Development of the Gonads of Mice after Intense Incorporation of Tritiated Thymidine During the Period of Oogenesis

^{3}H-Thymidine incorporation in the germ cells of female mouse embryos has been investigated by RUDKIN and GRIECH[1], LIMA DE FARIA and BORUM[2], PETERS et al.[3] and CRONE et al.[4]. LIMA DE FARIA and BORUM[2] found a labelling of the female germ cells of the mouse after a ^{3}H-thymidine pulse on the 10th and 12th day of pregnancy, corresponding to the period of premitotic DNA synthesis in the oogonia. This premitotic DNA synthesis should, however, still continue during the following days, since BORUM[5], in a cytological study, reports the presence of the last oogonial divisions together with leptotene and zygotene figures on the 14th and 15th day of the fetal life. On the other hand, BORUM[6], in a study about the origin of mature ova in the mouse, was able to label 83–100% of the oocytes by successive (every 12 h) i.p. injections of ^{3}H-thymidine in the mother from the 13th to the 16th day of gestation (chiefly the premeiotic DNA synthesis period). In contrast to what has been found in the ovary of the chicken[7–9], the timing and the localization of the premitotic DNA synthesis period in the mouse does not seem clearly separable from the premeiotic DNA synthesis period. For these reasons in order to label all the female germ cells during their successive ^{3}H-thymidine incorporating stages, it will be necessary to apply several ^{3}H-thymidine injections during the period of intensive oogonial multiplication (premitotic DNA synthesis) and during the ensuing period of premeiotic DNA synthesis.

We therefore started our i.p. injections already after 10 days of gestation. Since the premeiotic S phase in the germ cells of the female mouse was found by CRONE et al.[4] to be approximately 10.5 h, we chose to inject the mothers every 8 h with ^{3}H-thymidine.

In the present work 3 virgin inbred female BALB/c$^+$ mice, 3 months old, have been mated with males of the same strain. From the 11th (15.00) to the 16th (07.00) day of pregnancy, these mice received 15 successive (every 8 h) i.p. injections of 100 μCi ^{3}H-thymidine. The thymidine 6-^{3}H (S.C.K.-C.E.N. Mol) with a specific activity of 12–14 Ci/mM was dissolved in a sterile Hanks balanced salt solution to a concentration of 200 μCi/ml. At birth, 19–20 days after the observation of the vaginal plug, 3 new-born female mice were killed and their ovaries fixed in acetic-alcohol (1:3 vol). At the 60th day of age the last 4 females were allowed to mate with different untreated males. In spite of the fact that new males were used every 14 days, no pregnancy could be detected. Four months after birth, these females were killed and their ovaries fixed.

The volume of these ovaries has been found to be much lower than normal (Figure 1). In only one of the ovaries was a corpus luteum seen. After fixation, the ovaries were embedded in paraffin and sectioned at 5 μ thickness. After deparaffination and rehydration the acid soluble precursors were extracted by treatment of the sections with 3% perchloric acid at 4 °C during 20 min. The slides were coated with nuclear emulsion L4 (Ilford, England) by the dipping method. After 14 days exposure in the dark and photographic development, the sections were coloured with Groat's iron hematoxylin and eosin. On the autoradiographs of the ovaries of the new-born female mice all the germ cells are found intensively labelled. In the sections of the ovaries fixed 4 months after birth no oocyte can be found.

This investigation is in agreement with the observed absence of pregnancy. Rudiments of follicles without oocytes have occasionally been seen but typical follicles

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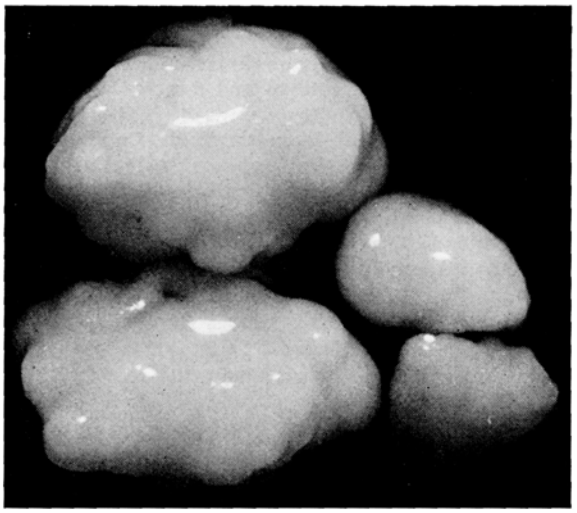

Fig. 1. Comparison of the volume and aspect of normal ovaries (left) of a 4-month-old control female mouse with the ovaries (right) of a female mouse of the same age but treated with successive thymidine-^{3}H pulses during the period of oogenesis. × 15.

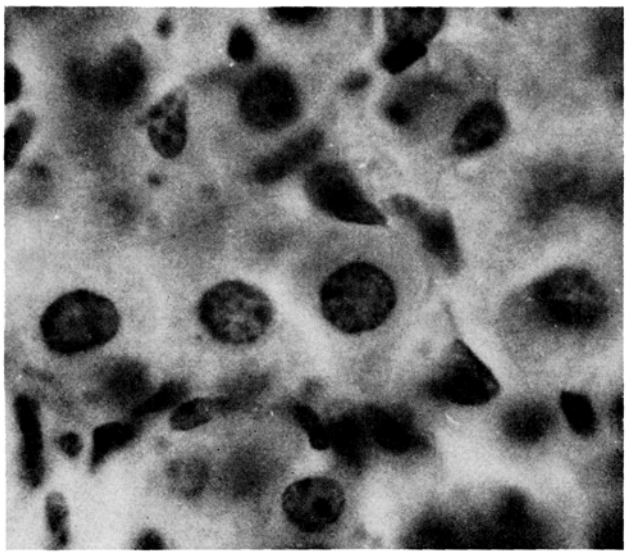

Fig. 2. Section through the ovary of a treated 4-month-old female mouse with a view of the polyedric cells with their well-developed eosinophilic cytoplasm. × 1100

do not appear to have developed. The largest part of these sterile ovaries is occupied by cords of polyedric cells with a well developed eosinophilic cytoplasm and a voluminous nucleus (probably interstitial cells; see Figure 2). In the ovary of new-born mice, most of the oocytes are in the pachytene or early diplotene stages but a few days later they have grown intrafollicular and are in the late diplotene (so-called 'resting' or dictyotene) stage, in which the oocytes remain for varying lengths of time[5].

In general the oocytes of different species (rat[10,11], mouse[15], chicken[12] and rhesus monkey[13]) are relatively refractory to Röntgen irradiation as they pass through leptotene, zygotene and pachytene stages. The radiosensitivity to ionizing radiations however increases as they pass from early to late diplotene or at the stage of early primordial oocytes (rat[11,14], mouse[15–17], rhesus monkey[13] and chick[18]).

These observations can probably explain why in the present study the ovaries of the new-born mice still contained numerous oocytes, whilst at puberty the mice have become sterile. However, the continuous internal β irradiation from the ^{3}H-thymidine, incorporated in the germ cell nuclei, may also have an integrating effect resulting in cell death before the oocytes mature. The present study also supports the view[6] that most if not all definitive ova in the mouse are formed before birth and is in favour of NUSSBAUMS thesis[19] of the germ-line continuity.

Five male mice, which also received the successive ^{3}H-thymidine pulses during their intrauterine life, were killed when mature, their testes were removed and meiotic preparations were made by an air-drying method[20]. The volume of the testes from 4 animals was found to be lower than normal. Fifty male germ cells at the diakinesis-first metaphase stage were examinated from each animal for the presence of multivalent configurations. No chromosome rearrangement at all was found and the population of the testes appeared to be normal.

It may be concluded that the decrease in the weight of the testes is not related to a loss of fertility, whilst female mice appear to be sterilized by the same treatment[21,22].

Résumé. Le développement des gonades de souris après une forte incorporation de thymidine-^{3}H, administrée pendant la période de l'oogénèse, a été étudié. Suite à ce traitement les souris femelles sont devenues stériles à l'âge de la puberté et le volume de leurs ovaires, dépourvues de cellules germinales, a fortement diminué. Les mâles traités de la même façon ne semblent pas être stérilisés malgré une diminution du volume de leurs testicules.

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Studies by the Newt Test on the Possible Importance of Conjugated Double-Bonds and Trans-Isomerization for Carcinogenic Properties of Lipids

The newt test[1] is a short-term test for screening substances suspected of having carcinogenic properties. A satisfactory specificity of the test when applied to lipophilic compounds was found by one of the present authors, and a number of heated and oxidized fats was tested[2–4]. Further studies on a broader spectrum of native and altered fats revealed a large number of newt-positive substances, but it could not be settled whether the introduction of a hydroxyl-group in an α-position to a double-bond, conjugation, or trans-isomerization was responsible for the induction of the carcinogen-like effects in unsaturated fats[5]. The results of a new series of tests are reported in the present paper.

The tests were carried out as described in the previous papers. The substances, in arachid oil, were injected s.c. into the newts, which were examined histologically after 15 days. Positive reactions consist in local hyperplasia of the epidermis, most often with infiltrative downgrowth of the epithelium. Arachid oil served as negative, 3-methylcholanthrene or dibenz(a,h)anthracene as positive controls.

Most of the tested substances were commercial preparations. Crystalline vitamin A-acetate and β-carotene were gifts from F. Hoffmann-La Roche & Co. A.G., Basel. The conjugated ethyl linoleate was prepared from ricinoleic acid by elaidinization, dehydration and esterification. It showed an optical density in cyclohexane at 233 nm of 94 calculated for 1 g/l. Certain of the substances to be tested were only sparingly soluble in arachid oil. A 2% ergosterol and a 1% sorbic acid solution could be prepared by gentle heating, but β-carotene was not even completely soluble in 0.1% concentration. For that reason the higher concentrations of the 3 substances indicated in the Table do not represent true solutions. The results are presented in the Table.

Discussion. The negative or dubious results for ethyl elaidate indicate that the presence of one trans-double-

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