bers of genetically-marked subfamilies[5,6]. Together, these results demonstrate genetic contributions to variability in worker behavior. These results were obtained even though the assay we used is relatively insensitive compared to one in which each allozyme-phenotype group corresponds to a single subfamily.

The observed differences in allozyme-phenotype frequencies between guards and honey-storage-area bees may be a consequence of changing patterns of sperm use over time if guards and honey-storage-area bees have different age distributions. Although workers from these groups are likely to be very similar in age, this relationship is not firmly established[3,10]. This argument is less tenable as an explanation for the differences between pollen foragers and nectar foragers. Workers performing these tasks have not been shown to represent different age groups, therefore, we suggest that the observed differences in this case are primarily a consequence of genotypic effects on worker behavior. These results demonstrate that genetic structure is an important component of the social organization in unselected honeybee colonies with naturally-mated queens.

Acknowledgments. We thank Tom Seeley for helpful comments on this manuscript and M. Kim Fondrk for technical assistance. This research was supported by an Ohio State University Presidential Fellowship to N. W. Calderone, an Ohio State University Postdoctoral Fellowship to G. E. Robinson, and grants from the National Science Foundation (USA) and The Ohio State University to R. E. Page. Publication charges were paid by The Ohio State University James I. Hambleton Apiculture Memorial Award Fund.

* Present address: U.S.D.A., Beneficial Insects Laboratory, BARC-EAST, Bldg 476, Beltsville, MD 20705, USA.

1 Oster, G. F., and Wilson, E. O., Caste and Ecology in the Social Insects. Princeton University Press, Princeton 1978.
2 Wilson, E. O., The Insect Societies. Harvard University Press, Cambridge 1971.
3 Seeley, T. D., Behav. Ecol. Sociobiol. *11* (1982) 287.
4 Calderone, N. W., and Page, R. E. Jr, Behav. Ecol. Sociobiol. *22* (1988) 17.
5 Robinson, G. E., and Page, R. E. Jr, Nature *333* (1988) 356.
6 Frumhoff, P. C., and Baker, J., Nature *333* (1988) 358.
7 Page, R. E. Jr, A. Rev. Ent. *31* (1986) 297.
8 Contel, E. P. B., Mestriner, M. A., and Martins, E., Biochem. Genet. *15* (1977) 859.
9 Moore, A. J., Breed, M. D., and Moore, M. J., Anim. Behav. *35* (1987) 1159.
10 Seeley, T. D., Honeybee Ecology. Princeton University Press, Princeton, N. J., 1985.
11 Gary, N. E., and Lorenzen, K., J. Apic. Res. *15* (1976) 73.
12 Lindauer, M., Bee Wld *35* (1955) 81.
13 Ribbands, C. R., Proc. Roy. Soc. London Ser. B *140* (1952) 32.
14 Lindauer, M., Bee Wld *34* (1953) 63.
15 Lindauer, M., Bee Wld *34* (1953) 85.
16 Sekiguchi, K., and Sakagami, S. F., Hokkaido natl agric. Exp. Stn Rept *69* (1966).
17 Sokal, R. R., and Rohlf, F. J., Biometry. Freeman, New York 1981.

0014-4754/89/080765-03$1.50 + 0.20/0

Cadmium effects and biochemical status in hamsters following acute exposure in late gestation

D. P. Hanlon and V. H. Ferm

Department of Anatomy, Dartmouth Medical School, Hanover (New Hampshire 03756, USA)
Received 24 January 1989; accepted 6 April 1989

Summary. A normally teratogenic dose of cadmium ions administered to hamsters late in gestation does not cross the placental barrier nor does it result in placental pathology. Our finding, which differs from data for other rodents, may be due to differences in the chemical status of cadmium in the placental cytosols.
Key words. Placenta; cadmium ion; biochemical status; hamster.

The cadmium ion (Cd^{++}) is a potent teratogen in mammals and other vertebrates[2]. The deleterious effect of Cd^{++} on in utero development is not restricted to the production of fetal abnormalities but also produces profound toxic responses in both placentas and fetuses of certain rodents during late gestation[3–7]. This paper reports our findings for the feto-toxic and placento-toxic effects of Cd^{++} administered to hamster dams late in gestation. Our study includes an appraisal of the distribution and chemical status of Cd in the maternofetal system.

Materials and methods

Pregnant hamsters (LKV strain) were obtained from the Charles River Breeding Labs on day 5 of gestation. The dams were held individually in cages and fed rat chow and water ad libitum. Individuals were injected i.p. with 7.80 μmoles of Cd^{++} (as the sulfate salt) per kg on the morning of day 14 of gestation (about 36 h before birth). Carrier-free $^{109}Cd^{++}$ (New England Nuclear) served as the radiotracer (5–10 μC/μM Cd). Animals were sacrificed 24 h post-injection using CO_2 inhalation. Maternal

blood was obtained by cardiac puncture using heparinized vacutainers. Liver and fetal tissues (fetus and placentas) were removed by dissection, weighed, and placed on ice prior to radioassay. Radioassays were performed using a previously described method [8].

The biochemical status of Cd in maternal liver, maternal kidneys and placentas was determined by gel filtration chromatography of tissue cytosols, following the procedure of Hanlon et al. [8]. In some cases the eluting buffer contained 1.0 mM EDTA. In other gel experiments, cytosols were incubated with 1 mM EDTA at 4 °C for 1 h prior to gel chromatography.

In another part of this study, six hamsters were given an i.p. injection of 7.80 μmoles Cd^{++}/kg on day 14 of gestation. 24 h post-injection the animals were sacrificed by CO_2 asphyxiation. Placentas and fetuses were removed by dissection and examined immediately for pathology, e.g., necrosis, hemorrhage and edema. Placentas were transferred to Bouin's solution. Paraffin embedded tissues were sectioned, stained with hematoxylin-eosin and examined under the light microscope.

Results

Table 1 shows the concentration of Cd in maternal blood, maternal liver, placentas and fetuses of day 15 hamsters 24 h after a single i.p. dose of 7.80 μmoles Cd^{++}/kg dam. Net counts in the fetus samples were no different than background which indicates a maximum Cd concentration of 0.001 μmoles Cd/kg in the fetal tissues. Calculation of Cd body burden indicated nearly 100% of the injected Cd resides in the maternal liver.

Typical Sephadex G-75 elution profiles for the Cd components of hamster maternal liver cytosols and placental cytosols are depicted in the figure. Elution profiles were highly reproducible. Over 95% of the applied counts were recovered in the fractions between the excluded volume (V_o) and the included volume. Two significant Cd peaks are present, one at V_o and a second near $V_o \times 2$. A small shoulder eluting at $V_o \times 1.4$ may be an unresolved Cd component.

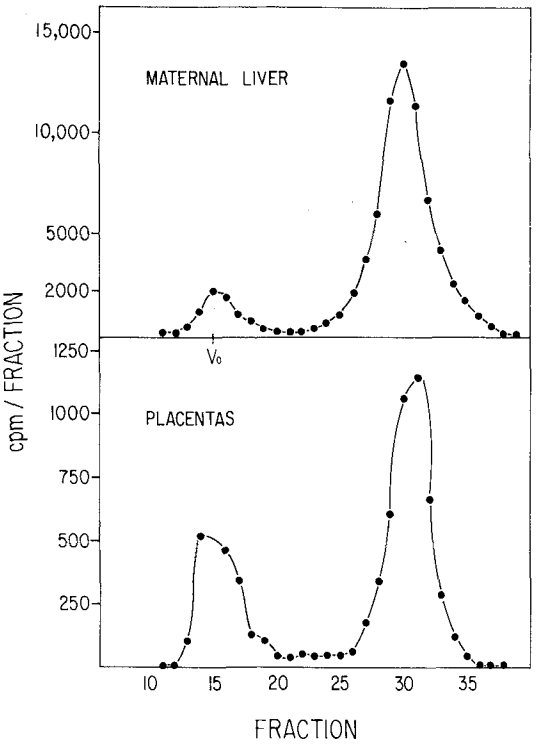
Table 2 contains data for the partitioning of Cd in maternal liver and placentas. Soluble Cd is 92% of the total in maternal liver samples and 65% of the total in placentas. The Cd component eluting near $V_o \times 2$ is dominant in both tissue cytosols. Neither the presence of 1 mM EDTA in the eluting buffer nor 1-h preincubation with 1 mM EDTA had any effect on the elution profile of the tissue cytosols.

Gross examination of the day-15 placentas, 24 h after exposure to cadmium, showed no necrosis or hemorrhaging. Examination of fixed and stained sections of the placental tissues by three individuals using light microscopy showed normal architecture. Day-15 fetuses were normal.

Table 1. The concentration of Cd in the maternal and fetal tissues of the Syrian hamster on day 15 of gestation 24 h after an i.p. injection of 7.80 μmoles Cd^{++}/kg dam. Numerical values represent means ± standard errors.

Tissue	μmoles Cd/kg tissue[a]
Maternal blood	0.105 ± 0.020
Maternal liver	142 ± 6
Placentas	16.6 ± 2.4
Fetuses	< 0.001

[a] Values are based on 6 animals. Placental values represent pooled placentas from each day to 10 placentas per individual.



The Sephadex G-75 elution profiles for ^{109}Cd in maternal liver and placental cytosols of hamsters treated with 7.80 μmoles Cd^{++}/kg on day 14 of gestation and sacrificed 24 h later.

Table 2. The biochemical status of Cd in maternal livers and placentas of day-15 Syrian hamsters 24 h after an i.p. injection of 7.80 μmoles Cd ion/kg dam. Numerical values represent means ± standard errors.

	μmoles Cd/kg[a]			
	Whole tissue	Cytosol	V_o[b] component	$V_o \times 2$[b] component
Maternal liver	142.0 ± 6	131.0 ± 2.0	17.0 ± 0.8	111.0 ± 3
Placentas	16.6 ± 2.4	10.8 ± 1.0	3.70 ± 0.37	6.07 ± 0.77

[a] Values are based on data from 6 dams. Placental data represent pooled tissues from each dam (4 to 10 placentas per individual). [b] The V_o component comprises fractions 12–18 on the gel columns. The $V_o \times 2$ component comprises fractions 27–33 on the gel columns.

Discussion

In this study we show that hamster dams injected with a dose of Cd^{++}, which is teratogenic if administered on day 8 of gestation, manifest no deleterious effects if injected late in gestation. The absence of pathology [9] in the

fetal unit of hamsters is in marked contrast to findings reported by others[3-7,10]. Since a toxic response must depend in part on the concentration and chemical status of a toxic agent, we will consider our own data in the light of other studies of similar design, particularly those in which pathology was found.

The Cd concentration of placental cytosols in our study (16.6 μmoles/kg) is close to the value of 17.1 μmoles Cd/kg calculated from the data of Samarawickrama and Webb[9] for Wistar rats 24 h after an i.v. injection of 9.38 μmoles Cd^{++}/kg on day 20 of gestation (term in the rat is day 21). Samarawickrama and Webb reported placental necrosis, fetal deaths and vaginal bleeding. In a similar study, Sonowane et al.[6] found extensive placental pathology and fetal mortality in Cd-treated rats. Placental Cd concentration was 8.39 μmoles/kg 24 h after an i.v. injection of 14.1 μmoles Cd^{++}/kg dam. Collectively, our findings do not support the notion that placental pathology correlates directly with the total Cd concentration. Fetal concentrations were not given by Samarawickrama and Webb, but the data of Sonowane et al. yield a value of 0.217 μmoles Cd/kg compared to the hamster value of less than 0.001 μmoles Cd/kg (table 1). The very low fetal Cd concentration of day-15 results from the impressive ability of the late placenta to act as a barrier to Cd in the hamster. Previous studies of our own, as well as those of others, have provided evidence that the hamster placenta becomes virtually impermeable to Cd sometime on day 9 of gestation. For example, all of the Cd found in day-12 hamster fetuses can be accounted for by that which crosses the placenta during the 24 h following administration on day 8 of gestation[11].

Our chemical status data provide an indication of the availability and chemical associations of Cd in the late hamster placenta. The chemical status of Cd in fetuses was not examined for obvious reasons. Maternal liver cytosols and placental cytosols produced Sephadex G-75 elution profiles which exhibited 2 major Cd fractions (fig.). One or more Cd molecular species eluted at V_0 and possessed 13% of the Cd content of maternal liver cytosols and 34% of the placental cytosols (see table 2). We have not characterized this Cd component beyond establishing that it has an apparent molecular weight greater than 80,000 (the minimum molecular weight of proteins excluded from Sephadex G-75) and that it binds Cd with sufficient avidity to prevent Cd exchange to EDTA in the presence of 1 mM EDTA in the elution buffer or by 1-h incubation prior to gel filtration. We have ruled out hemoglobin, which elutes at $V_0 \times 1.15$, on Sephadex G-75, as a contributor to this Cd component for two reasons. First, hemoglobin contamination of the cytosols was minimal and, second, the very low concentration of Cd in whole blood (table 1) must reflect a very low contribution for Cd bound to hemoglobin. Serum albumin could also contribute to the high molecular weight Cd fraction since it elutes at $V_0 \times 1.08$ on Sephadex G-75. However, 1 mM EDTA immediately chelates Cd bound to serum albumin (Hanlon and Ferm, unpublished finding). The Cd component eluting as a shoulder at $V_0 \times 1.4$ represents another unidentified Cd macromolecule with an apparent molecular weight near 30,000. High molecular weight polymer forms of metallothionein can be generated during gel chromatography if antioxidants, such as mercaptoethanol, are not included in the eluting solvent[12]. Since our buffer system contained 5 mM mercaptoethanol, we do not believe that the Cd species eluting at V_0 and $V_0 \times 1.4$ are polymers of CdMT. The dominant Cd component of day-15 maternal liver cytosols and placental cytosols elutes as a single peak near $V_0 \times 2$ (fig.). This component comprises 85% of the maternal liver cytosol Cd and 56% of the placental cytosol Cd (table 2). Since hamster metallothioneins elute near $V_0 \times 2^8$ and 1-h incubation of maternal liver and placental cytosols with 1 mM EDTA did not reduce the Cd content or change the elution characteristics of this component, we have tentatively identified this Cd species as Cd metallothionein. Our placental cytosol profiles have the same qualitative Sephadex G-75 elution characteristics as placental cytosols from rats receiving Cd^{++} repeatedly in mid-late gestation[13]. Samarawickrama and Webb[9] and Wolkowski[4] also found gel elution profiles in placental cytosols of rodents treated late in gestation similar to those seen in the day-15 hamster placental cytosols. Both of these studies reported extensive pathology in the fetal system. The findings of Samarawickrama and Webb are particularly interesting in that they show a significantly greater contribution of the high molecular weight Cd component to the total Cd content of placental cytosols (49%) than we found for day-15 hamster placental cytosols (34%) They also reported a Cd hemoglobin component (29% of the placental cytosol), which we did not see in hamster placental cytosols. Presumptive Cd metallothionein comprised 21% of the day-20 rat placental cytosol Cd, whereas we found it contributed 56% to the total hamster placental cytosol Cd. It is possible that the absence of placental pathology in the hamster and its presence in the other rodents is due to different routes of administration, intraperitoneal (i.p.) in the hamster, intravenous (i.v.) in the others. Intraperitoneal injection does result in a faster initial distribution of agent in maternal and fetal compartments, but this would not account for the greater total placental cadmium concentration, relative to dose, in the hamster 24 h post injection. Pertinent here is the finding of Levin et al.[14] that a single subcutaneous dose of cadmium ions (40 μmoles/kg) delivered to rat dams late in pregnancy is almost completely cleared from the maternal blood 4 h post injection. These data collectively suggest that there is probably not much difference between i.p. and i.v. routes as far as placental exposure to cadmium 24 h post injection. Perhaps the greater capacity of the late hamster placentas to sequester Cd in the form of Cd metallothionein is responsible for the absence of pathology in the fetal system. In any event, our study of the Cd concentra-

tion status of the aged hamster placenta provides useful information regarding the chemical forms and bioavailability of Cd present. In concert with the findings of Samarawickrama and Webb it suggests a mechanism to account for the presence (and absence) of pathology in the fetal system as a response to Cd^{++} administration.

1 This project has been financed in part with Federal funds from the U. S. Environmental Protection Agency under grant number R 81 1078. The contents do not necessarily reflect the views and policies of the Environmental Protection Agency nor does mention of trade names or commercial products constitute endorsement of recommendation for use.

2 Ferm, V. H., and Layton, W. M., in: Cadmium in the Environment, Part 2: Health Effects, p. 746. Ed. J. O. Nriagu. John Wiley and Sons, New York 1981.
3 Parizek, J., in: Reproductive and Development Toxicity of Metals, p. 301–313. Eds T. W. Clarkson, G. F. Nordberg and P. R. Sager. Plenum Press, New York 1983.
4 Wolkowski, R. M., Teratology *10* (1974) 243.
5 Wolkowski-Tyl, R. M., and Preston, S. F., Teratology *20* (1979) 341.
6 Sonowane, B. R., Nordberg, M., Nordberg, G. F., and Lucier, G. W., Envir. Hlth Perspect. *12* (1975) 97.
7 Levin, A. A., and Miller, R. K., Teratology *22* (1980) 1.
8 Hanlon, D. P., Ferm, V. H., and McLain, G., Reprod. Toxic. (1989) in press.
9 Ferm, V. H., and Hanlon, D. P., in: Reproductive and Developmental Toxicity of Metals, p. 383. Eds T. W. Clarkson, G. F. Nordberg and P. R. Sager. Plenum Press, New York 1983.
10 Samarawickrama, G. R. I., and Webb, M., J. appl. Toxic. (1981) 264.
11 Ferm, V. H., Hanlon, D. P., and Urban, J., J. Embryol. exp. Morph. *22* (1969) 107.
12 Minkel, D. T., Pulsen, K., Weilgus, S., Shaw, C. F., and Petering, D. H., Biochem. J. *191* (1980) 475.
13 Arizono, K., Ota, S., and Ariyoshi, T., Envir. Contam. Toxic. *27* (1981) 671.
14 Levin, A. A., Klipper, R. W., and Miller, R. E., Teratology *36* (1987) 163.

0014-4754/89/080767-04$1.50 + 0.20/0

An immune response-dependent mechanism for the vertical transmission of an entomopathogen

A. Warburg* and K. Ostrovska

Department of Epidemiology and Public Health, Yale University School of Medicine, 60 College Street, P. O. Box 3333, New Haven (Connecticut 06510, USA)
Received 6 January 1989; accepted 9 March 1989

Summary. An exceptionally efficient mechanism for the vertical transmission of a parasitic gregarine is dependent on the insect host's immune response. Gametocysts of *Ascogregarina chagasi* on the genital accessory glands of adult female sand flies (*Lutzomyia longipalpis*) become encapsulated through hemocyte-mediated immune reactions. Oocysts of *A. chagasi*, ejected into the lumen of the glands owing to pressure exerted by this capsule, become glued to eggshells and are subsequently ingested by larvae. In *L. longipalpis* with an experimentally suppressed encapsulation reaction, fewer accessory glands contained oocysts of *A. chagasi*.
Key words. Humoral encapsulation; sand fly; gregarine; vertical transmission; parasitic life cycle.

Humoral encapsulation is an immune mechanism of dipteran insects, which has been described in *Plasmodium*-infected mosquitoes[1,2] as well as in several other host-parasite combinations[3–6]. The prophenoloxidase-activating cascade, triggered by invasive organisms, releases phenoloxidase which transforms phenolic compounds into quinones. These bind to amino groups of proteins creating a resistant complex of macromolecules, which is deposited around the foreign bodies[5,6]. The involvement of hemocytes in this process has been documented; however, their exact role remains unclear[7,8].

Phlebotomine sand flies (Diptera: Psychodidae) are vectors of the leishmaniases, a group of protozoan diseases which affect humans and occur in many regions of the world. *Lutzomyia longipalpis* is the vector of human visceral leishmaniasis in Latin America[9]. Gregarines are parasites of invertebrates which have been reported in over 20 species of sand flies[10], as well as numerous other arthropods[11]. *Ascogregarina chagasi* (Apicomplexa: Lecudinidae) was originally described in Brazilian sand flies[12], and has since been identified in a laboratory colony of *Lutzomyia longipalpis*, originating in Colombia[13].

Preliminary studies with the above *L. longipalpis* colony verified that 94%–100% of the adults were infected with *A. chagasi*. This high rate of infection was maintained despite the routine removal of dead adults from the rearing containers. It must therefore be attributed to oocyst contamination of eggshells. The earliest larval stage in which oocysts were observed was the third instar. Therefore horizontal transmission in a synchronized laboratory colony could not play a major role in maintaining high infection rates. Irrespective of gonotrophic status, most gamonts and gametocysts of *A. chagasi* in adult female *L. longipalpis* selectively adhere to the accessory glands of the genital apparatus (fig. 1.) Infective-stage oocysts from adherent gametocysts are inoculated through the wall of the accessory glands into their lumen. During oviposition, the accessory gland fluid containing the oocysts is discharged into the common oviduct, smearing