TABLE I.

				No. 1-K		No. 2-M		No. 3-G	
	1	2	3	Control	After oxygen	Control	After oxygen	Control	After oxygen
Cord—Vol. %									
Vein—O_2 content	3.53	10.4	10.5	6.13	8.57	6.8	12.6	12.79	14.73
Saturation %			50.5	30.8	43.1	37.0	69.0	64.3	74.0
CO_2 content			45.9	46.53	43.10	44.55	41.3	42.2	39.10
Artery—O_2 content	0.94	3.40	3.3	2.14	2.83	2.6			
Saturation %			15.8	10.8	14.2	14.0			
CO_2 content				50.13	48.55	50.1			
O_2 capacity			20.8	19.85		18.2		19.9	
Maternal—Vol. %									
Vein—O_2 content			11.0	8.51		10.4		8.10	10.40
Saturation %			71.3	70.7		70.0		54.0	68.5
CO_2 content			43.3	46.04		40.7		40.52	39.24
O_2 capacity			15.4	12.03		14.9		14.8	

Haselhorst and Stromberger—2. Average vaginal. 1. Average cesarean delivery. Eastman—3. Average vaginal delivery.

stripped and clamped close to the fetus. This technic ensures a normal fetal blood volume. The blood for oxygen and carbon dioxide content is obtained with oiled syringes using 22-gauge needles. The usual amount of neutral potassium oxalate is used. The blood samples are kept in an ice bath and the analyses are completed as quickly as possible.

Data for the oxygen content of fetal blood before and after administration of 100% oxygen to the mother for five minutes are given in Table I and demonstrate the practicability of the method. Appreciable increases in the oxygen content are noted in the umbilical vein blood (arterial). The 3 patients were not in labor.

Data for the CO_2 content are also given. The Van Slyke and Neill constant volume apparatus was used. The oxygen capacity was calculated from the hemoglobin which was determined by the carbon monoxide method of Palmer. Eleven patients have been studied. Every experiment cannot always be completed; hemostasis of the uterine wall is at times impossible, the cord may become compressed, cord blood clots rapidly, etc.

Summary. A method is described by which repeated samples of human cord blood can be obtained over a short period of time. Undue prolongation increases the risk of the operation. The oxygen content of fetal blood is appreciably increased by the administration of oxygen to the mother.

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Phosphatase Activity in Chromatolytic Nerve Cells.*

DAVID BODIAN AND ROBERT C. MELLORS. (Introduced by H. A. Howe.)

From the Poliomyelitis Research Center, Department of Epidemiology, School of Hygiene and Public Health, Johns Hopkins University.

In nerve cells with axons extending into peripheral nerves, it is possible by simple nerve section to produce profound alterations in cytoplasmic structure and activities without causing cell destruction. These changes are fairly constant in their time course when pro-

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duced in this manner, and are generally completely reversible, so that they may be studied at any given time of the cycle in a readily controllable fashion. Such an experimental preparation therefore lends itself not only to study of a fundamental neuropathological process but also to analysis of controllable changes in the basic physiological properties of the cell.

As a consequence of axon section there is initiated a series of morphological changes in the cell body. These retrograde changes, the "axon reaction," reach a climax after one or 2 weeks, when active axon regeneration is in process. The cellular reaction as seen in stained histological sections was first described by Nissl in 1892 and is commonly known as "chromatolysis," since the most obvious visible change is a loss of chromophilic or stainable material in the cytoplasm. This chromophilic material is a nucleoprotein of the ribose type, and its degradation and resynthesis in the chromatolytic cycle has recently been studied with the quantitative histochemical method of Caspersson[1] by Gersh and Bodian.[2] The important role of enzymatic activity in these cellular reactions suggested that demonstrable changes in phosphatase activity could be found associated with the considerable turnover of nucleoprotein, and of chemical energy, in the chromatolytic process. The development of a histochemical method for phosphatase determination by Takamatsu[3] and by Gomori,[4] and the finding of demonstrable acid phosphatase activity in the cytoplasm of normal nerve cells with this method has led us to a study of the changes in, and localization of, phosphatase activity during the chromatolytic cycle. These studies are still in progress, but our early findings may be of sufficient general interest to merit a preliminary report.

The details of our methods have been modified from those published by Wolf. Kabat, and Newman[5] for the study of acid phosphatase activity. The procedure consists essentially in the incubation for 5 to 24 hours of paraffin sections of acetone-fixed spinal cord in a buffered bath containing a phosphate ester and lead nitrate. Inorganic phosphate released by enzyme activity is precipitated *in situ* by the lead reagent, and a dark lead sulfide is subsequently formed by treating the sections with dilute ammonium sulfide. The sites of phosphatase activity thus appear as brown-to-black "stained" areas in the microscopic section. Control sections in which phosphatase activity has been inhibited by dilute sodium fluoride in the incubating mixture show very little or no staining. Other control sections give excellent staining with toluidin blue and gallocyanin and permit a quite satisfactory comparison of the degree of chromatolysis as visualized with these standard histological methods.

A high degree of consistency of staining throughout the block of spinal cord from which paraffin sections were made was obtained by vascular perfusion of the fixative (chilled acetone) through the anesthetized animal. Chromatolysis was studied in the anterior horn cells of rhesus monkeys in which the sciatic nerve on one side had previously been sectioned. Nerve cells of the sciatic nerve groups on the opposite side of the same spinal cord sections served as normal controls. By varying the time of incubation in the substrate mixture an optimum degree of "staining" could be obtained for comparison of chromatolytic and normal nerve cells.

The substrates used which gave most satisfactory results were sodium glycerophosphate, adenylic acid, a mixture of pentose nucleotides from ribonucleic acid, and nucleic acid. Wolf. Kabat, and Newman[5] have shown that phosphatase activity at pH 5.0 is present in the cytoplasm of nerve cells, whereas at pH 9.0 it is absent in this site but present in mesodermal structures such as blood vessels. Consequently, most of our experiments were carried out on incubating solutions buffered at pH 5.0. When difficulty was encountered in preparing the incubating solution at pH

1 Caspersson, T., *Skand. Arch. f. Physiol.*, 1936, **73** (Suppl.), 1.

2 Gersh, I., and Bodian, D., *J. Cell. and Comp. Physiol.*, 1943, **21**, 253.

3 Takamatsu, H., *Tr. soc. path. Jap.*, 1939, **29**, 492.

4 Gomori, G., PROC. SOC. EXP. BIOL. AND MED., 1939, **43**, 23.

5 Wolf, A., Kabat, E. A., and Newman, W., *Am. J. Path.*, 1943, **19**, 423.

5.0, such as when nucleic acid was used as a substrate, the substrate mixture was buffered at pH 7.0. At this pH level phosphatase activity characteristic of both the acid and alkaline ranges occurred, with staining of neurones as well as of blood vessels.

The results obtained gave unequivocal and precise information under the conditions described above, and it is possible at the present time to summarize the changes in phosphatase activity occurring in the chromatolytic cycle from the 1st to the 46th day after nerve section. With standard histological methods the degradation of Nissl substance in our material is first clearly visible at 3 days and reaches its height at 10 days. Following this period, restitution of the Nissl material occurs progressively. This regeneration is clearly apparent at 3 weeks and is well advanced at 46 days, the longest interval which we have thus far studied with the phosphatase method. It is known that complete restoration of Nissl bodies occurs in most cells after several months, although a small number of cells may fail to recover.

A marked increase in phosphatase activity occurs in the regions of the cytoplasm of chromatolytic nerve cells where Nissl material is deficient. This increase is roughly parallel in onset with earliest histological changes and is very marked at the height of chromatolysis, precisely in the region of the cytoplasm in which Nissl bodies can no longer be seen in the control sections stained with toluidin blue. Moreover, at 24 days when restitution of Nissl bodies, and presumably increased synthesis of ribonucleotides, is well under way, a marked increase of phosphatase activity still persists in chromatolytic regions. After 46 days, when many cells show well advanced restoration of Nissl bodies, the phosphatase activity in these cells is only slightly increased above normal. At this stage, cells which have lagged behind in recovery show greater phosphatase activity, and some cells which have achieved far advanced recovery of Nissl bodies show little if any increase of phosphatase activity over normal levels. It is thus likely that the phosphatase activity in chromatolytic cells is proportional to the degree of chromatolysis, during both the period of decrease and of restitution of Nissl substance. The analysis will be continued into later stages of the restitution cycle up to the period of complete morphological recovery and functional axon regeneration.

In general, similar results were obtained with the several substrates employed. The increase of phosphatase activity obtained when nucleic acid is used is a measure also of concomitant ribonuclease activity in chromatolytic cytoplasm, since phosphatase activity alone would be incapable of releasing free phosphate from nucleic acid. No increase of phosphatase activity was found in the nuclei at any stage of chromatolysis. This serves to confirm the findings of Gersh and Bodian[2] that the nucleus plays little if any role in the replacement of Nissl substance.

Previous indicators of physiological and pathological changes in the nerve cell have been alterations of stainable Nissl substance studied by staining methods or the ultraviolet absorption method. Changes in the quantity of nucleoprotein material within the cell as determined by these methods can now be supplemented by a simple histochemical enzyme analysis, so that observed changes in phosphatase and ribonuclease activity can be correlated with changes in stainable nucleoprotein material. It is hoped that the unique opportunity of interpreting nucleoprotein turnover in terms of localizable intracellular biochemical kinetics, will contribute to the analysis of the dynamic equilibrium of nucleoprotein synthesis and of energy utilization in the cell.

Summary. Markedly increased acid phosphatase activity has been found in regions of the cytoplasm of nerve cells in which chromatolysis has been produced by axon section. This increased activity is proportional to the degree of chromatolysis, and is thus probably associated with increased nucleoprotein synthesis or degradation.