over P_2O_5 gave carbon 51.4, nitrogen 9.0 and phosphorous 0.87 per cent. By extraction with alcohol-ether (3–1) and reextraction of the lipids with petroleum ether,[5,6] the lipid fraction was 24 and the non-lipid fraction 77.76 per cent. of the material. The total lipid fraction was made up of 10.64 per cent. phospholipid, 5.67 per cent. cholesterol and 7.64 per cent. neutral fat. Of the total phosphorous, 52 per cent. were found in the lipid fraction. If the remainder of the phosphorous was present in nucleic acid, the virus would contain about 4.0 per cent. nucleic acid of the desoxypentose type.

The total carbohydrate[7] (as glucose) content of the whole virus was 10 per cent. The carbohydrate, apparently firmly bound in complex form, was greatly in excess of that expected for the amount of nucleic acid present. Subtracting this value from that of the non-lipid fraction, 77.76 per cent., the probable protein content of the virus was about 67.76 per cent.

A similar finding has been encountered with influenza virus B (Lee strain), in which the total carbohydrate was 9.3 per cent.

The infectious unit of the virus when inoculated in 0.05 ml volumes in chick embryos was $10^{-12.16}$ to $10^{-13.1}$ grams with an average of $10^{-12.74}$. One concentrate, purified by adsorption, elution and centrifugation and titrated in fivefold dilutions employing 40 embryos per dilution, gave the value $10^{-13.11}$ grams; another concentrate purified by ultracentrifugation alone and titrated similarly in 40 embryos per dilution gave $10^{-12.75}$ grams. The hemagglutinative activity of the concentrates was such that $10^{-6.16}$ to $10^{-6.46}$ grams with an average of $10^{-6.29}$ grams gave the 2 plus end point.

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[5] E. Kirk, *Jour. Biol. Chem.*, 106: 191, 1943.
[6] E. Kirk, I. H. Page and D. D. Van Slyke, *Jour. Biol. Chem.*, 106: 203, 1943.
[7] J. Tillmans and K. Philippi, *Biochemische Zeitschrift*, 256: 36, 1929.
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DECREASE IN LACTIC ACID CONTENT OF THE BRAIN IN POLIOMYELITIS[1]

RACKER and Kabat[2] demonstrated that anaerobic breakdown of glucose is significantly decreased in mouse brain infected with poliomyelitis virus, while oxidation of glucose by the infected tissue is apparently unimpaired. Brodie and Wortis[3] reported some determinations of the lactic acid content of brain and spinal cord in poliomyelitic monkeys. Their data indicate a decrease in lactic acid content of the brains infected with poliomyelitis as compared to normal monkey brains, although there were too few determinations to be conclusive. It therefore appeared of interest to determine the lactic acid content of the brain in mice infected with poliomyelitis.

Swiss albino mice four to six weeks of age were inoculated intracerebrally with a 10 per cent. suspension of tissue of the central nervous system from mice infected with the Lansing strain of poliomyelitis virus. When the animals showed definite paralysis, they were sacrificed by sudden immersion in a mixture of solid CO_2 and ether and left in the mixture for at least ten minutes. The brains were removed carefully using chilled instruments; the tissue was crushed and finely ground in a chilled mortar or tissue crusher and added to cold trichloroacetic acid in a tared glass-stoppered flask. The flask was reweighed and placed in an ice bath. After fifteen minutes, the solution was filtered. Lactic acid of the filtrate was determined by the Miller-Muntz method using the photoelectric colorimeter.[4] Lactic acid content of the normal mouse brain was determined by the same procedure. Three brains were pooled for each determination, giving a total wet weight of brain tissue of approximately one gram.

Data on lactic acid content of the brain in normal mice and in mice infected with the viruses of poliomyelitis and encephalitis are presented in Table I. The lactic acid content of the normal mouse brain is similar to the results reported by Stone,[5] who obtained a mean value of 18.9 mg per cent. and a range of 12–25 mg per cent. after freezing in liquid air. It is apparent that brain lactic acid is decreased in poliomyelitis, since the mean value is only 16.0 mg per cent. as compared to 20.7 mg per cent. for the normal controls, a decrease of 22.7 per cent. By use of the "t distribution,"[6] this difference has been found to be

[1] Aided by a grant from the National Foundation for Infantile Paralysis. Dr. Kabat is at present with the Division of Chemotherapy, National Institute of Health, Bethesda, Md.
[2] E. Racker and H. Kabat, *Jour. Exp. Med.*, 76: 579, 1942.
[3] M. Brodie and S. B. Wortis, *Arch. Neurol. and Psychiat.*, 32: 1159, 1934.
[4] R. H. Koenemann, *Jour. Biol. Chem.*, 135: 105, 1940.
[5] W. E. Stone, *Biochem. Jour.*, 32: 1908, 1938.

statistically significant. On the other hand, a preliminary study of brain lactic acid content in mice infected with encephalitis (3 determinations on Western equine encephalomyelitis and 2 on St. Louis encephalitis) showed no decrease from normal. A decrease in lactic acid content of the brain tissue in poliomyelitis is probably related to the decreased ability of the brain to break down glucose to lactic acid under anaerobic conditions demonstrated by Racker and Kabat.[2] That this effect of poliomyelitis may be specific and not produced by other neurotropic viruses is suggested by recent metabolic studies.[7]

TABLE 1

LACTIC ACID CONTENT OF THE BRAIN OF NORMAL, POLIOMYELITIC AND ENCEPHALITIC MICE*

	Normal	Poliomyelitis	Encephalitis	
			Western equine	St. Louis
	19.9	16.5	23.0	15.9
	16.9	17.9	34.0	21.0
	22.9	13.2	15.0	
	19.3	16.4		
	20.5	15.0		
	20.2	11.8		
	22.4	7.9		
	12.6	16.4		
	25.9	21.2		
	21.7	20.6		
	18.9			
	19.8			
	26.7			
	16.3			
No. of determinations	15	11	(both types) 5	
range	12.6–26.7	7.9 to 21.2	15.0–34.0	
mean	20.7	16.0	21.5	
t		3.04	0.43	

* All values in mg per 100 grams of brain tissue.

In another set of experiments, normal and poliomyelitic mice were decapitated and the heads kept at 37° C. for definite time intervals and then frozen in dry ice-ether mixtures. The progressive increase in lactic acid in the brain following decapitation is illustrated in Fig. 1. These results are preliminary, since each point on the curves represents an average of no no more than three determinations. The production of lactic acid under anaerobic conditions from substrates present in the brain tissue appears to be at least as rapid in poliomyelitis as in normal controls, with the possible exception of the period immediately following decapitation. Kerr and Ghantus[8] studied the production of lactic acid during autolysis in the dog brain and found that sugar disappears within 3 to 5 minutes and 80–85 per cent. of the glycogen is lost within 15 minutes. Even in the early stages of autolysis, however, glycogen breakdown provides a considerable portion of the lactic acid produced. Since the rate of formation of lactic acid in autolysis depends on the concentration of at least two substrates in the brain tissue, glucose and glycogen, as well as on many other factors, the experiment illustrated in Fig. 1 may not be considered evidence against a decreased anaerobic metabolism of glucose in the brain in poliomyelitis.

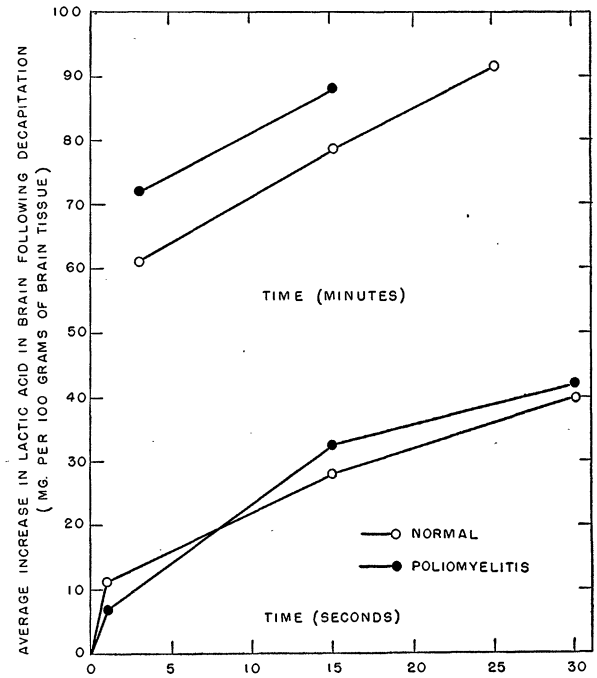

FIG. 1. Increase in lactic acid content of the brain of normal mice and of mice infected with the virus of poliomyelitis during postmortem autolysis. The abscissa for the lower curves represents seconds and for the upper curves represents minutes.

Another type of experiment is presented in Table 2. Mice were sacrificed by freezing in a dry ice-

TABLE 2

EFFECT ON BRAIN LACTIC ACID OF STORAGE OF MICE IN DRY ICE BOX

Time after death (hours)	Normal 20.7*	Poliomyelitis 16.0*
0		
3	22.4	....
3.5	29.5	....
5	41.5	....
6.5	31.9	....
12	33.4	16.9
24	53.1	13.8
48	30.2	22.7
72	40.3	....
80	31.0	12.1
120	30.3	20.7
No. of determinations	10	5
range	22.4 to 53.1	12.1 to 22.7
mean	34.4	17.2
t†	5.48	0.545

* Average.
† Compared to mean of determinations on unstored brain.

[6] F. E. Croxton and D. J. Cowden, "Applied General Statistics," Prentice-Hall, Inc., New York, 1940.

[7] M. Nickle and H. Kabat. Unpublished observations.

[8] S. E. Kerr and M. Ghantus, *Jour. Biol. Chem.*, 117: 219, 1937.

ether mixture and then stored for various periods of time in a dry ice box before removal of the brains and analysis for lactic acid. There is a statistically significant though variable increase in lactic acid content in the stored normal brain while no significant change was noted in the stored poliomyelitic brain. This experiment requires confirmation before speculation on the mechanism would be advisable.

Summary: Lactic acid content of the brain is significantly decreased in mice infected with the virus of poliomyelitis. This appears to be additional evidence for the view that the virus may interfere in a specific manner with cell metabolism.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A RAPID IRON HEMATOXYLIN TISSUE STAIN FOR ROUTINE LABORATORY USE

THE Heidenhain iron hematoxylin method for tissue staining is one of the most important and satisfactory cytological stains for the demonstration of fine nuclear and cellular detail. Its use as a routine stain would be highly desired. However, the length and relatively difficult technique of the iron hematoxylin stain restricts this method to the more or less special, non-routine procedures. Although it is possible to stain tissues with Heidenhain iron hematoxylin in as little as one to two hours, the most satisfactory results require from twelve hours to two days (Guyer,[1] Bensley and Bensley[2]). In view of the desirability of the use of this stain routinely, we have attempted to find a suitable modification whereby the technique might be shortened considerably and made more adaptable for common laboratory use. By incorporating a single change in the usual procedure, to be described below, the time necessary for staining the tissue is shortened to even less than that entailed for Delafield's or Harris's hematoxylin stain.

The modification simply consists in fixing and mordanting the tissue simultaneously in Bouin's fixative containing one and a half grams of ferric alum (Ferric ammonium sulphate) in 100 cc of fixative. The Bouin's acts as the fixative and the ferric alum functions as the mordant. The ferric alum does not impair the excellent fixative qualities of Bouin's, while Bouin's does not interfere with the mordanting of the tissue by ferric alum. The combined fixative and mordant has no deleterious effect upon the tissue.

After removal of the tissue from the combined fixative-mordant, it is prepared for mounting and staining by following the procedure usually employed for hematoxylin stains. Either ethyl alcohol or dioxan may be used for the dehydrating agent and 0.5 per cent. hematoxylin to stain. Inasmuch as mordanting has already been accomplished during fixation, the sectioned tissue may be placed directly into the hematoxylin after passing the mounted sections from xylol to water, using either dioxan or alcohol in the intermediate steps. The tissue will overstain in from 3 to 5 minutes and destaining may be carried out as desired in 0.1 per cent. HCl (aqueous or 35 per cent. alcoholic solution). The acid destain is followed by an alkaline aqueous or alcoholic wash prepared by the addition of several cc of a saturated solution of lithium carbonate or 1 per cent. solution of sodium bicarbonate to 100 cc of water or 35 per cent. alcohol, respectively.

The modification of the accepted iron hematoxylin procedure has been used successfully on many different types of tissues and the resulting cytological picture is comparable to that observed after use of the lengthy Heidenhain iron hematoxylin method. There are, however, two cautions worthy of note: One is that although this technique is very adaptable for small pieces of tissues (5 to 6 mm cubed), larger pieces of tissue require longer fixation. This is to allow for sufficient penetration of the ferric alum to complete the mordanting process. For the smaller pieces of tissue, the combined fixative-mordanting process is completed by the time the tissue would be normally fixed when placed in the fixative alone. The second point is that in the process of staining the slides it is important that the transfer of tissue from dioxan or alcohol to the dye should be accomplished as rapidly as possible. If the tissue stays in the intermittent water wash for too long a period of time the ferric alum may be completely removed from the tissue and remordanting will be necessary.

Summary: A modification of the standard Heidenhain iron hematoxylin stain is described in which the process of fixation (in Bouin's) and mordanting (ferric alum) are executed simultaneously instead of independently. Consequently, mordanting just prior to

[1] M. F. Guyer, "Animal Micrology," University of Chicago Press, Chicago, 1936.

[2] R. R. Bensley and S. H. Bensley, "Handbook of Histological and Cytological Technique," University of Chicago Press, Chicago, 1938.