The Distribution of Cytochrome-Oxidase Activity in Rabbit Brain

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1. The cytochrome-oxidase activity in eleven structures of the rabbit brain has been investigated. 2. Kinetic data suggest that the same enzyme occurs in all brain structures but that the activity on a tissue-weight basis differs from structure to structure, being in general higher in the phylogenetically newer structures. 3. When the kinetic data are related to cell density (as measured by DNA concentration) all the neuron-containing structures of the cerebrum and brain stem show the same activity but the corpus callosum and the cerebellum show much less. 4. A special study was made of the distribution of activity and cell density within the diencephalon. 5. The results suggest that all neurons in the cerebrum and brain stem have a similar cytochrome-oxidase activity, which is about 80 times that of glia, and that the cerebellum is relatively richer in glia or that it contains neurons with a much lower cytochrome-oxidase activity, or both.

One of the most striking events in the ontogeny of the mammalian brain is the change from a predominantly anaerobic to an aerobic metabolism during late embryonic life or immediately *post partum* (Himwich, 1951; McIlwain, 1966). Since embryonic development closely parallels phylogenesis in many respects it is of interest to enquire whether the phylogenetically older parts of the brain possess a lower cytochrome-oxidase (cytochrome *c*-oxidoreductase, EC 1.9.3.1) activity than the 'newer' structures. A comparison has therefore been made of the activity of this enzyme in some of the principal anatomical regions of the brain.

There is a wide variety of size and shape of cells in most brain regions and all regions contain a greater or less proportion of axons, which have a sluggish oxidative metabolism (Heller & Elliott, 1955). To relate the activity of cytochrome oxidase to the structures primarily responsible for it, i.e. the cell bodies, it has been expressed in terms of the DNA concentration, as well (for comparative purposes) as in terms of tissue weight.

The regions examined were the cerebral and cerebellar cortices, the caudate nucleus, the hippocampus, the corpus callosum, the central white matter of the cerebellum, the pons and the medulla oblongata. In the particular case of the diencephalon, which is such a complex region anatomically, a single experimental value is of limited significance. An attempt was therefore made to investigate the distribution of cytochrome-

oxidase activity and DNA concentration within the region.

Tolani & Talwar (1963) have reported that cytochrome-oxidase preparations from different parts of the brain of the rhesus monkey exhibited different kinetic characteristics. This finding, if confirmed, could have great significance. It was therefore decided to search for such differences in the rabbit, a more readily available and widely used species.

EXPERIMENTAL

Material. Female New Zealand White rabbits weighing at least 7 lb. were used. They were killed by an intravenous injection of air. With the exception of the diencephalon (i.e. thalamus and hypothalamus) the brain structures were dissected out freehand. The part of the cerebral cortex used was that lying dorsal to the diencephalon.

Excision and dissection of the diencephalon. Blocks of tissue bounded by planes A3, P4, L3, R3, D3 and the ventral surface of the brain were excised by stereotactic dissection (Ridge, 1964). These blocks contained all the hypothalamus, the anterior and medial parts of the thalamus and parts of certain non-diencephalic structures such as the optic tract and the anterior commissure (Fig. 1). The lateral thalamus was excluded because its inclusion would, by the nature of the dissection, have included much other non-diencephalic tissue also. The blocks were cut into slices as described by Ridge (1964): in one series of experiments in the frontal planes, in the second in the sagittal planes and in a third in the horizontal planes. Each slice was analysed separately and provided information about the variation of DNA concentration and cytochrome-oxidase activity along the rostrocaudal, lateral and dorsoventral axes respectively.

All slices were 1 mm. thick, except the most ventral horizontal slice, which, owing to the shape of the brain in this region, was conical with a height of about 2.5 mm.

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Fig. 1. (a) A sagittal silhouette of part of a rabbit skull showing the positions relative to the stereotactic grid (Ridge, 1964) of the cerebrum and (cross-hatched) the diencephalon (see text). (b) Frontal sections of the cerebrum to the same scale as (a) in planes A3, AP0 and P3 showing the position of the diencephalon (cross-hatched). Diagrams (c), (d) and (e) (to be read in conjunction with Fig. 2) are enlarged parasagittal sections, in the planes indicated, of the diencephalon showing the positions of the principal structures. Abbreviations: Ac, anterior commissure; OT, optic tract; M, mammillary bodies; H, hypothalamus; Th, thalamus. In (a) and (b) each scale interval represents 2 mm.; in (c), (d) and (e) each scale interval represents 1 mm. A frontal slice, e.g. P0,1, contains the structures in the stippled areas of all three sections; a horizontal slice, e.g. V2,3, contains the structures in the cross-hatched areas of all three sections and a sagittal slice, e.g. L0,1, contains the structures in (c).

Slices were identified by naming the planes of the two principal faces; e.g. A2,1 is the frontal slice bounded by A2 and A1.

Estimation of cytochrome-oxidase activity. The unit of enzymic activity is defined as that quantity which will catalyse the oxidation of 4 moles of reduced cytochrome *c*/min. at $20 \pm 1^\circ$.

The excised tissues were transferred to tared Griffiths tubes (Baird and Tatlock Ltd.), weighed by difference and homogenized in 60 times their weight of ice-cold water. The homogenate was then immediately diluted with an equal volume of ice-cold 1.1% (w/v) sodium deoxycholate solution. Such homogenates retained their activity for at

least 3 hr. at 0° . A portion of the homogenate was added to a cuvette containing a solution of reduced cytochrome *c* ($25 \mu\text{M}$ in Sørensen's 0.09 M-phosphate buffer, pH 7.2). The contents of the cuvette were immediately mixed and readings of the extinction at $550 \mu\text{m}$ were made (Unicam SP.500) every 15 sec. for 120 sec. The rate of decrease of extinction was plotted against time and extrapolated back to give the initial rate of change of extinction/15 sec. Each estimation was duplicated.

It was established in preliminary experiments that the rate of reaction between cytochrome *c* and its oxidase was directly proportional to the fraction of the cytochrome in the reduced form. Reaction rates were therefore corrected

to the value which would have obtained if the cytochrome *c* in the reaction mixture had been initially fully reduced.

Michaelis constants and maximum velocities were calculated by Wilkinson's (1961) method.

Catalytic reduction of cytochrome *c*. The cytochrome was dissolved in phosphate buffer (0.09M, pH 7.2) in a two-necked flask. A piece of platinum foil, coated with platinum black (Reilly & Rae, 1943), was immersed in the solution and a fast stream of hydrogen passed in and out through one neck. Frothing was prevented by smearing a trace of Silicone MS Antifoam A (Hopkin and Williams Ltd.) on the gassing tube above the solution. After 1 hr. over 95% of the cytochrome *c* was reduced. To determine the degree of reduction and the concentration of cytochrome *c* a portion was removed through the second neck of the flask and the extinction at 550m μ compared with that of the same sample in the fully reduced and in the fully oxidized states (Umbreit, Burris & Stauffer, 1959).

Estimation of DNA. DNA was extracted by the method of Schneider (1945) and estimated by the *p*-nitrophenylhydrazine reaction (Webb & Levy, 1955). As the purple colour faded fairly rapidly several timed readings of the extinction were taken and extrapolated back to zero time.

RESULTS

Factors affecting the spectrophotometric assay of cytochrome-oxidase activity

Preparation of homogenate. Homogenates of brain in water alone were active but the addition of sodium deoxycholate produced a preparation that was less turbid and more active. For maximum activity the final enzyme preparation required a liquid:tissue ratio 120:1 and a sodium deoxycholate concentration 0.55% (w/v). Provided that these conditions could be attained the initial water:tissue ratio was unimportant. Homogenates in sodium chloride (0.9%, w/v), phosphate buffer (0.09M, pH 7.2) or sucrose (0.25M) were less active than similarly prepared water dispersions. The addition of EDTA (final concentration 1mM) to either the solution or the water in which the tissue was initially dispersed caused a slight inactivation of the enzyme.

pH and ionic strength. A combination of low pH and low ionic strength gave maximum activity but under these conditions deoxycholic acid tended to precipitate. A satisfactory rate of reaction was obtained in 0.09M-phosphate buffer, pH 7.2.

Enzyme concentration. Over a wide range of enzyme concentrations there was a linear relationship between the initial reaction velocity and the enzyme concentration.

Interfering enzymes. There was no interference from cytochrome *c* reductases: the rate of reduction of oxidized cytochrome *c* by a brain homogenate after its cytochrome-oxidase activity had been inhibited by 5mM-potassium cyanide was zero.

Freezing. Freezing (-20°) and thawing the tissue before homogenizing had no effect on

cytochrome-oxidase activity. The enzymic activity was retained indefinitely in the intact tissue at -20° .

Mechanical comminution. Comparison of Griffiths tubes and Potter-Elvehjem homogenizers showed that the latter gave a homogenate having lower activity (about 60% of that prepared with a Griffiths tube) and a much higher turbidity.

Cytochrome-oxidase activity in relation to DNA

DNA of the brain. The DNA concentrations of the structures examined are listed in Table 1 together with the cell densities derived from these values. In the derivation it was assumed that each cell nucleus contained 6.1×10^{-12} g. of DNA (Vendrelly, 1955). When due allowance is made for differences in the analytical methods used and the species examined the DNA and cell-density values in Table 1 agree well with earlier results for the brain: human (normal and neoplastic), cat and dog (Heller & Elliott, 1954, 1955), monkey (Robins, Smith & Eyd, 1956), rabbit (Kissane & Robins, 1958), lamb (Korey & Orchen, 1958), human (Hess, 1961), calf and rat (Zamenhof, Bursztyn, Rich & Zamenhof, 1964).

The apparent Michaelis constant for cytochrome *c* was the same in all the brain structures examined (Table 1). The maximum velocity of the reaction between cytochrome oxidase and cytochrome *c* when expressed on a fresh-weight basis (V_w) was high in grey matter, low in white and decreased steadily from the cerebral cortex towards the spinal cord. However, if the maximum velocity was expressed in terms of the DNA concentration (V_{DNA}) the variation between the structures of the cerebrum, excluding the corpus callosum, was negligible. The V_{DNA} values for corpus callosum and for cerebellum were both lower than the V_{DNA} for the cerebral structures. No cytochrome-oxidase activity could be detected in the optic tract.

Activity in the diencephalon. The DNA concentrations and cytochrome-oxidase activities in horizontal, frontal and sagittal slices cut from the diencephalon are presented in Fig. 2. Cytochrome-oxidase activities did not vary significantly laterally (Fig. 2*b*) but along the rostrocaudal axis the activity increased steadily from A3,2 to P0,1, and remained steady thereafter (Fig. 2*a*); along the dorsoventral axis there was a plateau between V4,3 and D0,1 (Fig. 2*c*). The changes in DNA concentration along each of these axes were opposite to the changes in cytochrome-oxidase activity; there was a decrease from A3,2 to P0,1 with steady values thereafter (Fig. 2*a*), a trough from V4,3 to D1,2 (Fig. 2*c*) but no significant lateral variation (Fig. 2*b*).

Reference to Fig. 1 (or, for greater detail, to the

Table 1. *DNA concentration, cell density and kinetic data for cytochrome oxidase in various regions of rabbit brain*

DNA values are expressed as the mean $\pm$ s.e.m. (with no. of animals). K_m is the Michaelis constant for cytochrome *c*, V_w is the maximum velocity on a weight basis; the variation of the results is expressed as s.e. $\pm$ the number of experimental points (Wilkinson, 1961). V_{DNA} is the maximum velocity in terms of DNA and the figures in parentheses after V_{DNA} values are the probable limits of V_{DNA} based on $(V_w + \text{s.e.m.})/(\text{DNA} - \text{s.e.m.})$ and $(V_w - \text{s.e.m.})/(\text{DNA} + \text{s.e.m.})$.

	DNA ($\mu\text{g./mg.}$ fresh wt.)	Estimated no. of cells/mg. fresh wt.	K_m ($\mu\text{mole of reduced}$ cytochrome <i>c</i> /ml.)	V_w (μmoles of cytochrome <i>c</i> oxidized/g. fresh wt./min.)	V_{DNA} (m $\mu\text{moles of}$ cytochrome <i>c</i> oxidized/ $\mu\text{g. of}$ DNA/min.)
Cerebral cortex	0.90 ± 0.05 (14)	148 000	0.017 ± 0.003 (18)	89.8 ± 7.8 (18)	99.8 (114.8–86.3)
Caudate nucleus	1.24 ± 0.07 (13)	203 000	0.019 ± 0.004 (18)	103.1 ± 14.4 (18)	83.2 (100.4–67.7)
Hippocampus	0.96 ± 0.04 (14)	157 000	0.017 ± 0.004 (18)	79.0 ± 10.4 (18)	82.3 (97.2 $\pm$ 68.6)
Diencephalon	1.08 ± 0.06 (6)	177 000	0.023 ± 0.005 (16)	84.5 ± 11.9 (16)	78.2 (94.5–63.7)
Mesencephalon	0.65 ± 0.04 (6)	107 000	0.025 ± 0.005 (13)	56.9 ± 11.7 (13)	87.5 (112.5–66.2)
Pons	0.70 ± 0.05 (6)	115 000	0.027 ± 0.008 (13)	62.5 ± 12.7 (13)	89.3 (115.7–66.4)
Medulla oblongata	0.68 ± 0.02 (10)	111 000	0.025 ± 0.005 (13)	59.7 ± 8.7 (13)	87.8 (103.6–72.9)
Cerebellar cortex	3.76 ± 0.12 (14)	616 000	0.020 ± 0.003 (18)	97.6 ± 8.7 (18)	26.0 (29.2–22.9)
Central white matter of cerebellum	1.43 ± 0.07 (13)	234 000	0.016 ± 0.007 (17)	55.8 ± 13.3 (17)	39.0 (50.8–28.3)
Corpus callosum	1.19 ± 0.08 (13)	195 000	0.016 ± 0.004 (16)	19.6 ± 2.3 (16)	16.5 (19.7–13.6)

Fig. 2. Cytochrome-oxidase activity (O) and DNA concentration (●) in (a) frontal slices, (b) sagittal slices and (c) horizontal slices cut from the diencephalon of the rabbit. Vertical bars are twice s.e.m. in length. Values along the abscissae are stereotactic co-ordinates (Ridge, 1964). For morphology of slices see Fig. 1(c, d, e).

stereotactic atlas given by Ridge, 1964) shows that relatively high cytochrome-oxidase activity and low DNA concentrations are associated with the thalamus, that the ventral, dorsal and rostral regions of the diencephalon are regions of relatively

high cell density and low oxidase activity and that the hypothalamus was intermediate in both these respects.

K_m , V_w and V_{DNA} were estimated in a series of frontal slices only. K_m did not vary significantly

Table 2. *DNA concentration, cell density and kinetic data for cytochrome oxidase in frontal slices cut from the diencephalon of the rabbit*

Expression of results and symbols are as given in Table 1.

Slice	DNA ($\mu\text{g./mg. fresh wt.}$)	Estimated no. of cells/mg. fresh wt.	K_m ($\mu\text{mole of reduced}$ cytochrome <i>c</i> /ml.)	V_w ($\mu\text{moles of cytochrome}$ <i>c</i> oxidized/g./min.)	V_{DNA} (μmoles of cytochrome <i>c</i> oxidized/ $\mu\text{g. of}$ DNA/min.)
A3,2	1.30 $\pm$ 0.05 (6)	213000	0.023 $\pm$ 0.004 (14)	54.6 $\pm$ 7.9 (14)	42.0
A2,1	1.17 $\pm$ 0.03 (6)	192000	0.016 $\pm$ 0.003 (16)	62.0 $\pm$ 5.0 (16)	53.0
A1,0	1.08 $\pm$ 0.04 (5)	177000	0.018 $\pm$ 0.010 (16)	85.5 $\pm$ 23.4 (16)	79.2
P0,1	1.07 $\pm$ 0.05 (7)	175000	0.022 $\pm$ 0.007 (16)	99.6 $\pm$ 18.3 (16)	93.1
P1,2	1.04 $\pm$ 0.03 (7)	171000	0.015 $\pm$ 0.004 (16)	84.7 $\pm$ 12.1 (16)	81.4
P2,3	1.06 $\pm$ 0.04 (7)	174000	0.023 $\pm$ 0.007 (15)	95.3 $\pm$ 17.9 (15)	90.0
P3,4	1.04 $\pm$ 0.06 (7)	171000	0.018 $\pm$ 0.003 (16)	91.4 $\pm$ 5.7 (16)	86.9

along the rostrocaudal axis but both V_w and V_{DNA} increased from A3,2 to A1,0 and thereafter remained steady (Table 2).

DISCUSSION

It is surprising, in view of the importance attaching to oxidative processes in brain metabolism, that the cytochrome oxidase of the central nervous system has received so little attention. The only studies comparable to the present ones are those of Mokrasch & Grady (1964) and Tolani & Talwar (1963). The former authors were concerned, *inter alia*, with the activities of cytochrome oxidase in the brains of alert and hibernating hamsters. A shortage of experimental details prevents a detailed comparison of our respective results but Mokrasch & Grady placed the brain structures of the alert animals in the following order of decreasing oxidase activity (fresh-weight basis): brain stem > diencephalon > cerebellum > cerebral cortex > hippocampus. This order is different from that in Table 1.

Tolani & Talwar (1963) estimated the cytochrome-oxidase activity of mitochondrial preparations from the cerebellum, the cerebral cortex, the thalamus and the corpus callosum of the rhesus monkey. The order of decreasing activity was the same as that in Table 1. They also claimed that in spite of some similarities (e.g. the effect of pH and inhibition by inorganic phosphate) the enzymes from neuron-rich and neuron-free tissues had different Michaelis constants. I found no such differences in the rabbit; in fact, the cytochrome-oxidase preparations from many different parts of the rabbit brain had identical Michaelis constants.

Attempts have already been made to determine the quantitative distribution of certain biochemically important compounds in the hypothalamus and neighbouring regions: e.g. nor-adrenaline by Vogt (1954), serotonin by Amin,

Crawford & Gaddum (1954), histamine by Adam (1961), catecholamines by Bertler (1961). In all these cases the hypothalamus was dissected out freehand and divided, at most, into three or four pieces with the result that only a vague relationship between the structure of the region and the distribution of the amines could be obtained. With stereotactic dissection it becomes possible to map, e.g. diencephalic, distributions more accurately. In this respect the method cannot compare with histochemistry but it does allow quantitative methods of proven analytical specificity to be employed. The attainable degree of localization depends upon the thinness of the slice and the anatomical complexity of the region. The distribution of cytochrome-oxidase activity and of DNA in the diencephalon was a severe test of the method, but the results obtained were in good agreement with the histochemical results of Shimizu, Morikawa & Ishi (1957).

The values of V_w (Table 1) might be taken as supporting other evidence (Himwich, 1951) which suggests that the phylogenetically newer parts of the brain exhibit a more oxidative metabolism than the older parts and that this reflects an evolutionary tendency to adopt the more efficient oxidative pathways. But much of the evidence for this conclusion is expressed, as is V_w , in terms of tissue weight and this fails to take into account the increasing contribution made to the weight of the lower structures by fibre tracts and bundles. These oxidatively sluggish features dilute the activity of the relatively few cell bodies (Table 1), reducing the activity for the region as a whole to a low value. But V_{DNA} (Table 1) shows that the average cell in, for example, the pons, the medulla or the mesencephalon, is no less active than the average cell in the cerebral cortex. However, this in itself is not enough to demolish the above hypothesis as a few simple calculations can show.

V_{DNA} is proportional to the activity of the

average cell in the structure and the average cell is a function of the numbers and activities of all types of neurons and glia present. (Ependymal and epithelial cells may be ignored.) To simplify matters neurons and glia will be considered as separate but homogeneous groups. If one now assumes that the glia to neuron ratio (R) in the cerebral cortex is 12 (Hild, 1964) and that the glia have the same cytochrome-oxidase activity as in the corpus callosum then it can be shown (on the basis of the values in Table 1) that, at a first approximation, the number of neurons can vary by $\pm 20\%$ without significantly altering the value of V_{DNA} (i.e. translating V_{DNA} outside the probable limits of Table 1) for the average cortical cell. The same conclusion is reached if R is as low as 3 (Hess, 1961). In the same way it may be shown that for any given value of R the activity of the neurons, or of the glia, can also vary by $\pm 20\%$ without significantly altering the value of V_{DNA} . Both factors may operate independently and to different extents in the different brain structures so that quite wide variations in the relative numbers and enzymic activities of glia and neurons are possible within the limits of accuracy of V_{DNA} . Thus it is not possible to draw any definite conclusions about the biochemical evolution of the brain from the values in Table 1, but it does seem unlikely that in so many structures so many factors should be mutually exclusive. On balance it would seem that a neuron, wherever it occurs (but see below) and regardless of its size and function, is capable of very high rates of oxidation. Values of about 80 times that of a callosal glial cell emerged from the above calculations.

The exception to this broad conclusion appears to be the cerebellum. Not much can be said about the central white matter: its relatively high cell population is probably related to the difficulty of dissecting it out entirely free from the granular cell layer of the cortex and its V_{DNA} value (greater than that for corpus callosum but less than for the cerebral structures) is probably related to the dentate and other nuclei embedded in it. But if one assumes that the average cerebellar cortical neuron possesses a cytochrome-oxidase activity as high as the average cerebral neuron and that the cerebellar glia are similar to the callosal glia then it may be shown that R for the cerebellar cortex is about 100. Alternatively if R is fixed at 12 and the cerebellar glia are as active as those in the

corpus callosum then it can be shown that the average cerebellar cortical neuron is about nine times as active as a glial cell and if R is 3 only about four times as active. From this it is clear that either the assumptions which seem valid in the cerebrum do not hold in the cerebellum or, if they do, that the cerebellar neuron differs from neurons in other parts of the central nervous system.

REFERENCES

- Adam, H. M. (1961). In *Regional Neurochemistry*, p. 293. Ed. by Kety, S. S. & Elkes, J. London: Pergamon Press.
- Amin, A. H., Crawford, T. B. B. & Gaddum, J. H. (1954). *J. Physiol.* **126**, 596.
- Bertler, A. (1961). *Acta physiol. scand.* **51**, 97.
- Heller, I. H. & Elliott, K. A. C. (1954). *Canad. J. Biochem. Physiol.* **32**, 584.
- Heller, I. H. & Elliott, K. A. C. (1955). *Canad. J. Biochem. Physiol.* **33**, 595.
- Hess, H. (1961). In *Regional Neurochemistry*, p. 200. Ed. by Kety, S. S. & Elkes, J. London: Pergamon Press.
- Hild, W. (1964). In *Brain Function*, vol. 2, p. 109. Ed. by Brazier, M. A. B. Berkeley and Los Angeles: Univ. of California Press.
- Himwich, H. E. (1951). In *Brain Metabolism and Cerebral Function*, p. 124. Baltimore: Williams & Wilkins.
- Kissane, J. M. & Robins, E. (1958). *J. biol. Chem.* **233**, 184.
- Korey, S. R. & Orchen, M. (1958). *J. Neurochem.* **3**, 277.
- Mellwain, H. (1966). In *Biochemistry and the Central Nervous System*, p. 289. London: J. and A. Churchill Ltd.
- Mokrasch, H. & Grady, H. J. (1964). In *Comparative Neurochemistry*, p. 213. Ed. by Richter, D. London: Pergamon Press.
- Reilly, H. & Rae, W. M. (1943). In *Physicochemical Methods*, vol. 2, p. 433. London: Methuen.
- Ridge, J. W. (1964). *J. Neurochem.* **11**, 765.
- Robins, E., Smith, D. E. & Eydt, K. M. (1956). *J. Neurochem.* **1**, 54.
- Schneider, W. C. (1945). *J. biol. Chem.* **161**, 293.
- Shimizu, N., Morikawa, M. & Ishi, Y. (1957). *J. comp. Neurol.* **108**, 1.
- Tolani, A. J. & Talwar, G. P. (1963). *Biochem. J.* **88**, 357.
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1959). In *Manometric Techniques*, p. 301. Minneapolis: Burgess Pub. Co.
- Vendrey, R. (1955). In *Nucleic Acids*, vol. 2, p. 166. Ed. by Chargaff, E. & Davidson, J. M. New York: Academic Press Inc.
- Vogt, M. (1954). *J. Physiol.* **123**, 451.
- Webb, J. M. & Levy, H. M. (1955). *J. biol. Chem.* **213**, 107.
- Wilkinson, G. N. (1961). *Biochem. J.* **80**, 324.
- Zamenhof, S., Bursztyn, H., Rich, K. & Zamenhof, P. J. (1964). *J. Neurochem.* **11**, 505.