Pediat. Res. *10*: 67–70 (1976)

Adenosine deaminase deficiency
fetus
liver
severe combined immunodeficiency (SCID)
thymus

Hereditary Severe Combined Immunodeficiency and Adenosine Deaminase Deficiency

CORNELIA ACKERET, H. J. PLÜSS, AND W. H. HITZIG[84]

Department of Paediatrics, University of Zürich, Zürich, Switzerland

Extract

A retrospective study aiming at detection of heterozygous carriers of blood adenosine deaminase (ADA) deficiency was carried out in nine families known to us because children had died of combined immunodeficiency (SCID). The trait was found in 3 of 9 parent couples, and in 14 other relatives. In two families one homozygous patient was identified. A total of 54 family members and 60 healthy control subjects were tested. Clinically, the patients were all characterized by marked lymphopenia, nearly normal immunoglobulin levels, and inability to produce antibodies. One homozygous patient recovered after transplantation of fetal liver and thymus and is immunologically normal 1.5 years afterwards.

Speculation

A cause-effect relation between ADA deficiency and SCID is likely (8, 15, 30). *In vitro* the accumulation of toxic metabolites as a consequence of the enzyme deficiency inhibits division and/or function of lymphoid cells. *In vivo*, the same mechanism might lead to SCID by impairing the lymphoid cells. In fact, clinical, immunologic and histologic findings are suggestive of a progressive destruction of lymphatic organs (14). Therefore, this particular kind of SCID is probably not caused by a primary defect of the lymphoid stem cell.

During the last two decades an increasing number of different diseases with permanent insufficiency of immune mechanisms have been identified and usually classified, as recommended by a WHO committee, on the basis of the T- and B-cell concept (7). An entirely new possibility, *i.e.*, a biochemical approach, was raised by the detection of adenosine deaminase (ADA) deficiency (5) in the erythrocytes, lymphocytes, and serum of patients with combined immunodeficiency (SCID). The lack of this enzyme is transmitted as an autosomal recessive trait from heterozygous "healthy" parents to a homozygous and mortally sick child. Because the ADA assay is sensitive enough to detect heterozygous carries (27), it was possible to reinvestigate the families of a number of our patients who had died of SCID (12, 13, 28). Among nine families, three parent couples had significantly reduced ADA activity. Two of these had each one living infant with SCID, who indeed had practically absent ADA activity.

METHODS

ADA activity was measured in hemolyzed erythrocytes (duplicate), in serum and in lymphocytes (single measurements) by a spectrophotometric method (6, 15, 18) using a Zeiss PM Q II spectrophotometer with thermostatized quartz cuvettes (1 cm depth) and automatic recording.

ADA ACTIVITY IN HEMOLYZED ERYTHROCYTES (15)

Fifty microliters of sedimented erythrocytes (three times washed with saline) were suspended in 15 ml 0.05 M phosphate buffer solution, pH 7.5, and sonicated (Braun Sonic 300; 270 W) for 5 sec. To 3 ml hemolysate 5 μl xanthinoxydase (Böhringer) were added and allowed to equilibrate for 15 min at 37°. The reaction was initiated by adding 75 μl adenosine solution (1 mg/ml); immediately the increase in extinction due to formation of uric acid was measured at 293 nm for 10 min at 37°. The enzyme activity is expressed as μmol adenosine deaminated/min/mmol Hb/liter.

ADA ACTIVITY IN SERUM (6, 18)

Goldberg's method was slightly modified, the decrease of extinction being measured at 265 nm for 30 min at 37°. One hundred microliters of serum + 3 ml phosphate buffer, 0.05 M, pH 7.5, were equilibrated for 15 min at 37°. Then 75 μl adenosine solution (1 mg/ml) were added, and the extinction was measured for 30 min. Calculation is based on the molar extinction coefficient of 9.1 $cm^2/\mu mol$, and the enzyme activity is expressed as micromoles of adenosine deaminated per min per liter of serum (18).

ADA ACTIVITY IN SONICATED LYMPHOCYTES (18)

Lymphocytes are separated over a Ficoll-Hypaque gradient (26), washed three times with normal saline, and counted; a suspension containing 130,000 cells/ml was prepared with phosphate buffer 0.05 M, pH 7.5, and sonicated with 240 W for 30 sec. Three milliliters of the sonicated debris were again equilibrated at 37°. The enzyme reaction was started by adding 75 μl adenosine solution, and the decrease of the extinction at 265 nm was measured for 30 min at 37°. Enzyme activity was calculated, using the molar extinction coefficient of 8.1 $cm^2\mu mol$, and activity is given as micromoles of adenosine deaminated per min per 10^6 lymphocytes.

PROBANDS AND PATIENTS

A control group of 30 male and 30 female healthy persons (laboratory and hospital personnel, aged 19–54 years) provided normal values. Nine pairs of parents of affected children were available for study. The relatives of the three parent couples with intermediate ADA deficiency were tested, a total of 22 persons in *family B* (Figs. 1 and 4), 20 in *family K* (Figs. 2 and 4), and of 12 in *family Sch* (Figs. 3 and 4), *i.e.*, 54 people, including 2 children with SCID.

RESULTS

Normal ADA values with 2 SD (30 males, 30 females) and standard error of the mean as calculated from multiple measurements of the same specimen are given in Table 1. Values for the same person determined on different days remained very stable. ADA activities of all family members are charted in Figure 4.

FAMILY B

This family living in a remote mountain valley of southeastern Switzerland, has lost three boys to SCID at ages 6, 9, and 15

Table 1. *Adenosine deaminase in normal adults*[1]

	Erythrocytes μmol ADA/ mMol Hb/ liter/min	Serum μmol ADA/ liter/min	Lymphocytes μmol ADA/ min/10^6 cells
Males	74.5 ± 33.6	3.36 ± 2.3	1.31 ± 0.96
Females	65.4 ± 24.3	3.00 ± 1.6	1.32 ± 0.76
SE of mean	6.46	0.31	0.29

[1] Thirty males; 30 females. Mean ± 2 SD.

months; these cases have been published (14). The surviving two siblings are healthy. Multiple consanguinity of their parents was detected by interviewing family members and certified by inquiries in the official church books and civil state registers. One of the surviving siblings, both parents, three siblings of the parents, the maternal grandmother, and one brother of the paternal grandfather were identified as heterozygous carriers. The probable gene track is outlined by *heavy lines* in the pedigree (Fig. 1).

FAMILY K

This family from a secluded pre-alpine valley of Central Switzerland, shows similar multiple consanguinity (Fig. 2). The second child (*IX 3*) died of SCID in 1973, but before his death complete deficiency of ADA could be ascertained (32).

The father (*VIII 3*) and one of his brothers (*VIII 4*) have significantly diminished ADA activity, but the mother's (*VIII 6*) and two of her sisters' (*VIII 7* and *VIII 9*) ADA activity is well within the normal range. The paternal grandfather (*VII 3*) is heterozygous, and so is one of his brothers (*VII 4*). In contrast to the normal result in the mother, the situation in her parents seems clear, her father (*VII 7*) being normal, but her mother (*VII 10*) presenting diminished ADA activity. Multiple consanguinity of the parents is evident from the pedigree, once in the 5th degree, once in the 7th degree, three times in the 11th, and once in the 12th degree.

FAMILY SCH

Family Sch (Figs. 3 and 4), was discovered at the beginning of this study. The young parents are not consanguineous. Their only children were binovular twin girls (Fig. 3). One of these was admitted at the age of 5 months to our hospital and died within 4 days of severe lung infection. Autopsy revealed a rudimentary thymus and all the other typical findings of SCID. When her sister was admitted for evaluation she already suffered from severe lung infection. ADA deficiency was found in three successive blood specimens. Her lung condition necessitated artificial respiration for 6 days, but after implantation of one fetal liver (16×10^8 cells given intravenously, fetal gestational age 12 weeks) and thymus the child recovered and all her immunologic functions and ADA activity of her lymphocytes (but not of her erythrocytes) became slowly normal. At the present time, 18 months after this transplant, the child is healthy and well developed in every respect, and her immune functions are normal.

The mother is clearly heterozygous, and so is one of her brothers and her father. The father of the patient, however, has only slightly reduced ADA activity, but since his mother (*I 2*) is unequivocally a heterozygote, he is with high probability a gene carrier.

DISCUSSION

The activity of the enzyme adenosine deaminase (ADA) seems to be individually rather constant and age-independent. We could confirm that the day-to-day variation in the same person is not significant. Changes due to disease, pregnancy, or drugs are not known. Therefore the severe diminution of ADA activity in patients with SCID (3, 5, 19, 20, 24, 25, 29, 31) is most remarkable, and until now only one apparently healthy individual with lack of erythrocyte ADA has been detected (17). According to Scott *et al.* (27), the heterozygous carriers of the deficient gene can be recognized in most cases, and this seems very important for genetic counseling. However, the mother in *family K* (Fig. 2, *VIII 6*) presents an exception to this rule, which is explained by different penetrance of the gene, since her mother (Fig. 2, *VII 10*) has unequivocally diminished levels.

Comparison of clinical and laboratory findings of patients with and without ADA deficiency reveals some differences: most patients with lack of the enzyme have a tendency to very marked lymphocytopenia, but less pronounced decrease of the immunoglobulins. This striking dissociation prompted us in 1971 to publish *family B* as special form of "normogammaglobulinemic antibody deficiency syndrome with severe impairment of the cellular immune reactions" (14). We suspected that the Nézelof syndrome

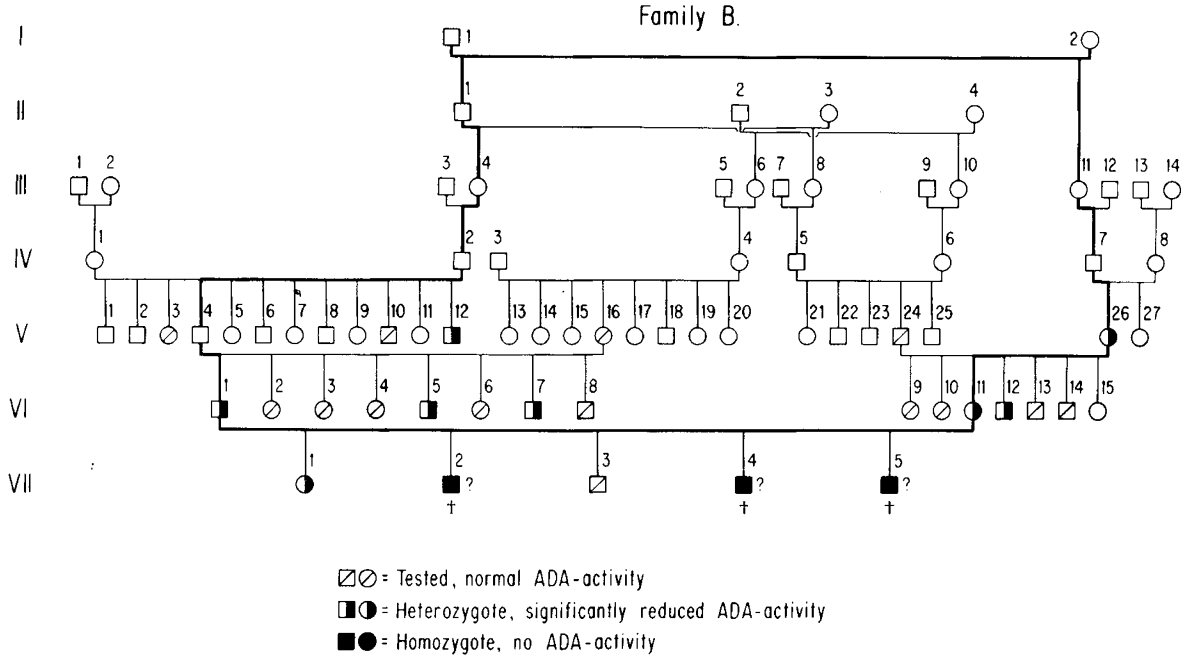

Fig. 1. Pedigree of *family B*.

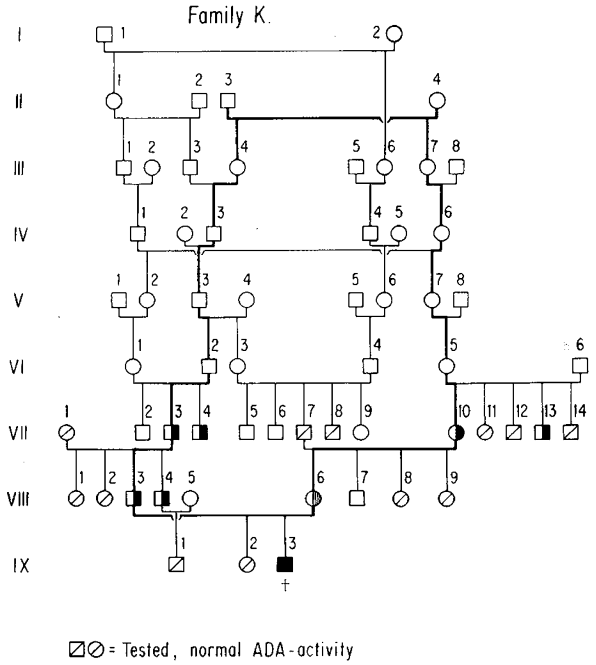

Fig. 2. Pedigree of *family K*.

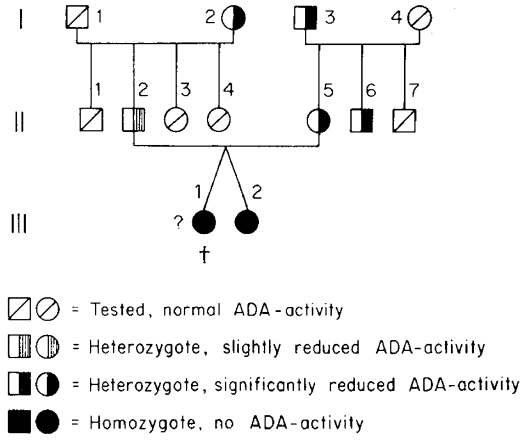

Fig. 3. Pedigree of *family Sch*.

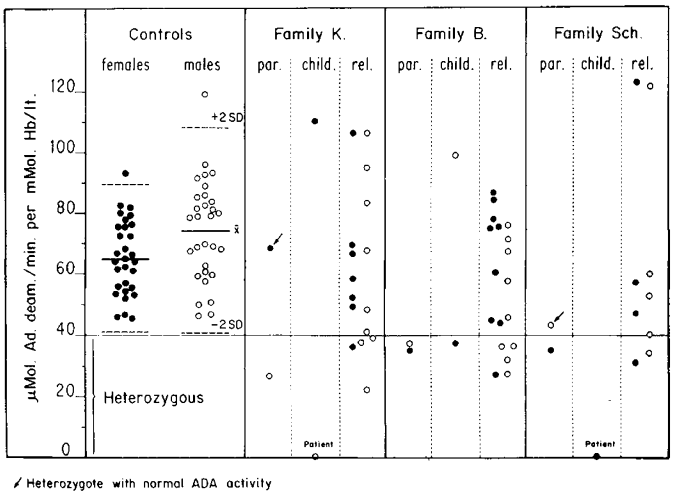

Fig. 4. ADA activities in the members of *families B*, *K*, and *Sch*. Each circle represents one individual. Females: ●; males: ○.

(23) might belong into this category. Meuwissen *et al.* (19, 20) came to similar conclusions.

Histologic findings raise the possibility of secondary involution or destruction of a primarily normal lymphatic system (14); although all autopsied children had a hypoplastic thymus, we found one weighing 4.1 g, which is unusually high for SCID. The thymic involution in the three sons of *family B* was more pronounced the longer the patient survived; in two boys who died early (at 6 and 9 months of age) some Hassal's bodies could be found, while in the third boy, who survived to 15 months, no such structures could be seen. The peripheral lymphatic system presented a severe depletion of lymphocytes and complete absence of germinal centers. In contrast, however, plasma cells were unequivocally present. This is in keeping with the essentially normal immunoglobulin levels, but specific antibody functions were absent. The parallel of the morphologic dissociation between plasma cells and disturbed lymph node architecture and functional discrepancy between immunoglobulin levels and deficiency of antibodies is striking. It is another hint for a regressive change within the lymphoid system during the course of the disease leading to increasing sclerosis.

Little is known as yet concerning the connection between ADA and the immune system or immune function. Meuwissen and Pollara (20, 24, 25) have reviewed the presently available evidence.

1. After local antigenic stimulation the efferent lymphatics contain increased activity of ADA and higher immunoglobulin concentrations.

2. Adenosine and its metabolites, especially the monophosphate, are toxic for cell cultures in high concentrations ($>2 \times 10^{-4}$ M), and mainly for lymphoid cells (16). They might therefore be deleterious to rapidly proliferating lymphoid organs. Equally, inosine, which results from the deamination of adenosine by ADA, seems to play an important role in the metabolism of lymphocytes, especially of the T-cells (2, 8, 9, 22, 30).

3. Molecular heterogeneity of ADA is well known (4). Erythrocytes contain the small molecular form exclusively, but in most tissues polymers are found (4, 10, 29). These two forms can be transformed into each other by a tissue factor isolated from lung (1).

For the time being, the determination of ADA activities is of considerable practical diagnostic value for the detection of SCID, since both the heterozygous gene carriers and the homozygous patients can be identified.

SUMMARY

A new approach to characterize SCID biochemically was recently found by the detection of ADA deficiency. This enzyme assay is sensitive enough to distinguish normal subjects, heterozygous gene carriers, and homozygous patients. A retrospective study of families known for patients who had died of SCID revealed ADA deficiency in three out of nine. Extensive pedigrees of these three families show the distribution of the deficient gene: 6 SCID patients, presumaly homozygotes for ADA deficiency (proven in 2), 6 heterozygote parents, 14 other heterozygote relatives, and 32 homozygote normal subjects. The possible connections between metabolic pathways of adenosine and pathogenesis of SCID are discussed.

REFERENCES AND NOTES

1. Akedo, H., Nishihara, H., Shinkai, K., Komatsu, K., and Ishikawa, S.: Multiple forms of human adenosine-deaminase. Biochim. Biophys. Acta, *276:* 257 (1972).
2. Chan, T., Ishii, K., and Green, H.: Purine excretion by mammalian cells deficient in adenosine kinase. J. Cell. Comp. Physiol., *51:* 315 (1973).
3. Dissing, J., and Knudsen, B.: Adenosine-deaminase deficiency and combined immunodeficiency syndrome. Lancet, *ii:* 1316 (1972).
4. Edwards, Y. H., Hopkinson, D. A., and Harris, H.: Adenosine deaminase

isoenzymes in human tissues. Ann. Hum. Genet., *35:* 207 (1971).
5. Giblett, E. E., Anderson, J. E., Cohen, F., Pollara, B., and Meuwissen, H. J.: Adenosine-deaminase deficiency in two patients with severely impaired cellular immunity. Lancet, *ii:* 1067 (1972).
6. Goldberg, D. M.: Serum adenosine deaminase in the differential diagnosis of jaundice. Brit. Med. J., *i:* 353 (1965).
7. Good, R. A., Peterson, R. D. A., Finstad, J., and Gabrielsen, A. E.: Ser. Haematol., *8:* 1 (1965).
8. Green, H., and Chan, T.-S.: Pyrimidine starvation induced by adenosine in fibroblasts and lymphoid cells: Role of adenosine deaminase. Science, *182:* 836 (1973).
9. Green, H., and Ishii, K.: On the existence of a guanine nucleotide trap: The role of adenosine kinase and a possible cause of excessive purine production in mammalian cells. J. Cell. Sci., *ii:* 173 (1972).
10. Hirschhorn, R., and Levytska, V.: Alterations in isozymes of adenosine deaminase during stimulation of human peripheral blood lymphocytes. Cell Immunol., *12:* 387 (1974).
11. Hirschhorn, R., Levytska, V., Pollara, B., and Meuwissen, H.: Evidence for control of several different tissue-specific isozymes of adenosine-deaminase by a single genetic locus. Nature New Biol., *246:* 200 (1973).
12. Hitzig, W. H., Biro, Z., Bosch, H., and Huser, H. J.: Agammaglobulinämie und Alymphozytose mit Schwund des lymphatischen Gewebes. Helv. Paediat. Acta, *13:* 551 (1958).
13. Hitzig, W. H., Barandun, S., and Cottier, H.: Die Schweizerische Form der Agammaglobulinämie. Ergebn. Inn. Med. Kinderheilk., *27:* 79 (1968).
14. Hitzig, W. H., Landolt, R., Müller, G., and Bodmer, P.: Heterogeneity of phenotypic expression in a family with Swiss-type agamma-globulinemia: Observations on the acquisition of agamma-globulinemia. J. Pediat., *78:* 968 (1971).
15. Hopkinson, D. A., Cook, P. J. L., and Harris, H.: Further data on the adenosine deaminase (ADA) polymorphism and a report of a new phenotype. Ann. Hum. Genet., *32:* 361 (1969).
16. Ishii, K., and Green, H.: Lethality of adenosine for cultured mammalian cells by interference with pyrimidine biosynthesis. J. Cell. Sci., *13:* 429 (1973).
17. Jenkins, T.: Red-blood-cell adenosine deaminase deficiency in a "healthy"!Kung individual. Lancet, *ii:* 736 (1973).
18. Kalckar, H. M.: Differential spectrophotometry of purine compounds by means of specific enzymes. III. Studies of the enzymes of purine metabolism. J. Biol. Chem., *167:* 461 (1974).
19. Meuwissen, H. J., Pickering, R. J., Moore, R. E., and Pollara, B.: Impairment of adenosine deaminase activity in combined immunological deficiency disease. (Submitted for publication).
20. Meuwissen, H. J., and Pollara, B.: Adenosine deaminase deficiency: The first inborn error of metabolism noted in immunodeficiency disease. J. Pediat., *84:* 315 (1974).
21. Meuwissen, H. J., Pollara, B., and Pickering, R. J.: Combined immunodeficiency disease associated with adenosine deaminase deficiency. J. Pediat., *86:* 169 (1975).
22. Murray, A. W.: The biological significance of purine salvage. Ann. Rev. Biochem., *40:* 811 (1971).
23. Nézelof, C., Jammet, M. L., Lortholary, P., Labrune, B., and Lamy, M.: L'hypoplasie héréditaire du thymus. Arch. franç. Pédiat., *21:* 897 (1964).
24. Pollara, B., and Meuwissen, H. J.: Combined immunodeficiency disease and ADA-deficiency. Lancet, *ii:* 1324 (1973).
25. Pollara, B., Pickering, R. J., and Meuwissen, H. J.: Combined immunodeficiency disease and adenosine deaminase deficiency: An inborn error of metabolism. Pediat. Res., *7:* 362 (1973).
26. Schwenk, H. U., Gimpert, E., Plüss, H. J., Pilgrim, U., and Hitzig, W. H.: Lymphocyte markers for B- and T-cells in primary immunodeficiency disease. Klin. Wschr., *52:* 426 (1974).
27. Scott, C. R., Chen, S.-H., and Giblett, E. R.: Detection of the carrier state in combined immunodeficiency disease associated with adenosine deaminase deficiency. J. Clin. Invest., *53:* 1194 (1974).
28. Tobler, R., and Cottier, H.: Familiäre Lymphopenie mit Agamma-globulinämie und schwerer Moniliasis—Die "essentielle Lymphocytophthise" als besondere Form der frühkindlichen Agammaglobulinämie. Helv. Paediat. Acta, *13:* 313 (1958).
29. Van der Weyden, M. B., Buckley, R. H., and Kelley, W. N.: Molecular form of adenosine deaminase in severe combined immunodeficiency. Biochem. Biophys. Res. Commun., *57:* 590 (1974).
30. Wolberg, G., Zimmermann, T. P., Hiemstra, K., Winston, M., and Chu, L.-C.: Adenosine inhibition of lymphocyte-mediated cytolysis: Possible role of cyclic adenosine monophosphate. Science, *187:* 957 (1975).
31. Yount, J., Nichols, P., Ochs, H. D., Hammar, S. P., Scott, R., Chan, S.-H., Giblett, E. R., and Wedgwood, R. J.: Absence of erythrocyte adenosine deaminase associated with severe combined immunodeficiency. J. Pediat., *84:* 173 (1974).
32. We thank Dr. Dooren, Department of Pediatrics, University of Leiden, Holland, for these data, obtained at the Central Laboratory of the Dutch Red Cross, Amsterdam.
33. This research was supported by Schweiz Nationalfonds Grant no. 2.8820.72.
34. Requests for reprints should be addressed to: W. H. Hitzig, M.D., Kinderspital, 8032 Zürich (Switzerland).
35. Accepted for publication September 15, 1975.

Printed in U.S.A.

Pediat. Res. *10:* 70–75 (1976)

Brain development
cytochrome oxidase
inorganic lead
mitochondria
NAD-linked respiration
oxidative phosphorylation

Early Effects of Inorganic Lead on Immature Rat Brain Mitochondrial Respiration

D. HOLTZMAN[44] AND J. SHEN HSU

Departments of Neurology and Pediatrics, Stanford University School of Medicine, Stanford, California, USA

Extract

Inorganic lead, added to the diet of the suckling rat in high doses, produces an encephalopathy similar to that seen in the immature human. Pathologic changes of edema and hemorrhage are seen earliest and are most prominent in the cerebellum. In this study, we measured respiration in cerebral hemisphere and cerebellar mitochondria isolated from led-fed and age-matched normal rat pups. Lactating mothers were begun on *ad libitum* feedings containing 4% lead carbonate when their pups were 2 weeks old. Mitchondria were isolated by differential centrifugation. Oxygen consumption was measured polarographically. NAD-linked respiration was measured with oxidation of the substrate pair, glutamate and malate. Cytochrome oxidase (cytochrome *c* oxidase, EC. 1.9.3.1) activity was measured in the presence of tetramethyl-*p*-phenylenediamine dihydrochloride (TMPD) and ascorbate. Within 2 days of starting lead feedings, rat pups showed a significant loss in body weight ($P < 0.02$) and, after 1 week, a significant loss in cerebral hemisphere wet weight ($P < 0.01$) compared with controls. Overt encephalopathy appeared in pups from two of nine litters receiving lead feedings for 1 week and in half of the litters after 2 weeks of feedings. None of the lead-fed mothers developed encephalopathic signs. With oxidation of the NAD-linked substrate pair, there was a progressive decrease, relative to controls, in ADP/O ratios in both cerebellar