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Iron and copper accumulation in the brain of coxsackievirus-infected mice exposed to cadmium

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Abstract

Cadmium (Cd) is a potentially toxic metal widely distributed in the environment and known to cause adverse health effects in humans. During coxsackievirus infection, the concentrations of essential and nonessential trace elements (e.g., iron (Fe), copper (Cu), and Cd) change in different target organs of the infection. Fe and Cu are recognized cofactors in host defence reactions, and Fe is known to be associated with certain pathological conditions of the brain. However, whether nonessential trace elements could influence the balance of essential trace elements in the brain is unknown. In this study the brain Fe, Cu, and Cd contents were measured through inductively coupled plasma mass spectrometry and their distributions determined by nuclear microscopy in the early phase (day 3) of coxsackievirus B3 (CB3) infection in nonexposed and in Cd-exposed female Balb/c mice. In CB3 infection the brain is a well-known target that has not been studied with regard to trace element balance. The brain concentration of Cu compared with that of noninfected control mice was increased by 9% ($P<0.05$) in infected mice not exposed to Cd and by 10% (not significant) in infected Cd-exposed mice. A similar response was seen for Fe, which in infected Cd-exposed mice, compared to noninfected control mice, tended to increase by 16%. Cu showed an even tissue distribution, whereas Fe was distributed in focal deposits. Changes in Cd concentration in the brain of infected mice were less consistent but evenly distributed. Further studies are needed to define whether the accumulation and distribution of trace elements in the brain have an impact on brain function.

Keywords: Brain; Cadmium; Copper; Iron; Mass spectrometry; Nuclear microscopy; Virus

1. Introduction

Many trace elements, including copper (Cu) and iron (Fe), are essential and required for optimal functioning of the central nervous system (CNS) (Zheng et al., 2003). Changes in the concentrations of trace elements, especially of Cu, Fe, and zinc (Zn), have been observed in the aging brain and in pathological conditions of the brain (e.g., Parkinson's and Alzheimer's diseases) (He et al., 1996; Takahashi et al., 2001; Tiffany-Castiglioni and Qian, 2001; Waggoner et al., 1999). Fe is normally present in the brain, particularly in the forebrain and cerebellum, but it is widely accepted that increased Fe concentrations are associated with neurodegenerative processes (Ke and Ming, 2003), although whether the Fe accumulation is the initial event that causes neurodegeneration or whether it is a consequence of a disease process is not always clear.

Also in several infections associated with neuropathological changes, including human immunodeficiency virus (HIV)/acquired immune deficiency syndrome, Fe accumulation seems to occur in the brain (Boelaert et al., 1996; Kim et al., 2000). Acute infectious diseases in general, regardless of etiology and target organs of microorganisms, are associated with altered dynamics of Fe, Cu, and Zn, resulting in changed concentrations in the blood (Beisel et al., 1974; Funseth et al., 2000; Ilbäck et al., 2003). These trace elements are crucial for host defence

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(Cunningham-Rundles, 1991; Pekarek and Engelhardt, 1981), including the development of inflammation (Milanino et al., 1993). Yet, not all trace element changes are favorable to the host, including the progressive increase of Fe in the brain during HIV infection (Boelaert et al., 1996).

Strong support in favor of an etiologic role of environmental factors in Parkinson's disease has been given (Di Monte et al., 2002). Nonessential trace elements, such as cadmium (Cd) and mercury (Hg), can compete and interact metabolically with essential trace elements in the body (Goyer, 1997; Ilbäck et al., 2005). Cd is an environmental pollutant to which humans are exposed, resulting in gradual accumulation in certain tissues (e.g., the kidney) (Nordberg and Nordberg, 2000). In coxsackievirus infection Cd is redistributed to the kidneys and accumulates in target organs of the infection (e.g., the liver and kidneys), resulting in aggravated disease (Ilbäck et al., 1992, 1994, 2004; Wicklund-Glynn et al., 1998).

Virtually all humans acquire several enteroviral infections during a lifetime, including coxsackievirus infections, but the majority of these infections are mild and either pass unrecognized or result in only minor symptoms from the upper respiratory or gastrointestinal tract. In some instances, however, the coxsackieviruses cause myocarditis, pancreatitis, or meningoencephalitis, all of which are well-known manifestations of such infections (Gear, 1984; Woodruff, 1980). The murine model of coxsackievirus type B3 (CB3) infection has a pathogenesis closely resembling this common disease in humans (Huber, 1993; Woodruff, 1980).

The aim of this study was to determine whether CB3 infection affects the brain concentration and distribution of the two essential trace elements Fe and Cu. Furthermore, the influence of exposure to the nonessential trace element Cd, which is known to interact with essential trace elements and to be redistributed during infection, was studied.

2. Material and methods

2.1. Mice

Adult female Balb/c mice were purchased from Charles River (Copenhagen, Denmark) and maintained at the Animal Department, Biomedical Centre, Uppsala University, Uppsala, Sweden. The mice were randomly assigned to groups of similar initial mean body weight (bw) and housed individually at 23 ± 1 °C on a 12-h light/dark cycle behind hygienic barriers with free access to food (R3; Ewos, Södertälje, Sweden) and water. Control, Cd-exposed, infected, and infected Cd-exposed mice, all of whom were sacrificed at day 3, were studied simultaneously.

The animal experiments described in this publication took into account all ethical aspects of the welfare of animals following the recommendations in "Guide for the Care and Use of Laboratory Animals" of the Swedish National Board for Laboratory Animals. The study was approved by the local Ethical Committee for Experimental Use at the Faculty of Medicine, University of Uppsala, Uppsala, Sweden.

2.2. Virus

A myocarditic coxsackievirus type B3 of the Nancy strain was used (Woodruff and Kilbourne, 1970). The virus was propagated in HeLa cells, which were grown in Eagle's minimal essential medium supplemented with 5% fetal calf serum and antibiotics (kanamycin). Virus titers were determined on HeLa cells as plaque-forming units (pfu) and a stock solution was stored at −20 °C until use. The stock solution of 10^7–10^8 pfu/mL was diluted with phosphate-buffered saline (PBS) to obtain 10^5 pfu/mL.

2.3. Infection, tissue sampling and tissue preparation

On day 0 of the experiment, each mouse was inoculated intraperitoneally (i.p.) with approximately 2×10^4 pfu of CB3 virus. In prestudies of adult female Balb/c mice this dose and route of administration had been shown to produce 30% lethality from 7 to 9 days after inoculation (Ilbäck et al., 1989). Additional mice were sham-inoculated with a similar volume of HeLa cell medium to serve as uninfected controls.

On day 1 of the experiment, half of the noninfected and half of the infected mice were randomly selected and then administered (i.p.) 0.2 mL of a $CdCl_2$ solution corresponding to a dose of 50 μg/kg bw. The remaining mice were similarly administered an equal volume of PBS.

Infected ($n = 3$) and infected Cd-exposed ($n = 3$) mice were anesthetized with Hypnorm/Dormicum and sacrificed at day 3. Sham-inoculated control ($n = 3$) and Cd-exposed ($n = 3$) mice were concomitantly sacrificed to serve as controls. The whole brain was excised quickly; a biopsy from the left part of the cerebrum was taken, immediately frozen in isopentane, and cooled in liquid nitrogen for nuclear microscopy and histological processing. The remaining brain was frozen at −70 °C and used later for analysis of total brain trace element contents.

2.4. Histologic examination

The brain tissue was cut into 5-μm-thick serial sections that were used either for standard histologic examination following hematoxylin–eosin (HE) staining or for nuclear microscopy. HE sections were used only for localization of brain segments of interest for nuclear microscopy of trace element distribution.

2.5. Assessment of trace elements

To determine the concentrations of the trace elements Fe, Cu, and Cd, the tissue samples were decomposed using ultrapure nitric acid (Scan Pure; Chem Scand AS, Elverum, Norway) in a steel bomb (MeAna-Konsult, Uppsala, Sweden). Tissue samples of about 0.1 g were weighed and put in quartz tubes; 1 mL of 65% nitric acid/0.1 g sample dry weight was added and the tubes were sealed with a Teflon lid and put into the steel bombs which were sealed with exactly the same momentum. The bombs were then heated in an oven to 180 °C for 4 h. After decomposition, an internal standard (indium) was added and the samples were diluted in 10 mL of high-purity water from an ElgaStat UHP (Elga Ltd., High Wycomb, Buckshire, England). The water quality was maintained at more than 18 MΩ cm. All handling of the samples was done in a clean room. The trace element content of the samples was then measured by inductively coupled plasma mass spectrometry (ICP-MS; Perkin–Elmer SCIEX ELAN 6000; Perkin–Elmer Corp., Norwalk, CT, USA). The detection limit for Fe, Cu, and Cd was <10 ppb. For quality control, every fifth sample was a Certified Reference Material of Bovine muscle (BCR-184; Community Bureau of Reference, Brussels, Belgium), resulting in an overall precision of less than 5% and an overall accuracy of less than 8%.

2.6. Nuclear microscopy of tissue trace element distribution

The tissue distribution of metals was unveiled using the National University of Singapore Nuclear Microscope (Watt, 1997). Unstained, freeze-dried brain tissue sections from control and infected mice were raster-scanned with a microbeam (1 μm) of 2-MeV protons. The limits of trace element detection using this technique approach 0.5 μg/g dry weight.

The tissue distributions of Fe, Cu, and Cd were studied with nuclear microscopy in different areas of the brain of control, noninfected Cd-exposed, infected, and infected Cd-exposed mice.

2.7. Statistical analysis and data evaluation

The nonparametric Mann–Whitney U test was used to determine whether any differences in the mass spectrometry data existed between the mice groups ($n = 3$ per group). The objective of nuclear microscopy studies is to unveil tissue distribution of certain elements and make possible association of elements to specific structures. It is not meaningful to make statistical comparisons of morphological data from different areas of a tissue or from tissues of different animals.

3. Results

During the early phase of CB3 infection, i.e., on day 3 postinoculation, neither mortality nor body weight loss occurred. Virus can be detected in the pancreas and heart on days 1–5 and inflammatory lesions start to develop on day 2 in the pancreas and on days 5–6 in the heart in this infection model (Funseth et al., 2000; Huber, 1993; Ilbäck et al., 1996). Clinical signs of disease (e.g., ruffled hair and inactivity) appear from day 2 in this experimental infection, corresponding to the time of viremia, which peaks around days 3 to 4. On day 3, all infected animals had developed expected clinical signs of disease.

The mean trace element concentrations in the brain are shown in Figs. 1A–C and a map of the distribution of Fe obtained from nuclear microscopy of the brain is shown in Fig. 2.

The Fe concentration in the brain (Fig. 1A) of the infected mice (24.09 ± 2.78 g/kg ww) tended to be increased compared with that of the noninfected control mice (20.81 ± 2.16 mg/kg ww). The Fe concentration also tended to be increased in the noninfected Cd-treated mice (23.43 ± 1.96 g/kg ww) and in the infected Cd-treated mice, although with substantial interindividual variation (22.90 ± 24.67 g/kg ww). The Cu concentration in the brain (Fig. 1B) of the infected mice (4.38 ± 0.81 g/kg ww) was increased ($P < 0.05$) as compared with that of the noninfected control mice (4.03 ± 0.14 g/kg ww). Cd exposure in the noninfected mice also tended to increase the Cu concentration (4.20 ± 0.04 g/kg ww), a concentration that tended to be further, but not significantly, increased in the infected Cd-treated mice (4.43 ± 0.46 g/kg ww). Somewhat unexpectedly, the noninfected Cd-exposed mice exhibited lower ($P < 0.05$) concentration of Cd in the brain than did the noninfected control mice (Fig. 1C). However, the infected Cd-exposed mice had a higher ($P < 0.05$) Cd concentration in the brain than the noninfected Cd-exposed mice, although the concentration was not significantly higher than that of the noninfected control mice.

Thus, the total brain concentration of Fe in the infected and Cd-exposed mice (Fig. 1A) tended to be increased, though with considerable interindividual variation. Nuclear microscopy of brain tissue slides from the cerebrum showed Fe to be deposited focally in the infected Cd-treated mice (Fig. 2), whereas Cd-treated noninfected mice had a distribution of Fe comparable to that found in noninfected control mice with no focal deposits of Fe. The focal accumulations of Fe were present in samples from different parts of the brain in infected Cd-exposed mice. Although the concentration of Cu (Fig. 1B) in the brain was increased ($P < 0.05$) in both the infected (9%) and the infected Cd-exposed mice (10%, not significant), no focal deposits were observed. Cd exposure in infected mice tended to increase the Cd concentration in the brain, but it was not deposited focally.

4. Discussion

During the early 3-day course of coxsackievirus type B3 infection, the concentration of Cu was found to increase in the brain and a similar suggestive response was seen for Fe. This infection-associated accumulation was of the same magnitude in combination with Cd exposure. However, a striking difference was observed between Cu and Fe in the pattern of accumulation in the Cd-treated mice. Cu showed an even distribution with this infection, whereas Fe showed a scattered accumulation in focal deposits throughout the brain. Cd tended to increase in the brain in infected Cd-exposed mice, but it was not deposited focally.

Many trace elements are essential components in metabolic processes in health and disease, including host defence against microorganisms. When an infectious process proceeds and concomitant immune activation occurs, alterations in plasma trace elements have been described in bacterial, viral, rickettsial, and parasitic infections (Beisel et al., 1974; Beisel, 1998). The most consistent responses are associated with acute-phase responses and include a decrease in plasma levels of Fe and Zn and an increase in Cu, which has been used to indicate ongoing infection in experimental animal models (Beisel, 1998). These trace elements are important in the regulation of cellular immune function and are transported by acute-phase proteins, i.e., ferritin (Fe), ceruloplasmin (Cu), and metallothionein (Zn) (Beisel, 1998).

Data on trace elements in infected organs and tissues are rather scarce. In one study on CB3-infected mice a decrease was found in Zn contents in the infected/inflamed heart in mice exposed to mercury (Hg), suggesting a competition between the nonessential toxic trace element Hg and the essential trace element Zn (Ilbäck et al., 2000). In addition, this change was associated with an altered disease pathogenesis in which heart lesions, virus persistence, and cytokine responses tended to be influenced by Hg in a direction compatible with the development of chronic disease (Ilbäck et al., 1996). In animals pretreated with Cd, elevated viral titers, an up-regulation of inflammatory cytokines, and maximum encephalitis were observed in encephalomyocarditis, semliki forest, and Venezuelan equine encephalitis virus infections (Seth et al., 2003). Furthermore, treatment with Cd has experimentally been

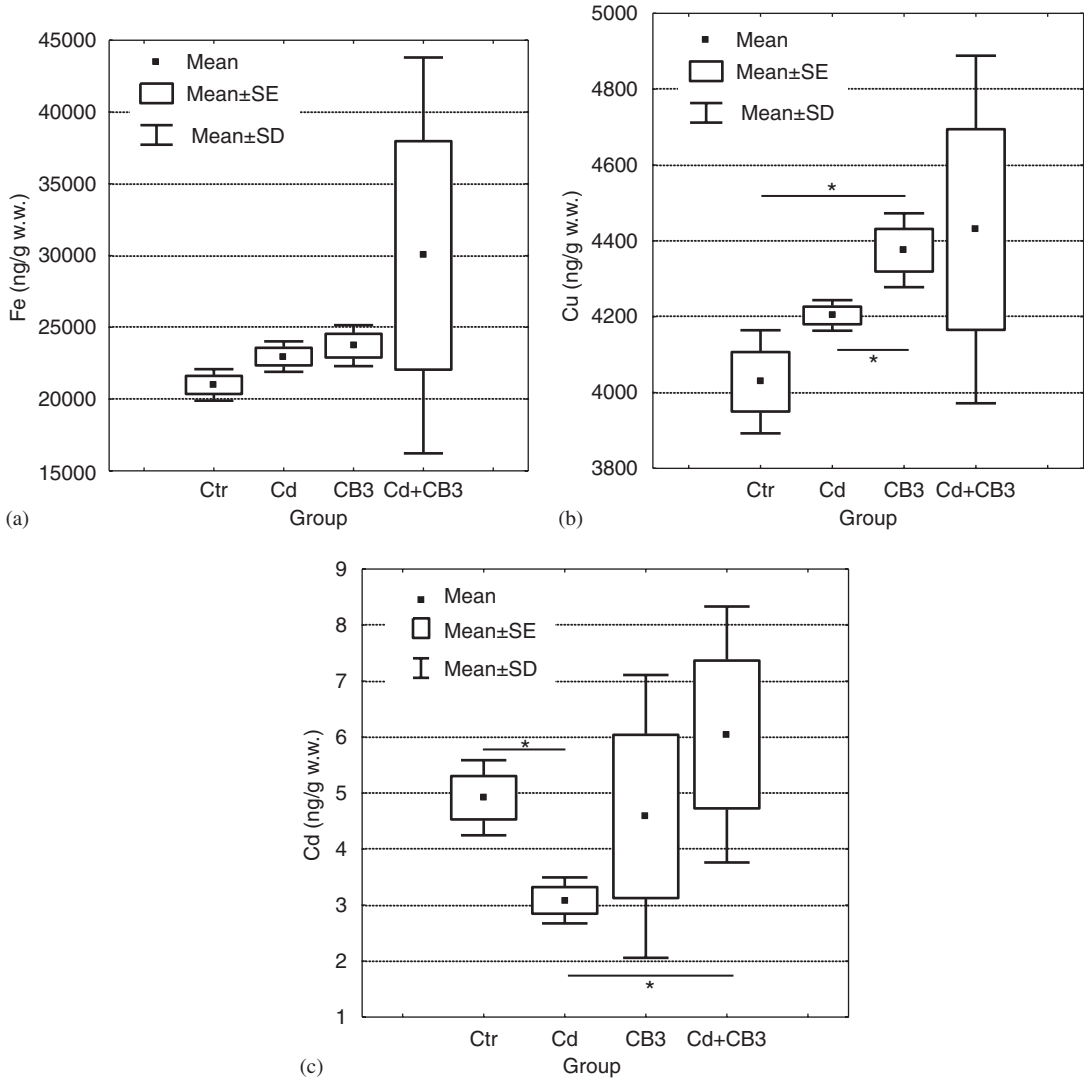

Fig. 1. Total concentration of Fe (A), Cu (B), and Cd (C) in the brain of nonexposed noninfected control (Ctr), Cd-exposed noninfected (Cd), CB3-infected (CB3), and Cd-exposed CB3-infected (Cd + CB3) mice. Each group consists of three mice. A box indicates the interquartile range between the 25th and the 75th percentiles while the point inside the box represents the median. The whiskers indicate the total range within each group. A significant difference between each of the treatment groups is indicated by an asterisk ($*P<0.05$).

shown to reactivate latent herpes simplex virus type 1 in the rat (Fawl et al., 1996).

Metallothioneins are found in various tissues of the body, including the liver, kidney, and brain (Nordberg and Nordberg, 2000; Palmiter, 1998). These metal-binding proteins predominantly bind Zn but also nonessential metals such as Cd and are highly inducible by Cd and cytokines (Nordberg and Nordberg, 2000; Palmiter, 1998). In CB3 infection there is increased production of cytokines (Ilbäck et al., 1993), increased synthesis of metallothionein (Funseth et al., 2002; Ilbäck et al., 2004), increased gastrointestinal absorption of Cd (Wicklund-Glynn et al., 1998), and redistribution of essential (Zn) and nonessential (Cd) trace elements to the target organs of the infection (Funseth et al., 2000; Ilbäck et al., 1992, 2003, 2004). In the present study, Cd concentration in the brain of noninfected mice showed an unexpected pattern of response with a decreased concentration as a result of Cd exposure. Moreover, in the brain of infected Cd-exposed mice the Cd concentration was similar to that of noninfected mice not exposed to Cd. The brain seems to be less responsive than the liver to the induction of metallothioneins (Aschner et al., 1997; Kägi and Schäffer, 1988; Waalkes and Goering, 1990). Thus, it is possible that Cd-induced induction of liver metallothionein increased liver accumulation of Cd and concomitantly reduced the blood level of Cd, resulting in a reduced amount of Cd available to be taken up by vital organs (e.g., the brain).

The most important pathway of Fe transport into the brain is receptor-mediated endocytosis of Fe-transferrin (Zheng et al., 2003). Moreover, Fe and other divalent ions (e.g., Cu) may also be delivered to the CNS via a nonspecific metal transporter (DMT1) protein (Zheng et al., 2003). One of the responses to infection, and part

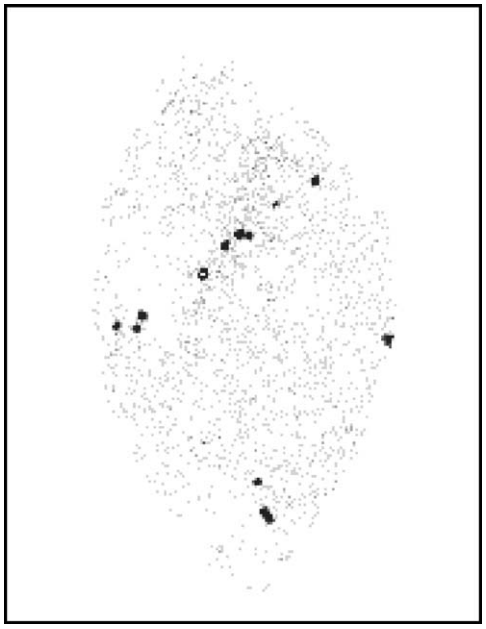

Fig. 2. Elemental maps of a brain tissue section from a CB3-infected mouse exposed to Cd. The map shows the distribution of Fe with a set of hot spots in the tissue.

of the accompanying acute-phase reaction, is an increased blood level of Cu-transporting ceruloplasmin and a corresponding increase in the Cu concentration (Beisel, 1998). The associated increase in both Cu and Fe contents in the brain of infected and Cd-exposed mice may thus reflect an infection-induced increase in the transport of these elements from plasma into the brain.

It is widely known that oxidative stress induced by free radicals is associated with Fe accumulation (Kim et al., 2000; Weinberg, 1999) and that Fe is a nutrient for invading microbial cells, such as bacteria (Cockayne and Arbuthnot, 1992; Weinberg, 1999). In the scrapie infection it has been suggested that brain metal changes, especially the increase of Cu, are associated with altered antioxidative functions of the Cu/Zn superoxide dismutase (SOD) enzyme (Wong et al., 2001). The amino acid cysteine has well known Fe-chelating properties that could account for regional brain Fe accumulation (Ke and Ming, 2003). Furthermore, cysteine undergoes rapid autooxidation in the presence of Fe and then induces free radical reactions, which have been suggested to be a common pathway in neurodegeneration (Zhou et al., 2001). The observed increase of Cu in the brain of CB3-infected mice, being an essential cofactor for SOD, may therefore partly reflect an increased protective capacity of antioxidative enzymes.

In conclusion, this study, in a limited number of mice ($n = 3$ per group), suggests that Fe and Cu are accumulated in the brain during coxsackievirus infection. Furthermore, with concomitant Cd exposure, the distribution of Fe in the brain, showing focal deposits, seems to be disturbed. It is not known at this time whether these changes are reversible. Humans contract several enterovirus infections during the course of a lifetime, including coxsackievirus infections. Accordingly, it cannot be ruled out that these, and possibly several other infections, may cause stepwise metal accumulation in the brain. Further studies are needed to define the role of trace element changes in the brain during infections and whether the accumulation and distribution of elements have an impact on brain function.

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References

Aschner, M., Cherian, M.G., Klaassen, C.D., Palmiter, R.D., Erickson, J.C., Bush, A.I., 1997. Metallothioneins in brain—the role in physiology and pathology. Toxicol. Appl. Pharmacol. 142, 229–242.

Beisel, W.R., 1998. Metabolic response of the host to infections. In: Feigin, R.D., Cherry, J.D. (Eds.), Textbook of Pediatric Infectious Disease. WB Saunders, Philadelphia, pp. 54–69.

Beisel, W.R., Pekarek, R.S., Wannemacher, R.W., 1974. The impact of infectious diseases on trace element metabolism in the host. In: Hoekstra, G., Gauther, H.E., Mertz, W. (Eds.), Trace Element Metabolism in Animals. University Park Press, Baltimore, pp. 217–240.

Boelaert, J.R., Weinberg, G.A., Weinberg, E.D., 1996. Altered iron metabolism in HIV infection: mechanisms, possible consequences, and proposals for management. Infect. Agents Dis. 5, 36–46.

Cockayne, A., Arbuthnot, J.P., 1992. Bacterial pathogenicity. In: Greenwood, D., Slack, R.C.B., Peutherer, J.F. (Eds.), Medical Microbiology. A Guide to Microbial Infections: Pathogenesis, Immunity, Laboratory Diagnosis and Control. Churchill Livingstone, London, p. 97.

Cunningham-Rundles, S., 1991. Zinc modulation of immune function: specificity and mechanism of interaction. J. Lab. Clin. Med. 128, 9–11.

Di Monte, D.A., Lavasani, M., Manning-Bog, A.B., 2002. Environmental factors in Parkinson's disease. NeuroToxicology 23, 487–502.

Fawl, R.L., Gesser, R.M., Valyi-Nagi, T., Fraser, N.W., 1996. Reactivation of herpes simplex virus from latently infected mice after administration of cadmium is mouse-strain-dependent. J. Gen. Virol. 77, 2781–2786.

Funseth, E., Lindh, U., Wesslén, L., Friman, G., Ilbäck, N.-G., 2000. Trace element changes in the myocardium during coxsackievirus B3 myocarditis in the mouse. Biol. Trace Elem. Res. 76, 149–160.

Funseth, E., Påhlman, M., Eloranta, M.-J., Friman, G., Ilbäck, N.-G., 2002. Effects of coxsackievirus B3 infection on the acute-phase protein metallothionein and on cytochrome P450 involved in the detoxification processes of TCDD in the mouse. Sci. Total Environ. 284, 37–47.

Gear, J.H., 1984. Nonpolio causes of polio-like paralytic syndromes. Rev. Infect. Dis. 6 (Suppl. 2), 379–384.

Goyer, R.A., 1997. Toxic and essential metal interactions. Annu. Rev. Nutr. 17, 37–50.

He, Y., Tong, P.S., Lee, T., Leong, S.K., Chi, C.Y., Wong, P.T., Yuan, S.Y., Watt, F., 1996. Increased iron in the substantia nigra of 6-OHDA induced Parkinsonian rats: a nuclear microscopy study. Brain Res. 30, 149–153.

Huber, S.A., 1993. Animal models: immunological aspects. In: Banatvala, J.E. (Ed.), Viral Infections of the Heart. Hodder & Stoughton, London, pp. 82–109.

Ilbäck, N.-G., Fohlman, J., Slorach, S., Friman, G., 1989. Effects of the immunomodulator LS 2616 on lymphocyte subpopulations in murine coxsackievirus B3 myocarditis. J. Immunol. 142, 3225–3228.

Ilbäck, N.-G., Fohlman, J., Friman, G., Wicklund-Glynn, A., 1992. Altered distribution of ^{109}Cd in mice during viral infection. Toxicology 71, 193–202.

Ilbäck, N.-G., Wesslen, L., Pauksen, K., Stålhandske, T., Friman, G., 1993. Effects of the antiviral WIN 54954 and the immune modulator LS2616 on cachectin/TNF and γ-interferon responses during viral heart disease. Scand. J. Infect. Dis. 88, 117–123.

Ilbäck, N.-G., Fohlman, J., Friman, G., Ehrnst, E., 1994. Immune responses and resistance to viral-induced myocarditis in mice exposed to cadmium. Chemosphere 29, 1145–1154.

Ilbäck, N.-G., Wesslén, L., Fohlman, J., Friman, G., 1996. Effects of methyl mercury on cytokines, virus and inflammatory heart lesions in a common viral infection. Toxicol. Lett. 89, 19–28.

Ilbäck, N.-G., Lindh, U., Wesslén, L., Fohlman, J., Friman, G., 2000. Trace element distribution in heart tissue sections studied by nuclear microscopy is changed in coxsackievirus B3 myocarditis in methyl mercury exposed mice. Biol. Trace Elem. Res. 78, 131–147.

Ilbäck, N.-G., Benyamin, G., Lindh, U., Friman, G., 2003. Sequential changes in Fe, Cu and Zn in target organs during early coxsackievirus B3 infection in mice. Biol. Trace Elem. Res. 91, 111–124.

Ilbäck, N.-G., Glynn, A.W., Wikberg, L., Netzel, E., Lindh, U., 2004. Metallothionein is induced and trace element balance changed in target organs of a common viral infection. Toxicology 199, 241–250.

Ilbäck, N.-G., Lindh, U., Minqin, R., Friman, G., Watt, F., 2005. Selenium and mercury are redistributed to the brain during viral infection in mice. Biol. Trace Elem. Res. 108, 215–224.

Kägi, K.J.R., Schäffer, A., 1988. Biochemistry of metallothionein. Biochemistry 27, 8509–8515.

Ke, Y., Ming, Q.Z., 2003. Iron misregulation: a primary cause of neurodegenerative disorders. Lancet Neurol. 2, 246–253.

Kim, N.H., Park, S.J., Jin, J.K., Kwon, M.S., Choi, E.K., Carp, R.I., Kim, Y.S., 2000. Increased ferric iron content and iron-induced oxidative stress in the brains of scrapie-infected mice. Brain Res. 884, 98–103.

Milanino, R., Marrella, M., Gasperini, R., Pasqualicchio, M., Velo, G., 1993. Copper and zinc body levels in inflammation: an overview of the data obtained from animal and human studies. Agents Actions 39, 195–209.

Nordberg, M., Nordberg, G.F., 2000. Toxicological aspects of metallothionein. Cell. Mol. Biol. 46, 451–463.

Palmiter, R.D., 1998. The elusive functions of metallothioneins. Proc. Natl. Acad. Sci. USA 95, 8428–8430.

Pekarek, S., Engelhardt, J.A., 1981. Infection-induced alterations in trace metal metabolism: relationship to organism virulence and host defense. In: Pekarek, R.S., Engelhardt, J.A. (Eds.), Infection: The Physiologic and Metabolic Responses of the Host. Biomedical Press, Elsevier/North-Holland, Amsterdam, pp. 131–146.

Seth, P., Husain, M.M., Gupta, P., Schoneboom, A., Grieder, B.F., Mani, H., Maheshwari, R.K., 2003. Early onset of virus infection and up-regulation of cytokines in mice treated with cadmium and manganese. Biometals 16, 359–368.

Takahashi, S., Takahashi, I., Sato, H., Kubota, Y., Yoshida, S., Muramatsu, Y., 2001. Age-related changes in the concentrations of major and trace elements in the brain of rats and mice. Biol. Trace Elem. Res. 80, 145–158.

Tiffany-Castiglioni, E., Qian, Y., 2001. Astroglia as metal depots: molecular mechanisms for metal accumulation, storage and release. NeuroToxicology 22, 577–592.

Waalkes, P., Goering, P.L., 1990. Metallothionein and other cadmium-binding proteins: recent developments. Chem. Res. Toxicol. 3, 281–288.

Waggoner, D.J., Bartnikas, T.B., Gitlin, J.D., 1999. The role of copper in neurodegenerative disease. Neurobiol. Dis. 6, 221–230.

Watt, F., 1997. The nuclear microprobe: a unique instrument. Nucl. Instrum. Methods B 130, 1–9.

Weinberg, E.D., 1999. Iron loading and disease surveillance. Emerging Infect. Dis. 5, 346–352.

Wicklund-Glynn, A., Lind, Y., Funseth, E., Ilbäck, N.-G., 1998. The intestinal absorption of cadmium increases during a common viral infection (coxsackievirus B3) in mice. Chem. Biol. Interact. 113, 79–89.

Wong, B.S., Brown, D.R., Pan, T., Whiteman, M., Liu, T., Bu, X., Li, R., Gambetti, P., Olesik, J., Rubenstein, R., Sy, M.S., 2001. Oxidative impairment in scrapie-infected mice is associated with brain metal perturbations and altered antioxidant activities. J. Neurochem. 79, 689–698.

Woodruff, J.F., 1980. Viral myocarditis. A review. Am. J. Pathol. 101, 424–479.

Woodruff, J.F., Kilbourne, E.D., 1970. The influence of quantitated post weaning undernutrition in coxsackievirus B3-infection of adult mice. I. Viral persistence and increased severity of lesions. J. Infect. Dis. 121, 137–163.

Zheng, W., Aschner, M., Ghersi-Egea, J.-F., 2003. Brain-barrier systems: a new frontier in metal neurotoxicological research. Toxicol. Appl. Pharmacol. 192, 1–11.

Zhou, B., Westaway, S.K., Levinson, B., Johnson, M.A., Gitschier, J., Hayflick, S.J., 2001. A novel panthothene kinase gene (PANK2) is defective in Hallervorden–Spatz syndrome. Nat. Genet. 28, 345–349.