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Inositol and the Respiration of Brain.

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By means of manometric measurements using the Warburg apparatus Das and Guha[1] claim to have shown that the oxygen consumption of various rat tissues is greater when inositol is present in the suspension medium than in control experiments in which it is absent. This effect was found in the case of brain, heart, kidney and liver tissue, being most marked in the case of brain tissue. After experiments of 2-3 hours' duration Das and Guha found that when inositol was present unwashed brain tissue showed an oxygen consumption about 35% greater than that of the control (one experiment reported) and in the case of washed brain tissue (3 experiments reported) increases up to about 90% were found.

Since it has not been possible to ascribe any biochemical significance to the considerable amounts of inositol shown to be present in brain (Thudichum,[2] Young[3]) the following work was undertaken to confirm and extend the above findings of Das and Guha.

The experimental procedure adopted in the case of the results recorded in Series I and II was that used by Das and Guha. White rats were used, and after decapitation the brains were removed as rapidly as possible, sliced, and the slices cut into small pieces. The tissue was transferred to Warburg flasks containing 1 ml. of Ringer-Locke solution, 1 ml. of Na_2HPO_4-NaH_2PO_4 buffer (pH 7.4) and either 0.5 ml. of water or 0.5 ml. of 0.1 M inositol solution. The CO_2 was absorbed with 0.2 ml. of 20% KOH solution in the inset of the flask, and the temperature of the thermostat was 37.3°C. In experiments in which washed brain was used, the finely cut tissue was washed twice in a centrifuge tube with Ringer-Locke solution as described by Das and Guha. Washing the finely cut brain several times was not found to affect the results greatly.

It must be recognized that with the above technique certain factors are involved which can introduce variations in replicate experiments. When the Warburg flasks contain air the brain tissue does not re-

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1 Das, N., and Guha, B. C., *Z. physiol. chem.*, 1935, **231**, 157.

2 Thudichum, J. L. W., *Die chemische Konstitution des Gehirns des Menschen und der Tiere,* Tübingen, 1901.

3 Young, L., *Biochem. J.*, 1934, **28**, 1435.

ceive as much oxygen as it can use. This is demonstrated in Series III in which parallel experiments to those in Series II were performed using oxygen instead of air. Furthermore with low oxygen tensions variations in the state of division of the tissue in different flasks tend to cause larger variations in the oxygen consumption than when pure oxygen is used. Since the oxygen uptake of cerebral cortex is greater than that of white matter, experiments in which whole brain is used are open to variation due to varying proportions of grey and white matter in different flasks. In view of these facts it is desirable that conclusions be drawn only from the mean results of a sufficient number of experiments to eliminate these possible variations.

The results given in Series I and II show that when this was done it was not possible to confirm the findings of Das and Guha on rat brain. Furthermore no inositol effect was found in the case of similar experiments with washed whole brain in oxygen (Series III) or with unwashed cerebral cortex in air. In the control experiments of Das and Guha there was a rapid falling off in the rate of oxygen consumption which has not been found in the present work. The most marked effect with added inositol was obtained by Das and Guha in those experiments in which the rate of oxygen uptake of the controls fell off to the greatest extent. The writer has not found either in this or other work that the respiration rate of brain tissue in the presence of glucose decreases to any marked degree over periods of 3 hours, and the control experiments for rabbit cerebral cortex reported in Series IV indicate little change in rate even over a period of 5 hours.

Other experiments were performed in which the more satisfactory phosphate medium described by Dickens and Greville[4] replaced the Ringer-Locke-phosphate medium used in the above experiments. Furthermore since it is desirable that the smallest possible changes should be made in the concentration of the suspension medium 0.2 ml. of 0.25 M inositol solution (or water) was added instead of the 0.5 ml. of 0.1 M inositol solution (or water) added in the above experiments. Under these conditions the oxygen uptakes in control experiments with either rat cerebral cortex or rat whole brain in either air or oxygen did not show any significant differences from those found in experiments when inositol was present. A similar result was obtained (See Series IV) in a series of experiments with rabbit cerebral cortex in which tissue slices were allowed to respire for 5 hours in oxygen and in which 0.2 ml. of 0.9% NaCl solution was added to the controls instead of 0.2 ml. of water.

[4] Dickens, F., and Greville, G. D., *Biochem. J.*, 1935, **29**, 1468.

TABLE I.

Series	No. of animals used	No. of pairs of exp.	Hr.	Mean oxygen uptake in cmm. per mg. (dry wt.) of tissue: Controls	Inositol added
I. Unwashed whole brain (rat) in air	4	5	1	5.2	5.1
			2	5.1	5.1
			3	4.7	4.7
			Total	15.0	14.9
II. Washed whole brain (rat) in air	3	6	1	5.3	5.6
			2	5.1	5.5
			3	4.7	5.1
			Total	15.1	16.2
III. Washed whole brain (rat) in oxygen	3	4	1	10.3	9.9
			2	10.0	10.1
			3	8.2	8.4
			Total	28.5	28.4
IV. Unwashed cerebral cortex (rabbit) in oxygen	1	5	1	13.9	13.7
			2	14.5	14.6
			3	14.2	14.4
			4	13.5	13.9
			5	13.2	13.7
			Total	69.3	70.3

It seems likely that even if inositol is involved in brain respiration, the above experiments might give no evidence of this. Inositol is not easily extracted from brain and it is possible that there is sufficient inositol present in brain, even after washing, to supply the needs of the tissue over the period of the experiments. Quastel and Wheatley[5] described a technique by which the effect of a substrate on the brain was studied by adding it after the tissue had been allowed to respire for some time in the absence of any added substance capable of being oxidized by the brain. Under such conditions the brain became depleted of its own oxidisable material before the addition of the substrate under investigation. This procedure was used in the present work. Rat whole brain was allowed to respire in oxygen in Dickens-Greville phosphate medium (2.0 ml.) from which glucose was absent. After a period of depletion the following solutions were added to the tissue from the side arms of the Warburg flasks in 4 parallel experiments: (1) 0.2 ml. water, (2) 0.2 ml. 0.1 M glucose solution, (3) 0.2 ml. 0.1 M inositol solution, (4) 0.1 ml. 0.2 M glucose solution and 0.1 ml. 0.2 M inositol solution. An example of the results obtained in such experiments is

[5] Quastel, J. H., and Wheatley, A. H. M., *Biochem. J.*, 1932, **26**, 725.

shown graphically in Fig. 1. It is seen that the oxygen consumption of the tissue to which inositol was added continued to fall off at the same rate as the control without substrate. Addition of glucose alone caused some increase in the rate of oxygen consumption with subsequent stabilization at this level. When inositol was added at the same time as glucose no greater effect was detectable than in the case of glucose alone. A negative result was also obtained in a similar experiment in which rabbit cerebral cortex was used.

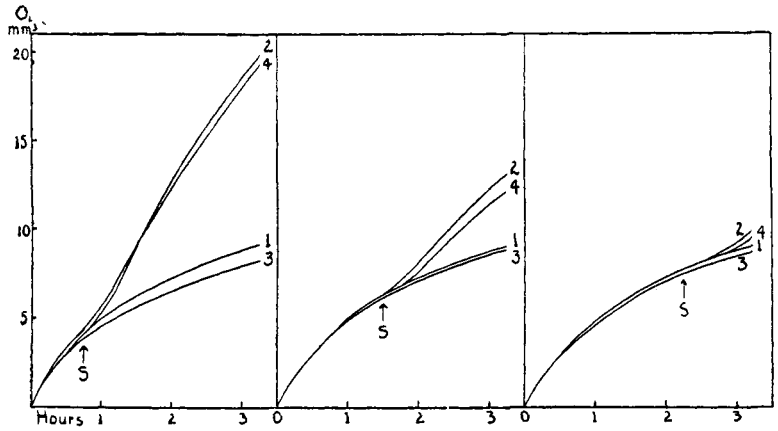

FIG. 1.

Graphs showing the effect on oxygen consumption obtained on adding (1) water, (2) glucose solution, (3) inositol solution, (4) glucose + inositol solution, to rat whole brain which had been allowed to respire without added substrate for varying periods. The additions were made at the points marked S. The oxygen consumption is expressed as cmm. of oxygen per mg. (dry weight) of tissue.

Even if inositol does play a part in the aerobic processes occurring in the brain, experiments of the type described above might well fail to give evidence of this. The purpose of this communication is merely to show that it has not been possible to confirm the findings of Das and Guha with regard to brain tissue and to report experiments under other conditions which have likewise failed to indicate that inositol has any significant effect on the respiration of brain.