CCXXII. RESPIRATION OF BRAIN.

By THEODORE FREDERIC DIXON¹ AND ALFRED MEYER.²

*From the Central Pathological Laboratories of the London
County Mental Hospitals.*

(Received 13 March 1936.)

FEW facts are known concerning the metabolism of parts of the brain other than cerebral cortex. Holmes [1930] has shown that grey matter is more active than white in its oxidising systems. Respiration rates of roughly differentiated parts of the brain in Ringer-phosphate-glucose have been studied by Gavrilescu & Peters [1931] in the pigeon and by Himwich & Fazikas [1932] in the rat. Experiments have been started in this laboratory to extend further this differentiation of brain regions by studying respiration rates with different substrates, and parts have been selected which are known to be especially susceptible to pathological change. Thus we have chosen the striate body, globus pallidus, cerebellar cortex and the cornu ammonis. We have also included the hypothalamus and medial parts of the thalamus because of their alleged specific affinity for narcotics, particularly the barbiturates.

The present investigation deals with the metabolism of these parts in normal ox brain.

EXPERIMENTAL.

Respiration rates were measured in Barcroft differential manometers at 37.5°. The medium was Ringer-phosphate as used by Gavrilescu & Peters [1931]. The manometers were filled with oxygen. The ox brains were received about 1 hour after death of the animal and the first manometer readings were normally taken about 2 hours after death. After removal of the membranes, slices of the various parts were cut with a small arrow-shaped scalpel and were then either transferred to Ringer solution or mashed for about 30 sec. on a glass plate with a bone spatula. Slices of cerebral cortex about 1 cm. square were easily cut by this method, but from cerebellar cortex only thin strips could be cut owing to its structure. About 4 or 5 such strips, usually cut from folds of the vermis, were used in experiments with slices. The cornu ammonis and corpus striatum (caudate nucleus) were minced or sliced after removal of the ependyma. The thalamus with the hypothalamus and corpus callosum were easily dissected out *en bloc* for mincing or slicing. The globus pallidus is so small and its respiration so low that it was necessary to pool tissue from six ox brains for a satisfactory experiment. Normally about 120 mg. tissue were used.

Table I and Fig. 1 summarize the results obtained on minced tissue from different brain regions with or without the addition of glucose, lactate, succinate etc., in Ringer-phosphate solution.

It is seen that minced cornu ammonis and globus pallidus show no extra oxygen uptake in presence of glucose and little in presence of lactate, although these parts show an increased respiration in presence of succinate. The respiration of cerebellar cortex, corpus striatum and thalamus in glucose is also affected

¹ Maudsley Research Fellow.

² Under a grant from the Rockefeller Foundation.

Table I. *Effect of substrate on oxygen uptake of minced brain tissues.*

		$\mu\text{l. O}_2/\text{g. fresh tissue/hour.}$											
Exp.	Substrate	Cerebral cortex			Cerebellar cortex			Thalamus and hypothalamus			Corpus striatum		
		1 hr.	2 hr.	3 hr.	1 hr.	2 hr.	3 hr.	1 hr.	2 hr.	3 hr.	1 hr.	2 hr.	3 hr.
1	None	702	500	368	936	507	352	574	483	374	806	594	440
2	Glucose	876	811	750	1010	783	622	—	—	—	1000	829	692
3	"	898	878	820	—	—	—	602	520	430	919	827	624
4	Lactate	939	848	700	1145	870	680	—	—	—	1011	788	568
5	"	1295	1136	1008	1708	1360	1092	—	—	—	—	—	—
6	"	920	848	752	—	—	—	863	664	537	1158	841	639
7	Succinate	1740	1130	745	1833	1117	706	—	—	—	2092	1316	800
8	"	—	—	—	2065	1400	960	—	—	—	2049	1600	1112
9	"	1910	1380	970	—	—	—	1340	1040	800	2365	1600	970
10	Lactate-glycero-phosphate	1450	1070	815	—	—	—	1160	880	625	1670	1080	680
11	"	1620	1440	—	2080	1480	—	—	—	—	1760	1460	—
12	Glutamate	806	622	540	880	568	392	658	512	416	890	636	492

Exp.	Substrate	Cerebral cortex			Other brain region		
		1 hr.	2 hr.	3 hr.	1 hr.	2 hr.	3 hr.
16	None	705	528	408	Corpus striatum		
	Glucose	767	711	650	667	532	408
17	None	761	524	390	667	532	408
	Glucose	880	824	750	740	608	464
32	None	829	692	500	Corpus callosum		
	Na butyrate	692	538	400	100	66	60
34	None	692	476	282	100	64	55
	Na glutamate	816	624	560	Corpus striatum		
35	None	672	488	380	679	556	412
	Na glutamate	776	628	520	487	360	256
34	None	692	476	282	Cerebellar cortex		
	Na glutamate	816	624	560	933	500	344
35	None	672	488	380	936	520	372
	Na glutamate	776	628	520	812	372	280
30	None	980	550	380	872	540	360
	Cerebellar cortex	980	550	380	Cerebellar cortex		
21	Cornu ammonis	543	394	268	933	500	344
	Globus pallidus	228	138	96	936	520	372
25	None	672	488	380	812	372	280
	Na glutamate	776	628	520	872	540	360
30	None	980	550	380	Cerebellar cortex		
	Cerebellar cortex	980	550	380	933	500	344
34	None	692	476	282	936	520	372
	Na glutamate	816	624	560	812	372	280
35	None	672	488	380	872	540	360
	Na glutamate	776	628	520	Cerebellar cortex		
30	None	980	550	380	933	500	344
	Cerebellar cortex	980	550	380	936	520	372
21	Cornu ammonis	543	394	268	812	372	280
	Globus pallidus	228	138	96	872	540	360
25	None	672	488	380	Cerebellar cortex		
	Na glutamate	776	628	520	933	500	344
30	None	980	550	380	936	520	372
	Cerebellar cortex	980	550	380	812	372	280
34	None	692	476	282	872	540	360
	Na glutamate	816	624	560	Cerebellar cortex		
35	None	672	488	380	933	500	344
	Na glutamate	776	628	520	936	520	372

Exp.	Brain region	None			Glucose			Lactate		
		1 hr.	2 hr.	3 hr.	1 hr.	2 hr.	3 hr.	1 hr.	2 hr.	3 hr.
21	Cornu ammonis	543	394	268	550	334	228	678	454	280
25	Globus pallidus	228	138	96	206	140	90	250	172	104
30	Cerebellar cortex	980	550	380	1100	880	670	1680	1340	1010

though to a less extent. It is of course well known that most tissues have very powerful succinoxidase systems which can be freed from cell debris [compare Cohen & Gerard, 1934].

It was tempting to ascribe these results to real differences in the biochemistry of these regions. When, however, slices of these parts were used a constant respiration rate was always obtained, in glucose or lactate, and the usual falling off of oxygen uptake in absence of added substrates. Table II summarizes the results obtained for the respiration of sliced tissue in Ringer-phosphate with glucose or lactate.

The oxygen uptake values given in Table II were roughly constant during the 3-hour period of the experiment.

Fig. 1. ●—● Ringer-phosphate + glucose; ×---× Ringer-phosphate.

In the absence of added substrate the oxygen uptake of each part decreased rapidly from an initial value which was roughly the same as that with glucose to about 1/3 of this in 1½ hours. The slices of tissue were removed from the bottles and the dry weights found in the usual way. The dry weight of cerebral and cerebellar cortex is about 17% of the wet weight and those of corpus striatum, thalamus and globus pallidus, about 18%. These ratios have been used to calculate the oxygen uptakes in μl./g. wet weight/hour from the dry weight.

Table II. *Oxygen uptakes of sliced tissue in μl./g. wet weight/hour.*

	Glucose	Lactate
Cerebral cortex	1700	2040
Cerebellar cortex	2550	3060
Corpus striatum	1980	2160
Cornu ammonis	1260	1440
Thalamus	1170	1440
Globus pallidus	360	450

Further experiments were carried out in which about 30 slices of cerebral cortex were cut from a single fresh ox brain. Half of these slices were then minced in the usual way and the respiration of the mince so prepared was compared with that of sliced tissue unminced. About 100 mg. of tissue were used for each Barcroft bottle. It is easy to obtain duplicate respiration rates of minced tissue agreeing within 5% with this amount of tissue, but results on single cortex slices may vary by 20–30%. To obtain good agreement of duplicates in the slicing series, three or four representative slices were used for each bottle and duplicates then agreed to within 10%. One of three such experiments is recorded here.

Medium: Ringer-phosphate + glucose 0.2%.

Oxygen uptake in $\mu\text{l.}$ per g. wet weight over 2-hour period during which respiration rate of tissue did not change appreciably:

Minced	1600
Sliced	4000

In the case of cerebral cortex then, the respiration of the slice ($2000 \mu\text{l./g./hour}$) is about double that of the mince ($800 \mu\text{l./g./hour}$) and this difference between sliced and minced tissues is even more marked in other parts of the nervous system. This does not agree with the work of Holmes [1930] and of Quastel & Wheatley [1932] who state that mincing the brain tissue does not alter the respiration rate.

As the Ringer-phosphate solution used in the above experiments contained a rather large amount of potassium it was considered that this factor might possibly account for these results, since Dickens & Greville [1935] have shown that $M/10$ KCl added to Ringer solution increases the respiration of rat cortex slices by about 100 % in glucose or lactate. It was found that such an addition of potassium to $M/60$ sodium phosphate-Ringer produced a slight inhibition of the respiration of minced ox cortex or minced rat whole brain. The respiration of ox cortex slices was increased about 30 % in glucose as against 100 % in the rat. The difference between the mince and the slice was therefore not due to the large potassium content of the medium.

Oxygen uptake of cerebral cortex in Ringer-phosphate-glucose with and without $M/10$ KCl in $\mu\text{l./g.}$ wet weight/hour.

	Without KCl	With KCl
Minced	850	800
Sliced	1700	2380
		(KCl added after 40 min.)

Duration of experiment 80 min. 0.3 ml. of M KCl contained in "danglers" and added to the 3 ml. of medium in the Barcroft cups by shaking after 40 min. Medium: NaCl 0.70 %, KCl 0.018 %, CaCl_2 0.019 %, NaH_2PO_4 0.04 %, Na_2HPO_4 0.21 %, as used by Warburg [1923].

Differences in the magnitudes of the respiration rates of the various parts are not necessarily indicative of relative cellular metabolic activities, since it is not known how much is due to variation in cell density. It is however interesting to note that the respiration rate of cerebellar cortex is initially about 50 % higher than that of cerebral cortex. In the minced tissue experiments, diminution of the respiration rate was most marked in those regions which contain specially large ganglion cells like the cornu ammonis and the cerebellar cortex. It was therefore supposed that these large ganglion cells suffered more damage on mincing. Confirmation of this was obtained by histological examination of films of minced tissue stained with toluidine blue, since the large pyramidal cells of the cornu ammonis and the Purkinje cells of the cerebellum were often nearly completely destroyed, whereas the smaller cells of the cerebral cortex preserved their shape better. Examination of slices of tissue revealed no such damage to the large ganglion cells. This explanation then may account for some of the differences observed in the minced tissue experiments, although it must be pointed out that the large ganglion cells in the cerebellar cortex and cornu ammonis constitute a fraction of the total ganglion cell content of these regions which is difficult to assess since there are both large and small ganglion cells. The same applies to the thalamus, the hypothalamus and the striate body, whilst the globus pallidus contains only large cells which however do not attain the size of the Purkinje cell.

It was then considered possible that injury to the large cells of the gasserian ganglion might have similarly damaged their respiration in the experiments of Holmes [1932] who, using chopped tissue, found that their respiration was very low in glucose, being actually less than that of the cranial nerve. On the basis of these experiments Holmes suggested that the seat of metabolic activity is the dendrite rather than the cell body, since the gasserian ganglion cells have no dendritic structures. The point was therefore investigated in the ox by removing the two ganglia and cutting slices of them with a razor or chopping them with scissors. Simultaneously the brain was removed and slices of cerebral cortex and cerebral white matter used for comparison. It was found that even the slight injury to the ganglion resulting from chopping with scissors caused the respiration rate in glucose to fall rapidly to one-half of its initial value in 2 hours. The respiration of the sliced ganglion, on the other hand, was not only much higher than that of the minced ganglion or of sliced white matter, but was constant over the 2-hour period of the experiment in glucose and fell rapidly in its absence, resembling that of cerebral cortex in this respect. Although the magnitude of the oxygen uptake of the sliced ganglion is low ($150 \mu\text{l./g./hour}$) when compared with that of cortex ($1700 \mu\text{l./g./hour}$) yet it must be concluded that in the ox the cells of the gasserian ganglion oxidize glucose, even though they have no dendritic structures, and that they are more efficient in this respect than white matter.

*Oxygen uptakes of gasserian ganglion and corpus callosum in
 $\mu\text{l./g. wet weight/hour.}$*

Minced		Sliced	
		With glucose	Without glucose
Gasserian ganglion	100	300	90
White matter (corpus callosum)	60	180	70

The above figures represent average oxygen uptakes over the 2-hour period of the experiment.
Medium: Warburg's [1923] Ringer-phosphate + glucose.

The steady maintenance of the respiration rate of mammalian brain when minced is a point often cited when the metabolism of brain is compared with that of other tissues. The respiration of the liver for example is almost completely destroyed by mincing. The experiments here recorded show that even if the respiration of cerebral cortex appears to be undamaged by mincing it is in reality injured and the injury to other parts is more marked. As a whole brain tissue may be less damaged than, say, liver tissue, not because of any essential biochemical difference but because it is less susceptible to mechanical injury. The above investigation illustrates the necessity for accurate morphological control in the interpretation of biochemical findings.

SUMMARY.

Respiration rates of different parts of the brain which are known to be especially vulnerable have been studied in the presence of different substrates. In the minced tissue, unlike cerebral cortex, certain regions showed little or no extra oxygen uptake in the presence of glucose or lactate, whereas, when slices of these regions were used, constant and much higher respiration rates were observed. Cerebellar cortex has a greater metabolic activity than any other part of the brain.

The authors desire to thank Dr F. Golla, Dr S. A. Mann and Prof. R. A. Peters for their advice and encouragement, and Mrs Meyer for technical help during this investigation.

REFERENCES.

- Cohen & Gerard (1934). *Amer. J. Physiol.* **109**, 22.
Dickens & Greville (1935). *Biochem. J.* **29**, 1468.
Gavrilescu & Peters (1931). *Biochem. J.* **25**, 1391.
Himwich & Fazikas (1932). *Proc. Soc. exp. Biol., N.Y.*, **30**, 366.
Holmes (1930). *Biochem. J.* **24**, 914.
—— (1932). *Biochem. J.* **26**, 2005.
Quastel & Wheatley (1932). *Biochem. J.* **26**, 725.
Warburg (1923). *Biochem. Z.* **142**, 317.