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Cardiac and Non-cardiac Malformations Produced by Mercury in Hamsters

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The susceptibility of the developing mammalian embryo to the adverse effects of mercury is well documented. A variety of organic mercury compounds have been demonstrated to produce embryotoxic effects in experimental animals (MURAKAMI et al. 1956; RAMEL 1967; OHARAZAWA 1968; SPYKER and SMITHBERG 1972). HARADA (1978) recently summarized the reports of human intrauterine methylmercury poisoning ie., congenital Minamata disease, resulting from the ingestion of contaminated food. Ongoing studies in this laboratory have involved several different aspects of the embryotoxicity produced by inorganic mercury in hamsters including a dose response study (GALE and FERM 1971), the interaction of mercuric acetate with cadmium and zinc (GALE 1973), the effect of different routes of administration (GALE 1974), the placental permeability of ^{203}Hg (GALE and HANLON 1976) and the embryotoxic response in several different hamster strains (GALE 1980).

Little is known regarding a human syndrome of congenital malformations characterized by ectopia cordis, internal cardiac defects and abnormalities of the diaphragm and ventral body wall. Most papers regarding this human syndrome are clinical reports describing the characteristics and management of specific cases; only speculative information is provided regarding etiology and possible embryopathic mechanisms (TOYAMA 1972). The observation that a similar syndrome, which will be designated CNC for cardiac and non-cardiac malformations, can be produced by mercury in hamsters (GALE 1974) prompted the present study. The specific goals of this study were: 1) to study the effect of treating pregnant hamsters at different times during embryonic organogenesis to determine the time which produces the highest incidence of the CNC syndrome and whether different treatment times modify the morphological characteristics of the inclusive malformations and 2) to study the structural features of all mercury-induced external and internal abnormalities of the CNC syndrome in late gestation fetuses.

MATERIALS AND METHODS

The timed-pregnant LVG hamsters (Lakeview Hamsters, Inc.) utilized in this study were housed individually in solid bottom cages in a room at 72°F with a 12-hr light and 12-hr dark lighting cycle and were provided with food and water and libitum. Females were bred in the evening and separated from the males

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the following morning. The separation time was considered as the first gestation day. The treated animals were injected with a single dose (15 mg/kg, SC) of mercuric acetate (certified, Fisher Scientific Co.) at 8 AM, Noon or 5 PM on day 7,8, or 9 of gestation. Control animals received demineralized distilled water (5 ml/kg, SC). The pregnant animals were killed by an overdose of ether on either gestation day 12 or 15. All fetuses were examined for external malformations and the number of resorption sites was recorded. All of the 12th gestation day and half of the 15th gestation day fetuses were fixed in Bouin's solution. The 15th gestation day, Bouin's fixed fetuses were dissected in order to determine the types and frequency of internal malformations. Tables of binomial confidence limits (MAINLAND et al. 1956) were utilized to determine the statistical significance of the incidence of resorptions and malformations among the different treatment times of mercury-treated animals and between the treated vs. control animals. Differences were significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

The tables summarize the significant manifestations of mercuric acetate-induced embryotoxicity following the administration of this salt at nine times during the period of embryonic organogenesis in hamsters. Tables 1 and 2 present the data on the frequency of resorptions and the incidence of external abnormalities in fetuses examined on gestation days 12 and 15 respectively. While the severity of the fetal edema varied from slight to marked distention of the entire animal, in most of the fetuses the edema was most noticeable on the dorsal region between the neck and tail. Retarded fetuses are those in which the stage of development is delayed from 0.5 to 2 days when compared with controls. The "ventral wall defect" category includes a whole spectrum of abnormalities. In the simplest form a small opening is detectable in the ventral midline of the thorax through which a small portion of the heart is visible. An intermediate condition exists in which the heart protrudes through a larger opening i.e., ectopia cordis, and the intact diaphragm is visible. In the most severe cases the defect involves the thorax and the wall of the abdomen cranial to the site of attachment of the umbilical cord, both the heart and liver are ectopic and the diaphragm is defective ventrally. Another abnormality is characterized by different degrees of distention of the ventral thoracic wall in the region of the pericardial cavity. A comparison of the data on resorptions as well as abnormal, edematous and retarded fetuses in Tables 1 and 2 demonstrates that in general most of the externally detectable manifestations of mercury embryotoxicity are more severe in the fetuses examined on gestation day 12 vs. 15. In fact two categories of embryotoxicity ie., ventral wall defects and pericardial cavity distention were detected only in the 12th gestation day fetuses.

TABLE 1. EXTERNALLY VISIBLE EFFECTS ON MERCURY ON 12TH GESTATION DAY HAMSTER FETUSES

Treatment Day-Time	No. Females	No. of Implantation Sites	No. Live Fetuses	Percent*					
				Resorption Sites	Abnormal Fetuses	Edematous Fetuses	Retarded Fetuses	Ventral Wall Defects	Pericardial Cavity Distended
				Mercuric acetate-treated animals					
7 8AM	8	110	54	51	49	46	49	10	36
7 Noon	8	122	77	37	62	58	30	11	14
7 5PM	8	98	62	37	63	63	59	10	30
8 8AM	7	82	65	21	78	65	70	20	37
8 Noon	8	107	82	23	75	72	44	14	22
8 5PM	8	97	51	47	52	49	35	10	31
9 8AM	8	102	62	39	61	42	54	10	19
9 Noon	8	116	90	22	78	78	53	8	36
9 5PM	8	101	55	46	54	52	28	9	21
				Controls					
7 8AM	4	55	53	4	4	2	0	0	0
7 Noon	3	38	38	0	3	0	0	0	0
7 5PM	3	25	22	12	0	0	0	0	0
8 8AM	3	36	30	17	3	0	3	0	0
8 Noon	3	41	40	2	0	0	0	0	0
8 5PM	2	26	25	4	0	0	0	0	0
9 8AM	3	40	37	8	0	0	0	0	0
9 Noon	4	55	53	4	2	0	0	0	0
9 5PM	3	42	39	7	0	0	0	0	0

* % = No. ÷ No. Implantation sites X 100
Underlined % are significantly different statistically from corresponding controls.

TABLE 2. EXTERNALLY VISIBLE EFFECTS OF MERCURY ON 15TH GESTATION DAY HAMSTER FETUSES

Treatment Day-Time	No. Females	No. Implantation Sites	No. Live Fetuses	Percent* Resorption Sites	Abnormal Fetuses	Edematous Fetuses	Retarded Fetuses
				Mercuric acetate-treated animals			
7 8AM	6	74	56	24	5	3	0
7 Noon	7	86	42	51	21	13	2
7 5PM	7	89	55	38	24	18	0
8 8AM	7	76	67	12	16	12	0
8 Noon	6	73	61	16	51	51	32
8 5PM	6	58	50	14	5	2	2
9 8AM	6	65	56	14	2	2	0
9 Noon	6	83	69	17	8	8	0
9 5PM	6	56	43	23	0	0	0
				Controls			
7 8AM	3	34	34	0	0	0	0
7 Noon	3	38	37	3	0	0	0
7 5PM	3	42	42	0	0	0	0
8 8AM	3	27	27	0	0	0	0
8 Noon	3	45	38	16	0	0	0
8 5PM	4	50	49	2	0	0	0
9 8AM	3	39	39	0	0	0	0
9 Noon	3	26	26	0	0	0	0
9 5PM	3	33	32	3	0	0	0

* % = No. ÷ No. Implantation sites X 100
Underlined % are significantly different statistically from corresponding controls

Table 3 demonstrates that mercury also produces several internal malformations, which are detectable in fetuses late in gestation ie., on day 15 of the 16-day gestation period. The mercury-induced cleft palate involved varying degrees of incomplete fusion of the secondary palatal shelves with one another and the posterior edge of the intact primary palate. Hydrocephalus consisted of slight to moderate dilation of the lateral brain ventricles. The most significant internal malformation involved the heart. This cardiac damage was characterized by varying degrees of dilation of the ventral wall of the conus cordis and right ventricular portions of the heart. In a few of the most severely dilated hearts the truncus arteriosus was also affected.

Several conclusions can be drawn from this study. 1) The administration of mercuric acetate to pregnant hamsters at nine different times during the period of organogenesis produces marked embryotoxicity including embryonic death as well as external and internal abnormalities in living fetuses examined on gestation day 12 or 15. Based on the incidence figures in the tables, the most significant categories of embryotoxicity include resorptions, retardation, edema, pericardial cavity distention (Table 1) and abnormal hearts (Table 3). Some of the other categories including ventral body wall defects (Table 1), cleft palate and hydrocephalus (Table 3) were present but at much lower frequencies which were often not significantly different from control values. 2) No single treatment time appeared to be the optimal time for producing embryonic damage ie., the incidence of each of the categories of embryotoxicity varied among the different treatment times. 3) For those categories of embryotoxicity compared between day 12 and day 15 of gestation the frequencies are generally much lower in the older fetuses. In fact the ventral body wall defects and the distended pericardial cavity abnormalities were detected exclusively in the day 12 fetuses. One possible explanation is that such abnormalities are transient in nature. Another possibility could be that many of the abnormal day 12 fetuses die within the following three day interval. The generally lower incidence of resorptions for each of the different treatment times in the day 15 group vs the day 12 group argues against this latter possibility. 4) A high incidence of abnormal hearts characterized by a dilation and attenuation of the wall of the conus cordis and right ventricle is produced by the mercury exposure. The underlying cause for this defect is not presently known. While there is some evidence in the literature regarding possible causes for this type of selective heart dilation (WARKANY 1971) discussion will be deferred until the completion of more detailed histological study of the malformed hearts.

Malformations of the ventral body wall with and without ectopic thoracic and abdominal organs include a wide spectrum of abnormalities which are given the general term celosomias (DUHAMEL 1963). For an understanding of some postulated mechanisms of formation of celosomias the reader is referred to the descriptions by PATTEN (1953), CANTRELL et al. (1958), KANAGASUNTHERM and VERZIN (1962) and DUHAMEL (1963). The celosomias produced by

TABLE 3. INTERNAL MALFORMATIONS PRODUCED BY MERCURY IN 15TH GESTATION DAY HAMSTER FETUSES

Treatment Day-Time		No. Females	No. Implantation sites	No. Fetuses	Percent* of Fetuses with: Cleft Palate	Hydrocephalus	Abnormal Hearts	Dilated Conus Cordis	Right Ventricle
					Mercuric acetate-treated animals				
7	8AM	3	32	18	9	0	47	47	31
7	Noon	3	40	16	10	8	38	38	35
7	5PM	4	44	30	18	16	59	55	34
8	8AM	3	35	30	0	0	74	74	34
8	Noon	3	31	27	48	16	77	74	39
8	5PM	2	23	19	26	17	70	70	35
9	8AM	4	39	36	31	26	79	79	46
9	Noon	3	43	31	7	5	60	60	23
9	5PM	3	29	20	3	0	69	69	41
					Controls				
7	8AM	3	34	34	0	0	0	0	0
7	Noon	3	38	37	0	0	0	0	0
7	5PM	3	42	42	0	0	0	0	0
8	8AM	3	27	27	0	0	0	0	0
8	Noon	3	45	38	0	0	2	2	2
8	5PM	4	50	49	0	0	2	2	0
9	8AM	3	39	39	0	0	10	10	8
9	Noon	3	26	26	0	0	0	0	0
9	5PM	3	33	32	0	0	0	0	0

* % = No. ÷ No. Implantation sites X 100

Underlined % are significantly different statistically from corresponding controls

mercury in hamsters include a wide spectrum of defects ranging from small openings with no ectopic organs to large openings involving the thorax and upper abdominal wall through which both the heart and liver protrude. The literature includes cases encompassing a comparable range of severity in man and experimental animals (GREIG 1926; BLATT and ZELDES 1942; MILLHOUSE and JOOS 1959; KANAGASUTHERAM and VERZIN 1962; FRANKLIN 1971). At the cellular level mercury alters the function of the plasmalemma (PASSOW et al. 1961), lysosomes and mitochondria (GOLDWATER 1971) and inhibits enzymes (PASSOW et al. 1961) and mitosis (BERLIN et al. 1969). One or more of these factors selectively acting on the mesoderm of the cranial fold of the developing ventral body wall could be the initial event leading to the production of the celosomias, pericardial cavity distention and heart dilation observed in this study.

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