ANAEROBIC GLYCOLYSIS OF THE BRAIN IN EXPERIMENTAL POLIOMYELITIS*

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(Received for publication, October 5, 1944)

It has been reported by Racker and Kabat (1) and Nickle and Kabat (2) that the rate of anaerobic glycolysis of mouse brain is substantially and regularly reduced in brain experimentally infected with poliomyelitis. These findings were offered in support of the theoretical assertion that the virus may act by interfering with "certain specific metabolic activities of the nerve cell." In the more recent report (2), it is concluded that when the glucose concentration is 37.5 mg. per cent, the anaerobic metabolism of encephalitic brain (W.E.E.) is significantly below the normal whereas that of poliomyelitic brain is not and with a glucose concentration of 229.5 mg. per cent, the anaerobic metabolism of both encephalitic and poliomyelitic brain is significantly below the normal. It is the purpose of the present investigation to determine the reproducibility of the experimental findings, and some of the factors responsible for the results.

Methods

Two strains of mice were used: ABC mice from the colony of Dr. John Bittner, Division of Cancer Biology, University of Minnesota, and Swiss albino mice furnished to us by Dr. Carl M. Eklund, State Board of Health, Minneapolis. The Swiss albinos were of the same strain used by Racker and Kabat. A few Swiss albino mice from our own colony were also used, and seemed to differ in no way from the others.

The experimental animals were litter mates selected on a weight as well as age basis. Those used had weights lying mostly between 13 and 16 gm.; in this way complicating effects of differences not only of age but of initial nutritional state were limited. The initial body weights of the animals were recorded at the time of inoculation, and weight change was recorded when they were sacrificed.

The animals to be infected were inoculated intracerebrally with 0.03 ml. of a 10 per cent suspension of spinal cord tissue from mice infected with the Lansing strain of virus. The animals were sacrificed when they showed definite paralysis; the location of the paralysis and time elapsed since inoculation was noted. Both infected animals and normal controls were killed by decapitation with scissors, and the complete brain was removed, immediately weighed, and prepared for the experiment.

In the experiments using ABC mice, the procedure developed by Elliot and Libet (3) was followed. The brain tissue was homogenized in a grinder (4) after addition of an isotonic saline solution in the proportion of 20 ml. to each gram of brain. (Composition of isotonic saline: 100 ml. 0.154 M NaCl, 10 ml. 0.014 M $MgSO_4$, 7.9 ml. phosphate buffer of pH 7.4, consisting of 4 parts 0.25 M Na_2HPO_4 and 1 part 0.25 M KH_2PO_4.) One ml. of the brain sus-

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

pension was added to each vessel of the Warburg apparatus, the vessel being previously filled with 1.4 ml. of the isotonic saline solution, 0.48 ml. of 0.155 M $NaHCO_3$ solution, and 0.12 ml. of 0.24 M glucose solution (100 ml. 0.284 M glucose, 7.9 ml. of 0.25 M phosphate buffer, pH 7.4, and 10 ml. 0.014 M $MgSO_4$). This gave approximately 48 mg. of brain and 5.19 mg. of glucose in each vessel. Each brain suspension was run in duplicate. Preliminary experiments had shown that the 1:20 dilution gives maximal carbon dioxide values under strictly anaerobic conditions. The observed increase in glycolysis with dilution may be related to dilution of an inhibitor released by homogenization (Geiger (5)).

In the experiments with Swiss albino mice, the brain tissue was finely minced with razor blades mounted in a rubber stopper. The tissue was suspended in standard Locke's solution using 10 ml. of solution to 1 gm. brain. The Locke's solution was made up as follows: 0.9 gm. NaCl, 0.024 gm. $CaCl_2$, 0.042 gm. KCl, 0.02 gm. $NaHCO_3$, 0.25 gm. dextrose, and distilled water to 100 ml. One ml. of this suspension was added to the Warburg vessel, which already contained 0.2 ml. of 0.16 M glucose solution, 0.5 ml. of 0.16 M $NaHCO_3$ solution, and 1.3 ml. of physiological saline (0.9 per cent NaCl). This gave approximately 91 mg. of brain tissue and 7.91 mg. of glucose in each vessel. Each brain suspension was run in duplicate.

In all experiments, the dry weight was determined on an aliquot of the brain suspension, which was dried for 24 hours at 105° C. 4 ml. aliquots were taken from the 1:20 suspension, and 2 ml. aliquots from the 1:10 suspension. A correction was made for the weight of the solids of the isotonic saline or Locke's solutions, which was directly determined in the same way. The dry weight of the brain is reported in milligrams per milliliter of the brain suspension, which is the same as the amount used per flask in the respirometer measurements. The relative volume occupied by brain tissue in the suspension was taken into account approximately in calculating dry weight percentage and dry weight of entire brain assuming density of tissue to be 1; this is not a negligible correction.

When the vessels were placed in the Warburg bath, nitrogen gas containing 5 per cent carbon dioxide was passed through them for 7 minutes at a pressure of about 9 cm. of Brodie's solution; traces of oxygen were removed by passing the gas over heated (250°) metallic copper, which was previously reduced by a stream of hydrogen gas. The copper treatment was omitted in the experiments with minced brain, since this procedure had not been followed by Racker and Kabat. After the vessels were closed, 5 minutes more were allowed for equilibration before readings were commenced. Readings were usually taken within half an hour of the killing of the first mouse.

The shaking rate was about 120 oscillations per minute. The bath temperature was approximately 38°C. Temperature variation was followed by means of a Beckmann differential thermometer, and controlled by a variable resistance in the heating coil circuit. The temperature change usually remained within 0.005°C. during the time required for making one reading, and within 0.01–0.02°C. during the entire time of the experiment. These precautions were taken because it was found that rapid temperature variations occurring while the entire set of manometers was read, even over so small a range as 0.03°C., caused disagreement among blanks (vessels containing only saline or water).

Readings were taken every 15 minutes during the 1st hour, and at the half-hour periods during the 2nd hour. Total evolution of CO_2 was calculated for each hour.

One set of controls which deserves special emphasis concerns the manner in which the brain samples were arranged on the bath from day to day. Eight mouse brains could be, and usually were, run in duplicate at one time. The following procedure was strictly observed: When several paralyzed animals were sacrificed, usually an equal number of normal mice from the control group were sacrificed at the same time. The vessels containing infected and normal brain were alternated in position on the bath. Duplicates from the same brain suspension were placed on opposite sides of the bath. Moreover, on successive determinations, the order of infected and normal brains was shifted (the series beginning with infected

TABLE I

Comparison of the Brains of Normal and Infected ABC Mice

Normal									Infected									
	Body weight		Brain weight		Anaerobic CO_2 per 100 mg. of brain					Body weight		Brain weight		Anaerobic CO_2 per 100 mg. of brain				
Time	Initial	Change	Wet	Dry weight per flask	1st hr.		2nd hr.		Time	Initial	Change	Wet	Dry weight per flask	1st hr.		2nd hr.		Paralysis*
days	*gm.*	*gm.*	*mg.*	*mg.*	*µl.*	*µl.*	*µl.*	*µl.*	*days*	*gm.*	*gm.*	*mg.*	*mg.*	*µl.*	*µl.*	*µl.*	*µl.*	
4	13.4	2.43	353	10.23	101	107	71	76	4	14.5	−1.58	402	9.90	101	90	76	69	LH
4	12.7	3.97	442	10.15	88	101	63	74	4	16.6	−1.63	369	10.18	116	95	88	74	RSh
4	14.7	4.62	429	10.23	88	88	76	74	4	15.4	−2.00	408	9.65	92	95	71	71	LF
4	11.8	2.97	384	9.83	86	82	71	67	5	15.8	−0.80	426	9.63	113	113	101	84	RF, RH
5	15.4	2.05	421	10.35	109	103	88	86	5	15.7	0.92	435	9.60	109	111	84	92	RH
5	14.6	2.40	419	10.38	103	99	88	78	5	13.8	0.33	414	10.10	103	92	88	80	BH
5	14.5	2.71	380	10.33	99	101	84	88	5	14.0	1.31	407	10.20	101	92	82	76	BF, LH
5	16.5	2.03	372	10.80	122	118	90	80	5	15.7	−1.70	374	10.35	113	103	78	74	RF
5	14.3	5.94	424	10.33	84	107	74	82	5	15.0	0.35	435	10.00	103	99	92	78	LF, LH
5	13.5	4.09	384	9.95	94	90	88	90	5	13.6	−0.02	407	10.43	103	95	95	84	RF
6	14.8	3.17	428	10.33	113	107	101	97	5	12.0	3.08	400	10.10	101	97	92	80	LSh
6	14.9	2.53	421	9.95	111	107	92	101	6	14.8	−0.50	388	10.05	109	118	105	99	RH
6	12.8	3.37	395	10.40	103	99	92	103	6	14.8	−1.30	430	10.05	116	124	101	109	LF
6	15.5	3.71	428	10.50	97	97	97	109	6	14.3	−0.20	398	10.33	111	103	99	97	BH
7	15.0	0.04	399	10.73	109	122	55	55	6	14.5	−0.27	400	10.17	109	99	109	105	RF
7	15.4	−1.70	410	10.60	103	111	53	59	7	12.7	−1.27	378	10.07	107	101	57	53	LF
7	16.2	−0.78	408	10.13	111	103	69	65	7	14.0	2.01	432	11.03	95	101	55	48	LSh
7	15.7	0.07	388	10.27	101	95	69	67	7	11.1	−0.07	404	10.37	103	99	65	63	LSh
7	15.4	1.82	406	10.57	97	95	86	80	7	12.1	2.00	378	10.23	92	90	50	57	RF
7	15.8	1.72	393	10.60	88	97	84	74	7	15.0	−0.74	431	10.23	92	97	78	88	BF
7	12.8	5.93	413	10.40	80	84	69	78	7	16.0	−1.62	433	10.33	82	80	76	71	LF
7	15.5	1.23	425	10.57	69	71	65	78	7	15.2	1.53	400	10.40	78	78	78	78	LF
8	15.0	1.70	401	10.20	82	99	86	86	8	14.7	0.20	421	10.13	107	84	92	84	LF
8	14.5	1.23	435	10.53	80	88	80	82	8	15.8	0.15	397	9.83	86	88	86	80	BF
3	15.1	2.29	398	10.17	92	101	90	97	3	13.3	−2.25	367	10.07	90	86	97	86	BF, LH
3	16.6	1.53	422	9.93	84	84	90	88	3	12.6	1.45	403	9.83	78	76	78	78	BH
4	13.4	3.60	402	9.63	78	74	55	53	4	14.3	−4.91	394	10.17	74	71	46	40	BF
4	15.4	3.03	427	10.30	71	78	61	48	4	14.4	0.05	403	9.27	69	74	46	50	LF
4	13.6	2.30	390	9.90	67	71	50	50	4	13.1	−1.55	413	10.13	65	71	44	42	BF
4	13.3	1.20	403	10.27	78	78	46	48	6	15.0	−4.20	402	10.57	65	74	50	44	BH
6	14.6	3.80	399	9.97	63	71	42	48	6	13.0	−0.74	405	10.93	67	69	48	46	BH
6	15.6	1.49	443	11.40	73	75	48	54	6	14.1	−0.58	415	10.97	82	71	61	52	LH
6	15.8	3.70	434	10.97	82	76	76	74	6	13.5	0.03	424	8.53	55	57	42	44	BH
6	13.4	2.60	416	10.93	76	82	53	53	11	12.9	4.50	377	9.73	74	80	63	59	RF
11	12.8	5.20	408	10.10	76	67	65	57	11	16.5	−2.15	388	10.20	63	82	53	69	RF
11	13.3	6.02	411	10.23	61	63	59	48	6	15.2	−0.13	441	10.30	78	74	67	71	BH
6	15.6	3.89	434	10.17	84	71	65	59	6	13.8	0.64	394	9.80	82	78	65	61	BF
6	14.7	4.72	438	10.40	61	63	61	67										

* B, both; F, fore leg; H, hind leg; L, left; R, right; Sh, shoulder.

on one day, and with normal on the next), so that no vessel should always contain tissue from only one of the groups.

A glance at the data presented in the following pages will show the justification for these precautions. They were especially necessary because the glycolytic rate being measured was so small—of the order of 10 to 20 mm. of Brodie's solution in 15 minutes. It is our experience that day-to-day variations (sometimes even morning-to-afternoon variations) are such as to demand the procedure described if reliable results are to be obtained. It is reasonably certain, with the randomization procedure employed in these experiments, that any sources of systematic error were eliminated.

TABLE II

Effect of Inoculation of Non-Infected Cord on the Brains of ABC Mice

Normal									Inoculated								
Time	Body weight		Brain weight		Anaerobic CO_2 per 100 mg. of brain				Time	Body weight		Brain weight		Anaerobic CO_2 per 100 mg. of brain			
	Initial	Change	Wet	Dry weight per flask	1st hr.		2nd hr.			Initial	Change	Wet	Dry weight per flask	1st hr.		2nd hr.	
days	*gm.*	*gm.*	*mg.*	*mg.*	*μl.*	*μl.*	*μl.*	*μl.*	*days*	*gm.*	*gm.*	*mg.*	*mg.*	*μl.*	*μl.*	*μl.*	*μl.*
4	13.1	−0.92	401	10.48	118	95	109	76	4	13.0	3.36	431	10.33	99	74	99	78
4	15.0	2.95	436	10.05	82	82	74	74	4	13.7	0.01	418	10.18	88	80	86	69
4	15.2	2.80	397	10.45	90	86	78	78	4	13.0	2.99	430	10.45	90	84	78	80
4	15.2	1.50	404	10.38	71	71	69	61	4	13.5	4.26	427	10.15	84	82	74	74
5	12.8	2.78	408	10.20	109	103	61	61	5	14.6	2.72	406	10.25	109	122	74	69
5	15.9	3.07	414	9.88	95	99	59	65	5	13.0	2.75	402	10.13	99	109	59	61
5	12.7	3.25	421	10.43	99	99	61	65	5	13.7	1.36	404	10.40	107	105	67	71
5	13.9	4.25	416	10.33	111	105	65	65	5	16.4	−0.82	454	9.38	97	88	63	61
6	13.6	3.74	408	10.43	55	63	55	50	6	14.0	0.85	406	9.98	69	61	53	53
6	14.6	3.79	440	10.00	59	61	48	50	6	14.2	0.55	411	10.18	61	59	48	50
6	15.6	2.38	425	10.63	74	65	48	50	6	14.1	2.40	392	10.25	67	78	50	53
6	13.3	4.09	392	10.30	59	67	46	46	6	13.6	2.61	378	10.43	74	59	50	46
7	17.0	3.00	410	10.63	118	103	82	84	7	18.0	0.63	404	10.73	95	99	82	76
7	17.1	4.60	407	10.20	88	101	78	80	7	16.8	1.43	382	10.53	105	101	78	88
7	12.7	2.69	400	10.58	97	92	82	78	7	14.0	1.55	390	10.50	107	101	78	76
7	13.8	3.95	410	10.28	99	101	61	65	7	16.5	2.02	408	10.43	95	95	69	74

RESULTS

The results of three series of experiments are presented in Tables I to III and summarized in Table IV. The duplicate readings for each hour are given.

In these tables, "incubation time" for infected animals refers to the interval from inoculation to the appearance of paralysis; this fixes its meaning for normal animals, since a corresponding number of controls were sacrificed with each group of infected animals.

Table I gives the data from normal and infected ABC mice in which the brain was homogenized and sugar concentration was 173 mg. per cent. Table

II shows the results from a similar experiment in which no virus was used, the inoculation being made with suspension of non-infected cord. This experiment

TABLE III

Comparison of the Brains of Normal and Infected Swiss Albino Mice

Normal									Infected									
Time	Body weight		Brain weight		Anaerobic CO_2 per 100 mg. of brain				Time	Body weight		Brain weight		Anaerobic CO_2 per 100 mg. of brain				Paralysis
	Initial	Change	Wet	Dry weight per flask	1st hr.		2nd hr.			Initial	Change	Wet	Dry weight per flask	1st hr.		2nd hr.		
days	*gm.*	*gm.*	*mg.*	*mg.*	*µl.*	*µl.*	*µl.*	*µl.*	*days*	*gm.*	*gm.*	*mg.*	*mg.*	*µl.*	*µl.*	*µl.*	*µl.*	
2	14.5	−0.7	392	17.0	165	164	89	110	2	16.8	−6.1	429	16.2	122	118	80	73	RF
2	13.1	0.5	386	15.9	109	130	64	84	2	15.7	−1.4	417	18.2	145	112	78	74	LF
2	13.4	0.3	378	17.4	98	94	84	75	2	13.1	1.0	403	18.7	119	132	84	94	RF
3	20.5	0.4	402	16.1	141	136	88	89	3	19.7	−2.4	408	15.7	138	131	97	81	LF
3	21.1	−0.1	414	17.1	116	103	75	65	3	16.0	−0.9	374	15.9	111	95	75	57	LH
3	15.3	1.2	399	16.3	111	101	76	58	3	12.0	−0.2	416	16.4	108	—	76	—	LH
3	17.1	1.1	367	17.3	98	101	75	61	3	15.5	−2.2	356	18.9	98	101	66	58	BF
4	16.7	2.4	391	16.9	—	105	—	76	4	14.5	0.1	383	16.3	111	109	88	84	LF
4	13.0	−0.6	368	16.2	107	103	90	83	4	15.7	−1.0	400	16.9	33	111	98	97	LF
4	15.8	0.7	391	18.1	117	89	78	69	4	17.0	−4.5	378	16.1	103	97	72	76	LF
5	15.2	2.5	338	17.1	101	95	73	62	5	10.2	3.3	376	18.8	114	109	77	72	RF
5	15.6	2.4	398	17.1	112	96	75	72	5	14.0	−2.1	399	17.9	113	95	81	63	LH, LF
5	13.8	−0.1	327	19.1	106	94	69	69	5	18.6	−4.9	404	16.6	97	72	66	55	RH, RF
5	17.8	−0.1	322	21.3	114	89	75	70	5	14.8	−1.4	332	20.1	98	83	70	66	LH, LF
6	15.7	−2.1	376	17.9	98	86	69	57	6	19.5	−1.7	431	17.1	92	91	68	64	LF
6	19.5	0.6	415	16.6	110	94	74	63	6	19.3	−2.1	370	18.6	111	96	77	65	BF
6	19.6	−1.8	410	18.7	101	88	74	61	6	16.1	−1.2	419	17.5	106	81	69	53	BF
6	15.5	1.0	374	16.9	88	69	64	50	6	15.6	−0.6	393	16.3	89	84	67	59	LF
6	15.7	0.7	424	17.2	99	95	66	70	6	10.4	0.1	406	16.4	95	81	72	62	RH
6	—	—	414	19.2	111	92	80	68	6	14.9	0.4	415	17.0	105	110	70	75	LF
6	—	—	382	15.7	90	81	64	63	6	13.9	−1.4	413	19.0	105	86	80	63	LF
6	—	—	388	17.4	100	96	69	65	6	15.8	−1.3	403	18.3	100	89	70	58	RF
7	—	—	401	17.9	103	95	70	67	7	18.0	−3.9	429	18.0	100	101	66	66	LF
7	—	—	382	17.4	102	100	69	62	7	16.9	−1.4	419	19.8	98	89	68	63	LF
7	—	—	395	18.1	94	80	66	55	7	14.8	0.1	380	18.3	108	99	77	73	RF
7	—	—	380	15.4	102	103	68	64	7	14.5	−0.3	404	17.6	96	81	69	61	RF

served as a partial control for the experiment of Table I, and was a measure of the effect of inoculation in the absence of infection.

The experiment shown in Table III was designed to duplicate as closely as possible the experiments of Racker and Kabat. The same strain of mice (Swiss albino), concentration of brain suspension, salts, and substrate were used; the brain was minced rather than homogenized, and the nitrogen and carbon

dioxide mixture was not treated with copper to remove oxygen. The glucose concentration was 263 mg. per cent.

The anaerobic glycolysis of the brain is presented in two ways in the summarized results in Table IV: one on the basis of dry weight of brain, $Q_{CO_2}^{N_2}$, the other on the basis of wet weight of brain. The latter is the form used by Racker

TABLE IV

Summary of Data

	Experiment 1 (ABC mice, homogenate, virus)		Experiment 2 (ABC mice, homogenate, no virus)		Experiment 3 (Swiss albino mice, mince, virus)	
	Normal	Infected	Normal	Inoculated	Normal	Infected
Initial body weight, *gm*	14.6	14.3	14.4	14.5	16.3	15.5
Final body weight, *gm*	17.3	13.9	17.4	16.2	16.7	14.1
Increase in body weight, *gm*	2.7	−0.39	2.99	1.79	0.84	−1.5
Incubation time, *days*	5.8	5.9	5.5	5.5	4.8	4.8
Rate of body weight increase, *gm./day*	0.51	−0.10	0.55	0.36	0.15	−0.37
Brain weight, *mg*	410	405	412	409	385	398
Ratio brain to body weight at death, *mg./gm*	23.9	29.6	24.0	25.3	23.6	28.7
Dry weight of suspension, *mg./ml.*	10.34	10.10	10.32	10.26	17.4	17.6
Dry weight percentage of brain	21.7	21.2	21.7	21.5	19.1	19.3
Total dry weight of brain, *mg*	88.8	86.0	89.3	88.1	73.4	76.9
$Q_{CO_2}^{N_2}$, 1st hr., *μl./mg. dry weight*	4.12	4.26	4.05	4.10	5.45	5.36
$Q_{CO_2}^{N_2}$, 2nd hr., *μl./mg. dry weight*	3.35	3.44	3.06	3.16	3.75	3.74
$Q_{CO_2}^{N_2}$, difference 1st and 2nd hrs.	0.77	0.80	0.99	0.94	1.70	1.63
CO_2 output, 1st hr., *μl./100 mg. wet weight*	89	90	88	89	103	103
CO_2 output, 2nd hr., *μl./100 mg. wet weight*	73	73	66	68	71	72
CO_2 output, difference 1st and 2nd hrs	17	18	22	21	32	31
No. of animals	38	37	16	16	26	26

and Kabat. The calculations based on dry weight and wet weight conceivably might differ because of differences in moisture content of the brain.

In contrast to the results obtained by Racker and Kabat, no obvious difference in anaerobic glycolysis was found between normal and infected mice by either method of calculation. This was true whether homogenized brain was used from ABC mice, or the minced brain from Swiss albino mice with conditions the same as those of Racker and Kabat (1). They found that infection reduced the anaerobic glycolysis by 6.6 to 47.5 per cent, with an average of 16.5 per cent. In experiment 1 the average CO_2 output on a wet

weight basis in the 1st hour was 89 μl. for normal mice and 90 μl. for infected mice, it was the same for both, 73 μl., in the 2nd hour. With the Swiss albino mice the values for normal and infected mice were identical, 103 μl., in the 1st hour, and 71 μl. and 72 μl. respectively in the 2nd hour. The $Q_{CO_2}^{N_2}$ values show a somewhat greater variation: 4.12 for normal as compared to 4.26 for infected mice in experiment 1, and 5.45 compared to 5.36 for experiment 3. Considering the obvious difference in nutritional state of the infected and non-infected animals, this agreement was better than anticipated, however.

Animals in all stages of illness were tested; some had paralysis in only one limb and were very lively, others were paralyzed in two or more limbs, some were prostrate and obviously quite sick, and a few died as they were about to be sacrificed. Examination of the data from the individual mice (Tables I and III) shows that even in the cases of gross involvement there was no marked difference from the normal mice tested at the same time.

DISCUSSION

No difference in glycolysis between normal and infected brain is found when results are calculated on a wet weight basis. The small difference in $Q_{CO_2}^{N_2}$ is probably not significant. It is noted that the dry weight percentage of the brain varies inversely as the Q values in all the experimental series, as between normal and infected animals. The dry weight is lower and the Q is higher in experiment 1, while the reverse is true in experiment 3. Since the Q values are obtained by dividing the wet weight glycolysis, which is the same for normal and infected, by the dry weight per milliliter, which is different for normal and infected, it follows that the difference in Q values is entirely attributable to the difference in dry weight values. It is highly unlikely, however, that these changes in brain dry weight are fundamentally associated with poliomyelitis, for the dry weight is increased by infection in a series of animals of one strain, and decreased in a series of animals of another strain.

It is noted (Table IV) that the mean dry weight percentage of the brain of the Swiss mice is conspicuously lower than for the ABC mice. It was considered that the difference may have arisen because of differences involving the homogenate and mince. However, the same results were obtained when the procedure was reversed and dry weight was determined on the minced brain from ABC mice and homogenized brain from Swiss mice. The difference between the dry weight percentage of brain of Swiss and ABC mice is clearly not an artefact. It is not necessarily a strain difference but may result from different conditions and feeding methods of the two colonies.

In all investigations of metabolic activity of a tissue which are designed to throw light on the state of a specific enzyme system of that tissue, the problem of a standard of reference arises. What one would like is the activity per unit amount of "active" tissue—of tissue which is in some sense *relevant* to the en-

zyme system in question. Wet weight might give fallacious results because of changes in water content. The activity on a dry weight basis is likewise subject to error; for example, if there were loss of enzymatically inert material, such as a fat, but no loss of enzyme activity. Total nitrogen has sometimes been used as a standard but in many respects it is subject to the same limitations. In our case no difference between normal and infected mice was found in nitrogen content expressed as per cent of dry weight of the brain; glycolytic activity on a per cent nitrogen basis would therefore give the same results as on a dry weight basis.

The problem of standards is particularly important for comparison of the enzymes of diseased and normal animals, since here especially there may be alteration in the tissue components due to difference in nutritional state or to the disease itself.

In any case, it should be emphasized that in our data the difference in glycolysis between normal and infected brains is in no way comparable to that reported by Racker and Kabat. This is true even if the $Q_{CO_2}^{N_2}$ results are taken at face value and no consideration is given to dry weight change. The difference is a very small one, and in experiment 1 it is even in the opposite direction ($Q_{CO_2}^{N_2}$ increased with infection).

At present there is no obvious explanation for the discrepancy between our results and those of Racker and Kabat. Because of the variability of the material, it seems likely that reliable values will be obtained only with the rigorous controls employed in the foregoing series of experiments. Nickle and Kabat (2) point out that in their experiments the normal brains were not always studied at the same time as the infected ones. Also, they state, "Because of the small number of brains investigated and the wide variation in metabolic activity *in vitro* of both normal and infected brains, it would be hazardous to consider these observations conclusive."

The speculation that the virus of poliomyelitis has its effect on some enzyme system is not questioned. There are, however, a number of practical problems which from the standpoint of methodology should be considered in judging the reliability of the above results for determination of whether or not poliomyelitis affects the anaerobic glycolysis of the brain. The virus of poliomyelitis, as judged from histological evidence, produces the most extensive lesions in the anterior horn cells of the spinal cord. By methods so far derived the anaerobic glycolysis of the cord is so feeble that it would be difficult to demonstrate variations in it with any degree of reliability. The damage to the brain is usually minor, or at most, very spotty. Certainly a great majority of the cells of the whole brain are undamaged, at least as determined by histological methods. It is questionable whether the enzyme methods so far used in poliomyelitis studies are adequate to demonstrate the damage to such a small part of the total number of cells.

This conclusion is especially true because there is no assurance that the methods employed give a true measure of the enzymic content of the tissue. Geiger (5) has prepared extracts from brain which on an equivalent basis give $Q_{CO_2}^{N_2}$ equal to 50, whereas our homogenates and minces had $Q_{CO_2}^{N_2}$ equal to approximately 5. The implication is that only one-tenth the maximal enzyme activity is measured in brain minces. A comparison made with minces would not necessarily indicate a change in the glycolytic "capacity," but perhaps a change in the proportion of the total glycolytic activity that is measured in infected as compared to normal brain.

It is apparent that no decision can yet be made as to the effect of poliomyelitis on the anaerobic glycolysis of the nerve cells. Further studies are needed in which adequate methods of enzyme measurement are employed, and tissues are used in which there is demonstrated extensive involvement. The report by Victor and Huang (6) that Western encephalomyelitis reduces the anaerobic glycolysis of chick embryo is of interest, and the studies should be extended. Their results encourage the belief that a similar change may occur in poliomyelitis, and that present methods are at fault.

SUMMARY

The rate of anaerobic glycolysis of brain tissue was compared for normal animals and animals with experimentally induced poliomyelitis, using two different strains of mice and two different procedures. The report of interference of poliomyelitis with anaerobic glycolysis of brain was not confirmed. In one series there was a small increase and in the other series a small decrease in the brain $Q_{CO_2}^{N_2}$ calculated for infected animals as compared to normal animals. When the calculations were made on the basis of wet weight of brain there was no difference in glycolysis.

It is considered that the methods so far used for study of the enzymes may be inadequate, and that no decision is as yet possible on the effect of poliomyelitis on anaerobic glycolysis.

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