# Influence of Lansing Virus Infection on Phosphorus Metabolism of Monkey Brain Tissue.* (17789)

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Recently there has been considerable interest in studies on the influence of viral growth on the metabolism of the host cell. Studies on the origin of phosphorus found in the bacteriophage virus particles(1,2), and studies on the influence of viral growth on the anaerobic glycolytic metabolic cycle of host cells(3,4,5), have been done using $P^{32}$ as the tracer substance. Unfortunately, apart from the bacteriophage-bacterial cell studies, most of these investigations have been done *in vitro* using minced tissue preparations as culture media for murine strains of virus. As the transfer of phosphorus as phosphate ($^{-}PO_4$) appears to be of great importance in the transfer of energy, both in the synthesis of nucleoprotein and in the metabolism of carbohydrate, it seemed advisable to study the metabolism of phosphorus in the intact animal during the development of the disease poliomyelitis. It was felt that determination of the amount of inorganic and total acid soluble organic phosphates in brain tissue and the rate of turnover of $P^{32}$ into these phosphate fractions would provide information on the influence of a poliomyelitis virus on the metabolism of brain tissues.

*Method.* For these experiments, young conditioned healthy rhesus monkeys were given a single intracerebral inoculation of Lansing poliomyelitis virus (Poliomyelitis Standard Commission). Both control and virus inoculated animals were then sacrificed at 12, 24, 48, 72, 96, 120, 144, 168, and 192 hours following inoculation. Fifty minutes before sacrificing, about one millicurie of $P^{32}$ was injected intravenously in the form of an inorganic salt ($NaH_2PO_4$). Animals were sacrificed by bleeding from the neck. The brain, brain stem and cord were then removed rapidly and aseptically. Adjacent transverse slices from the medulla, pons, thalamus, spinal cord and cerebellum were obtained for (1) analysis of the content of acid soluble phosphate compounds, (2) determination of the specific activity of these phosphate compounds, (3) pathology, and (4) viral assay.

*Results.* These experiments demonstrate that associated with the development of pathologic changes and growth of the virus in the various nerve tissues studied, there were significant changes in the rate of turnover of phosphorus ($P^{32}$) in the inorganic phosphate fractions (IP) and in the total acid soluble organic phosphate fractions (TASOP). Table I presents the changes in $P^{32}$ turnover rate of the various phosphate fractions in the virus inoculated animals as compared with the phosphate fractions obtained from tissues of control animals. The presence of virus, the presence of significant pathology and symptomatology are indicated in the table.

There appeared to be no significant change in the $P^{32}$ turnover rate in the phosphate fractions obtained from any of the brain stem tissues of animals that were sacrificed at or before 72 hours following inoculation of the virus. At 96 hours, however, significant increases in the $P^{32}$ turnover rate in the IP and the TASOP fractions were evident. These were first most striking in the medulla and in the pons tissues. Significant early pathology and evidence of viral growth were pres-

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TABLE I.

Change in the Rate of Phosphorus Uptake Into the Inorganic and Total Acid Soluble Organic Phosphate Fractions Prepared from Poliomyelitis (Lansing) Infected Monkey Tissues.

| Time of sacrifice after virus inoc., hr | No. monkeys | % change in specific activity of phosphate fractions above controls | | | | | | | | Pathology | Virus | Symptoms |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Medulla | | Pons | | Spinal cord | | Cerebellum | | | | |
| | | IP | ASOP | IP | ASOP | IP | ASOP | IP | ASOP | | | |
| 12, 24 | 4* | 25 | 30 | 2 | 0 | —30 | —40 | —20 | 32 | 0 | T† | None |
| 48, 72 | | | | | | | | | | | | |
| 96 | 3 | 100 | 180 | 70 | 120 | 130 | 0 | —40 | —10 | + | P, M | " |
| 120 | 3 | 420 | 350 | 250 | 260 | 140 | 160 | 60 | 80 | ++ | P, M | Tremors |
| | | | | | | | | | | | | Weakness |
| 192 | 3 | 196 | 195 | 220 | 115 | 426 | 403 | 160 | 156 | + | | Paralysis |

T, P, M = Virus present in thalamus, pons, or medulla.
IP = Inorganic phosphate.
TASOP = Total acid soluble organic phosphate.
* One monkey at each indicated time.
† Virus in thalamus only of 12 hr animal.

ent in these two tissues particularly by this time. At 120 hours after virus inoculation, when early symptomatology was manifesting itself, a most striking increase in the $P^{32}$ turnover rate in the phosphate fractions obtained from the medulla, pons, and spinal cord was now observed. At this time, only tremors and slight weakness of the extremities was noted. By 192 hours, when advancing paralysis and increasing weakness were now evident, the changes in the phosphate fractions from the medulla and the pons seemed to decrease, but the fractions obtained from the spinal cord continued to show increases in $P^{32}$ turnover rates. The fractions obtained from the cerebellar tissues now also showed significant increases.

A comparison of the $P^{32}$ turnover rates in the phosphate fractions obtained from control and from experimental animals is justified on the basis of the fact that the diffusion curves of $P^{32}$ from blood to spinal fluid was almost the same for control animals as for the inoculated animals. Further, the mean concentration of the specific activity in the blood and in the spinal fluid of control animals as compared with the blood and spinal fluid of inoculated animals was practically the same at the time of sacrifice. The relationship between blood and spinal fluid specific activity was 1:2.6 for control animals and 1:2.5 for the inoculated animals.

*Discussion.* It would appear from these studies that coincident with the early pathologic changes produced by a Lansing poliomyelitis virus in monkey nerve tissues, there is a marked increase in the turnover of $P^{32}$ in the IP and the TASOP fractions of brain stem and spinal cord tissues. An increase in the rate of turnover of $P^{32}$ in the IP fractions in these virus infected parts may be due to cell damage and associated changes in permeability of the tissues to phosphorus. However, the concomitant and equal increases in the $P^{32}$ turnover in the TASOP fractions must be related to an increased metabolic activity within the cell. Whether this increased metabolism is due to an increased glycolytic metabolism or to increased protoplasmic synthesis (including the synthesis of the virus particles) remains to be studied.

It is of interest to note that the increase in metabolic activity was present first in the medulla, second in the pons, and third in the spinal cord. This sequence parallels the usual sequence of development of pathology and increased viral concentration in the intracerebrally inoculated animal.

*Summary.* Coincident with the development of viral growth and earliest pathology in the brain stem of Lansing poliomyelitis virus inoculated monkeys, there appears to be a marked increase in the metabolic activity of these tissues as regards the phosphate ion. This increase in metabolic activity of the affected brain stem tissues and spinal cord continues to increase during and throughout the period when clinical evidence of weakness, paralysis, and neurologic signs of clinical poliomyelitis exist.

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