d'enzyme dans le sérum et le poids de la rate représenterait peut-être le degré de destruction de ces cellules dans la rate, étant donné que le lysozyme ne se libère dans la circulation qu'après la mort de la cellule productrice de celui-ci[15].

1 Adresse actuelle: 1st Department of Internal Medicine, University of Athens School of Medicine, Vassilevs Pavlos Hospital, Athens 609, Greece.
2 B. Halpern, Bull. Ass. Fr. Vét. Microbiol. Immunol. *7*, 3 (1971).
3 C. Adlam, E.S. Broughton et M.T. Scott, Nature New Biol. *235*, 219 (1972).
4 R.M. Fauve, dans: Corynebacterium parvum. Applications in experimental and clinical Oncology, p.77. Ed. B. Halpern. Plenum Press, New York and London 1975.
5 B. Halpern, A.R. Prévot, G. Biozzi, C. Stiffel, D. Mouton, J.C. Morard, Y. Bouthillier et C. Decreusefond, J. reticuloendoth. Soc. *1*, 77 (1964).
6 G. Litwack, Proc. Soc. exp. Biol. Med. *89*, 401 (1955).
7 R.S. Briggs, P.E. Perillie et S.C. Finch, J. Histochem. Cytochem. *12*, 167 (1966).
8 M.E. Carson et A.M. Dannenberg, J. Immunol. *94*, 99 (1965).
9 E.F. Osserman et D.P. Lawlor, J. exp. Med. *124*, 921 (1966).
10 M.E. Fink et S.C. Finch, Proc. Soc. exp. Biol. Med. *127*, 365 (1968).
11 G. Eliopoulos, Thèse d'Etat, Paris 1977.
12 A.E. Stuart, dans: Reticuloendothelial system, p.156. Livingstone, London 1970.
13 B. Halpern, A. Fray, Y. Crepin, O. Platica, A-M. Lorinet, A. Rabourdin, L. Sparros et R. Isac, Ciba Found. Symp. *18*, 217 (1973).
14 L. Dazord, L. Toujas, M.P. Ramee et J. Guelfi, Annls Immunol. Inst. Pasteur (Paris) *124C*, 375 (1973).
15 C.G. Craddock, dans: Hematology, p.593. Ed. W. Williams. McGraw-Hill Book Comp., New York 1972.

Effects of cadmium on the immune system of mice*

S. Müller, K.-E. Gillert, Ch. Krause[1], G. Jautzke[2], U. Gross[2] and T. Diamantstein[3,4]

Robert Koch-Institut, Nordufer 20, D-1000 Berlin 65 (West), und Klinikum Steglitz, Hindenburgdamm 30, D-1000 Berlin 45 (West), 4 October 1978

Summary. Chronic oral exposure of mice to Cd^{++} inhibits cell-mediated immunity of delayed type hypersensitivity induced by sheep red blood cells (SRBC). No effect was detected on humoral immune response to SRBC. Spleen cells derived from mice exposed to Cd^{++} showed in vitro enhanced response to T and B cell mitogens. These results demonstrate that Cd^{++} exposure alters the immune system of mice.

It has been reported that cadmium, known as an environmental contaminant, alters the antibacterial defense mechanisms[5,6]. This alteration seems to be due especially to an impairment of the function of phagocytic cells[6]. As tested in rabbits, Cd^{++} given in subtoxic doses results in decreased circulating antibody titers[7] and diminishes the humoral immune response of mice to sheep red blood cells (SRBC) as measured by the number of antibody forming cells in the animal spleen. Therefore, it has been claimed that the decreased antibody titers after Cd^{++} treatment might be due to the impairment of the clonal expansion of the specific B lymphocytes[8]. Recently, we showed an impairment of the T cell-mediated, macrophage-dependent, cellular immune response of mice by subtoxic doses of lead[9].

In this study we present results concerning the effect of chronic Cd^{++} exposure a) on cell-mediated immunity (delayed type hypersensitivity = DTH) induced by SRBC, b) antibody production to SRBC and c) function of spleenic T and B lymphocytes in vitro. Cd^{++} was given orally, since this route can be considered as relevant for intoxication with Cd^{++} as an environmental agent. In the form of cadmium acetate 30, 300 and 600 ppm Cd^{++} were administered to mice ad libitum with drinking water for 10 weeks. This dose range is reported to be subtoxic and to be inhibitory for humoral immune response in mice[8]. Histological studies revealed no morphological changes of kidneys, small and large intestine, thymus, and lymph nodes, but a dose-dependent decrease of the white spleen pulp, cell necrosis in liver and inflammatory infiltrates and edema in the cardia, especially in mice, which showed the highest cadmium level in the serum.

Cd^{++} resorption was measured in the sera of specimens by atomic absorption spectrophotometry (AAS) in a range of 0.2 up to 4.0 µg/100 ml serum. The cadmium range of normal individuals is between 0.17 up to 0.23 µg/100 ml serum[10,11]. This reveals the possibility that the Cd^{++} doses detected in mice after exposure is in a similar range as in Cd^{++}-burdened humans.

Groups of Cd^{++}-treated and control mice were sensitized with SRBC and challenged by a single intracutanous footpad injection of the antigen to provoke a specific DTH-reaction. The intensity of the DTH-reaction was measured by the footpad swelling due to the inflammatory response[11,12]. In parallel, the influx of ^{125}J-HSA into the inflammatory area was determined[13].

As shown in figure 1, DTH reactions were inhibited in mice fed with Cd^{++}. The inhibition of DTH by Cd^{++} was related to the concentration detected in the serum of the animals.

To test the effect of Cd^{++} on the humoral immune response, groups of mice treated as described above were injected i.p. with 2×10^8 SRBC. 5 days after immunization, the number of IgM and IgG antibody producing cells in the spleen of Cd^{++}-treated and non-treated mice was determined by the plaque test according to Cunningham and Szenberg[14], as described in detail by Diamantstein et al.[15]. Mice treated even with 600 ppm of Cd^{++} showed no significantly reduced quantity of anti-SRBC-plaque forming spleen cells (IgM: $446{,}285\pm45{,}000$/spleen, IgG: $566{,}000\pm154{,}000$/spleen) as compared to the non-treated mice (IgM: $504{,}571\pm49{,}000$/spleen, IgG: $740{,}000\pm100{,}450$/spleen). In another experiment, mice were fed with 100 ppm Cd^{++} for 10 weeks and then immunized with 2×10^8 SRBC. No influence of Cd^{++} on the circulating antibody titers could be observed after the primary immune response against SRBC and secondary challenge with SRBC 4 weeks later (data not shown). These results are in contrast to those reported by Koller et al.[8].

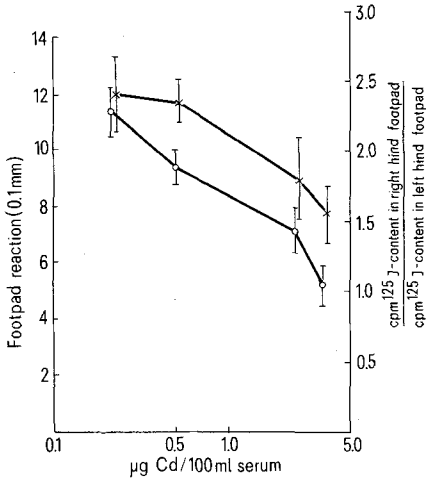

Fig. 1. Suppression of delayed type hypersensitivity by Cd^{++}. Female NMRI-mice, 6–8 weeks old, received orally 30, 300 and 600 ppm Cd^{++} of cadmium acetate dissolved in drinking water for 10 weeks until sacrifice. The average consumption of water was measured twice a week. The x-axis represents the Cd^{++} content in sera detected by AAS. Mice were sensitized with 10^5 SRBC i.v. and challenged 4 days later i.c. in the right hind footpad with 10^8 SRBC. 48 h later the footpad swelling was measured by means of a gauge caliper (left y-axis, O——O). Additionnally the mice were injected with 1 µCi ^{125}J-HSA (Behring) 1 h after SRBC challenge, 48 h later they were killed, the joint of each leg distal to the femur was severed and both hind feet were counted for radioactivity. The ratio of radioactivity between the right, challenged footpad and the left, unchallenged footpad was estimated (right y-axis, ×——×). Each point represents the mean±SEM of 10 mice (left y-axis) and 5 mice (right y-axis), respectively.

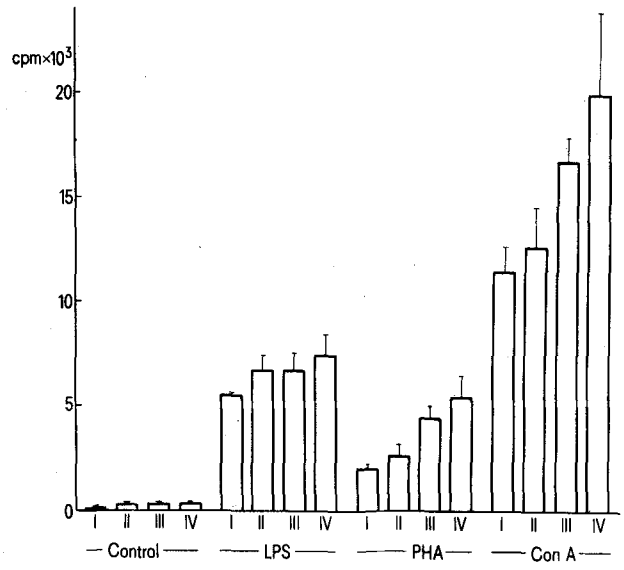

Fig. 2. Influence of Cd^{++} exposure on mitogenic stimulation of spleen cells in vitro. Groups of NMRI-mice were treated with Cd^{++} as described in the legend of figure 1. Spleen cell suspensions obtained from individual mice were cultured in a concentration of 2×10^6 spleen cells per ml in RPMI-1640 medium supplemented with 5% fetal calf serum, 2 mM L-glutamine, 100 units penicillin and 100 µg streptomycin/ml for 48 h at 37 °C in an atmosphere of 5% CO_2 in air. The cells were incubated with either 10 µg/ml PHA (Difco), 2 µg/ml Con A (Serva) or 50 µg/ml LPS (E.coli 055B5, Difco). ^{3}H-thymidine (0.1 µCi; 2 Ci mM^{-1}; Radiochemical Centre, Amersham) was added to the cultures for the last 4 h of incubation period. Cells were collected on glass fibre filters using a Skatron multiple cell culture collector. Incorporation of ^{3}H-thymidine into the nuclear DNA was determined as described previously[18]. I:0, II:30, III:300, IV:600 ppm Cd^{++}.

Using cell cultures, Shenker et al.[16] stated a dose-dependent enhancement or inhibition of DNA-synthesis of murine spleen cells by Cd^{++}. Similary, Cd^{++} enhanced or inhibited, respectively, stimulation of cell proliferation by a B lymphocyte activator (lipopolysaccharide, LPS)[16]. For the question whether Cd^{++} administered in vivo might affect the function of B and T lymphocytes, we examined the reactivity of spleen cells derived from Cd^{++} treated and non-treated mice to various T and B cell mitogens in vitro. Spleen cells derived from Cd^{++}-treated animals gave a significantly higher response to the T cell mitogens phythemagglutinin (PHA) and concanavalin A (Con A) than spleen cells of non-treated mice (figure 2). A similar but less pronounced effect was observed by stimulating the spleen cells with the B cell mitogen lipopolysaccharide (LPS).

The results presented here demonstrate that the immune system is altered by Cd^{++} exposure. It might be of interest to consider that p.o. administration of lead influenced neither DTH nor humoral immune response to SRBC (Müller et al., unpublished data). In contrast to cadmium, lead, even when administered i.p. in doses which inhibited DTH and humoral immunity, failed to affect the capacity of the spleen cells in their response to LPS, Con A and PHA (Müller et al., unpublished data).

* Dedicated to Prof. Georg Henneberg on the occasion of his 70th anniversary on 12.10.1978.

1 Institut für Wasser-, Boden- und Lufthygiene, Corrensplatz 1, D-1000 Berlin 33 (West).
2 Institut für Pathologie, Klinikum Steglitz, Freie Universität Berlin, Hindenburgdamm 30, D-1000 Berlin 45 (West).
3 Immunologische Forschungseinheit, Klinikum Steglitz, Freie Universität Berlin.
4 Acknowledgments. Supported by a grant from the Umweltbundesamt. We thank Ms Odenwald, Ms Schulz, Klinikum Steglitz, and Ms Emeis, Robert Koch-Institut, Abt. Immunologie, for the excellent technical assistance.
5 D.W. Fassett, A. Rev. Pharm. *15,* 425 (1975).
6 D.E. Gardner, F.J. Miller, J.W. Illing, J.M. Kirtz, Bull. Eur. Physiopath. respiratoire *13,* 158 (1977).
7 L.D. Koller, Am. J. vet. Res. *34,* 1457 (1973).
8 L.D. Koller, J.H. Exon and J.G. Roan, Archs environm. Health *30,* 598 (1975).
9 S. Müller, K.-E. Gillert, Ch. Krause, U. Gross, J. l'Age-Stehr and T. Diamantstein, Experientia *33,* 667 (1977).
10 D. Szadkowski, Problems of the contamination of Man and his Environment by Mercury and Cadmium, Proc. int. Symp. Luxemburg, 1973, p. 495.
11 K.H. Falchuk, M. Evenson and B.L. Vallee, Analyt. Biochem. *62,* 255 (1974).
12 T.E. Miller, G.B. Mackanness and P.H. La Grange, J. natl Cancer Inst. *51,* 1669 (1973).
13 M.F. Franco and I. Morley, J. immunol. Meth. *11,* 7 (1976).
14 A.J. Cunningham and A. Szenberg, Immunology *14,* 599 (1968).
15 T. Diamantstein, W. Keppler, E. Blitstein-Willinger and S. Ben-Efraim, Immunology *30,* 401 (1976).
16 B.J. Shenker, W.J. Matarazz, R.L. Hirsch and J. Gray, Cell. Immunol. *34,* 19 (1977).
17 T. Diamantstein and A. Ulmer, Nature *256,* 418 (1975).
18 T. Diamantstein, H. Rühl, W. Vogt and G. Borchert, Eur. J. Immunol. *3,* 488 (1973).