AN EXPERIMENTAL STUDY ON TERATOGENIC EFFECT OF CADMIUM

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Administration of 5 mg per kilogram of cadmium chloride to pregnant mice on 7 days of gestation produced a variety of malformations in the surviving fetuses.

Exencephaly was found most among the fetuses and such exencephalic fetuses also often exhibited open eye.

Clefts palate and lack of tail or rachischisis were also found in some other fetuses.

Skeletal malformations were observed in the skull region, vertebral parts, ribs and tails.

The total rate of malformation appearance exceeded about 80%.

The less the dosis of cadmium chloride, the less the rate of malformation appearance: 19% for 2.5 mg/kg 1.0% or less for 0.63 mg/kg and none for 0.33 mg/kg.

The quantities of cadmium in the liver, the kidneys and the placentas of the mother animals and fetuses were measured by atomic absorption spectrophotometry.

The cadmium content in the liver of cadmium chloride injected mother animal was 800 times greater than the control animal, that in the kidneys was 450 times and that in the placenta 10 times, but measurable amount of cadmium was not detected among fetuses of cadmium chloride injected mice.

Above mentioned fact expresses that cadmium chloride in the late pregnancy does not transfer through the placenta and stays in the mother’s side.

A number of experiments were conducted on the teratogenic effect of drugs and food additives in the past. As for the teratogenic effect of polluting substances, such as herbicides, insecticides and many other industrial poisons, however, it was only recently that attention was paid to them.

It was because these poisons rarely affected the general public in the past, even in specific workshops it was prohibited for woman laborers to handle poisons, and there was no possibility of accident.

As environmental pollution became severer lately, the general public became suffered from it not seldom, and information on unfortunate examples in Japan, such as occurrence of fetal Minamata Disease, and on pregnant women affected by
herbicides and insecticides in the south made people concerned very much about this problem.

In other countries symposiums on polluting substances and congenital abnormalities were already held by medical societies. These problems were also taken up in related societies in Japan since last year.

Previously the authors studied the sexual difference in the effect of industrial poisons. Paying attention to the fact that cadmium, which came into serious problems lately1), attacked a sexual organ of a male animal (the testicles) selectively, the authors observed its effect on female sexual organ and found that cadmium had no peculiar noci-influence under normal conditions.

In this report the authors studied the effect of cadmium administered to female mice during pregnancy and found that cadmium attacked fetuses strongly and produced a variety of malformations in the surviving fetuses. Malformations induced by cadmium were studied morphologically in detail2,3) and the distribution of cadmium in the mother animals, the placentas and the fetuses was also measured.

**EXPERIMENTAL METHODS**

Four-week-old male and female mice of ICR-SLC strain were purchased and raised to 13 weeks old by feeding chaws (by CA-I of Clea Co. Ltd.) and tap water freely.

A male and a female mice were placed in a cage for 16 hours at night and mated while observing the vaginal smear of the female. The day when a vaginal plug was noticed was defined as Oth day of gestation. On 7th day of gestation, cadmium chloride saline solutions of different concentrations, namely 5.0 mg/kg (group A), 2.5 mg/kg (group B), 0.63 mg/kg (group C) and 0.33 mg/kg (group D), were injected once subcutaneously. Each group consisted of 20 mice.

A) **Morphological studies of the fetuses**

In a control group only the same amount of saline as in experimental groups was administered.

On 18th day of gestation the mother animals were killed by dislocation of cervical vertebra and their abdomens were opened. Appearances of uteri and amniotic fluid were observed and the conditions of the fetuses were tested in the following orders.
1) Position of a fetus in the uterus.
2) Sex difference and body weight of the fetus.
3) Presence of external malformations.
   Head, face, mouth, oral cavity, back, body, tail, limbs and toes.
4) Number of macerated fetuses, retention of placenta and nidation.
5) Skeletal malformations and malformations of entrails.

After checking the external malformations, the skeletal malformations and malformations of entrails were observed.
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To observe the skeletal malformations, the fetuses were soaked in anhydrous alcohol for 24 hr to dehydrate, the skin was removed, fat on a shoulder and the neck was removed by hand, washed and cleaned in 1% KOH solution for 24 hr.

After washing thoroughly with water, the fetuses were soaked in a mixture of 0.3 ml of 0.1% alizarin S solution and 100 ml of 20% Mall's solution for 24 hr for staining. The stained fetuses were kept in glycerin and skeletal abnormalities were observed by a stereoscopic microscope.

To observe the malformations of entrails, the fetuses were fixed in Bouin's fluid for 1 week and sliced 1 mm thick. The slices were floated in 70% alcohol and observed by the stereoscopic microscope.

B) Determination method for cadmium

The livers and the kidneys of the mother animals were placed in Kjeldahl flasks separately after weighing and washing. The fetuses from a mother mouse were divided into two groups (4 to 5 fetuses on an average), weighed and placed in a Kjeldahl flask. The liver of a mother animal was handled in the same manner as other samples. Two kidneys (right and left) were combined and treated as a sample.

Placentas from a mother animal were combined (about 12 to 13), weighed, treated similarly and placed in a Kjeldahl flask.

One and a half ml of conc. H$_2$SO$_4$ and then 30 to 50 ml of conc. HNO$_3$ were added to each sample.

About 5.0 ml of saturated ammonium oxalate were added and the flask was heated till fume came out.

After cooling off, about 20 ml of water were added and the flask was heated again.

After cooling off, the content was transferred to a test tube, washings of the flask were combined and the volume was adjusted to about 35 ml.

Five ml of 25 g/dl Rochelle salt were added to the above solution and pH was adjusted to about 3.5 with dilute ammonia.

Ten ml of saturated sulfuric acid and 1 ml of 2% ammonium pyrolidine dithiocarbamate were added to it. Cadmium was extracted with about 5 ml of MIBK and measured by Hitachi 208 type atomic absorption spectrophotometer.

Cadmium solutions of known concentrations (0.1, 0.5 and 1.0 ppm) were prepared each time as standards.

RESULTS

A. 1) Observation of the mother animals

When cadmium chloride saline solutions of various concentrations, namely 5.0 mg/kg (group A), 2.5 mg/kg (group B), 0.63 mg/kg (group C) and 0.33 mg/kg (group D), were injected subcutaneously once on their back on 7th day of gestation, 8 out of 20 animals in group A, 2 out of 20 in group B, 3 out of 20 in group C and 2 out of 20 in group D miscarried and increase in body weight due to
pregnancy could not be observed as shown in Table 1. In the control group, all 20 animals became pregnant and no miscarriage was observed. No other changes in general state were observed.

<table>
<thead>
<tr>
<th>Group</th>
<th>Doses of CdCl₂</th>
<th>No. of mother animal</th>
<th>Abortion</th>
<th>Dead fetus</th>
<th>Live fetus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Actual No.</td>
<td>%</td>
<td>Actual No.</td>
<td>%</td>
<td>Actual No.</td>
</tr>
<tr>
<td>A</td>
<td>5.0 mg/kg</td>
<td>20</td>
<td>8</td>
<td>26</td>
<td>122</td>
</tr>
<tr>
<td>B</td>
<td>2.5</td>
<td>20</td>
<td>2</td>
<td>28</td>
<td>205</td>
</tr>
<tr>
<td>C</td>
<td>0.63</td>
<td>20</td>
<td>3</td>
<td>28</td>
<td>206</td>
</tr>
<tr>
<td>D</td>
<td>0.33</td>
<td>20</td>
<td>2</td>
<td>25</td>
<td>227</td>
</tr>
<tr>
<td>Control</td>
<td>Saline</td>
<td>20</td>
<td>0</td>
<td>22</td>
<td>204</td>
</tr>
</tbody>
</table>

Table 1. Effect of cadmium chloride on pregnant mice.

†: Total of macerated fetuses, retentions of placenta and nidations.

2) Observation of the fetuses

When the mother animals were killed by dislocation of cervical vertebrae and their abdominal cavities were opened on 18th day of gestation, hemorrhagic amniotic fluid was observed in many of those administered cadmium.

When presence of macerated fetuses, retention of placenta and nidation was checked by dissection of uteri, dead fetuses were about 10% in the control group, while in group A the rate was a little higher and was 18% as shown in Table 1.

When live fetuses were observed, exencephaly as shown in Fig. 1 and the external malformations such as cleft palate, rachischisis, lack of tail and vaginal or anal atresia as shown in Figs. 2, 3 and 4 were found.

Exencephaly was found most, in 70 fetuses out of 122 in group A (57.13%), in 39 out of 205 in group B (19.02%), and in 2 out of 206 in group C (0.97%). No exencephaly was found in group D and the control group.

Open eyelid was observed in 35 out of 122 fetuses in group A (28.69%), in 25 out of 205 in group B (12.19%) and in 1 out of 206 in group C (0.48%). Group D and a control group didn't have any fetuses with open eyelid.

Malformations such as rachischisis, lack of tail and vaginal atresia were found only in group A; the excerpt is shown in Table 2.

Fig. 5 shows malformation appearing rates in each group.

The result showed that cadmium higher than 0.63 mg/kg caused malformations in the fetuses, while dosage lower than that didn't induce malformations.

After observing external malformations, skeletal malformations were observed using specimens stained with alizarin. As shown in Table 3, Figs. 6 and 7, skeletal malformations were found mostly in the skull region, such as lack of parietal bone, interparietal bone and occipital bone, and were found in 59% of the fetuses in group A, in 19.5% in group B and in 0.97% in group C. Group D and a control group didn't show any of these malformations.

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Fig. 1. Exencephaly induced by cadmium chloride.
The fetus was taken out on 18th day of gestation from the mother animal administered 5.0 mg/kg of cadmium chloride on 7th day of gestation.

Fig. 2. A normal fetus taken out on 18th day of gestation from the mother animal administered saline on 7th day of gestation.

Fig. 3. Rachischisis and lack of tail induced by cadmium chloride. The fetus was taken out on 18th day of gestation from the mother animal administered 5.0 mg/kg of cadmium chloride on 7th day of gestation.

Fig. 4. Clefts palate (shown by an arrow head) induced by cadmium chloride. The left fetus is normal. The fetuses were taken out on 18th day of gestation from the mother animal administered 2.5 mg/kg of cadmium chloride on 7th day of gestation.
Table 2. Kinds of external malformations induced by cadmium chloride and their appearing rates (in mice).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose of CcCl₂</th>
<th>No. of mother animals</th>
<th>No. of live fetuses</th>
<th>External malformation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Exencephaly</td>
</tr>
<tr>
<td></td>
<td>Actual No.</td>
<td>%</td>
<td>Actual No.</td>
<td>%</td>
</tr>
<tr>
<td>A</td>
<td>5.0 mg/kg</td>
<td>20</td>
<td>122&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70</td>
</tr>
<tr>
<td>B</td>
<td>2.5</td>
<td>20</td>
<td>205</td>
<td>39</td>
</tr>
<tr>
<td>C</td>
<td>0.63</td>
<td>20</td>
<td>206</td>
<td>2</td>
</tr>
<tr>
<td>D</td>
<td>0.33</td>
<td>20</td>
<td>227</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>Saline&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20</td>
<td>204</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> The number was small as 8 out of 20 mother animals miscarried.

<sup>b</sup> Saline alone was injected subcutaneously.

<sup>ε</sup> Including lack of vagina, anus and tail.
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![Graph showing incidence of malformations due to CdCl₂ injection.](image)

**Fig. 5.** Incidence of malformations due to CdCl₂ injection.

<table>
<thead>
<tr>
<th>Cd injected (mg/kg)</th>
<th>Malformation in skull region</th>
<th>Malformation in vertebral parts</th>
<th>Malformation in ribs</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>70, 57.13</td>
<td>17, 13.9</td>
<td>49, 40.1</td>
</tr>
<tr>
<td>2.5</td>
<td>40, 19.50</td>
<td>16, 7.8</td>
<td>38, 18.5</td>
</tr>
<tr>
<td>0.63</td>
<td>2, 0.97</td>
<td>2, 0.97</td>
<td>1, 0.48</td>
</tr>
<tr>
<td>0.33</td>
<td>0, 0</td>
<td>0, 0</td>
<td>0, 0</td>
</tr>
<tr>
<td>Control</td>
<td>0, 0</td>
<td>0, 0</td>
<td>0, 0</td>
</tr>
</tbody>
</table>

**Table 3.** Kinds of skeletal malformations induced by cadmium chloride.

![Image of skeletal specimens stained with alizarin.](image)

**Fig. 6.** Skeletal specimens stained with alizarin. The left specimen is normal. The right specimen has rachischisis and lack of tail (shown by an arrow head). The specimens were obtained from the fetuses taken out from the mother animal administered 5.0 mg/kg of cadmium chloride.
Skeletal malformations in vertebral parts, such as adhesion or abnormal conditions of cervical vertebrae, dorsal vertebrae and coccygeal vertebrae, were found in 13.9% of the fetuses in group A, in 7.8% in group B and in 0.97% in group C. Group D and a control group didn't have any fetuses with this type of malformations.

Skeletal malformations in ribs, such as adhesion or ramification of ribs, were found in 40.1% of the fetuses in group A, in 18.5% in group B and in 0.48% in group C. They also could not be found in group D and a control group.

Malformations of entrails were studied only in a small number of animals, and no significant differences were found between experimental groups and the control group.

On malformations of entrails, however, more studies have to be done in detail using larger number of animals in future.

B. Cadmium contents of the livers and the kidneys of the mother animals, the fetuses and the placentas.

Eleven days after administering cadmium, the livers and the kidneys were removed from the mother animals, weighed and analyzed for their cadmium contents. As shown in Table 4, an average cadmium content of the livers from 11 animals was 16.77 µg/g and that of the kidneys was 10.00 µg/g.

The livers from control animals contained 0.0125 µg/g of cadmium and their kidneys contained 0.022 µg/g. In experimental groups the livers contained cadmium 800 times more than that found in the control group and the kidneys contained cadmium about 450 times more that found in the control group.
When fetal cadmium content was measured, an average of experimental animals was 0.0039 μg/g and that of 22 control animals was 0.0032 μg/g as shown in Fig. 8. There found almost no differences.

When placental cadmium contents in Fig. 8 were compared, an average of experimental groups was 0.193 μg/g and that of a control group was 0.019 μg/g. The former value was about 10 times higher than the latter.

This showed that most of cadmium administered to the mother animals stayed...
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in the placentas right before delivery and did not transfer to the fetuses, though its distribution in the early stage of gestation was not known.

Cadmium contents in the placentas and the fetuses were not necessarily related to the presence of external malformations. Almost the same amount of cadmium was detected both in apparently normal fetuses and placentas and in the fetuses with external malformations.

**DISCUSSIONS**

It was Parizek et al.\(^4\) that observed abnormalities in the placentas and the fetuses after administering cadmium compounds to pregnant animals for the first time. Their purpose was to study the specific effect of cadmium ion on estrogen producing organs\(^5\).

Therefore they administered 0.04 mM/kg of cadmium to the rats in their late stage of pregnancy (17 to 21 days) and observed primarily morphological changes of the placentas and counted live and dead foetuses discriminately.

Chiquoine, A. D.\(^6\) administered cadmium chloride to mice in various stages of pregnancy covering 6 to 17 days of gestation. When cadmium was administered in an early stage of pregnancy, macerated fetuses and retention of placenta were mostly observed. When cadmium was administered in a late stage of pregnancy (14 to 17 days), however, most fetuses died. Endo et al.\(^19\) also reported many cases of premature delivery and abortion by administering cadmium chloride in various stages of pregnancy.

Later Ferm et al.\(^7,8\) used golden hamsters and studied on fetal abnormalities in detail by administering cadmium sulfate to mother animals in their early stage of pregnancy. Two mg/kg of cadmium sulfate were injected intravenously on 8th day of gestation, and 11, 12-and 13-day-old fetuses were removed and observed. Most of the 11-day-old fetuses showed incomplete closing of the 1st branchial arch, exencephaly and cardiocele. On 12th day of gestation, strengthening of facial cleft in the sagital plane and facial malformations were observed. On 13th day of gestation, cleft of upper and lower jawbones, exencephaly, rachischisis, microphthalmia and encephalocele into foramen occipitalis magnus were observed.

In our experiments, when large amount of cadmium, such as 5.0 mg/kg, was administered on 7th day of gestation, 8 out of 20 mice miscarried. Even with lower dose of cadmium, miscarriage was observed in a few cases.

When the fetuses were taken out on 18th day of gestation, more than half of them showed external malformations, mainly exencephaly.

When skeletal examination was conducted, many of the fetuses, which showed no external abnormalities, had skeletal malformations. Therefore total malformation appearing rate reached to about 80%.

Malformation appearing rate, however, decreased significantly by reducing dose of cadmium to one half (2.5 mg/kg), one eighth (0.63 mg/kg) or to one six-
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teenth (0.33 mg/kg). The rate was lower than 1% with 0.63 mg/kg of cadmium and was zero with 0.33 mg/kg.

Therefore malformations induced by cadmium seemed to be in proportion to dosage of cadmium taken into the body, which would be a matter of course. According to our experimental method, malformation causing limit of cadmium was presumed to be 0.5 mg/kg.

Quantities of cadmium in the livers, the kidneys and the placentas of the mother animals and the fetuses were measured when pregnancy was completed. Cadmium contents in the livers was 16.8 μg/g on the average, 10.0 μg/g in the kidneys and 0.19 μg/g in the placentas. While these values were much higher than those found in the control group, almost no cadmium was detected in the fetuses.

This experimental result suggested that cadmium administered to the mother animals hardly transfer to the fetuses and stayed in the placentas. Recently Ferm et al. administered ¹⁰⁹CdCl₂ to pregnant hamsters, and studied the distribution of cadmium to the placentas and the fetuses after 24 and 96 hr. They found 11.40 ±1.32 of cadmium in the placentas and 1.93±0.69 in the fetuses after 24 hr when taking the concentration of cadmium per 1 volume of blood. After 96 hr the cadmium concentration in the placentas reduced significantly to 3.09±1.31 and that in the fetuses to 0.328±0.0144. Therefore it could not be said that cadmium did not transfer to the fetuses at all.

Judging from the amount of cadmium distributed to the placenta and the state of its storage in the placenta, however, the main acting place of cadmium was thought to be the placenta after all.

Anyhow it was true that cadmium administered on 7 to 8th day of gestation had some bad effects on embryos, though it was not clear whether the effect was direct or indirect. And this was probably why exencephaly was caused mostly.

According to the papers¹⁰,¹¹), exencephaly or encephalocele is the most common external malformation that can be induced by irradiating X ray or affecting other factors on 7 to 8th day of gestation for a mouse, on 7 to 9th day of gestation for a rat and on 7th day of gestation for a rabbit.

According to experimental data by Hood et al.¹²) published lately, when 45 mg/kg of sodium arsenate were administered to mice intraperitoneally, exencephaly and encephalocele were observed only in mice administered sodium arsenate on 8th and 9th day of gestation. No case with exencephaly was found in mice administered the same amount of sodium arsenate intraperitoneally on either 6th, 10th, 11th or 12th day of gestation.

It is not necessarily true, however, that any agent administered on 7th, 8th or 9th day of gestation induces exencephaly. For example, when 50 mg/kg of a certain lead compound were administered to hamsters on 8th day of gestation, no case with exencephaly was observed. Abnormalities found were only lack or shortening of tail¹³,¹⁴).

With a phenylmercury compound, malformation of central nervous system was
observed but cases with exencephaly were small. With tellurium, only hydrocephalus was observed mostly.

Therefore it can be said that the site of malformation depends on the kind, dosage, absorption rate, namely reaction rate of chemicals administered in various stages of gestation and shows complicated aspects.

Some interesting experimental facts on the mechanism of teratogenic effect of these heavy metals have been clarified. For example, Ferm et al. administered the same amount of zinc sulfate as that of cadmium simultaneously and found that malformation appearing rate was significantly reduced to 1/10 or less. According to them this anticadmium effect was not specific to zinc; cobalt acetate and selenium also had the similar effect and malformation appearing rate by sodium arsenate was reduced to about 1/2 by administering selenium simultaneously.

Though the mechanism of this antagonism was not known yet, it was also demonstrated by other experimental data that there were antagonisms between metallic compounds. It is an interesting problem which has to be studied further in future.

The authors reported above on teratogenic effect of cadmium on the fetuses by injecting cadmium chloride subcutaneously to the animals in their early stage of pregnancy. There were no such cases reported on human beings. The reason is probably that cadmium will actually be taken into a body through respiratory tract or digestive organ and therefore cadmium higher than a certain concentration, which will be the case of subcutaneous or intravenous injection, will never circulate in blood.

In fact the authors tried inhalation of cadmium chloride to the pregnant mice, however, all of them showed toxic symptoms and miscarried and the effect of cadmium on the fetuses could not be studied in this way.

The allowable concentration of cadmium for inhalation, with which no toxic symptoms appear on the mother animals, is now being studied.

CONCLUSION

1) Cadmium chloride (5 mg/kg as cadmium) was injected subcutaneously to mice on 7th day of pregnancy. When abdominal cavity was opened and appearance of the fetuses was observed on 18th day, exencephaly was found most, and lack of tail, rachischisis and vaginal atresia were also found, though number of these cases was small.

2) The fetuses were treated and stained with alizarin, and skeletal malformations in the skull region, vertebral parts and ribs were observed. When external malformations were combined, the total malformation appearing rate exceeded 80%.

3) When dosage of cadmium was reduced to 2.5 mg/kg, 0.63 mg/kg or 0.33 mg/kg, both external and skeletal malformations were reduced significantly, malformation appearing rate was less than 1% with 0.63 mg/kg and zero with 0.33 mg/kg.
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4) When the quantities of cadmium in the liver, the kidneys and the placentas of the mother animal were measured by an atomic absorption spectrophotometer, the cadmium content in the liver of cadmium chloride injected mother animal was 800 times greater than that of the control animal, the cadmium content in the kidneys was 450 times and that in the placenta was 10 times, but there was no measurable amount of cadmium in the fetuses of cadmium chloride injected mice.

The above mentioned fact shows that administered cadmium stays mostly in the placenta at least in the late pregnancy and does not transfer to the fetuses.

REFERENCES