CYTOCHROME OXIDASE IN NORMAL AND REGENERATING NEURONS*

By HOWARD A. HOWE, M.D., AND ROBERT C. MELLORS, M. D.

(*From the Poliomyelitis Research Center, Department of Epidemiology, Johns Hopkins University, Baltimore*)

(Received for publication, January 30, 1945)

The observation has been made that the motor cells of the monkey's spinal cord become resistant to destruction by poliomyelitis virus during most of the period of regeneration which follows the severance of a peripheral nerve (1, 2). This phenomenon, which can be easily induced experimentally, appears during the 3rd week of nerve regeneration and persists until functional recovery has been effected. The motor neuron, therefore, offers a situation which is unique in providing an opportunity for the study of some of the factors which make for cellular resistance, and it should be possible by chemical means, as well as by ordinary histological techniques, to detect some differences, either qualitative or quantitative, between normally susceptible tissue and that which has been rendered resistant.

Chemical studies of nervous tissue have dealt largely with the processes of aerobic and anaerobic respiration. They have been limited in scope by a number of factors, for example, the fragility of the tissue, the admixture of grey and white matter which have divergent metabolic rates, the difficulty of measuring the activities of the individual groups of cells and of correlating them with different functional types or states. Efforts have been made by a few investigators to study different functional conditions in complex mixtures of central nervous tissue. One of the most successful of these is that of Flexner, Flexner, and Straus (3) who have been able to make a correlation between cytochrome-cytochrome oxidase activity and morphological differentiation in the embryonic nervous system. Less successful is the attempt of Racker and Kabat (4) and Wood (5) who studied oxidations in the CNS of the mouse infected with poliomyelitis virus. The latter two studies are in complete disagreement and illustrate clearly the difficulty of analysis aimed at the interaction of a virus with a small group of cells which are quantitatively outweighed by inflammatory exudates and essentially non-reactive elements. Inconclusive efforts have also been made by Racker and Kabat, and Turner and Turner (6) to study the O_2 consumption of spinal cord slices containing normal and chromatolytic cells.

It is obvious that in the ideal analysis it should be possible to make separate determinations per unit of weight or volume of white matter, of nerve cells, and of supporting tissues. The need for such a separation has driven Pearce and Gerard (7) to an attempt to estimate the mass of the neurons, whose metabolism

* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

Downloaded from http://rupress.org/jem/article-pdf/81/5/489/1183209/489.pdf by guest on 09 May 2021

they had measured, by counting the number of supposed nerve cell nuclei still remaining undestroyed in homogenized brain suspensions. The methods which we shall describe have made it possible to secure direct measurements of cytochrome oxidase activity in normal thalamic neurons and to determine differences between normal and refenerating anterior horn cells in the spinal cord without the necessity of having to determine specifically the mass of the neurons involved. Unfortunately, it has not been possible to apply these methods to the study of the total oxidation of glucose under aerobic and anaerobic conditions, but since cytochrome oxidase apparently forms an important link in the aerobic oxidative process, its fluctuations have yielded information of considerable interest.

Material and Methods

Observations have been made on the cat and the monkey. The cat was used for the development of the methods because of its cheapness and availability. Since work involving susceptibility to poliomyelitis has been limited to monkeys, the study of their nervous tissues has been the main objective of the investigation. The technical problems may be divided into two classes—those of sampling and those of chemical assay.

The sampling problem has been one of the most serious in past chemical studies of the nervous system, so that considerable effort was devoted in these experiments to developing new methods for separating the neurons from irrelevant white matter and supporting tissues. At the outset advantage was taken of the fact that removal of the cerebral cortex produces almost complete retrograde degeneration of those thalamic neurons which send axons to it (8). While there is some neuroglial response to the death of the injured neurons, the reaction is of relatively brief duration so that within 4 to 6 weeks the debris of degenerating nerve cells has largely been removed and neuroglia cells have ceased proliferating, although they are somewhat more numerous than normal. Cats were prepared under ether anesthesia with unilateral decortications, sparing the hippocampus and corpus striatum as well as the thalamus. The animals tolerated the operation well and there was no subsequent evidence that the thalmus suffered from primary trauma or from interference with blood supply. Six weeks after operation the animals were etherized, and bled out through the femoral or carotid artery. The brain was rapidly exposed, removed from the skull, and the hemispheres trimmed off to visualize the two thalami. With sharp scissors it was then relatively easy to excise corresponding portions of the dorsolateral thalamus of the normal and operated sides, or from both sides of a normal animal in such experiments as were designed to determine the ordinary sampling variation between two normal sides. The entire operation occupied an average of 20 minutes from the death of the animal until the tissue was ready to be processed for respiration studies.

In the study of the motor cells of the spinal cord a different sampling technique was devised. Here the problem was that of isolating the grey matter of the anterior horn from the surrounding fiber tracts. A method satisfactory for this purpose had to be capable of giving consistently comparable samples of the two anterior horns with a minimum delay following removal from the animal.[1] Under ether anesthesia the entire cord was exposed and the dura opened. The lumbosacral cord, which was then removed from the living animal by transection and snipping the spinal nerves with sharp scissors, was placed on a cake of dry ice. It was com-

[1] The method described is that used for most of the monkey cords and represents a more satisfactory modification of that used for the cat cords.

Downloaded from http://rupress.org/jem/article-pdf/81/5/489/1183209/489.pdf by guest on 09 May 2021

pletely frozen within 2 to 3 minutes after removal. The lower cervical cord was then removed and treated in a similar fashion. The frozen segments of cord were split longitudinally in the midline. Each half of the cord was then scraped free of white matter with a sharp knife while in the frozen state. The tissue rested directly on a block of dry ice while the work was in progress. By using slight magnification it was found quite simple to isolate the grey column of the anterior horn from the surrounding fiber tracts and from the posterior horn. One to 2 hours was required to complete the dissection of both sides of the two cord segments. The isolated anterior horns were then collected in a specimen bottle and thawed at a time convenient to the operator. Freezing produces some loss in the activity of cytochrome oxidase but the method made possible the rapid transfer of material to the frozen state (2 to 3 minutes), completely uniform treatment of normal and operated specimens, and satisfactorily accurate sampling. It also removed the necessity for determining the total mass of the neurons in the grey matter analyzed, since in each instance the experimental procedures employed had brought about changes only in the motor neurons themselves. It was thus possible to follow certain alterations in the nerve cells without having to determine any absolute values for them. Regenerating anterior horn cells were produced by section of peripheral nerves. In some cases extraspinal section under ether anesthesia of lumbar and sacral roots was combined with section of the sciatic nerve (since the root of S_2 could not be reached directly). In the same animal the brachial plexus was also sectioned. In the case of the cat, this extensive operation made the animals difficult to care for, so that finally only the brachial plexus was sectioned and the cervical cords of two cats were pooled in order to secure enough tissue for determinations. Monkeys tolerated a combined cervical and lumbosacral root section well, so that enough tissue could be secured from one animal. The animals were killed at times varying between 7 and 70 days. From many animals at the time of each experiment small segments of spinal cord from the areas in question were removed and fixed by immersion in 1 per cent acetoformol. These were subsequently sectioned in paraffin and stained with gallocyanine. Although freezing somewhat disturbed the usual cell morphology, the presence of a normal control made interpretation quite reliable. Histological controls were also prepared from the spinal cords of animals with nerve root sections of 4, 7, 10, 14, 30, and 110 days standing. These animals were perfused intravascularly with 1 per cent acetoformol which assured excellent fixation and staining with gallocyanine or toluidine blue.

The cytochrome oxidase activity was determined by a modification of the method of Stotz (9) based upon the principle that the rate of oxidation of hydroquinone under specified conditions in the presence of an excess of cytochrome C (measured in Warburg-Barcroft manometers) is proportional to the amount of oxidase. The Warburg flasks contained 1.0 ml. of isotonic saline phosphate (pH 7.0), 1.0 ml. of cytochrome C solution (1×10^{-4} mM cytochrome C per ml. isotonic saline phosphate), and 1.0 ml. of tissue suspension. The latter was prepared by homogenizing the tissue in distilled water in a special pyrex grinder so that 1 ml. of suspension contained approximately 50 mg. of fresh tissue (10). The side arm of the vessel contained 0.3 ml. of 0.22 per cent hydroquinone solution. Another vessel, containing the same reagents and a tissue suspension inactivated by heating at 100°C. for 10 minutes was used to determine the rate of autoxidation of the hydroquinone. All studies were carried out at 37.5°C. After temperature equilibrium was reached, readings were taken immediately before the hydroquinone was added (these showed a very small oxygen uptake) and every 10 minutes for 30 minutes thereafter. The dry weight of the samples was determined by drying aliquots of the tissue suspension in weighing bottles. Values are expressed as cytochrome oxidase units per milligram of dry weight of tissue, where 1 unit of oxidase is defined as the amount which will produce an increase in oxygen consumption of 10 c.mm. per hour over the autoxidation of hydroquinone under these specified conditions.

Downloaded from http://rupress.org/jem/article-pdf/81/5/489/1183209/489.pdf by guest on 09 May 2021

Results of the Quantitation

Thalamic Neurons—Cat.—Before attempting an evaluation of any observed changes in the quantity of cytochrome oxidase which might result from the experimental procedures, it was necessary to establish the range of variation in readings from the two thalami of normal animals. Table I shows the range of determinations in seven separate experiments (9 animals). The average of the right sides was 5.22 units and that of the left 5.59 units with an average difference of +0.37 units (7 per cent). In one experiment the left side was *greater* than the right by 1.32 units (cats 69–70) but in the majority of instances the

TABLE I

Cytochrome Oxidase Units in Thalamus (Cat)

Control series					Experimental series				
Experiment	Right	Left	Difference	Per cent difference	Experiment	Normal	Deneuronated	Difference*	Per cent difference
68	4.44	4.97	+0.53	+11	54	5.24	4.05	−1.19	−23
69, 70	5.03	6.38	+1.35	+21	64	6.15	5.15	−1.00	−16
71, 72	6.78	6.80	+0.02	+3	65	7.38	4.31	−3.07	−42
73	6.75	6.43	−0.32	−5	81	5.10	2.70	−2.40	−47
89	4.88	5.04	+0.16	+3	82	5.45	2.93	−2.52	−46
90	4.60	4.52	−0.08	−2					
92	4.11	5.03	+0.92	+18					
Mean......	5.22	5.59	+0.37	+7	Mean......	5.86	3.84	−2.04	−35
Sigma mean difference..			0.21		Sigma mean difference..			0.45	

* This column represents the cytochrome oxidase activity of the thalamic neurones.

differences were much less than this and were almost equally distributed on the positive and negative sides.

In the experimental animals, however (Table I), the situation was quite different. While the average value for the normal control side was 5.86 units, that of the operated, or deneuronated, side was 3.82 units, giving an average reduction of 2.04 units (35 per cent) which is well outside the range of sampling variation.[2] This difference thus represents the amount of cytochrome oxidase normally present in the neurons of the dorsal thalamus, since virtually all of

[2] The standard deviation of the difference between the means of the normal and operated animals is 0.5 unit whereas the difference observed was −2.41 units which is approximately 5 standard deviations. The formula is as follows:

Sigma mean difference between operated and control series

$$= \sqrt{(\text{Sigma of control mean difference})^2 + (\text{sigma of operated mean difference})^2}$$

Downloaded from http://rupress.org/jem/article-pdf/81/5/489/1183209/489.pdf by guest on 09 May 2021

them were subtracted by the experimental procedures. The value represents 35 per cent of the total cytochrome oxidase present in neurons, neuroglia, myelin, and blood vessels.

Spinal Cord—Cat.—In the case of the spinal cord (Table II) the sampling variation of the two anterior horns turned out to be less than in the thalamus (0.06 unit as contrasted with 0.21 unit), indicating that the method of sampling was slightly more accurate although the values for cytochrome oxidase were much lower in the cord. In seven determinations the mean difference between the right and left sides was −0.11 unit. Differences between the normal and regenerating sides were consistently negative and the average difference was

TABLE II

Cytochrome Oxidase Units in Anterior Horn of Cat Spinal Cord

Experiment	Right	Left	Difference	Per cent difference	Experiment	Normal	Operated	Differenec	Per cent difference*
76	2.76	2.81	+0.05	+2	98	1.97	1.55	−0.42	−21
85	2.18	2.02	−0.16	−7	100	2.82	2.36	−0.46	−16
86	2.14	1.90	−0.24	−11	101	2.24	1.53	−0.71	−32
90	1.52	1.58	+0.06	+4	102	2.14	1.53	−0.61	−29
92	1.25	1.13	−0.12	−8	103	2.12	2.00	−0.12	−6
93	2.55	2.58	+0.03	+2	104	1.56	1.47	−0.09	−6
99	3.27	2.91	−0.36	−10	107, 109	2.49	1.47	−1.02	−41
					108, 110	2.47	1.70	−0.77	−31
					114, 115	2.33	1.81	−0.52	−22
Mean......	2.24	2.13	−0.11	−5	Mean......	2.23	1.71	−0.52	−23
Sigma mean difference..			0.06		Sigma mean difference..			0.09	

* This column is the loss of cytochrome oxidase activity in the anterior horn cells.

−0.52 unit.[3] This value represents a decrease of 23 per cent in the cytochrome oxidase of the anterior horn whose motor cells were stimulated to regenerative activity by section of their axons.

Spinal Cord—Monkey.—The focal point of this investigation has been the anterior horn cells of the monkey spinal cord since they present a most favorable opportunity for the study of the virus refractory state in neurons which are ordinarily very susceptible. The preliminary findings from the cat indicated that reduction of 35 per cent in cytochrome oxidase might be expected by the virtual elimination of all neurons in the thalamus and reduction of the order of 25 per cent in the complex tissue of the anterior horn when the motor cells were

[3] Sigma of the difference = 0.11 unit; the difference observed is −0.41 unit or nearly 4 standard deviations.

Downloaded from http://rupress.org/jem/article-pdf/81/5/489/1183209/489.pdf by guest on 09 May 2021

exerting regenerative effort. While this reduction of 25 per cent does not at first sight appear to be very great, it is in reality quite remarkable when one

TABLE III

Cytochrome Oxidase Units in Anterior Horn of Monkey Spinal Cord

Control series				
Experiment	Right	Left	Difference	Per cent difference
509	0.88	0.92	+0.04	+4
575	0.50	0.64	+0.14	+21
588, 9	0.58	0.72	+0.14	+19
718	0.41	0.37	−0.04	−9
720	0.64	0.67	+0.03	+5
723	1.01	1.01	0	0
740	0.40	0.44	+0.04	+9
748	0.52	0.51	−0.01	−2
754	0.55	0.59	+0.04	−7
767	0.81	0.73	−0.08	−10
Mean.........	0.63	0.66	+0.03	+4.5
Sigma mean difference...			0.02	

Experimental series 7–9 days				
Experiment	Right	Left	Difference	Per cent difference
722	0.77	0.77	0	0
800	1.03	1.03	−0.09	−8
810	0.91	0.89	−0.02	−2
811	0.62	0.64	+0.02	+3
828	0.88	0.77	−0.10	−11
833	0.52	0.56	+0.04	+7
Mean.......	0.80	0.77	−0.03	−3.75
Sigma mean difference..			0.02	
10–20 days				
383	0.69	0.35	−0.34	−49
499	0.97	0.66	−0.31	−32
500	0.65	0.64	−0.01	−2
562	0.64	0.67	+0.03	+5
541*	1.52	0.60	−0.92	−61
813	0.74	0.73	−0.01	−1
Mean.......	0.74	0.61	−0.13	−17.5
Sigma mean difference..			0.14	
21 days and over †				
504	0.66	0.42	−0.24	−36
645	0.47	0.48	+0.01	+2
646	0.75	0.62	−0.13	−17
682	0.53	0.45	−0.08	−15
683	1.00	0.74	−0.26	−26
687	0.36	0.24	−0.12	−33
Mean.......	0.63	0.49	−0.14	−22
Sigma mean difference..			0.04	

* This reading, which is obviously out of line, was omitted in calculating the mean, but was included in the calculation of sigma.

† The probability that by chance alone in 5 of 6 tests, the figure for the left side would be lower than that for the right by 15 per cent or more is one in a hundred.

considers that the anterior horn cells remain viable and probably represent only about $^1/_{50}$ of the tissue involved in the test. Studies of cytochrome oxidase

Downloaded from http://rupress.org/jem/article-pdf/81/5/489/1183209/489.pdf by guest on 09 May 2021

activity in the monkey have been divided into four groups according to the time which elapsed between nerve section and sacrifice of the animal. These are seen in Table III and comprise (1) controls (no nerve section), (2) 7 to 9 days, (3) 10 to 20 days, (4) 21 days and over. By grouping the experiments in this fashion it is possible to see certain trends which were not apparent in the cat material which is more restricted in the time range represented. As in the cat cord, the two sides of the normal animal showed slight but unimportant differences, the average difference being 0.03 unit in favor of the left side (+4.5 per cent) and the standard error of this mean 0.02 unit. A group of six animals in which the roots had been cut 7 to 9 days previously showed a slight reduction of

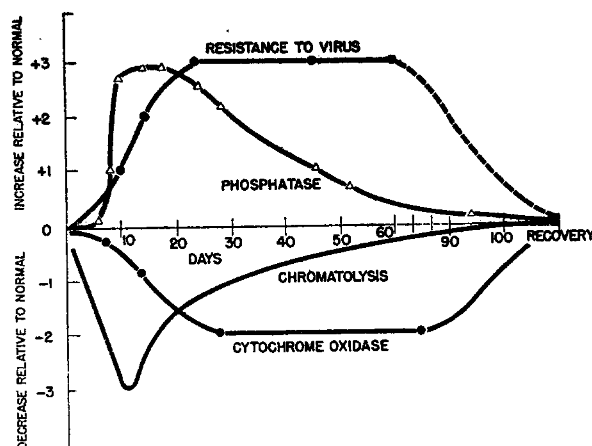

CHART 1. Diagram of changes in phosphatase and cytochrome oxidase in relation to the virus refractory and chromatolytic cycles of the nerve cell.

cytochrome oxidase activity (−3.75 per cent—see also Chart 1). This group, however, does not differ significantly from the controls (sigma of difference = 0.03; observed difference = 0.06). It is also important that none of the difference between the normal and operated sides exceeded 15 per cent. In the next group (10 to 20 days) which appears to be a borderline one, half of the observations show small differences while the other half show differences over 15 per cent. This inherent variability is reflected by a large value for the standard deviation of the average difference (sigma of difference = 0.13 unit; observed difference = 0.16 unit), which probably gives a poor picture of the biological realities of the situation. One can only say that the 10 to 20 day group shows a variable response with a tendency to striking reduction of cytochrome oxidase activity in some cases. Without the subsequent group these results could not be interpreted, although the average reduction is 17.5 per cent.

The last group (21 days and over) shows a consistent trend toward greater differences between normal and operated sides. Here five of the six observa-

Downloaded from http://rupress.org/jem/article-pdf/81/5/489/1183209/489.pdf by guest on 09 May 2021

tions differ from the control wide by more than 15 per cent, and the average difference is −22 per cent. (This difference is also significant since it exceeds sigma by four times; sigma of difference = 0.04 unit; observed difference = 0.17 unit.) No special importance is attached to the fact that the 10 to 20 day group shows a reduction of 17.5 per cent while the 21+ day group shows a 22 per cent reduction. These figures are both of the same order of magnitude and without more points on the curve it would be impossible to determine its highest point. One animal (Experiment 504) represents a root section of 70 days' duration which showed a reduction of 36 per cent on the chromatolytic side. While this is a single observation, the fact that reductions even approaching such magnitude were not observed in seventeen controls, suggests that this isolated experiment is valid. Such a finding indicates that the depression of cytochrome oxidase activity may be very enduring.

Correlation of Respiration Studies with Histological Changes and with the Virus Refractory State

Axon section produces in the nerve cell body a classical cytological reaction called chromatolysis. This consists essentialy of a modification of the Nissl bodies, the basic staining material in the cell cytoplasm. The reaction has recently been shown by ultraviolet absorption (11) to consist at least in part of reduction in the total amount of protein and nucleic acid, especially the latter, which parallels the increasing pallor of the staining reaction. During the first 24 to 48 hours little change is apparent, but by 5 to 6 days the aggregates of Nissl material are reduced in size and the staining has become pale. There is swelling of the cell body and beginning pallor and eccentricity of the nucleus. At 10 days these changes are maximal. The deeply staining Nissl material is now reduced to a ring at the cell periphery leaving a pale central zone, the nucleus is markedly eccentric and pale, and the cell appears to be swollen. As axonal regeneration proceeds these changes are gradually reversed. For example, at 30 days the Nissl bodies are recovering their normal staining reaction and form, though they are still smaller than normal. Complete restitution of Nissl material, however, does not occur for many weeks or until functional recovery has taken place. This entire 30 day cycle of changes is shown in Figs. 1 to 8 of the accompanying paper (12) where chromatolysis is represented in graded stages. The fact that chromatolysis may occur in pathological states which represent obvious damage to the nerve cell does not alter the proposition that it is a potentially reversible condition which is compatible with the life of the cell. It seems logical to consider chromatolysis as the visible manifestation of regenerative response on the part of the cell, since it most certainly does not always presage cell death. Chromatolysis may thus progress to necrosis or regress as the normal state of the cell is reestablished. The latter process is probably much more common than is generally realized.

Downloaded from http://rupress.org/jem/article-pdf/81/5/489/1183209/489.pdf by guest on 09 May 2021

It came as a surprise that cytochrome oxidase activity did not reach its maximum reduction at the period of most extreme chromatolysis. At 7 to 9 days when chromatolysis is far advanced there was an insignificant reduction in cytochrome oxidase activity. After 10 days chromatolysis begins to regress and it is precisely during this period that cytochrome oxidase activity begins its real decline. The fact that the groups "10 to 20 days and 20 days and over" show pregressive reductions to −17.5 per cent and −22 per cent respectively is probably not important and need not be stressed, since the lower average of the former is produced by the fact that half of the tests during this period showed almost no reduction. This is perhaps to be expected since chromatolysis does not proceed at a uniform rate in all individuals and the period of sharp decline would not be reached simultaneously in each case. The significant fact, however, lies in the finding that the amount of cytochrome oxidase is not regularly reduced when chromatolysis is maximal and reaches its most consistent depression during the period where chromatolysis has progressed well into the phase of resolution. These events are summarized in Chart 1 which also includes the phosphatase cycle (see 12).

TABLE IV

Motor Cell Counts in Sections of Monkey Spinal Cord
Comparison of Side of Root Section with Normal Side

Animal	No. of sections counted	Duration of operation	Normal side	Operated side	Difference
		days			*per cent*
A6-35 (cervical)	69	19	11.2	11.6	+3.4
A5-49 (cervical)	42	30	11.4	11.3	−0.9
A5-49 (lumbar)	33	30	11.4	11.7	+2.5
A7-83 (cervical)	37	30	11.0	11.3	+2.5
A7-83 (lumbar)	34	30	10.5	10.6	+0.9
A5-32 (cervical)	61	110	10.9	11.1	+1.8
A5-32 (lumbar)	55	110	10.5	10.3	−1.9

The reduction of cytochrome oxidase activity during the later phases of cell recovery could also be explained by the loss of cells which did not succeed in holding their own in the face of the trauma of axon section and the subsequent drain on their regenerative energies. Such cell losses do occur over long periods of time (16 to 18 months, Turner, 13), but at 30 days do not approach in magnitude the observed reduction of cytochrome oxidase. Table IV shows the results of counts of the motor cells in the normal and operated sides of monkeys 19 to 110 days after root section. In nearly every instance the operated side usually had a slightly higher cell count than the normal side. This is probably due to the fact that only cells with nucleoli in the plane of section were counted.

Downloaded from http://rupress.org/jem/article-pdf/81/5/489/1183209/489.pdf by guest on 09 May 2021

These small bodies are slightly more difficult to see in normal cells which may have resulted in some being omitted. This emphasizes the insignificance of the differences between the two sides.

Although there appears to be no correlation between cytochrome oxidase activity levels and visible chromatolysis, there is a striking parallel between the former and the virus refractory state. While this latter reaction has been described elsewhere (2) it may be briefly stated that after root section no resistance to destruction by virus is noted in the regenerating motor nerve cells until 6 or 7 days. Resistance becomes really substantial at 10 days and continues at a high level for many weeks or until functional recovery takes place, at which time the cells no longer show any resistance. There is thus a very suggestive parallel trend between these two phenomena, virus refractoriness and reduction of cytochrome oxidase. While, of course, these experiments do not show that the two *are* actually causally related it may not be entirely without profit to speculate as to how they *may* be related.

DISCUSSION

If cytochrome oxidase activity is reduced in the cell during the virus refractory period, the question naturally arises whether the total oxidative metabolism is likewise affected. Unfortunately our sampling methods have not been applicable to the study of tissue slices so that it has not been possible to make observations on the rates of aerobic and anaerobic glycolysis. Recent observations by Turner and Turner (6) on the O_2 consumption of chromatolytic cord are inconclusive. These authors were unable to demonstrate any significant difference between the oxygen consumption of tissue slices of the normal half of the lumbosacral cord of the guinea pig and the half involved in sciatic nerve section. Reductions of O_2 consumption were noted when both sciatic nerves were sectioned, but it is difficult to judge this finding since separate animals were used as controls and the authors give no data on which to determine how well they had made allowance for the great varition in the cytochrome oxidase levels characteristic of individual animals. The failure to find significant changes when one side of the whole cord was used as a control for the operated side only illustrates the difficulty of dealing with a complex mixture of different tissues all of which may not be involved in the particular process under consideration.

There is an increasing body of fact indicating that virus production is somehow geared to the metabolism of the host cell (14–21). Two examples which appear to bear in a measure upon our specific problem have been drawn from widely divergent fields. Spizizen (22) studied phage production in cultures of bacterial cells in which bacterial multiplication was inhibited by glycine anhydride. Despite these unfavorable conditions phage production still continued but was facilitated or inhibited by various substances which presumably

Downloaded from http://rupress.org/jem/article-pdf/81/5/489/1183209/489.pdf by guest on 09 May 2021

acted upon those cellular metabolic systems essential for phage production. Among other things, inhibition of phage growth was obtained when the culture was poisoned with NaCN. Even more suggestive is the work of Woods and DuBuy (23, 24) who showed that tobacco mosaic virus reduced the activity of a cyanide-sensitive respiratory mechanism in tobacco leaves. They were also able to demonstrate the converse, namely that leaves which had been reversibly poisoned with HCN were refractory to inoculation with virus but became subsequently susceptible after a period of 4 days. The identity of the cyanide-sensitive respiratory system with the cytochrome system seems to be sufficiently generally accepted among workers to require no specific documentation. It, therefore, appears to be more than mere coincidence that the activity of the cyanide-sensitive cytochrome system should be implicated with virus propagation in such widely divergent types of tissue as tobacco leaves, bacteria, and primate nerve cells. It is possible, of course, that our particular case is not strictly analogous to those just cited. For example, we do not know at present whether, as in these other instances, the virus fails to propagate or whether it continues to multiply but does not destroy the cell. While the latter seems unlikely it would force interpretation in another direction. Such questions and many others must be answered before it is possible even to hint at the mechanisms involved.

SUMMARY

Manometric determinations of cytochrome oxidase activity were carried out on grey matter from the thalamus and anterior horn of cats and monkeys under various experimental conditions. The thalamus of the cat was studied following the degeneration of virtually all the thalamic neurons secondary to decortication. In comparing the deneuronated thalamus with the normal one, it was found that approximately 34 per cent of the cytochrome oxidase activity was contributed by the neurons and the balance by neuroglia and mesodermal tissues which on the operated side remained comparable to that of the normal side. Total activity of the normal thalamus averaged 5.52 units per mg. of dry weight where 1 unit is defined as the amount of cytochrome oxidase required to produce a net oxygen consumption of 10 c.mm. per hour under the specified conditions of the experiment.

The grey matter of the anterior horns of the spinal cord was isolated by a special technique and its cytochrome oxidase activity was compared with anterior horns in which motoneurons had been stimulated to regenerative activity by section of peripheral nerves. Each animal was studied in relation to an anterior horn which was normal and one in which only the functional state of the motoneurons had been changed. Average normal levels of 2.23 units were found for cat anterior horn and 0.69 units for the monkey. Reductions of cytochrome oxidase activity in the range of 22 to 23 per cent were observed

Downloaded from http://rupress.org/jem/article-pdf/81/5/489/1183209/489.pdf by guest on 09 May 2021

for both cat and monkey following nerve section. In the latter the time sequence was carefully studied in relation to the cytological cycle known as chromatolysis and a virus refractory state previously described by us. It was found that maximal reduction of cytochrome oxidase activity coincided with maximal refractoriness of the cells to poliomyelitis virus (30 to 70 days following nerve section). Neither of these states could be correlated in time with maximal chromatolysis (10 to 15 days).

BIBLIOGRAPHY

1. Howe, H. A., and Bodian, D., Neural mechanisms in poliomyelitis, The Commonwealth Fund, New York, 1942.
2. Howe, H. A., and Bodian, D., *Bull. Johns Hopkins Hosp.*, 1941, **69,** 92.
3. Flexner, J. B., Flexner, L. B., and Straus, W. L., Jr., *J. Cell. and Comp. Physiol.*, 1941, **18,** 355.
4. Racker, E., and Kabat, H., *J. Exp. Med.*, 1942, **76,** 579.
5. Wood, H. G., *J. Lancet*, 1944, **64,** 240.
6. Turner, R. S., and Turner, M. L., *Fed. Proc.*, 1944, **3,** 49.
7. Pearce, J., and Gerald, R. W., *Am. J. Physiol.*, 1942, **136,** 49.
8. Bodian, D., and Howe, H. A., *Bull. Johns Hopkins Hosp.*, 1941, **68,** 58.
9. Stotz, E., *J. Biol. Chem.*, 1939, **131,** 555.
10. Elliott, K. A. C., and Libet, B., *J. Biol. Chem.*, 1942, **143,** 227.
11. Gersh, I., and Bodian, D., *J. Cell. and Comp. Physiol.*, 1943, **21,** 253.
12. Bodian, D., and Mellors, R. C., *J. Exp. Med*, 1945, **81,** 469.
13. Turner, R. S., *J. Comp. Neurol.*, 1943, **79,** 73.
14. Woolpert, O. C., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32,** 1430.
15. Sabin, A. B., and Duffy, C. E., *Science*, 1940, **91,** 552.
16. Friedewald, W. F., *J. Exp. Med.*, 1942, **75,** 197.
17. Parker, R. F., and Thompson, R. L., *J. Exp. Med.*, 1942, **75,** 567.
18. Sprunt, D. H., *J. Exp. Med.*, 1942, **75,** 297.
19. Foster, C., Jones, J. H., Henle, W., and Dorfman, F., *J. Exp. Med.*, 1944, **79,** 221.
20. Rasmussen, A. F., Jr., Waisman, H. A., Elvehjem, C. A., and Clark, P. F., *J. Infect. Dis.*, 1944, **74,** 41.
21. Toomey, J. A., Frohring, W. O., and Takacs, W. S., *Yale J. Biol. and Med.*, 1944, **16,** 477.
22. Spizizen, J., *J. Infect. Dis.*, 1943, **73,** 222.
23. Woods, M. W., and DuBuy, H. G., *Phytopathology*, 1942, **32,** 288.
24. Woods, M. W., and DuBuy, H. G., *Phytopathology*, 1941, **31,** 978.

Downloaded from http://rupress.org/jem/article-pdf/81/5/489/1183209/489.pdf by guest on 09 May 2021