Effects of Heavy Metal on the Immune Response. Preliminary Findings for Cadmium in Rats[1] (35762)

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(Introduced by Dr. Warren C. Eveland)

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There is general recognition that comparatively "large" doses of most heavy metals in animals cause acute physiologic (toxic) effects having various overt clinical manifestations. Likewise, individuals nutritionally deprived of certain heavy metals experience metabolic disorders of varying degrees (1–16).

Investigators (17–25) have reported physiological effects of acute and chronic cadmium (Cd) poisoning, studying humans mostly in specific occupational groups. Some of these effects are hypertension, emphysema, anosmia, testicular atrophy, renal damage, and proteinuria. The latter symptom is an extremely important metabolic consequence of chronic, low-level Cd^{2+} poisoning (26–28), especially today in view of the increased individual contact with heavy metal pollutants in water, air, and certain foods. Heavy metal contact no longer appears to be a problem for people in occupational groups only but for everyone in general.

It has been hypothesized (29–30) that Cd proteinuria is primarily due to an impaired tubular reabsorption of the serum proteins. Vigliani *et al.* (31) demonstrated 550–900 mg of gamma-globulin derived light-chain protein in urine samples from three people experiencing Cd^{2+} poisoning and excreting 1.5–2.5 g of total protein/day. The extremely high amount of light-chain protein excreted was thought (32) to be generally attributed to the inhibition by Cd^{2+} of the activities of catabolic enzymes, peptidases specifically. Recently, it was found (33) that platinum ions [$Pt(NH_3)_2Cl_4$(1 ppm), and $Pt(NH_2)_2(CH_2)_2(Cl_4$, or $Pt(CH_2)_2Cl_2$, (both 2–10 ppm)] inhibited or suppressed the immune response *in vitro* in dispersed spleen cells from rabbits previously immunized (10–24 months) with killed *Brucella abortus* cells (Ag). The metal ions were added to the cells the same time as the booster dose of Ag (10^8 killed cells). The purpose of the present study was not to investigate the various possible mechanisms of Cd^{2+} involvement, but to detect parametrically if Cd^{2+} affected synthesis of specific antibody.

Methods. Thirty-six Sprague-Dawley rats, each weighing 300 g, were distributed equally into six experimental groups (I–VI). Each animal in groups I–IV received two intravenous injections of 8 mg of human gamma-globulin (antigen—Ag), the second injection being given 21 days after the first. A stock solution containing 3 mg/ml of Cd^{2+} as $CdCl_2$ was prepared with demineralized glass distilled water, and was filter-sterilized and used throughout the study. Animals in groups II–IV received subcutaneous injections of Cd^{2+} [0.6 mg/kg) 5 days/week. The Cd^{2+} doses were initiated 1 and 2 weeks before the first Ag injection in animal groups III and II, respectively. In animal group IV, Cd^{2+} doses were initiated 2 days after the second

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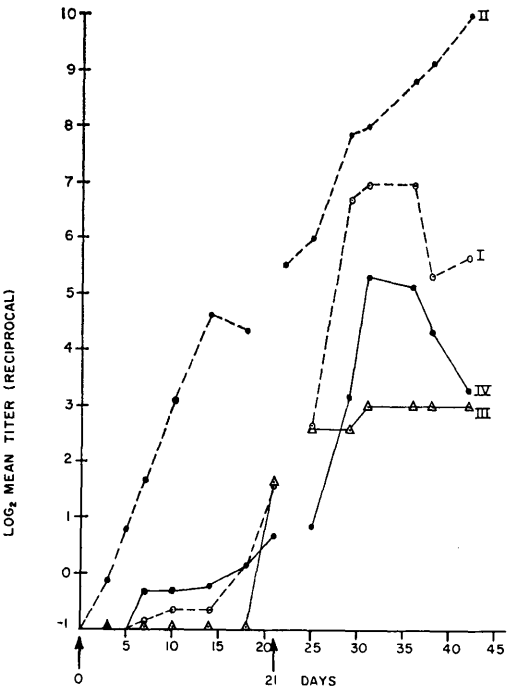

FIG. 1. Log_2 mean titers (reciprocals) of untreated and Cd^{2+}-treated Sprague-Dawley rats which received two Ag doses.

Ag injection. Animals in groups V and VI received Cd^{2+} only and nothing, respectively.

Serum samples were collected at various times via the tail vein and stored at −20°. All sera were analyzed for antibody (Ab) content at one time by the passive hemagglutination (PHA) microtiter test (33, 34).

Results and Discussion. Figure 1 shows that initiation of Cd^{2+} injections 2 weeks before the first Ag dose (group II) remarkably enhanced both the primary and secondary antibody responses as compared to the control (group I). On the contrary, Cd^{2+} doses initiated 1 week before the first Ag injection (group III) delayed and substantially depressed the primary and secondary Ab responses, respectively. An Ab response in group III animals was initially detected on the same day they received the second Ag injection. Since the blood samples were collected prior to Ag administration, such a titer was considered to be a delayed primary Ab response.

Animals receiving Cd^{2+} injections initiated 2 days after the second Ag dose was given (group IV), showed lower titers in the secondary response as compared to the control group. However, such differences were not considered as significant as those observed between I and II, I and III, and II and III (see Table I). A log_2 mean titer difference of ± 2.0 or more was considered significant because the standard error of the automated microhemagglutination system used is ± 1.0 log_2 titer (R.J. Porter, unpublished data). As expected, no antibody was detected in the control animals in groups V and VI.

Although the findings in the present study suggest that Cd^{2+} does have an effect on the immune response in relation to when Ag is given, the precise mechanism(s) of how Cd^{2+} is involved is currently unclear. It is possible that Cd^{2+} may be variously involved in such mechanisms as: (a) the reduction of the activity of leucine aminopeptidase (LAP—a catabolic enzyme), thereby decreasing or prolonging the breakdown of proteins (antibody) (32); (b) in small quantities, enhancement of the activity of certain enzymes as found for certain other heavy metals (35–37); or (c) modification of cell permeability or cell injury causing the release of nucleic acids.

Using a fluorometric system of analysis, it has been observed (unpublished data) that microgram (<100) and milligram (>1) quantities of Cd^{2+}, generally enhanced and depressed the activity of LAP, respectively.

Comparing the results obtained in the present study, it does not appear difficult to explain why there was such an enhanced Ab response in group II animals when mechanism (a) (above) is considered. Animals in group III received less Cd^{2+} (1.5 mg/animal) than group II animals and showed an overall depressed secondary Ab response. Perhaps an explanation for such an incredible difference in Ab response between groups II and III may embrace both mechanisms (a) and (b). However, explanations for the types of Ab responses obtained in groups II–IV, after receiving comparable accumulated quantities of Cd^{2+} (see Table II), would have to be inconsistent if (a) and (b) were the only mechanisms considered. Consequent-

TABLE I. Log_2 Mean Titers (reciprocals) of PHA Antibody in Untreated and Cd[a]-Treated Sprague-Dawley Rats (A), and Their Differences (B), Respectively.

	Animal group	Days												
		0[b]	3	5	7	10	14	18	21[b]	25	29	31	36	38	42
A															
	I	—[c]	—	—	−0.44	−0.36	−0.36	0.10	1.40	2.60	6.63	7.00	7.00	5.25	5.56
	II	—	−0.10	0.70	1.60	3.08	4.56	4.25	5.44[d]	6.00	7.80	8.00	8.76	9.09	10.00
	III	—	—	—	—	—	—	—	1.50	2.50	2.50	3.00	3.00	3.00	3.00
	IV	—	—	—	−0.20	−0.20	−0.14	0.10	0.60	0.80	3.13	5.25	5.13	4.25	3.25
B															
	II-I	—	+0.90	+1.70	+2.04	+3.44	+4.92	+4.15	+4.04	+3.40	+1.17	+1.00	+1.76	+3.84	+4.44
	III-I	—	—	—	−0.56	−0.64	−0.64	−1.10	+0.10	−0.10	−4.19	−4.00	−4.00	−2.25	−2.36
	IV-I	—	—	—	+0.24	+0.16	+0.22	0	−0.80	−1.80	−3.50	−1.75	−1.87	−1.00	−2.31
	II-III	—	+0.90	+1.70	+2.60	+4.08	+5.56	+5.25	+3.94	+3.50	+5.30	+5.00	+5.76	+6.09	+7.00
	II-IV	—	+0.90	+1.70	+1.80	+3.28	+4.70	+4.15	+4.84	+5.20	+4.67	+2.75	+3.63	+4.84	+6.75
	IV-III	—	—	—	+0.80	+0.80	+0.86	+1.10	−0.90	−1.70	+0.63	+2.25	+2.13	+1.25	+0.25

[a] CD doses initiated day −14, −7 and 23 for groups II, III, and IV, respectively.

[b] Ag (human gamma-globulin) given on days 0 and 21.

[c] Denotes no Ab titers measured in any of the animals.

[d] Samples taken on day 22.

TABLE II. Comparison of Log_2 Mean Titers (reciprocals) of PHA Antibody in Animals After Receiving Similar Accumulated Quantities of Cd^{2+}.

Animal group	Cadmium (mg/animal)								
	0	1.5	3.0	4.5	6.0	7.5	9.0	10.5	12.0
II									
Day[a]	—14	—7	0	7	14	21	28	35	42
Titer	—[b]	—	—	1.60	4.56	5.44[c]	7.34	8.61	10.00
Difference	0	0	2.60	2.96	0.88	1.90	1.27	1.39	
III									
Day	—7	0	7	14	21	28	35	42	
Titer	—	—	—	—	1.50	2.50	3.00	3.00	
Difference	0	0	0	2.50	1.00	0.50	0		
IV									
Day	23	30	37	42					
Titer	2.00	4.13	4.62	3.25					
Difference	2.13	0.49	—1.73						

[a] Day(s) in relation to time of first Ag administration.
[b] Denotes no Ab titers measured in any of the animals.
[c] Samples taken on day 22.

ly, Cd^{2+} seems to be similar to 6-mercaptopurine (6-MP) in that its effect on the immune response is relative to a time-factor relationship between metal treatment (and possibly extent of treatment) and Ag administration (38). Although platinum ions were tested *in vitro*, their inhibitory effects on the secondary immune response (primary was not tested) also appears to be related to time of Ag injection (33).

The enhancement of Ab synthesis by 6-MP was suggested to be attributed to effects mediated by the nucleic acids released from cells killed or injured by the drug (38). Later studies revealed that nucleic acids can enhance Ab synthesis by acting as adjuvants (39–42). Therefore, mechanisms (a) and (c) could together explain the unequivocally enhanced immune response seen in group II animals.

Perhaps reasons for the type of responses obtained in groups III and IV may include important metabolic functions other than those already mentioned.

Summary. Cadmium (Cd) injected into rats at various time intervals in relation to antigen (Ag) (human gamma-globulin) administration was shown to have a diverse but significant effect on the immune response, as measured by the passive hemagglutination test. Cd^{2+} doses (0.6 mg/kg) initiated 14 and 7 days prior to Ag injection enhanced and suppressed antibody synthesis, respectively. Mechanisms of Cd involvement are discussed.

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