EFFECT OF BUSULPHAN ON THE DEVELOPING OVARY IN THE RAT

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Summary. Female rats were exposed to Busulphan on selected days between the 5th day of foetal life and Day 15 post partum, inclusive. The drug apparently had no effect on the foetal sex cells when administered to the pregnant female between Days 5 and 7 post coitum. Later in pregnancy a destructive action became rapidly more powerful. So far as the developing ovary is concerned it would seem that the action of Busulphan at this dose level, i.e. 10 mg/kg intraperitoneally, is confined to the precursor of the oocyte, namely the oogonium and that when these cells enter meiotic prophase on about the 17th day of foetal life there is a marked decrease in their sensitivity to the action of the drug. The effect of the treatment on fertility has also been studied.

INTRODUCTION

The effect of Busulphan on the foetal ovary was described by Bollag (1954). He reported that a single dose given to pregnant rats 5 to 7 days ante partum resulted in sterility in the offspring. Later, Galton & Till (1956) stated that “earlier in pregnancy quite large doses do not interfere with its course and the F¹ and F² generations were apparently healthy”. In view of these studies, it was decided to investigate the sensitivity of the proliferating cell systems of the gonad during development. A preliminary report of these investigations has been published elsewhere (Hemsworth & Jackson, 1962a).

MATERIALS AND METHODS

ANIMALS

American Wistar rats of an inbred colony were fed on a diet supplied by the Scottish N.E. Agricultural Society. Females were placed separately in boxes and paired with fertile males. The day spermatozoa were found in the vaginal smear was regarded as Day 0, the males were then removed and the females allowed to go to term.

TREATMENT

Groups consisting of at least three animals were given an injection of Busulphan (10 mg/kg intraperitoneally, suspended in arachis oil) at selected times ranging from the 5th day of gestation to Day 15 post partum, inclusive. Litters from the
treated pregnant females were sampled on Days 1 and 15 post partum, the gonads from one F1 female from each of three litters being examined. The remaining females were kept to maturity, when their fertility was assessed by mating with males of proven fertility. After this they were killed at 6 months and their ovaries examined histologically.

Groups of nine young female rats also received a single injection of the drug between Days 1 and 15 post partum. From each group three were killed for examination of their ovaries at selected times and the remainder kept to adult life for fertility tests and later histological assessment of ovarian damage.

HISTOLOGICAL TECHNIQUES
Following fixation in Helly’s fluid and embedding in paraffin wax, sections were cut in the mid-region of each gonad at 7 μ, stained in haematoxylin and eosin and mounted in Xam.

EVALUATION OF HISTOLOGICAL CHANGES
The presence or absence of oocytes was recorded in each group of rats together with a classification of follicular development based on the schemes described by Slater & Dornfield (1945) and Beaumont (1961):

Stage 0  Oocytes without follicle cells
Stage 1  Oocytes + one layer of flattened granulosa cells
Stage 2  Oocytes + one layer of cuboidal granulosa cells
Stage 3  Oocytes + two layers of granulosa cells
Stage 4  Oocytes + three layers of granulosa cells
Stage 5  Oocytes + four layers of granulosa cells
Stage 6  Oocytes + follicles with Antrum

FERTILITY STUDIES
The treated animals were paired individually with proven fertile males until mating occurred, indicated by the presence of spermatozoa in the vaginal smear. Those groups of treated females which failed to mate, when left with males for at least 2 weeks, were classified as sterile.

RESULTS
BASED ON OVARIAN HISTOLOGY
The untreated ovary 1 day post partum contains oocytes in either meiotic prophase or the dictyate stage (Pl. 1, Fig. 1). The number of oocytes present 1 day post partum was greatly reduced by treatment on Day 9 or 11 and they were virtually absent when the drug was given on either Day 13, 14 or 16 of gestation (Pl. 1, Fig. 2). Treatment either before or after this period appeared to have no subsequent effect on the oocyte population.

Fifteen days post partum, oocytes are normally associated with Stages 0 to 6 of follicular development (Pl. 1, Fig. 3). Following an injection between the 13th and 16th day inclusive, oocytes were not seen associated with Stages 0 and 1 (Pl. 1, Fig. 4). The ovaries appeared histologically normal following treatment
on the 5th, 7th, 18th or 20th day of gestation. No histological abnormality was noted at 15 days post partum following treatment during the neonatal period. Oocytes were not seen in the ovaries of the 6-months-old rat following an injection between the 13th and 16th day of gestation, inclusive (Pl. 1, Fig. 6).

Following treatment both before and after this period the ovaries appeared histologically normal (Pl. 1, Fig. 5), oocytes being present and associated with all the stages of follicular development.

FERTILITY TESTS

The results of the fertility tests are shown in Text-fig. 1. Following treatment on either the 5th or 7th day of gestation inclusive, the fertility of the F1 females was normal. Treatment on Day 9 or 11 resulted in a reduction in average litter size to 6 and 2.6, respectively. Sterility in the F1 females was the rule following treatment during Days 13 to 16, inclusive. Females exposed to this treatment later in pregnancy or during the neonatal period were fully fertile.

DISCUSSION

The present study has shown that the effect of Busulphan (Myleran) on the ovary of the foetal and neonatal rat depends upon age at the time of treatment. The maximum effect, resulting in sterility of all female offspring, followed treatment between the 13th and 16th day of gestation inclusive. The majority of offspring treated on Day 11 of pregnancy and all those treated on Day 18 were fully fertile (Text-fig. 1). The greatly diminished number of oocytes observed 1 day post partum in the litter mates of the sterile females, suggests that the drug had a destructive effect on the oogonia. Alternatively, a cytotoxic action on these cells could result in their later disappearance as oocytes. This destructive effect parallels that which has been seen in their male counterparts (Hemsworth & Jackson, 1962b), but the foetal ovary differs from the testis in that the
period of maximum sensitivity to Busulphan happens to correspond with a high mitotic rate among the oogonia.

The increasing susceptibility of oogonia to Busulphan with time may be a reflection of inherent changes in sensitivity, although the resistance of the earlier germ cells might be explained on the basis of a regeneration which restores to some extent the germ cell numbers. The work of Mintz (1959) suggests that in the mouse, mitotic activity can be resumed in the gonocyte population if irradiation is performed on the 11th day post coitum.

The present work has also shown that the most susceptible cells to the action of Busulphan are the oogonia and that when these cells enter meiotic prophase on the 17th day of foetal life a notable decrease in sensitivity occurs. This recalls the sensitivity to Busulphan of spermatogonia in the adult rat testis and the insensitivity of spermatocytes in meiotic prophase (Jackson, Fox & Craig, 1959). In spite of the homogeneous appearance of the oogonal population between the 13th and 16th days of gestation, the results suggest that these cells exhibit different degrees of sensitivity. Some are able to continue development into oocytes and become associated with follicular development in the prepubertal period. Others, the precursors of oocytes which would undergo maturation in the adult animal, are affected by the drug. This would account for the absence of oocytes in the adult ovary.

Beaumont (1961) has studied the sensitivity to X-irradiation of oogonia and oocytes in the foetal rat. Her results have shown that the response is also related to foetal age at the time of treatment and to the dose administered, with maximum susceptibility on Day 15 post coitum. It is interesting to note that here too, the greatest damage was manifest by a marked reduction in the number of primordial follicles. It has been suggested by several workers (e.g. Hargitt, 1930; Sneider, 1940) that oocytes undergoing development during the prepubertal period become atretic. The study with Busulphan supports this view. Whilst treatment between the 13th and 16th day of gestation eliminated primordial oocytes by the 15th day post partum, oocytes in the more advanced stages of development were still numerous. These may well become atretic before puberty but it is intended to examine animals in early adult life before a definite conclusion is drawn.

REFERENCES

Effect of Busulphan on the developing ovary in the rat

EXPLANATION OF PLATE 1

CL = Corpus luteum  G = Germinal epithelium
GF = Graafian follicle  OC = Oocyte
PF = Primordial follicles  ST = Ovarian stroma

Fig. 1. Untreated ovary 1 day post partum showing numerous oocytes in either meiotic prophase or the 'resting' condition. ×200.

Fig. 2. An ovary 1 day post partum showing the effect of treatment on the 13th day of gestation. Oocytes are virtually absent. ×200.

Fig. 3. Untreated ovary 15 days post partum showing oocytes, primordial follicles, i.e. Stages 0 and 1 of follicular development and more advanced stages of follicle formation. ×200.

Fig. 4. An ovary 15 days post partum showing the effect of treatment on the 13th day of gestation. Primordial follicles are not present. ×200.

Fig. 5. Ovary in an untreated adult rat showing Graafian follicles and corpora lutea. ×40.

Fig. 6. An adult ovary showing the effect of treatment on the 13th day of gestation. Graafian follicles are absent. ×40.