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Morphological and functional changes in the gonads of the albino rat following administration of an alkylating cytostatic agent during intra-uterine life

by
V. PUGLIATTI

Even though the administration of radiomimetic cytostatic drugs to animals during gestation may not always bring about obvious somatic injury in the offspring, it may induce profound changes in the gonads, with more or less serious involvement of the functions of reproduction.

There is unanimous agreement concerning the cause of the action produced by cytostatics in the primary germ cells at the time when the gonadal primordia are being colonized ([1-9]). It is well know, in fact, that the mammalian gonads develop on the wall that forms the posterior border of the intra-embryonic coelomic cavity, from the genital tubercles which are situated longitudinally on each side, between the root of the mesentery and the mesonephric folds, and extending beyond the mesonephros or Wolffian body either upwards or downwards. At a stage of development which is predetermined for each animal species, the primitive germ cells, originating from the site where the yolk-sac is first formed in the dorsal endoderm, and later from the dorsal wall of the small intestine, arrive at the genital tubercles. The cells migrate by putting out pseudopodia along the root of the mesentery as far as the gonadal primordia; and as they develop, they undergo intensive multiplication ([10-11]). Although the amoeboid movements and the numerical growth of the gonocytes are in any case blocked, a more or less complete reversal takes place at the primordium for which they were destined, in that the gonocytes develop a process of induction upon the genital tubercles.

Since, in consulting the relevant literature, it was found that sometimes the morphological aspect of the injuries produced in the treated animals was taken into consideration, and sometimes only the functional aspect, and that the observations were often limited to one sex only, I tested groups of albino rats of both sexes, which had been exposed during embryonic and fetal development to the action of a radiomimetic antiblastic agent, which was administered to the mothers, during gestation, with the object of checking the short and long term effect.

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MATERIALS AND METHODS

The substance used was Mielucin (produced by the Simes Company, to which I express my thanks), whose chemical name is dimethylbusulfan (2,5-di[methanesulfonyloxy] hexane). Its action, as with all radiomimetic substances of this type, takes place by blocking some reactive groups in the cells, with the result that their metabolism and multiplication are disordered by agglutination and rupture of the chromosomes. Mielucin was reduced to a fine powder, suspended in olive oil, and introduced intraperitoneally in a dose of 10 mg/kg body weight to albino rats on a predetermined day of gestation. The days selected extended from the 10th to the 17th (inclusive) following conception, precisely because it is within this period, in the rat, that the principal phenomena occur that lead to the formation and sexual differentiation of the gonads.

The effects produced were observed 48 hours after administration, at birth, during the definitely pre-pubertal stage, after puberty and under gonadotrophic stimulation. The animals were not only examined from the morphological point of view; the age at which their puberty occurred, their sexual behaviour and their fertility (if any) were also checked.

A more detailed description of the material used, the histological methods whereby the gonocytes were recognized, and the results obtained, together with fuller illustrative material, has been reported in previous studies ([12-16]), of which the present description is a synthesis.

RESULTS

In general it can be stated that no gross anomalies occurred in the control animals, apart from a diminution in size and weight which was already evident at birth (Fig. 1) and was maintained thoughout the period of observation.

The changes in the gonads that could be observed after 48 hours (Fig. 2 b) were similar for both sexes, and may essentially be summarized by saying that the number of gonocytes that had migrated or were in the process of migrating

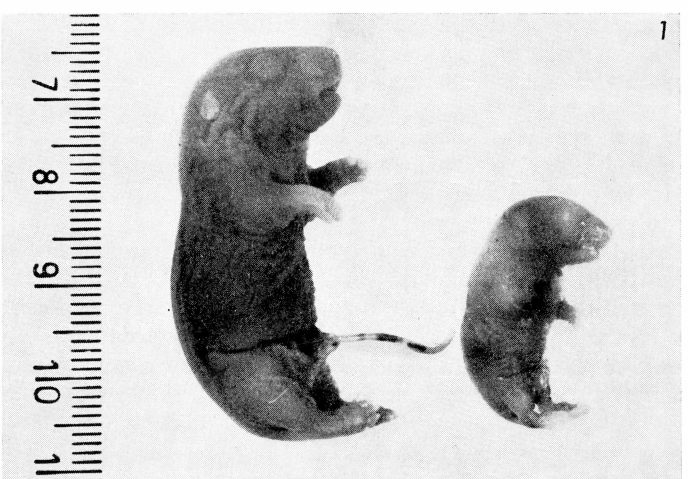

FIG. 1 - Comparison between a normal new-born rat (left) and one treated with Mielucin on the 12th day of intra-uterine life.

within the gonadal primordia had diminished, with a relative increase in the gonocytes contained within the juxta-enteric deposits, but with no apparent involvement of the gonadal arrangement characteristic of each sex. Examinations carried out at other stages of life (at birth, during the pre-pubertal phase, etc.) still demonstrated that the gonads were in a state of germinal and hormonal deficiency. To be precise, the testicle (Fig. 3 a, b) is almost normal as regards the architecture of this organ, but it is mostly characterized by the presence of Sertoli cells alone, with little or no participation by cells of the germinal line of descent, and by conspicuous clustering of the interstitial cells. Puberty, which is marked by the descent of the testicles into the scrotal pouch, usually occurs late, and any animals that have reached puberty are incapable of having sexual intercourses with the females.

In the ovary (Fig. 4 a, b) an alteration in the structure of this organ is seen, together with almost constant deficiency of ovocytes. Normal follicles are in fact present in very reduced numbers, while there is a predominance of somewhat rounded or tubular folliculoid cavities, covered with epithelium similar to the superficial epithelium of the ovary, and often clearly deriving from it. One frequently-recurring appearance is the abundant clustering of luteinic cells resembling small corpora lutea. Puberty, shown by the opening of the vulvar orifice, has often not made its appearance in many rats even four months after birth, and those animals that have become pubertal, not only remain in a permanent state of anoestrus but will not accept sexual relations. Stimulation with gonadotrophins had no effect in animals of either sex in improving sexual behaviour, nor in accelerating or determining puberty; it only increased the quantity of interstitial elements in the gonads.

CONCLUSIONS

The action of the alkylating cytostatic agent was undoubtedly a marked one on the gonocytes, in that it brought about a disorder of the inner structure of the gonads (and also of the architecture, as regards the ovary), while having no effect on the somatic cells in general. This link up precisely with what I said earlier in connexion with the more intense migratory and mitotic activity, and thus the greater sensitivity of the gonocytes just on the days when the cytostatic was administered. It may still be concluded that for normal testicular morphogenesis it is sufficient for these few gonocytes to reach the primordium, thereby succeeding in escaping from the injurious blocking agent, while as regards the ovary, each gonocyte has an induction action on the appropriate follicle; when the gonocytes are not present in sufficient number, they form cavities similar to the follicles, but not identical with them since, at least in my investigations, they have no hormonal activity.

The results obtained in the animals that were exposed during intra-uterine life to the action of the cytostatic agent recall certain morphological and functional conditions of the gonads that are not usually encountered in clinical practice, viz., cases of gonadal insufficiency in both sexes. In the male, Klinefelter's syndrome (otherwise described as testicular tubular dysgenesia), and in the female, or more precisely in subjects with a feminine phenotype, the various syndromes of gonadal dysgenesia (such as Turner's syndrome), are due to imbalance of the complement of sex chromosomes, usually traceable to meiotic nondisjunction (as in Klinefelter's syndrome) or to retarded migration of the chromosomes

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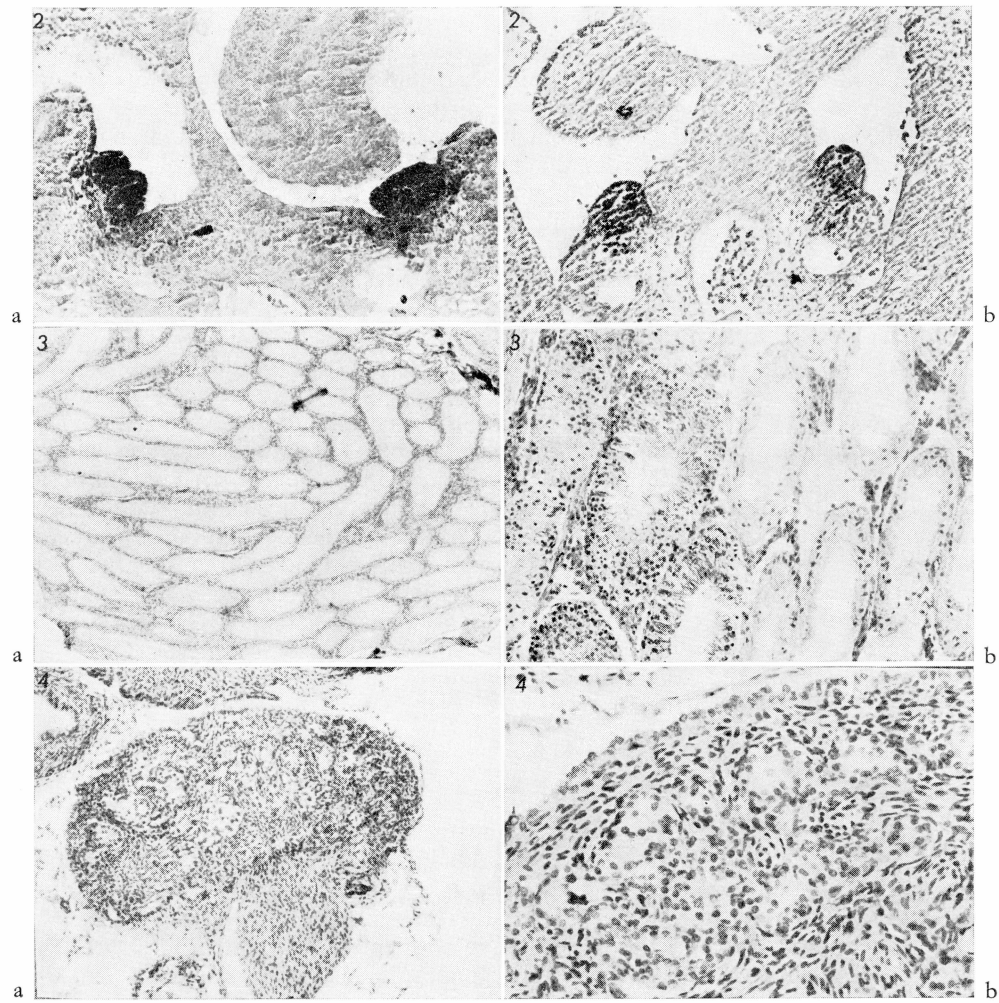

FIG. 2a. *Abdominal section of the rat embryo on the 12th day of intra-uterine life (untreated mother).* The gonadal tubercles, at the sides of the mesentery, are crammed with Gomori-positive germ cells. Other constituents are visible between the mesentery and the primordia (Gomori's method with alkaline phosphatase, x 100).

FIG. 2b - *Abdominal section of the rat embryo on the 12th day of intra-uterine life (mother treated with Mielucin on the 10th day of gestation).* The gonadal tubercles have few Gomori-positive cells (also x 100).

FIG. 3a - *Testicle of adult rat (mother treated on 14th day of gestation).* The tubules nearly all consist of Sertoli cells alone. (Haematoxylin-eosin, x 80).

FIG. 3b. *Ditto (mother treated on 15th day of gestation).* (Haematoxylin-eosin, x 100).

FIG. 4a, b. *Ovary of prepubertal rat (mother treated on 11th day of gestation).* Folliculoid cavities covered with epithelium similar to the superficial epithelium of the ovary. No folliculi of normal outline are seen. (Haematoxylin-eosin, x 80, x 100).

during the anaphase (anaphase lagging), as in Turner's syndrome. The two phenomena referred to may originate from the most diverse causes, but the sole result is that the gonocytes whose gonosomal heritage has been changed in this way do not migrate and do not multiply as they normally should, and they fail to

colonize the gonadal primordium adequately. On the basis of these premises, Mintz ([17]) attempted to obtain sterile gonads in the mouse, by means of a new mutant which produced mitotic arrest and hindered the migration of the gonocytes.

In addition to the syndromes referred to above (which from the chromosomal point of view are characterized by a whole range of conditions, some in the form of a mosaic, which always involve the XXY or the XO formula and appear in various situations from absolute sterility to normality), others have been recorded, in many respects quite similar, in which the inherited sex chromosomes are however normal; these are testicular germinal dysgenesia (Ullrich-Turner's syndrome, the male form of Turner's syndrome), del Castillo's syndrome or the syndrome in which only Sertoli cells are present, the syndrome of rudimentary testicles and, in the female, syndromes referable to various conditions of primitive hypoplasia of the ovary, and a few forms of pure gonadal dysgenesia with a normal complement of gonosomes.

In drawing comparisons with observations made in animals, it should be remembered that radiation, or a radiomimetic substance, or any other factor that paralyses the migratory power and mitotic potential of the gonocytes, may lead to changes that are not so much structural alterations of the gonads (at least in the male), as of the germinal heritage and its ability to function. In the human embryo the gonocytes migrate, according to Pinkerton *et al* ([18]), between the eighth and the twentieth week of development, but according to Witschi ([19]) migration begins as early as the end of the third week. A knowledge of these necessary phases in gonadal organogenesis, and of the sensitivity of the gonocytes to radiomimetic agents, which is particularly increased during these phases, should again counsel a prudent attitude when using such substances during pregnancy, with the aim of avoiding any injury to the foetus which, perhaps, might not be evident at birth and soon after, but would become so after a period of years, even after puberty. The safeguarding of the physical, psychic and reproductive integrity of the newborn infant, both in the future and at the time of birth, is the pivot upon which turns the whole of modern, intensive, perinatal medicine.

SUMMARY

Reference is made to the morphological and functional changes produced in the gonads of rats of both sexes on administering a radiomimetic cytostatic agent to the mother during gestation. As a result of a blocking action of this substance upon the gonocytes during the phase of colonization of the gonadal primordia, sterile or almost sterile gonads are obtained which are also deficient from the hormonal point of view.

Finally, the possible pathogenesis of some syndromes of gonadal insufficiency in the male is referred to.

Translated by Samil Pabyrn Foundation

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The Toluidine Blue Test in the early diagnosis of neoplasia of the vulva

by

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Malignant tumours of the vulva, although developing in an area readily accessible for normal means of diagnosis, are among the gynaecological neoplasias that are diagnosed at a very late stage.

The causes for this delay are attributed both to the absence of a serious and alarming symptomatology and to the difficulty of distinguishing clinically malignant lesions from those that are benign. Moreover, it is considered that this type of neoplasia is characteristic of old age, a time at which a woman's interest in her own genital area has greatly diminished.

The most frequently occurring symptom is pruritus, a symptom moreover that is very general and which for that reason is often underestimated by the patient and the doctor himself, who tends almost always to attribute it to a benign pathological condition.

This phenomenon is crearly of a certain seriousness since, as is verified by neoplasias in other organs as well as by cancer of the vulva, early diagnosis provides an important guide both for the therapeutic indications and above all for the prognosis. In fact survival for more than five years with invasive cancer, despite treatment that is both mutilating and complex, does not exceed 50%, while in intraepithelial cases, also with more restricted intervention, the survival rate varies between 92.5% ([1]) and 100% ([2]).

In order to achieve these objects it is absolutely necessary that the retarding factors mentioned above are reduced; in addition it is important that all the dystrophic lesions of the vulva are diagnosed and adequately treated, in parti-

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