oxygen uptake dropped almost immediately to the final low value, which was the same, no matter at which stage the borate had been added (Figs. 3 and 4).

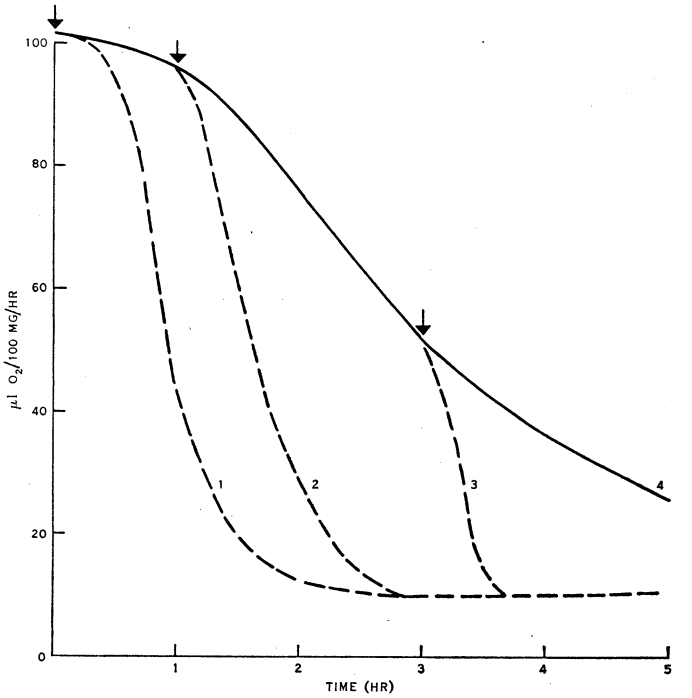

Fig. 4.—Rate of change in oxygen uptake for the experiment illustrated in Figure 3. Identical low, final rate in the presence of borate. Curves numbered as in Figure 3.

With lower concentrations of borate the delay in the onset of borate action was increased (Fig. 5). Under these conditions the final low oxidation rate was not always reached during the observation time. The lowest borate concentration at which this final rate was reached within 4 hr was found to be of the order of 0.05M.

(iii) *Reversibility of Borate Action.*—Fresh brain slices were incubated in borate-free and in borate-containing medium, removed after various time intervals, rinsed with Krebs-phosphate Ringer solution, and brought to manometric

observation in Krebs-phosphate Ringer containing 0.01M glucose. Slices which had been under the influence of borate for over 15 min. showed initially a lower respiration rate than the controls. After 20-30 min, however, their oxygen uptake increased slowly, to reach after 2-5 hr that of the control (Fig. 6). Partial recovery took place even when the tissue had been exposed to the action of borate for as long as 1 hr.

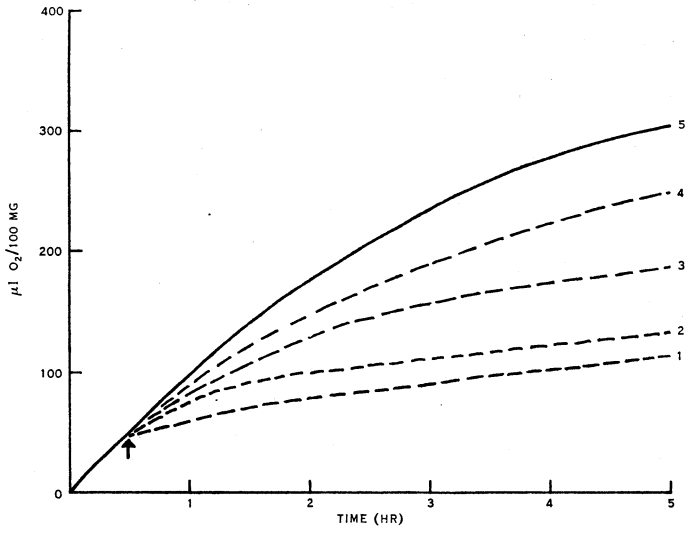

Fig. 5.—Effect of borate at various concentrations; no added substrate.

Curve 1: 0.26M borate.
Curve 2: 0.13M borate.
Curve 3: 0.065M borate.
Curve 4: 0.033M borate.
Curve 5: No borate.

(c) *Borate and Glucose*

(i) *Inhibition of Borate Effect.*—When borate was added to tissue already respiring in the presence of glucose sufficient to maintain its normal respiratory rate (above 0.002M), no early effects of borate were observed, no matter when the borate was added. In most cases the tissue continued to respire at its normal rate throughout the period of observation, but often a distinct, if slight, fall in the oxygen uptake was observed after 3-4 hr (Fig. 7, curve 3). This late effect of borate was noticed at low as well as at high glucose concentrations and could not be prevented by increasing the glucose concentration to 0.02M. Glucose in proportion of 1 part to up to 100 parts of borate prevented the early onset of depression by 0.13M borate.

With later addition of glucose of the same concentrations to tissue already respiring in the presence of borate, the effect of the sugar decreased with the delay in addition. Only during the first 15-20 min of borate action could glucose prevent the onset of respiratory inhibition, and only during this period

was the oxygen uptake the same, no matter whether borate or glucose was added first. On later addition the effect of glucose decreased rapidly, though it still caused an increased oxygen uptake and a constant oxidation rate (Fig. 7). There was only a slight delay in the onset of the glucose effect, and no late recovery was observed (cf. Fig. 6, representing an experiment in which the tissue was removed from the borate-containing into a borate-free glucose-containing medium). Even when the tissue was already respiring at the final low rate caused by borate, there was on addition of glucose still a small but distinct increase in the oxygen uptake, although usually not sufficient to double the prevailing rate of about 10 μl O_2/100 mg/hr.

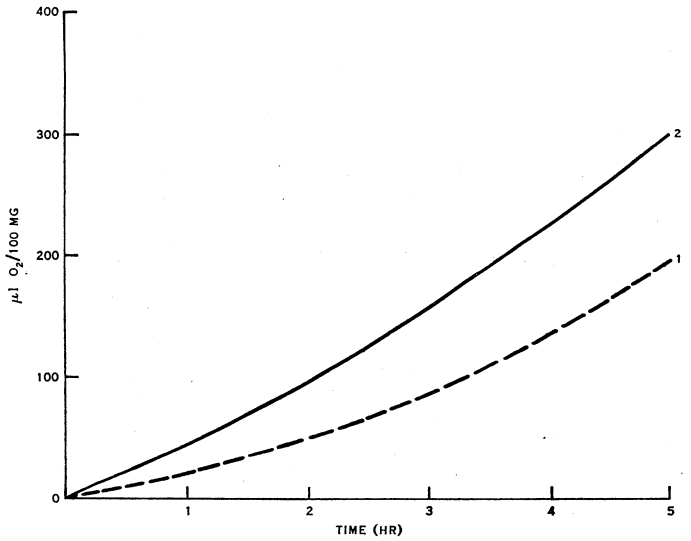

Fig. 6.—Recovery of brain slices in glucose phosphate Ringer after previous exposure to 0.13M borate in glucose-free medium. Glucose 0.01M.

Curve 1: Slices previously exposed to borate for 45 min, rinsed, and transferred into fresh medium.

Curve 2: Slices treated in the same manner without exposure to borate (control).

(ii) *Effect of Exhaustion.*—In parallel experiments tissue preparations were allowed to respire in substrate-free medium until a decline in the rate of respiration was distinctly observed. Then glucose (0.01M) was added to one flask, followed after 15 min by borate (0.13M); to the other flask the borate was added first, followed after the same time by glucose.

Although the media in both flasks were of the same final composition, the final respiration rates were different (Fig. 8). The tissue to which glucose had been added first restored and maintained the original respiration rate, while the tissue to which borate had been added first respired at a considerably lower, though also constant rate. Once the tissue became exhausted, the onset of the

borate-caused respiratory depression could be prevented only by almost simultaneous addition of glucose.

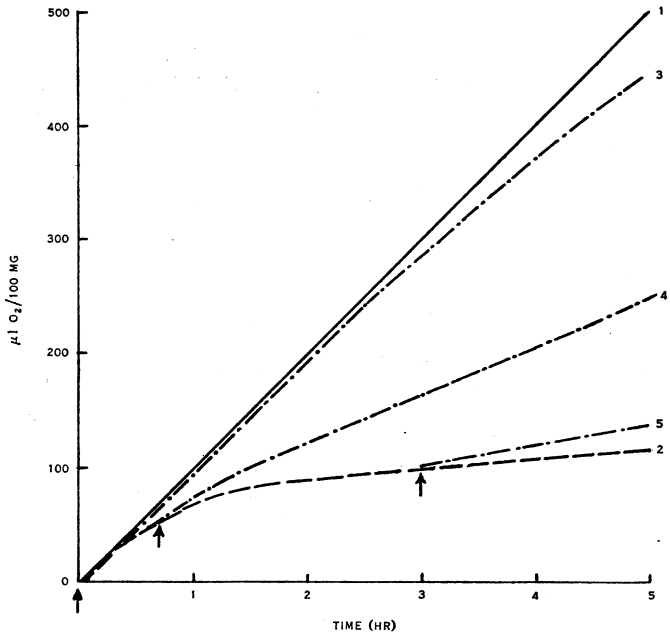

Fig. 7.—Effect of glucose added after various time intervals to brain cells respiring in borate from the start of measurement. The ability of glucose to abolish the effect of borate decreases rapidly with increase in the time of addition. Borate 0.13M; glucose 0.01M.

Curve 1: Respiration in glucose; no borate.
Curve 2: Respiration in borate; no glucose.
Curve 3: Glucose added simultaneously with borate.
Curve 4: Glucose added after 45 min of respiration in borate.
Curve 5: Glucose added after 3 hr of respiration in borate.

(iii) *Effect of Glucose Concentrations Lower than Sufficient for Maintenance.*—When glucose to concentrations below 0.002M was added early in the experiment together with 0.13M borate, two separate effects were observed (Fig. 9; compare also Fig. 2, curve 3′). One was a delay in the onset of borate action insofar as there was a period of unchanged respiration before, almost abruptly, a progressive decline in the oxygen uptake took place. The duration of this delay increased with the concentration of glucose and shifted the onset of the respiratory depression towards the end of the observation time. No simple relation between the duration of this delay and the concentration of glucose could be observed.

The second effect concerns the rate of oxygen uptake after the onset of the respiratory depression. At very low glucose concentrations the oxygen uptake followed almost that of the borate-containing, glucose-free control and the onset

of the respiratory depression showed itself as a distinct discontinuity in the curve. With increasing glucose concentrations the curves became steeper and approached finally the course of the oxygen uptake shown by the borate-free, glucose-containing control. At these higher concentrations the angle between the unchanged and the depressed rate of oxygen uptake was no longer observable as a distinct discontinuity.

Very similar curves were obtained when, in the presence of a constant low glucose concentration, the borate concentration was varied (Fig. 10). Lower borate concentrations showed a later onset of the respiratory depression and a higher rate of oxygen uptake after the discontinuity.

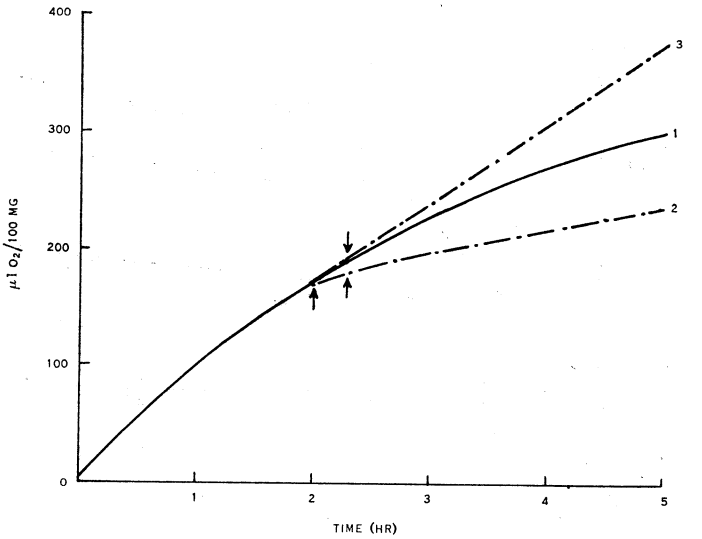

Fig. 8.—Effect of reversing the sequence of addition of glucose and borate to a preparation which had been respiring in the absence of added substrate for 2 hr. Glucose 0.01M; borate 0.13M.
Curve 1: No additions.
Curve 2: Borate added first, followed by glucose after 15 min.
Curve 3: Glucose added first, followed by borate after 15 min.

(*d*) *Borate and Fructose*

The experiments recorded in the previous section were repeated using fructose instead of glucose. The effect of 0.01-0.02M fructose was almost identical with that of the same concentration of glucose and the curves of oxygen uptakes were hardly distinguishable. It is not necessary to detail these experiments. Quantitative and qualitative differences between the two sugars were observed at lower concentrations only.

(i) *Maintenance Concentration.*—Figure 11, curves 3 and 4, represents the oxygen uptake of 100 mg tissue in the presence of 0.0025M glucose and fructose, slightly higher than the maintenance concentration. The addition of borate (0.13M) to the tissue with glucose (curve 2) had little effect on the respiratory rate, while its addition to that with fructose (curve 1) reduced the oxygen

uptake by over 50 per cent. over a period of 4 hr. Invariably fructose had to be present in several times its maintenance concentration, usually between 4 and 6 times, but up to 10 times, to counteract the early effect of borate. One part of fructose thus counteracted the effect of only 10-25 times excess of borate.

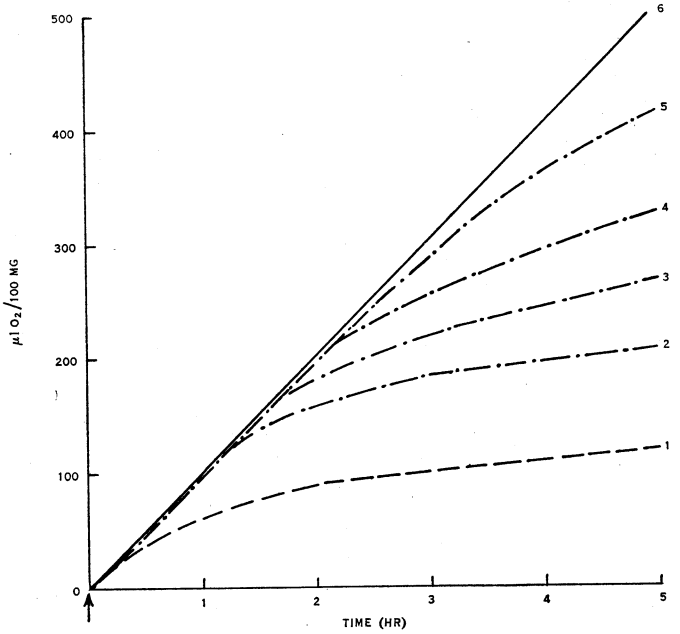

Fig. 9.—Effect of glucose in low concentrations on the respiration in the presence of 0.13M borate (cf. Fig. 2). Borate and glucose added simultaneously at start of measurement.

Curve 1: Borate alone; no glucose.
Curve 2: 0.00025M glucose.
Curve 3: 0.00050M glucose.
Curve 4: 0.0010M glucose.
Curve 5: 0.0020M glucose.
Curve 6: 0.0020M glucose; no borate.

(ii) *Low Concentrations of Fructose.*—Figure 12 records an experiment in which the same brain preparation was used as in the corresponding experiment with glucose (Fig. 9). It is seen not only that in the presence of borate the effective fructose concentrations were higher than those of glucose, but also that the oxygen uptake with fructose declined directly from the moment of addition. In no case was a delayed onset of borate action observed, nor were there any discontinuities in the curves, which were very similar to those obtained with lower concentrations of glucose *after* the onset of the respiratory depression. The same difference was observed when the borate concentration was varied in the presence of a constant, low concentration of fructose (Fig. 13; cf. Fig. 10).

(iii) *Low Fructose with Very Low Glucose Concentrations.*—It was considered that the presence of small glucose concentrations might facilitate the

utilization of fructose in the presence of borate. No such effect could be demonstrated.

(iv) *Late Addition of Glucose and Fructose.*—When equal, low concentrations of the two sugars, 0.005M, were added to tissue respiring in the presence of borate, the difference between the effect of the two sugars decreased with increase in the time of borate action. At a time when the final low rate of oxygen uptake had become established, there appeared to be no longer any difference between the two sugars; but the increases in the rate of oxidation caused by very late addition were too slight to allow conclusive interpretation.

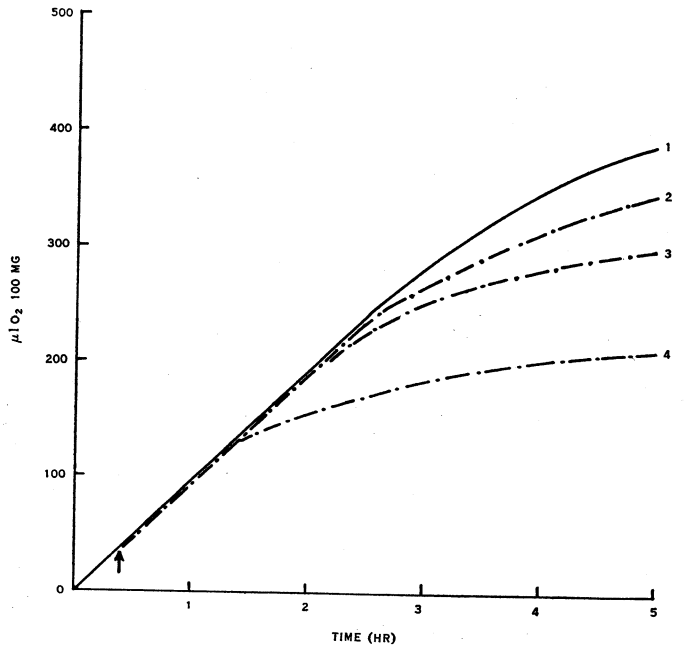

Fig. 10.—Effect of increasing concentration of borate on the respiration in the presence of a low concentration of glucose, 0.00050M. Borate and glucose added simultaneously after 25 min.
Curve 1: Glucose alone; no borate.
Curve 2: 0.065M borate.
Curve 3: 0.13M borate.
Curve 4: 0.26M borate.

(*e*) *Mannitol and Borate*

It seemed of interest to find to what extent the presence in the medium of fairly high concentrations of a strong, metabolically inert complex-former might reduce the effect of borate.

Mannitol to concentrations up to 0.03M was added to 0.13M borate and the pH readjusted after the mixture had been allowed to stand overnight. Since one molecule of mannitol probably combines with two of borate (Isbell *et al.* 1948) the amount of free boric acid in a solution of 0.13M concentration could

be expected to be reduced by almost 50 per cent. by 0.03M mannitol. However, within this range of concentrations, no significant influence on the reduction of oxygen uptake caused by borate could be detected, either in the presence or the absence of low concentrations of glucose or fructose.

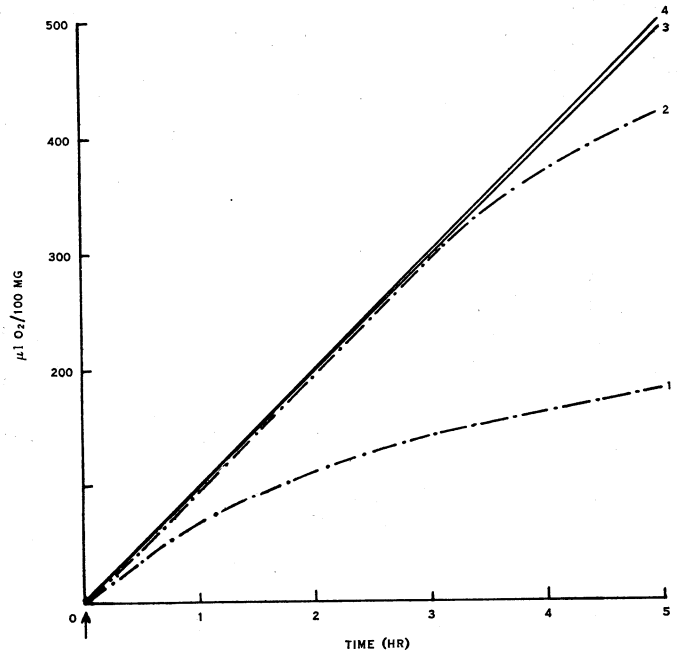

Fig. 11.—Effect of equal, low concentrations of glucose and fructose on the respiration in the presence and in the absence of 0.13M borate. Sugar and borate added simultaneously at start of measurement. Sugar concentration 0.0025M.

Curve 1: Fructose and borate.
Curve 2: Glucose and borate.
Curve 3: Fructose; no borate.
Curve 4: Glucose; no borate.

(*f*) *Respiratory Quotients*

Respiratory quotients (R.Q.) were measured in a series of experiments using the direct method of Warburg. Tissue respiring in the absence of any additional substrate showed during the first 2 hr R.Q.'s of about 0.99, later the value dropped below 0.95. (At the same time the pH in an unbuffered or only weakly buffered medium increased to above 7.5 and free ammonia could be determined, indicating that amino compounds were utilized as the exhaustion of the cell proceeded.) In the presence of borate and absence of additional substrate, the R.Q. dropped even more quickly, reaching values below 0.89 within 3 hr. These observations will be more thoroughly followed in connection with experiments on the utilization of a series of other metabolites in the presence of borate. For the present purpose, the only pertinent fact is that glucose prevents the sharp drop in R.Q. caused by borate alone. In the presence of a maintenance concen-

tration of glucose, borate affected the R.Q. but slightly, values between 0.95 and 0.98 having been observed. No difference in R.Q. could be noted between glucose and fructose in the presence of borate as long as the concentrations of the sugars were sufficient to maintain the normal rate of respiration.

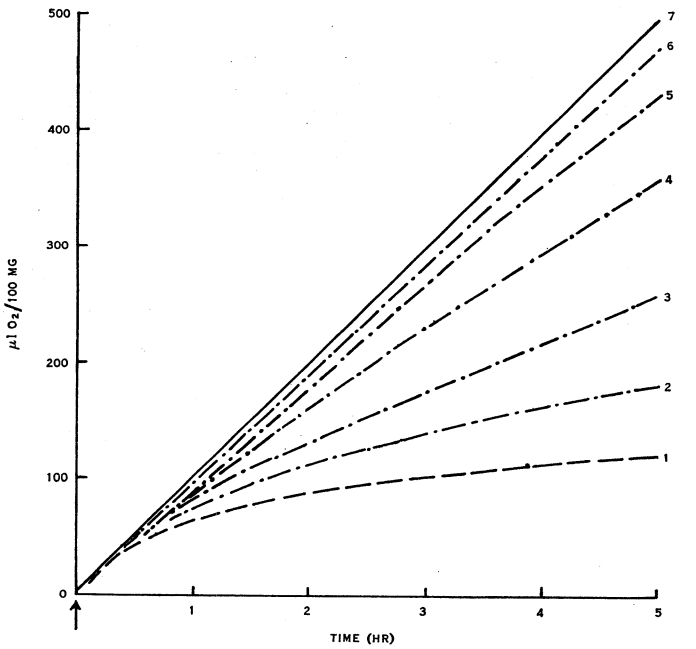

Fig. 12.—Effect of increasing concentrations of fructose on the respiration in the presence of 0.13M borate. Fructose concentrations are above the maintenance concentration. Same brain preparation as in the corresponding experiment with glucose, Figure 9. Borate and fructose added simultaneously at start of measurement.

Curve 1: Borate alone; no fructose.
Curve 2: 0.0025M fructose.
Curve 3: 0.0050M fructose.
Curve 4: 0.0075M fructose.
Curve 5: 0.010M fructose.
Curve 6: 0.020M fructose.
Curve 7: 0.020M fructose; no borate.

IV. Discussion

The experimental data presented show three points of interest: (i) in substrate-free medium borate depressed strongly the oxygen consumption of the surviving brain cell; (ii) the normal respiratory rate was, however, preserved if either glucose or fructose in concentrations of the order of 0.01M were present in the medium, but (iii) at lower hexose concentrations there was a pronounced difference between the effect of the two sugars in the presence of borate.

(i) Pfeiffer, Hallman, and Gersh (1945) observed that, on slow peritoneal irrrigation with 5 per cent. boric acid solution over 90 per cent. of the boric acid was absorbed during the first hour of irrigation, while after a period of 3 hr analysis of the brain, liver, and fat of the animals showed high borate concentrations. The drug freely penetrated the peritoneum and passed rapidly into the circulation and equally rapidly into distant tissues. Our observations were in accord with these findings insofar as already after 20 min of suspension in borate-containing medium the borate concentration in brain slices was equal to that of the medium. On early transfer of the tissue into borate-free medium, the normal rate of oxygen uptake was slowly restored; no irreversible damage appeared to be caused by a short borate action on the tissue. On prolonged action the cell lost the power of recovery.

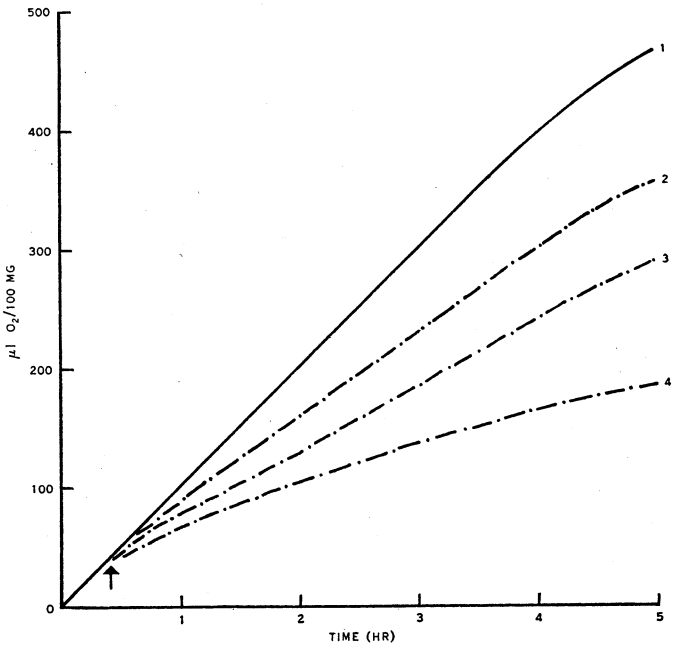

Fig. 13.—Effect of increasing concentrations of borate on the respiration in the presence of 0.005M fructose. Borate and fructose added simultaneously after 25 min. Compare Figure 10.

Curve 1: Fructose alone; no borate.
Curve 2: 0.065M borate.
Curve 3: 0.13M borate.
Curve 4: 0.26M borate.

The depressant effect of borate on the oxygen uptake of the cell may be caused through ionic reactions, or through complex formation between borate and suitable hydroxy compounds. Ionic reactions of borate may take place through formation of insoluble salts, e.g. with calcium, or by displacement, e.g. of phosphoric acid; Wiley (1904) reported increased secretion of inorganic phosphate after borate ingestion, and Pfeiffer, Hallman, and Gersh (1945) ob-

served that, possibly through formation of glyceroborates, borate displaces phosphate in a lipid fraction of the brain. Complex formation with the substrate is suggested in the inhibition of plant polyphenolase (MacVicar and Burris 1948); complexes are formed with pyridoxine (Scudi, Bastedo, and Webb 1940) and riboflavine (Frost 1942; cf. also the review by Zittle 1951). Furthermore, with muscle adenylic acid (Klimek and Parnas 1932), dehydroascorbic acid (Militzer 1945), adrenaline (Trautner and Messer 1952), and many other hydroxy compounds, particularly sugars and intermediates of sugar metabolism —unless one or more of the HO — groups concerned is protected by phosphorylation. (According to Boeseken (1949) the complexes with sugars are mainly those involving the potential aldo and keto groups. As seen in Figure 14, only β-glucose does not possess two free adjacent HO — groups in *cis*-position and cannot therefore form borate complexes involving carbons 1, 2, or 3.) The toxic effects of borate are thus likely to be spread over a multitude of metabolic processes; the depression of the O_2 uptake in substrate-free medium is only the most striking and possibly the earliest effect of the drug.

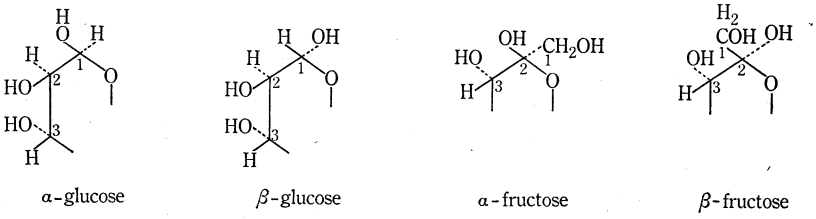

Fig. 14.—Steric positions of the hydroxyl groups at the 1, 2, and 3 carbon atoms of α and β glucose and fructose.

(ii) Glucose, added to the medium to a concentration above 0.01M, completely inhibited the respiratory depression caused by up to 100 times excess of borate; fructose in the same concentrations showed the same effect. Mannitol, however, although it forms complexes with borate, showed no effect even in the highest concentrations compatible with isotonic conditions. Obviously glucose and fructose do not restore the oxygen uptake by detoxicating the borate through complex formation. It is tentatively suggested that borate causes the fall in oxygen uptake initially by depriving the cell, through complex formation, of its supply of available hexose, thus accelerating the exhaustion of the cell and its final death. This interpretation accounts for the facts that (1) borate acts more slowly on the fresh cell which is still supplied with a certain store of carbohydrate, and more quickly on the aged cell whose stores are exhausted; (2) glucose counteracts the effect of a manifold excess of borate—since it is only necessary for it to raise the concentration of non-borate-combined sugar to the minimum required for continuance of normal respiration, and (iii) almost complete recovery occurs if the tissue is in time transferred into borate-free, sugar-containing medium since the unstable complexes hydrolyse on dilution. In liver, where there is an abundant supply of glycogen, borate does not cause any depression of the respiration (Feinstein and Stare 1940); the glycogen pre-

sent acts like the additional glucose in our experiments and its utilization does not appear to be interfered with.

Part of the symptoms of borate poisoning in man or animals, such as convulsions and coma, may thus be due to an acute lowering of the concentration of available hexose structures in tissues which, like brain and nerve, are not supplied with a big store of carbohydrate. Pfeiffer, Hallman, and Gersh (1945) observed, however, that borate poisoning in dogs is neither prevented nor cured by the administration of glucose. It must therefore be doubted whether the maintenance of the normal respiratory rate observed in the presence of hexose indicates a normal physiological respiration. In fact, this is not the case; investigations to be presented in a following publication showed, for example, that the toxic effects of borate on the nitrogen metabolism of the cell are not counteracted by hexose. The sugars only maintain the rate of oxygen uptake, but they do not prevent borate poisoning altogether.

(iii) Low concentrations of glucose or fructose (0.001M) have in the absence of borate an identical effect on the oxygen consumption of the brain cell; in its presence, however, significant differences were observed.

(1) The maintenance concentration of fructose, i.e. the concentration which just maintained a constant oxygen consumption during the experimental time, was in the presence of borate several times higher than that of glucose (about 0.01M compared with 0.002M for glucose). Borate forms complexes involving the potential aldo resp. keto group with all fructose structures and with α-glucose, but not with β-glucose. The site of this borate effect might therefore be placed at hexokinase level, where, at low hexose concentrations, borate may demonstrate the fact that brain hexokinase, like yeast hexokinase, reacts with all glucose structures, but only with β-fructo-furanose, which constitutes only 12-20 per cent. of the fructose supplied (Gottschalk 1943; Slein, Cori, and Cori 1950). The failure of fructose satisfactorily to substitute for glucose is, however, not confined to borate action or to extreme conditions, like low ATP concentrations (Meyerhof and Geliazkowa 1947). Even in high concentrations it may not be well utilized if the cell is in any way damaged. The best known instance of this failure is the fact that fructose is inferior to glucose in relieving insulin coma. It may therefore not be justified to ascribe the lower fructose utilization in the presence of borate to complex formation alone. Other borate effects may contribute to cause the phenomenon observed.

(2) At concentrations below those required for maintenance fructose increased with increasing concentration the oxygen uptake of the borate-treated cells until, at about 0.01M, it coincided with that of normal respiration. This behaviour is to be expected, if a metabolizing system is supplied with increasing substrate concentrations. Glucose, however, caused an initial period of normal oxygen consumption before the onset of respiratory depression. It will appear that glucose, even in very low concentrations, is capable of at least temporarily supporting normal cell respiration, thus causing a delay in the onset of respiratory depression, which increased with increasing concentrations of glucose until, at about 0.002M, the oxygen uptake remained constant during the observation

time. Fructose, though causing a fairly steady oxygen uptake, never effected this temporary maintenance of the normal respiratory rate. The causes for this difference may, tentatively, be looked for at oxo-isomerase level, where fructose, as the 6-phosphate, joins the path of glucose metabolism. This enzyme requires for its function free 1, 2, 3 carbon hydroxyl groups of the reacting hexose-6-phosphates. If borate forms complexes with the 6-phosphates to the same extent as with the free sugars, the Lohmann equilibrium will be affected and at low fructose concentrations in the medium there will inside the cell result a dearth of glucose structures for those mechanisms which require glucose as substrate, like glucose-6-phosphate dehydrogenase. The question whether one of these mechanisms is concerned with the maintenance of normal brain respiration is of great interest and may have a bearing on the fact that brain is so sensitive to lowered glucose concentrations. It is, however, not considered that the facts so far known allow any more detailed interpretation of the observations presented, nor any speculations as to the nature and site of hypothetical mechanisms possibly concerned with the maintenance of normal respiration and the way in which borate may affect them.

The investigation is being extended to nitrogen and phosphate metabolism in the presence of borate.

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