

THE METABOLISM OF THE CENTRAL NERVOUS SYSTEM IN
EXPERIMENTAL POLIOMYELITIS*

By E. RACKER, M.D., AND HERMAN KABAT, M.D.‡

(From the Anderson Institute for Biological Research and the Department of Physiology,
University of Minnesota, Minneapolis)

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Little is known of the mechanism of destruction of neurons by the virus of poliomyelitis. The older theory (1) that neurons of the spinal cord are injured as a result of impaired circulation is apparently not widely supported at present. Significant evidence has accumulated that the virus spreads through neurons (2) and that the mesodermal-glial changes are secondary to the involvement of nerve cells (3). It seems probable that the virus damages the neuron directly by its presence in the cell, disrupting some vital cellular function.

However, the mere presence of virus in a neuron does not signify that the cell will be destroyed. Poliomyelitis virus has been shown to spread through many neurons in the brain without producing cellular necrosis (4). Reversible neuronal injury may account to some extent for the recovery from paralysis frequently observed in clinical cases of the disease. The local pathological lesion produced by injection of poliomyelitis virus into the visual cortex in the monkey is insignificant in comparison to the reaction around a similar inoculum in the motor cortex (3). Recently, Bodian and Howe (5) have made the striking observation that highly susceptible neurons of the anterior horn of the spinal cord may, by interruption of their axons, be protected from poliomyelitis virus.

It is reasonable to suppose, as a working hypothesis, that the virus of poliomyelitis may interfere with certain specific metabolic activities of the nerve cell, and that the relative importance of these activities to a particular neuron may determine resistance or susceptibility to the virus. In beginning to explore this hypothesis, an investigation has been made of some aspects of the metabolism of the central nervous system of mice infected with poliomyelitis virus. In addition, normal tissues from regions of the central nervous system which have been shown to differ markedly in susceptibility to the virus have been examined to determine their metabolic activities.

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Methods

Swiss albino mice 4 to 6 weeks of age were inoculated intracerebrally with 0.03 cc. of a 10 per cent suspension of brain tissue from mice infected with the Lansing strain of poliomyelitis virus. When definite paralysis became evident, the animal was sacrificed, the brain removed, weighed, dispersed in a mortar or finely minced, and suspended in Locke's solution to make a 10 per cent suspension. All was done as rapidly as possible. In some instances, marked symptoms of encephalitis were present but in no case was the mouse moribund at the time it was sacrificed. A suspension of normal mouse brain was prepared at the same time and in an identical manner, serving as a control.

TABLE I
Metabolism of Mouse Brain Suspensions

Batch	Oxygen utilization					Anaerobic glycolysis				
	Normal		Poliomyelitis			Normal		Poliomyelitis		
	No. of mice	O ₂ uptake*	No. of mice	O ₂ uptake*	Deviation from the normal	No. of mice	CO ₂ † output	No. of mice	CO ₂ † output	Deviation from the normal
					<i>per cent</i>					<i>per cent</i>
1	5	92.8	3	97.1	+4.6	5	51.6	5	27.1	-47.5
2	4	120.4	4	119.9	-0.4	6	75.5	6	63.0	-16.5
3 and 4	5	86.5	4	86.2	-0.3	19	87.9	12	74.1	-15.7
5	8	98.9	6	104.0	+5.1	14	100.5	11	93.1	-7.3
6	5	71.2	6	78.8	+10.6	5	71.2	5	60.8	-14.5
7	8	87.3	8	80.0	-8.4	7	75.4	7	70.4	-6.6
8	3	128.6	3	107.6	-16.3	5	94.8	5	74.4	-21.5
Total . . .	38	95.0	34	93.4	-1.6	61	84.4	51	70.5	-16.5

* Average of oxygen consumption in c.mm. per 100 mg. wet weight in first hour.

† Average of CO₂ output in c.mm. per 100 mg. wet weight in first hour.

Oxygen utilization and anaerobic glycolysis of normal and poliomyelitic brain suspensions were measured by the usual manometric methods at 37°C. and pH 7.4, using the Barcroft-Warburg apparatus. Approximately 100 mg. wet weight of brain tissue was introduced into each vessel. In some instances, slices of mouse cerebrum were used and dry weight determined at the end of the experiment. For oxygen consumption, a phosphate-glucose-Locke medium was used; for anaerobic glycolysis, a bicarbonate-glucose-saline solution was used and the system filled with 95 per cent N₂ and 5 per cent CO₂.

RESULTS

The results of investigation of the oxygen utilization and anaerobic glycolysis of suspensions of surviving brain tissue from normal and poliomyelitic mice are tabulated in Table I. There was no significant difference in oxygen consumption of normal and infected brain with glucose as the substrate. This

was confirmed in experiments on slices of cerebrum: the average Q_{O_2} for normal brain was 10.4 and Q_{O_2} for poliomyelitic brain was 9.9. This is in conformity with the observations of Brodie and Wortis (6), who found no difference in oxygen consumption of normal and poliomyelitic spinal cord from monkeys. No difference in oxygen consumption by normal and poliomyelitic brain tissue was noted in experiments using other substrates, such as lactate, pyruvate, succinate, and glycogen. This was true even when these substrates were tipped into the vessel at the end of the third hour, allowing endogenous substrates to be depleted.

In contrast to the results on oxygen consumption, a consistent and significant decrease in anaerobic glycolysis was observed in tests of poliomyelitic brain suspension (Table I). The average values from a large number of experiments showed a percentage decrease of 16.5 per cent from the normal. The decrease was noted in practically every case although it varied in degree from 5 per cent to over 50 per cent in individual experiments. The percentage decrease in anaerobic glycolysis in the infected brain varied considerably for different batches of mice of the same age and strain, which may be related to the marked differences in susceptibility to poliomyelitis observed with different strains of Swiss mice (7). It was noted that the decrease of glycolysis was usually much greater when symptoms of encephalitis had been evident before the mouse was sacrificed, while simultaneously measured oxygen utilization remained unchanged. The wide variations in extent and severity of brain lesions in rodents infected with the Lansing strain of poliomyelitis virus (8) may help to explain the individual variations in the decrease of anaerobic glycolysis in infected brain tissue.

Suspensions of cerebrum, brain stem minus cerebellum, and spinal cord from four poliomyelitic mice were compared with the same regions of the central nervous system from normal mice. The inhibition of anaerobic glycolysis in cerebrum and brain stem was over 30 per cent, while in spinal cord, the inhibition was slightly less.

In an attempt to find the reason why the anaerobic breakdown of glucose in brain is less in poliomyelitis, experiments were performed using sodium fluoride as an inhibitor (Table II). The percentage inhibition resulting from fluoride was consistently higher in the normal than in the infected brain, particularly with lower concentrations of fluoride. The residual glycolysis in poliomyelitic brain treated with fluoride was equal to the glycolysis in normal brain with fluoride in every experiment. One of the major effects of fluoride is to inhibit glycolysis at the stage of breakdown of phosphoglycerate to phosphopyruvate. In contrast to the results with fluoride, preliminary experiments indicate that monoiodoacetate exerts an equal inhibitory effect on anaerobic glycolysis of normal and poliomyelitic brain suspensions.

Experiments were also performed to compare the dehydrogenase activity

of normal and poliomyelitic brain suspensions. The technique used was the Thunberg method of determination of rate of decolorization of methylene blue in an evacuated tube. Without added substrate, the methylene blue was uniformly decolorized more rapidly by the infected than by the normal tissue. The average decrease in decolorization time was 20 per cent, but was much higher in individual experiments. On the other hand, with addition of various substrates, for example glucose, lactate, and succinate, there was no significant difference observed between normal and poliomyelitic brain. It appears possible that the more rapid decolorization by the infected tissue may be related to the presence of a greater quantity of available substrate, due to changes in the chemical composition of the brain resulting from poliomyelitis. No quantitative correlation could be observed between the increase in dehydrogenase activity and the decrease in anaerobic glycolysis in infected brain.

TABLE II
The Effect of Sodium Fluoride on Anaerobic Glycolysis

Concentration NaF	Normal				Poliomyelitis			
	No. of mice	Without NaF	With NaF	Inhibition <i>per cent</i>	No. of mice	Without NaF	With NaF	Inhibition <i>per cent</i>
M/30	6	66.5*	8.9	86.6	7	56.9*	12.6	77.8
M/60	1	86.4	9.1	89.4	1	78.6	11.7	85.1
M/150	3	100.3	27.9	72.1	3	78.9	32.3	59.0
M/300	1	111.3	40.6	63.5	1	87.7	41.9	52.2

* CO₂ output in c.mm. per 100 mg. wet weight of brain per hour.

Preliminary observations have been made on the phosphatase activities of normal and poliomyelitic mouse brain suspensions, measured at pH 5.5 and pH 9.5, using veronal buffer. The substrates tested were β -glycerophosphate, nucleic acid isolated from mouse brain tissue, and adenosine triphosphate. The suspension was incubated for 2 hours at 37°C. in a shaking apparatus. Inorganic phosphate was determined colorimetrically. Although the number of experiments was small, the phosphatase activity of the infected brain appeared to be higher with mouse brain nucleic acid and adenosine triphosphate as substrates, while no difference from normal was noted with β -glycerophosphate as the substrate.

In an attempt to determine whether specific susceptibility of different brain regions to the virus of poliomyelitis might be related to differences in metabolism of these regions, a comparison has been made of oxygen consumption and anaerobic glycolysis of the motor cortex, visual cortex, and anterior horn of the spinal cord from normal animals. Bodian and Howe (3) have demonstrated in the monkey that visual cortex is much less susceptible than motor

cortex to inoculation of poliomyelitis virus, and it is well known that anterior horn cells are highly susceptible to this virus.

The technique used in these experiments was as follows:—

Dogs and cats were anesthetized with pentobarbital sodium, the calvarium carefully removed. In some cases motor cortex and in other instances visual cortex was excised and quickly sliced and washed in the medium containing glucose. The remaining cortex (visual or motor in different experiments) which had remained *in situ* with adequate circulation, was then excised and sliced in the same manner. In this way, the slices were obtained as fresh as possible. Slices of anterior horn of the lumbo-

TABLE III
Metabolism of Different Regions of the Central Nervous System
(Normal Dog and Cat)

Motor cortex			Visual cortex			Normal anterior horn			Chromatolytic anterior horn		
Q_{O_2}	Q_{CO_2}	$\frac{Q_{CO_2}}{Q_{O_2}}$	Q_{O_2}	Q_{CO_2}	$\frac{Q_{CO_2}}{Q_{O_2}}$	Q_{O_2}	Q_{CO_2}	$\frac{Q_{CO_2}}{Q_{O_2}}$	Q_{O_2}	Q_{CO_2}	$\frac{Q_{CO_2}}{Q_{O_2}}$
-6.4	+7.9	1.23	-6.9	+4.0	0.58	-1.7	+1.4	0.82	-1.4	+1.9	1.36
-5.0	+4.0	0.8	-6.6	+4.0	0.60	-3.0			-2.5		
	+4.0			+4.1		-2.0			-2.0	+1.7	0.85
-7.3			-6.3				+2.0				
	+2.7		+1.3			-4.3	+4.7	1.09			
-7.9			-9.0								
-10.7			-9.5								
-10.2			-8.1								
-7.8	+8.5	1.1	-8.0	+4.2	0.52	-3.9	+1.9	0.49			
-5.8	+5.0	0.86	-6.4	+3.3	0.51						
-6.0	+7.4	1.2	-5.9	+3.3	0.56						
Average... -7.4	+6.2	1.04	-7.4	+3.4	0.55	-3.0	+2.5	0.80	-2.0	+1.8	1.1

sacral spinal cord were obtained in a similar fashion. The Q_{O_2} and Q_{CO_2} were determined in the usual manner using the Barcroft-Warburg apparatus. It is now well established that barbiturates can be quickly removed from brain slices by washing (9) and do not exert a lasting effect on metabolism. While dog and cat are admittedly insusceptible to the virus of poliomyelitis, it is not known whether this resistance is based on immunological or chemical rather than metabolic factors.

The results of the experiments on slices of motor and visual cortex and anterior horn of the spinal cord are tabulated in Table III. There is apparently no difference in oxygen consumption between motor and visual cortex, while the anaerobic glycolysis is significantly lower for visual cortex. The oxygen utilization of anterior horn is only a fraction of that of cortical tissue. A few

experiments on chromatolytic anterior horn tissue obtained from dogs subjected to section of the sciatic and femoral nerves 3 weeks previously are also included, since chromatolysis greatly influences susceptibility of anterior horn cells to poliomyelitis. One must be cautious in interpretation of the results on anterior horn slices because of the low metabolic activity of this tissue. Of particular interest is the ratio Q_{CO_2}/Q_{O_2} , which is consistently higher for motor cortex than for visual cortex. Whether these results have any bearing on susceptibility of specific regions to poliomyelitis virus is still problematical.

DISCUSSION

The only previous study of metabolic activity of surviving nervous tissue infected with poliomyelitis virus was reported by Brodie and Wortis (6), who found that minced brain and spinal cord from infected monkeys showed no significant change from normal in oxygen consumption or respiratory quotient. It may be noteworthy that their data show a decrease in lactic acid content of the poliomyelitic brain.

All investigators agree that purified viruses do not utilize oxygen or carry out dehydrogenase activity (10-14). Phosphatase activity has been observed in bacteriophage (12), and tobacco mosaic virus (13), and phosphatase and catalase activities have been reported for elementary bodies of vaccinia (14). Of interest are the recent studies demonstrating the presence of biotin (15), copper (16), and flavin (17) in purified vaccinia virus.

SUMMARY

1. During paralysis, the brain of the mouse infected with poliomyelitis virus shows on test after mincing a decrease in anaerobic glycolysis with no significant change in oxygen utilization. The decrease in anaerobic glycolysis varies from 5 per cent to 50 per cent.
2. Sodium fluoride produces a greater inhibition of anaerobic glycolysis in normal than in poliomyelitic brain.
3. Dehydrogenase activity is higher for poliomyelitis-infected brain without added substrate. This difference from normal disappears when substrates are added.
4. The ratio of $\frac{\text{Anaerobic glycolysis}}{\text{Oxygen utilization}}$ for the sliced motor cortex is higher than for sliced visual cortex of the dog and cat.
5. The oxygen consumption of the anterior horn of the sliced spinal cord of dog and cat is much less than that of the cerebral cortex.
6. The findings are in keeping with the view that, at a certain stage of the infection, the nerve cells may be reversibly injured but not yet destroyed by the virus.

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