The effect of X-ray irradiation on the pituitary body of infantile rats treated with α-oestradiol monobenzoate

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Numerous investigations have been published dealing with the influence of X-ray and radium irradiations on the ovarian function. Both the effect of the direct irradiation of the ovaries and the influence of the X-ray irradiation of the pituitary body on its gonadotrophic hormone production were thoroughly investigated. However, the results of the latter are not unanimous as it may be seen from the data collected by Denniston (1942) and from our recently published survey of the corresponding literature (Baidins, Claesson & Westman, 1946). Some authors, among others Savasaki (1933) considered that premature oestrus could be elicited by weak irradiation of the pituitary body, while on the other hand others were unable to confirm his results. In one of our experiments it has been found that the irradiation of the pituitary body with X-ray doses from 2 to 45 r. did not damage to an appreciable extent the ovaries of infantile rats neither in function nor in anatomical structure. We have found further that even much higher doses i.e. 500—1000 r. did not produce any noticeable disturbance of the ovarian function.

On the other hand it is well known from clinical experiences that by using X-ray doses of the same magnitude of order the function of the pituitary gland can be influenced and its
gonadotrophic hormone production stimulated. The results of the experiments reported above are not in accordance with the clinical observations and are therefore rather surprising; but it must be taken into account that there are principal differences in the reaction of laboratory animals and human beings. Furthermore it must be emphasized that in nearly every case of therapeutic X-ray treatment pathologically altered and not quite normally functioning pituitary bodies are irradiated. Therefore it may be assumed that pathologically changed pituitary glands are more sensitive to the action of X-rays than the normal ones.

For the further elucidation of this both theoretically and practically important question experiments were carried out by us on juvenile rats treated with oestradiol* before X-ray irradiation (Baidins, Claesson & Westman 1948). In the animals, treated in this way, the pituitary body undergoes typical histological changes, which are well known in their characteristic features from the studies of numerous earlier investigators. It needs here only be recalled that the anterior lobe increases in size due to the increase in the number of the cells. Both the eosinophilic and the basophilic cells show signs of degranulation and their relative number decreases according to the increase of the chromophobe cells. Treatment with oestradiol causes a depression in the gonadotrophic hormone production of the pituitary body.

In our experiments referred to above a series of juvenile female rats were treated with oestradiol while an other series of animals were treated with oestradiol combined with X-ray irradiation. The doses administered were 600 and 2000 r. respectively. There was a characteristic difference between these two series; the ovaries of animals treated only with oestradiol were small with an average weight of 32.9 mg. By the examination of these ovaries only a very small number of mature follicles and newly formed corpora lutea were identifiable, and only a weak degree of luteinization was found. On the other hand the ovaries of the animals treated

*) In the text »oestradiol« means oestradiol monobenzoate.
with oestradiol combined with X-rays were large, their average weight being 59.3 mg. after the administration of 600 r. and 52.5 mg. after the administration of 2000 r. The follicles were well developed and large, newly formed corpora lutea were present.

Apart from a more expressed hyperemia of the pituitary body found in animals treated with X-rays combined with oestradiol, the microscopical appearances of the pituitary bodies in both groups were alike.

Hence, these our studies revealed the interesting fact that X-ray irradiation of the pituitary body does not produce any identifiable changes either in the histological appearance or in the function of the ovaries, whereas considerable changes could be observed in the ovaries after the X-ray irradiation of pituitary glands altered by previous treatment with oestradiol.

It seemed to us of interest to examine the question whether these changes in the response of the pituitary body to irradiation occur also in infantile animals, or are they restricted to the age of puberty and maturity.

**EXPERIMENTAL**

The present study was made on 25 female rats aged 8 days; of these 11 served as controls. Every control was of the same litter as the corresponding experimental animal. The vaginal smear of the animals was daily examined and their weight controlled on every third day. 200 I.B.U. oestradiol monobenzoate was administered subcutaneously to 14 experimental animals every day over a period of 8 days: the total amount administered was 1600 I. B. U. After this previous treatment the animals were irridiated with a single dose of 40 r. The 11 control animals were divided into 2 groups; in the first group 7 of the 11 animals were treated with oestradiol in the very same way as the irradiated ones, in order to study the reaction of the pituitary body to estradiol treatment. In the second group the remaining 4 animals did not get any treatment at all.
Before irradiation the animals were fixed in dorsal position and with the exception of a small area of the head, protected with lead plates. We are indebted to Dr. A. R. Forsberg, member of the Staff of the Radiopathological Department of the Radiumhemmet of Stockholm for the measurements of the dosage. Irradiation was performed with the use of a stabilovolt apparatus and metallix tubes: the conditions were 160 kv. 7 ma., 0.5 mm. Cu and 6.23 r/m.

After the irradiation the animals were observed over a period of 50 days and then sacrificed. The pituitary bodies were immediately weighed, fixed in Susa (Heidenhain) and embedded in paraffin. Serial sections were made, each sections measuring 4 μ in thickness, and the sections stained with Azan according to Heidenhain's description. The ovaries were weighed, and the sections stained with hematoxylin — eosin. The vagina and uterus were histologically also examined.

During this observation time the animals did not manifest any signs of disturbances attributable to the injurious effect of either oestradiol or X-rays. Their weight-curves were normal.

The effect of oestradiol treatment manifested in an immediate oestrus reaction in the vagina. Later this oestrus ceased, but reappeared when the animals reached the age of puberty. This oestrus reaction took place in both oestradiol treated and oestradiol treated + irradiated animals in the 5th week of their life, while in the untreated controls spontaneous oestrus did not occur before the 6th week. The cyclic processes were normal, excepting three cases of irradiated animals showing no typical cornified cells.

Pituitary body. The pituitary bodies of the animals treated with X-rays combined with oestradiol showed the characteristic changes which, as mentioned above, follow oestradiol treatment. As it is shown in Table 1, there was a slight increase in weight due to the increase in size of the anterior lobe. The number of the chromophil cells decreased while that of the chromophobes increased. Both the eosinophiles and basophiles showed degranulation.
Table 1. Average Weight of the Pituitary Body and the Ovaries of Infantile Rats treated with Oestradiol and X-rays.
(The animals were examined 58 days after the first injection and 51 days after irradiation).

<table>
<thead>
<tr>
<th>Material</th>
<th>Number of Animals</th>
<th>Average Weight of Pituitary Body in mg</th>
<th>Average Weight of Ovary in mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiated animals previously treated with oestradiol</td>
<td>14</td>
<td>7.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Controls treated with oestradiol</td>
<td>7</td>
<td>6.6</td>
<td>26.1</td>
</tr>
<tr>
<td>Untreated controls</td>
<td>4</td>
<td>5.2</td>
<td>27.5</td>
</tr>
</tbody>
</table>

X-ray dose: 40 r. Oestradiol: 1600 I. B. U.

Apart from a slightly higher degree of hyperaemia of pituitary glands found in animals treated with X-rays combined with oestradiol, the microscopical appearances did not show any other difference in these two groups.

Fig. 1.
Ovary of an Infantile Rat treated with a total Dose of 1600 I. B. U. Oestradiol over 8 days. × 28.
Ovaries. The ovaries of the animals treated with oestradiol weighed about the same as those of the untreated controls. The number of the Graafian follicles was reduced and large, intensively vascularized corpora lutea were in an increased number present (Fig. 1). The ovaries of the irradiated animals treated with oestradiol differed considerably from those of the non-irradiated ones. The ovarian weights in this group were significantly higher as shown in Table 1. No Graafian follicles were present and some of the growing follicles showed atretia. On the other hand a well developed interstitial gland was found. In this group the corpora lutea were entirely absent in 5 of the 14 animals and the ovarian parenchym was to a large extent constituted by interstitial gland (Fig. 2). In the ovaries of the remaining 9 animals of this group both the interstitial gland and the corpora lutea were to be found.

DISCUSSION

The present investigation and the results reported in our earlier communication (Baidins, Claesson & Westman, 1948) have shown that in infantile rats the X-ray irradiation of the
normal pituitary body with a single dose of 40 r. did not affect to an appreciable extent its production of gonadotrophic hormone. Hence, our studies have not brought forward evidence in supporting the assumption that X-ray irradiation of a normal rat pituitary could stimulate its production of gonadotrophic hormone and could thereby produce premature oestrus. X-ray irradiation combined with oestradiol treatment, however, yields different results. The ovaries increase in size and weight as compared to those of the corresponding controls. This increase in size and weight depends chiefly upon the intensive development of the interstitial gland. At the same time a marked degeneration of the follicles could be observed. The infantile animals show quite another reaction than the juvenile ones. The reason for this phenomenon may be cleared up by further investigations on the function of the interstitial gland under the given experimental conditions.

Some experiments of this type are in progress at this Institute.

SUMMARY
1. X-ray irradiation of the pituitary bodies of normal infantile rats in doses between 2—1000 r. does not influence their gonadotrophic hormone production.
2. Oestradiol treatment of normal infantile rats did not produce any changes in the gonadotrophic function of the pituitary gland examined two months after the treatment. The ovaries of these animals have normal weight and normal histological appearance.
3. X-ray irradiation of the pituitary bodies of normal infantile rats sensitized by previous oestradiol treatment affects the function of the pituitary gland; two months after the combined treatment the follicles show degenerative changes, and the ovaries are significantly increased in weight and size due to the intensive development of the interstitial gland.
4. The histological appearance of the pituitary bodies in the treated and non-treated animals was alike.
REFERENCES

Baidins, A., Claesson, L. & Westman, A.: Gynaecologica 125, 9, 1948

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