Radiation Malformations Belonging to the Cyclopia–Arrhinencephalia–Otocephalia Group in the Mouse Foetus

by UJIHIRO MURAKAMI, YOSHIRO KAMEYAMA, AKIO MAJIMA, and TSUNEYOSHI SAKURAI

From the Research Institute of Environmental Medicine, Nagoya University

WITH FOUR PLATES

INTRODUCTION

X-RADIATION is one of the most powerful agents in causing embryonic malformations and has been used recently by many investigators. Among various kinds of radiation malformations, those of the brain have been of special interest. Job, Leibold, & Fitzmaurice (1935), Kaven (1938), Warkany & Schraffenberger (1947), Russell (1950), Hicks (1953, 1954), Wilson & Karr (1951), Wilson, Jordan, & Brent (1953), Rugh & Grupp (1959), and Murakami & Kameyama (1958) studied teratogenic effects of X-rays on embryos by irradiating pregnant rats or mice. Exencephalia was listed as the chief malformation of the brain by most of these investigators.

Radiation malformation of the snout and nostril was reported by Russell (1950) as having some relationship to otocephalies. In the present paper, a series of malformations belonging to the cyclopia–arrhinencephalia–otocephalia group are described, and the critical period at which the pattern of malformations originates is discussed.

MATERIALS AND METHODS

Animals employed were the ddN strain of mice of 90 days or older. In each case a couple of oestrous females were kept overnight in a cage with one potent male and next morning those with vaginal plugs were taken to be in the 1st day of pregnancy. The pregnant mice were subjected to a single whole-body X-radiation of 200 r. on the 9th day of pregnancy. More accurately, the developmental stage corresponded to the 8½th day of pregnancy.

In order to follow the developmental process leading to malformations,

1 Authors' address: Research Institute of Environmental Medicine, Nagoya University, Furo-Cho, Chikusa-Ku, Nagoya, Japan.
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embryos and foetuses were removed after sacrificing mothers on the 13th to 19th day of pregnancy, and they were studied under a low-power microscope. Some embryos or foetuses with relevant malformations and some suspicious ones were examined in serial sections stained with haematoxylin and eosin after fixation with Bouin's fluid and embedding in paraffin.

X-radiation was done with a therapeutic X-ray machine. The beam was that produced by the factors: 200 kVP, 20 mA., 80 cm., and the filter was 0.5 mm. Al + 0.5 mm. Cu., 13·69/min.

RESULTS

As shown in Table 1, from the 48 treated mothers a total of 392 offspring, including 90 dead, was obtained. Among the dead individuals macerated or absorbed ones were found besides intact ones. Absorption was diagnosed if remnants of placentae were present. The incidence of deaths, 22.96 per cent. of the total number of the offspring, far exceeded that seen in control animals (8.18 per cent.). Among the 90 dead foetuses, there were two with characteristics of the relevant malformations. They were included in the subject for gross examinations, and therefore the examined number totalled 304. Many malformed offspring had more than two kinds of abnormalities. In Table 1 every malformation is listed separately.

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**Table 1**

<table>
<thead>
<tr>
<th>Examined stage</th>
<th>13th day</th>
<th>14th day</th>
<th>15th day</th>
<th>16th day</th>
<th>17th day</th>
<th>18th day</th>
<th>19th day</th>
<th>Total</th>
<th>Per cent.</th>
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<td>8</td>
<td>5</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>48</td>
<td></td>
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<tr>
<td>Total embryos and foetuses</td>
<td>58</td>
<td>67</td>
<td>60</td>
<td>45</td>
<td>52</td>
<td>47</td>
<td>63</td>
<td>392</td>
<td></td>
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<tr>
<td>No. of examined embryos and foetuses</td>
<td>47</td>
<td>49</td>
<td>56</td>
<td>40</td>
<td>48</td>
<td>22</td>
<td>42</td>
<td>304</td>
<td></td>
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<tr>
<td>Dead embryos and foetuses</td>
<td>12</td>
<td>19</td>
<td>6</td>
<td>7</td>
<td>4</td>
<td>20</td>
<td>22</td>
<td>90</td>
<td>22.96</td>
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<td>Univent. telencephalon (1st group)</td>
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<td>6</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>19</td>
<td>6.25</td>
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<td>3</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>19</td>
<td>6.25</td>
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<tr>
<td>Beak-like snout (3rd group)</td>
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<td>15</td>
<td>5</td>
<td>10</td>
<td>12</td>
<td>6</td>
<td>9</td>
<td>58</td>
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<td>...</td>
<td>...</td>
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<td>...</td>
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<td>...</td>
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<td>12</td>
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<td>28.95</td>
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<td>2</td>
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<td>7</td>
<td>4</td>
<td>10</td>
<td>11</td>
<td>37</td>
<td>12.17</td>
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<td>3</td>
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<td>4</td>
<td>9</td>
<td>3</td>
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<td>14</td>
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<td>7</td>
<td>11</td>
<td>10</td>
<td>...</td>
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<td>2</td>
<td>1</td>
<td>36</td>
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</tbody>
</table>

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**Note:** The data in Table 1 are rounded to the nearest whole number.

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**Table 1 (continued)***

*Pregnancy status and malformations of mouse foetuses X-irradiated on the 9th day of pregnancy*
Among those malformations occurring more frequently were eye-abnormalities (28.95 per cent.). In gross examinations many cases of microphthalmia and some cases of anophthalmia were detected. The detail of these eye-abnormalities will be described in another paper.

Further, harelips, cleft-palates, and tail abnormalities were also frequently encountered. Harelip and cleft-palate were often associated with the malformations which constitute the chief subject of this paper (Plate 1, figs. 4, 5).

The most conspicuous pattern of malformations found in the present experiments will be conveniently classified into the following three groups. The first is represented by an apparently univentricular telencephalon. The condition was detectable from the outside, and associated with 'beak-like appearance' of the snout, the latter having a configuration resembling the beak of some birds (Plate 1, figs. 1, 2, 3, 4). This condition of the snout was prominent in foetuses from the 14th to the 16th foetal day. The position of the eyes was also abnormal, for they approached the midline. One of the extreme cases (Plate 1, fig. 1; Plate 3, fig. 17) resembled a cyclopia with a rudimentary left eye. The condition is similar to monstra monophthalmia asymmetrica reported previously by Stockard (1909), but differs from the latter in the median approach of the eyes to each other. In this respect the present writers are inclined to interpret the case to be more closely related to cyclopia than monstra monophthalmia asymmetrica. However, no typical cyclopia was detected. Incidence of the typical univentricular telencephalon totalled 19, i.e. 6.25 per cent. The doubtful cases were confirmed by microscopic examinations.

The second group of malformations was thought to represent transitional conditions from the first to the third group, and they were characterized by a reduction of the cranium without severely affecting the laterality of ventricles. A pair of telencephalons was visible on gross examination. The abnormal shape of telencephalons, giving an appearance of a pair of cones with a common base, could be seen through the covering of the head. Although the snout was also malformed like that of the first group, the tip of the snout was less pointed (Plate 1, figs. 6, 7). The eyes were also somewhat shifted towards the median plane. There were 19 such cases.

In the third group the telencephalons were of almost normal appearance, whereas the shape of the snout indicated the abnormalities described for the second group. In some cases, especially at a later stage, the tip of the snout was also pointed, or nostrils were fused, giving an appearance of a single nostril (Plate 4, fig. 21). Fifty-eight such cases were registered (Table 1).

Several cases were chosen at random from the second and third groups for microscopic examinations. It was then revealed that the ventricles were incompletely separated, being connected with bilateral interventricular foramen which remained very broad even in the later stage of foetal life (Plate 3, fig. 15). A part of the telencephalon may even contain a single brain cavity (Plate 3, fig. 16), suggesting a transitional condition approaching the univentricular
telencephalon. The formation of the olfactory organ was also disturbed. In
general, the cartilage of the nasal septum was hypoplastic, and occasionally
fusion of nostrils or nasal cavities was noticed (Plate 4, figs. 19, 21, 22).

Besides the above-mentioned 96 malformations determined by gross examina-
tions, there were two cases with small-sized ears (microtias) (X3632-1 and 2). Since these could also be classified as a kind of otocephaly and be included in
the group with a characteristic shape of the snout, they are not listed separately
as otocephalies. All cases of the inscribed pattern detectable by gross examina-
tions totalled 98, making up 32·24 per cent. of the total number of examined
cases (Text-fig. 1).

Besides the above cases with externally distinguishable abnormal patterns, one
sectioned foetus of the 16th-day stage (X3738-8) indicated hypoplasia in the left
olfactory lobe as well as in the left nasal cavity (Plate 4, fig. 20). It should be
pointed out that the number of cases with the inscribed pattern recorded in
gross examinations probably does not include all the cases, and that more of
them may have been left unrecorded owing to their normal appearance.
DISCUSSION

The main malformation pattern observed in the present experiment may be presented as the following spectrum of abnormal conditions. The extreme condition is characterized by a univentricular telencephalon associated with tendencies towards cyclopia. This condition probably belongs to the category of cebocephalia in man. Through transitional steps the condition leads to a state where the effect of irradiation is only very weakly manifested, for instance by a tapered snout. Based on the microscopic findings, the present writers interpret this series of malformations as pertaining to the trait of the univentricular telencephalon, or the cyclopia–arrhinencephalia–otocephalia group defined by Ostertag in human beings. However, in contrast to the statement of Johnson (1926), no cases with defective lower jaws were detected in the present series.

This trait has been said to be the most frequent malformation of the nervous system in various animals (Potter, 1953). According to Johnson (1926), the corresponding morphology is found in a hereditary trait of the mouse (natural stock) called otocephaly. He described the occurrence of cyclopias with jaw abnormalities in the progeny of Little & Bagg’s X-rayed mice (1924) and also in the control line.

This conspicuous pattern of malformations was manifested very frequently in the series in which irradiation was made on the 9th day of pregnancy, but not in other series (Murakami, Kameyama, et al., 1961) irradiated on the 8th, 10th, or 11th day of pregnancy as shown in Text-fig. 1. The result reveals an evident stage-specificity. This is in general agreement with the notion of phasenabhängige Empfindlichkeit emphasized by Lehmann (1955).

In previous experiments treatment of pregnant mice with trypan blue solution at the 7th to the 9th day of pregnancy (Murakami, 1952) or with hypoxia at the 8th to 12th day of pregnancy (Murakami & Kameyama, 1962) did not lead to the malformations obtained in this experiment. Therefore, this malformation pattern may be influenced by an agent-specificity.

Russell (1950) stated that in the group treated with 200 r. on the 8½th day of pregnancy, abnormalities of snouts and nostrils did not resemble any known abnormalities in the mouse, but were similar to a guinea-pig abnormality appearing in the strain which showed a high incidence of otocephalia. We are convinced that these abnormalities of snouts and nostrils shown by Russell corresponded to those produced in our experiment, judged by the morphology, stage of treatment, and dose of X-radiation, though the incidence was higher in Russell’s experiments.

Further, the developmental stage at which the pattern of malformation is to be induced will be discussed. In lower vertebrates a number of embryologists succeeded in producing cyclopia in embryos either by surgical or by chemical means. The developmental stages of gastrulation and neurulation were thought to be
the sensitive period for the manifestation of the cyclopic condition (Ostertag, 1956).

It was suggested that disturbances in the process of inductive differentiation and growth, which are dependent on morphogenetic movements, were the cause of the malformation (Ostertag, 1956). Ostertag argued that *teratogenetische Terminationsperiode* of the cyclopia–arrhinencephalia–otocephalia group, including all their transitional forms, might correspond to the developmental phase at which from 3 to 5 brain vesicles were formed. This period was said to be in the 4th week of pregnancy in human embryos. In the mouse embryo, the approximate stage which corresponds to the 9th (8½th) day of pregnancy is the 6–10 somite stage, during which the closure of the anterior neuropore takes place (Otis & Brent, 1954). It appears, then, quite reasonable that the malformation had a high incidence because this developmental stage was treated in the present experiments.

Degenhardt (1959) reported a case of encephalocystocele and cyclopia in the rabbit produced by hypoxia of the mother on the 9th day of pregnancy. His experiment revealed that the most vulnerable stage for development of the vertebrae is found on the 9th day of pregnancy. This stage corresponds to the 10th day in the mouse (Murakami & Kameyama, 1962). The present experiment revealed that the 9th and 10th days of pregnancy were sensitive to irradiation, and this does not contradict Degenhardt.

Thus the present results fit in with the earlier data concerning the sensitive developmental phase according to experimental embryologists working on lower vertebrates, and the *teratogenetische Terminationsperiode* estimated by pathologists who have dealt with human material.

**SUMMARY**

1. Mice were subjected to a single whole-body X-radiation of 200 r. on the 9th (8½th) day of pregnancy and embryos and foetuses derived from them were examined from the 13th to 19th day of pregnancy.
2. Besides some exencephalies, hydrocephaluses, harelips and cleft-palates, a spectrum of malformations grossly classified into the following three groups was observed at comparatively high frequencies (over 32 per cent.). The first group: an apparently univentricular telencephalon accompanied by a beak-like appearance of the snout and eyes approaching the median. The second group: a reduction in the size of the telencephalon associated with a tapering of the snout and a tendency to synophthalmia. The third group: the tapering snout which may be accompanied by fusing nostrils. Besides, two cases of microtia were found.
3. Microscopic findings suggest that these three groups present a spectrum of malformations belonging to the cyclopia–arrhinencephalia–otocephalia group. However, no typical cyclopias were detected.
4. During the most sensitive developmental stage, the 9th (8½th) day of pregnancy, 3–5 brain vesicles are formed and the anterior neuropore is closed. That this phase of basic morphogenesis of the nervous system is most sensitive to teratogenic influences is in harmony with both the results of experimental embryologists employing lower vertebrates and those of human pathologists.

RESUMÉ

Malformations appartenant à la série cyclopie–arhinencéphalie–otocéphalie chez l'embryon de la Souris après irradiation

1. Des souris ont été soumises à une irradiation totale aux rayons X, le 9e jour (ou 8½) de la gestation, et les embryons et fœtus en provenant ont été examinés du 13e au 19e jour de la gestation.

2. Outre quelques cas d'exencephalie, d'hydrocéphalie, de bec-de-lièvre et de fissuration palatine, on a observé, à des fréquences relativement élevées (plus de 32 pour cent) un éventail de malformations grossièrement classées dans les trois groupes suivants. Premier groupe: télencéphale à ventricule apparemment unique, avec museau en forme de bec et yeux rapprochés vers la ligne médiane. Deuxième groupe: réduction de taille du télencéphale, associée à l'effilement du museau et à une tendance à la synophthalmie. Troisième groupe: museau effilé pouvant présenter des narines fusionnées. On a observé en outre deux cas de microtie.

3. Les observations microscopiques suggèrent que ces trois groupes présentent un éventail de malformations appartenant à la série cyclopie–arhinencéphalie–otocéphalie. Néanmoins, on n'a pas décelé de cyclopes typiques.

4. Pendant le stade de développement le plus sensible, c'est-à-dire le 9e jour (8½) de la gestation, il se forme trois à cinq vésicules cérébrales, et le neuropore antérieur se ferme. Le fait que cette phase fondamentale de la morphogénèse du système nerveux soit très sensible aux influences tératogènes est en harmonie avec tous les résultats obtenus expérimentalement chez les Vertébrés inférieurs ou observés en pathologie humaine.

REFERENCES


U. MURAKAMI, Y. KAMEYAMA, A. MAJIMA, and T. SAKURAI

Plate 1
U. MURAKAMI, Y. KAMEYAMA, A. MAJIMA, and T. SAKURAI

Plate 2


— (1962). Vertebra malformations of the mouse foetus caused by maternal hypoxia during the early stage of pregnancy. (To be published.)


**EXPLANATION OF PLATES**

**PLATE 1**

Fig. 1. (X3627-1.) A 14-day mouse foetus with a univentricular telencephalon and microphthalmia. Notice the peculiar shape of the head and snout.

Fig. 2. (X3732-a.) A 14-day mouse foetus with changes similar to those shown in fig. 1. Lateral view.

Fig. 3. (X3732-a.) The same foetus as shown in fig. 2; ventral view. Notice the shape of the telencephalon, a tapering snout and pointed tip of it.

Fig. 4. (X3724-a.) A 15-day mouse foetus with a univentricular telencephalon, median harelip and abdominal hernia. The shape of the head and snout is similar to those seen in figs. 1 and 2.

Fig. 5. (X3724-a.) The same foetus as shown in fig. 4; ventral view. Median harelip is presented.

Fig. 6. (X3735-b.) A 16-day foetus. The shape of the head is somewhat microcephalic. In gross examination, the size of the telencephalon is small but seemingly not univentricular. However, it presents a transitional condition in sections (Plate 3, figs. 15, 16).

Fig. 7. (X3735-b.) The same foetus as shown in fig. 6; ventral view. In contrast to the lateral view, the shape of the snout is tapering and the tip is pointed.

Fig. 8. (X3759-b.) A mouse foetus on the 17th day with a univentricular telencephalon. In this lateral view, the shape of the snout is almost normal.

Fig. 9. (X3759-b.) The same foetus as shown in fig. 8. A ventral view. The shape of the snout is tapering.

**PLATE 2**

Fig. 10. (X3766-b.) A 19-day mouse foetus with a univentricular telencephalon. The tip of the snout is somewhat rounded.

Fig. 11. (X3766-b.) The same foetus as shown in fig. 10; ventral view. The snout is tapering and the nostrils are united.

Fig. 12. (X3627-1.) Cross-section of the head of the mouse foetus shown in fig. 1. A univentricular telencephalon is presented.

Fig. 13. (X3759-b.) Cross-section of the head of the mouse foetus shown in figs. 8, 9. A univentricular telencephalon is presented.

Fig. 14. (X3766-b.) Cross-section of the head of the mouse foetus shown in figs. 10 and 11. A univentricular telencephalon is shown.
Plate 3

Fig. 15. (X3735-2.) Cross-section of the telencephalon of the mouse foetus shown in figs. 6 and 7. In this part, the telencephalon is incompletely separated.

Fig. 16. (X3735-2.) Cross-section of the telencephalon of the same mouse foetus as shown in figs. 6, 7, and 15. In this part, the telencephalon looks univentricular.

Fig. 17. (X3627-1.) A part of the cross-section of the head through the eye of the mouse foetus shown in fig. 1. Eyes are approaching the median. The left eye formed is rudimentary.

Fig. 18. (X3627-1.) A part of the cross-section of the upper jaw of the mouse foetus shown in fig. 1. A single nasal cavity is presented.

Plate 4

Fig. 19. (X3724-6.) A part of the cross-section of the upper jaw of a 15-day mouse foetus. A single nasal cavity is presented.

Fig. 20. (X3738-8.) A part of the cross-section of the upper jaw of a 16-day mouse foetus. This case is seemingly normal but the left nasal cavity shows only rudimentary development.

Fig. 21. (X3760-3.) A part of the cross-section of the upper jaw of a 17-day mouse foetus. A single nostril is evident.

Fig. 22. (X3766-5.) A part of the cross-section of the upper jaw of the mouse foetus shown in figs. 10 and 11. A single nostril is also evident.

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Plate 4