Miss O'Hara

INTERNATIONAL LEAD ZINC RESEARCH ORGANIZATION, INC.

292 MADISON AVENUE, NEW YORK, N.Y. 10017
TELEPHONE 679-8080 (AREA CODE 212)
CABLE ADDRESS: INTLZINC NEW YORK

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To: All Members of the Lead Environmental Health Committee

Re: Progress Report on LE-117 "Effects of Lead on Tissue Culture of the Central Nervous System"

Attached for your information is a copy of the report which covers the work done for us by Dr. Sobkovics at Columbia. I think you will find this quite interesting and that it opens up some important avenues for future investigation.

Dr. Sobkovics will prepare an article for publication in a medical journal for some future date. This article will, of course, be sent to us for review before it is published.

Very truly yours,

Don O. Fowler
Project Manager

DOF:sv
enclosure

PROGRESS REPORT

Project No: LH-117 — "Effects of Lead on Tissue Culture of the Central Nervous System"

Principal Investigator: Dr. Margaret R. Murray
Professor of Anatomy
Laboratory for Cell Physiology
Columbia University
College of Physicians and Surgeons

Advisor: Dr. Leonard J. Goldwater
Professor of Occupational Medicine
School of Public Health and Administrative Medicine
Columbia University

Project Director: Dr. Hanna M. Sobkowicz
Assistant Professor of Neurology
Tissue Culture Laboratory of the Dept. of Neurology
University of Wisconsin Medical School

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An investigation of the direct action of lead compounds upon Nervous Tissues in Culture was performed. The method of tissue culture was chosen for the study because of two main advantages:

1. The portion of the nervous system under study can be exposed directly to the experimental agent without the agent having been metabolised by other organs.

2. The blood-brain-barrier with its filtration function can be by-passed. Thus through the isolation of cultured tissue from the whole organism, secondary effects on the nervous system from poisoning of other organs can be eliminated. The long-term cultures from several areas of the nervous system maintain their cellular components with essentially normal structure and function for considerable periods (weeks, months). The cultures of central and peripheral nervous system were obtained from new born mouse and rat cerebellum and cerebral cortex, and from embryonic mouse spinal cord and sensory ganglia.

The study on the influence of lead upon the nervous system is concerned with three related but different problems considered separately as Parts : I, II, and III.

I. "Influence of Lead Acetate on the Development and Maintenance of Nervous Tissue in Culture." (H.M. Sobkowicz, M.R. Murray, L.J. Goldwater, E.R. Peterson and M.B. Bornstein)

For this study 417 cultures of newborn rat and mouse cerebellum and spinal cord and spinal ganglia were exposed to the heavy metal for periods of from 1 to 3 weeks. The influence of lead acetate in concentrations of from 10^{-7}M (0.037 γ/ml) to 10^{-3}M (379.00 γ/ml) of feeding solution was studied for its effect on myelin maintenance in mature cultures and on the pattern of development of young cultures compared with those where no heavy metal was present.

We found no evidence of primary morphological changes in nerve cell bodies, axons or myelin sheath in cultures exposed to lead acetate in vitro, even in high concentration (37000 γ/ml of nutrient medium up to one week of exposure). There was however a constant and marked degenerative reaction of macrophages followed by hypertrophy of glial cells. Similar results were obtained during preliminary exposures of cultures to triethyl tin sulfate and gold chloride. Cultures exposed to mercuric chloride (and thallium chloride and acetate - E.R. Peterson, M.R. Murray - 1965) showed evidence of direct toxic action by these metals on the nerve cell bodies or their axons, with secondary changes in the myelin sheath.

The above reactions of cultured nervous tissue seem to divide these metals into two groups: 1.- Those which cause a direct morphological injury to the nerve cell bodies or axons (mercuric chloride, thallium chloride and acetate) and, 2.- Those which provoke morphological changes primarily in cells of mesodermal origin and only secondarily in neuroglia (lead acetate, triethyl tin sulfate and gold chloride). Of the metals which were directly toxic to the nerve cell, the degree of toxicity was dependent on the concentration. The action of metals which did not produce direct morphological response in the nerve cell was dependent mostly on the duration of exposure. Also the degeneration of ecto-and mesodermal supporting tissue was common to this group of metals. Lead, which was tested most thoroughly, even facilitated the development and differentiation of nervous tissue in young cultures, although the mechanism and meaning of this observation is not clear to us.

II. "Absorption of Lead Acetate by Nervous Tissue in Culture" (H.M. Sobkowicz, E.R. Peterson, M.R. Murray, L.J. Goldwater)
The lack of primary morphological changes in nerve cells, their axons and myelin sheaths in cultures exposed to lead acetate, as well as the normal pattern of the development and myelination of exposed young cultures (4 - 6 days In Vitro) was rather intriguing to us. Using the histochemical staining technique of rhodizonic acid for the presence of lead we started a systematic study of the uptake of lead acetate from the feeding solution by nervous tissues In Vitro.

The mature myelinated cultures of new born rat and mouse cerebellum and embryo mouse spinal cord and spinal ganglia were exposed to 50 γ/ml of lead acetate in the feeding solution. As the results of our study we showed that there is no evidence of the absorption of lead by the nerve cell In Vitro from lead-containing medium. A heavy uptake of lead is however observed in macrophages, meningeal cells and to a less extent by the glial cells. Therefore it is strongly suggested that the toxicological responses of nervous tissue cultures exposed to lead acetate correspond to the histochemical pattern of absorption of lead by these cultures, as we observe it. The lack of evidence of lead uptake from the nutrient medium by the neurons could explain in fact the rather surprising phenomenon of the lack of toxicity of this metal to the nerve cell and fibre and the myelin sheath. On the other hand the cells which were involved in the process of lead absorption from the nutrient medium showed both toxic reaction and morphological damage during the time of exposure. The appearance of hypertrophied glial cells as observed during repeated experiments might be explained as a secondary effect resulting from the degeneration of macrophages and their possible liberation of more complex, organic compounds. The rather insignificant degree of lead absorption by the glial cells, as shown histochemically, does not seem to justify their marked involvement in the pathological process as it is observed in vitro.

III. "Influence of Serum from Laboratory Animals and Patients with Lead Poisoning on Nervous Tissue in Culture". (H.M. Bobkowicz, M.R. Murray, L.J. Goldwater E.R. Peterson and M.B. Bornstein).

Looking for a different approach to the problem of lead poisoning in vitro we tested sera obtained from laboratory animals and patients with lead poisoning, against mature myelinated cultures of the nervous system. The exposure of the cultures to these sera resulted in partial or complete degeneration and breakdown of the myelin sheath, which in most instances was accompanied by complete preservation of the nerve cell bodies and axons, but not of the glial cells (which are equally involved in myelin maintenance). We also demonstrated that the lysis and degeneration of central and peripheral myelin in response to the presence of the above sera is not correlated to the actual concentration of lead in these sera. Therefore it is possible that the observed myelin disturbance was not due to the actual presence of lead but rather to other substances secondarily present in these sera. Our conclusions therefore are summarized as follows:

1. There is evidence in vitro of central and peripheral myelin degeneration and lysis in response to the presence of serum from lead poisoned patients and laboratory animals.

2. There is evidence that the degeneration of the myelin sheath observed during exposure to lead poisoned sera in vitro was not related quantitatively to the actual concentrations of lead in these sera. Thus it is possible that the observed myelin disturbances were not due to the presence of the lead but rather to other disturbances secondarily operating. (cf. Part I).

3. Lead itself has no direct toxic effect on the nerve cell and the myelin sheath in vitro. This may be due to the demonstrated lack of uptake of the metal by the nerve cell from its environment. (cf. Part II)

4. It is also suggested that the excessive absorption of lead by mesenchymal tissue results in the degeneration of this tissue. (cf. Part II)

5. A secondary response of the glia due to the degeneration of mesenchymal tissue, as well as its moderate ability to absorb lead in vitro suggests the involvement of this tissue in the pathology of lead poisoning in vitro. (cf. Parts I and II)

6. The hypothesis is proposed that the direct toxicity of lead to the nervous system in vitro is correlable with assimilation of the metal by its various component tissues.