

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<sup>8</sup>.

In general the oocytes of different species (rat<sup>10,11</sup>, mouse<sup>15</sup>, chicken<sup>12</sup> and rhesus monkey<sup>13</sup>) 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<sup>11,14</sup>, mouse<sup>15-17</sup>, rhesus monkey<sup>13</sup> and chick<sup>18</sup>).

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  $\beta$  irradiation from the <sup>3</sup>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<sup>6</sup> that most if not all definitive ova in the mouse are formed before birth and is in favour of NUSSBAUMS thesis<sup>19</sup> of the germ-line continuity.

Five male mice, which also received the successive <sup>3</sup>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<sup>20</sup>. 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 examined 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<sup>21,22</sup>.

**Résumé.** Le développement des gonades de souris après une forte incorporation de thymidine-<sup>3</sup>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, dépourvus 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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<sup>21</sup> The author is very grateful to Dr. L. LEDOUX and Dr. J. HUGON for their advice and to Dr. A. LÉONARD for his contribution and investigations on the testes.

<sup>22</sup> This work was supported by a grant from the Fonds de la Recherche scientifique fondamentale collective.

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

The newt test<sup>1</sup> 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<sup>2-4</sup>. 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  $\alpha$ -position to a double-bond, conjugation, or trans-isomerization was responsible for the induction of the carcinogen-like effects in unsaturated fats<sup>5</sup>. 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  $\beta$ -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  $\beta$ -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-

<sup>1</sup> S. NEUKOMM, *Oncologia* **10**, 107 (1957).

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<sup>5</sup> E. ARFFMANN and J. GLAVIND, *Acta path. microbiol. scand.* **70**, 185 (1967).

bond is not a sufficient alteration of an unsaturated fatty acid molecule to give positive results in the newt test.

Negative results were also obtained for 2 of the substances containing conjugated double-bonds, sorbic acid and  $\beta$ -carotene. However, as mentioned above, both substances are sparingly soluble in arachid oil and  $\beta$ -carotene

Substance	Concentration % in arachid oil	No. of experi- ments	Results Positive reactions/ Effective No. of animals
Oleum arachidis		12	0/68
Tung oil	0.5	1	1/6
Tung oil	1.0	3	0/18
Tung oil	2.0	1	1/5
Tung oil	5.0	3	6/15
Tung oil	10.0	4	14/20
Tung oil	25.0	1	5/5
Ethyl linoleate	100.0	1	0/6
Conjugated ethyl linoleate	50.0	2	2/12
Conjugated ethyl linoleate	100.0	3	11/18
Ethyl elaidate	50.0	2	1/13
Ethyl elaidate	100.0	2	2/12
Sorbic acid	1.0	3	1/18
Sorbic acid	2.0	2	0/12
Sorbic acid	saturated (< 5.0)	1	0/6
$\beta$ -Carotene	0.1	1	1/6
$\beta$ -Carotene	0.2	1	0/6
$\beta$ -Carotene	0.5	1	0/6
Vitamin A-acetate	1.0	1	2/6
Vitamin A-acetate	5.0	2	4/12
Vitamin A-acetate	10.0	2	2/12
Vitamin A-acetate	20.0	1	3/6
Ergosterol	1.0	3	8/18
Ergosterol	2.0	3	10/18
Ergosterol	5.0	1	9/12
3-Methylcholanthrene	0.5	9	23/53
Dibenz(a,h)anthra- cene	0.1	2	5/12
Dibenz(a,h)anthra- cene	0.5	1	1/6

Results in the newt test with a trans-fatty acid and a number of compounds containing conjugated double-bonds. Each experiment (column 3) consisted of a group of newts, generally 6 animals, which were injected with the substance indicated in column 1. On the same day a series of experiments on a number of test substances, on pure arachid oil (negative controls) and on methylcholanthrene or dibenz-anthracene (positive controls) was started. The effective number of animals (column 4) was the number remaining after, in certain experiments, a few animals dying before the third day or showing severe necrosis, cadaverosis or skin inflammation had been discarded.

could be tested only in concentrations that were low in comparison with those required for positive results of a number of other conjugated compounds.

Positive results were obtained for vitamin A-acetate, ergosterol, tung oil, and conjugated ethyl linoleate. Since the 4 compounds all contain conjugated double-bonds, the results seem to support the hypothesis that the presence of conjugated double-bonds in a lipid brings about newt-positive properties. It should be mentioned, however, that conjugated ethyl linoleate, although highly active when tested undiluted, was only weakly active in 50% dilution whereas tung oil was active in much lower dilutions. Higher activities were also reported, in our first paper, for ethyl linoleate hydroperoxide and ethyl hydroxyoctadecadienoate. Structurally the 2 compounds are supposed to be conjugated linoleic acids containing a hydroperoxide- and an alcohol-group, respectively. Present results therefore do not permit us finally to decide whether other aspects of the chemical structure than the presence of conjugated double-bonds are essential for positive results.

The substances positive in the newt test are carcinogen-suspect. Immediately it seems improbable that a substance like vitamin A could be carcinogenic. On the other hand, vitamin A influences the growth of epidermal and other types of cells, and recently it has been claimed that excessive vitamin A increases the incidence of avian leukosis<sup>6</sup>, and that topical application increases the incidence of DMBA-induced carcinoma in the hamster cheek pouch<sup>7,8</sup>.

**Zusammenfassung.** Mittels der Salamander-Schnellmethode für Karzinogen-Prüfung wurde eine Reihe von Fettprodukten und verwandten Verbindungen untersucht. Äthylelaidat erwies sich als unwirksam, während Vitamin A, Ergosterol, Holzöl und konjugiertes Äthyllinoleat wirksam waren. Die Ergebnisse weisen auf eine grössere Bedeutung der konjugierten Doppelbindungen hin, während der quantitative Vergleich zeigt, dass auch andere Aspekte der chemischen Struktur von Bedeutung sein können.

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<sup>6</sup> B. E. MARCH and J. BIELY, *Nature* 214, 287 (1967).

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<sup>8</sup> This work was supported by a grant from the Danish Anti-Cancer League.

## Effect of Magnesium Pemoline on the Survival of Mice with Ehrlich Ascites Tumor

Magnesium pemoline, a central nervous system stimulant, has been reported to be a good radioprotective agent<sup>1</sup> for both short- and long-terms<sup>2</sup>. Some of the stimulants in this class such as dextroamphetamine, do not exhibit this property<sup>3</sup>. Recent studies have also indicated that the effect of magnesium pemoline in enhancing radio-resistance in mice seems to be independent of drug dosages (up to 2 weeks after irradiation) whether the animals are exposed to lethal amount of X-irradiation before or after the drug administration<sup>4</sup>. These interesting

properties of magnesium pemoline suggest the potential application of the drug in both radio-diagnosis and therapy and its possible use in civil defense and industrial accidents involving radiation exposures. The implications mentioned above have encouraged our explicit pilot study of the effect of this drug on the survival of Ehrlich ascites tumor mice with and without the effect of X-rays.

**Methods.** 360 CF<sub>1</sub> male mice, 50–60 days old (20–22 g) were used in 2 experiments. In the first experiment, 180 animals were divided randomly into 3 groups of