J. Pharm. Pharmac., 1967, **19**, 167–169 Received September 5, 1966

Effects on the reproductive organs of feeding the non-ionic surfactant Triton X-100 to mice

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A series of feeding experiments has shown Triton X-100 to induce cystic degeneration in the mouse ovary. Parallel experiments in ovariectomized mice revealed no evidence that Triton X-100 possesses oestrogenic activity. Long term exposure of female mice to this surfactant did not impair fertility.

THERE is widespread interest in the possible biological effects of surfactants. Chapman & Short (1965) have reported a marked thickening of the interfollicular epidermis of sheep subjected to long term topical treatment with polysorbate. This is similar to the effects obtained in mice by Dammert (1957) and Setala, Merenmies, Stjernvall, Ahoy & Kajanne (1959). Our previous findings (Goldhammer, 1956; Goldhammer & McManus, 1960) with non-ionic surfactants in plants and rodents we thought could be the result of an alteration in cell-membrane permeability. To explore this possibility the alkyl aryl polyether alcohol surfactant Triton X-100 has been administered in the food or drinking water of mice to enable its effects on the reproductive organs to be assessed. The mouse ovary is known to respond to the topical application of Triton X-100.

Experiments and results

The criteria of assessment were the number of Graafian follicles at least 400 μ in diameter, the presence of haemorrhagic points and the number of corpora lutea. A concentration of up to 0·5% of Triton X-100 in food was well tolerated and subsequently 0·3% of the surfactant was used. No diarrhoea was noted at these levels. In drinking water a suitable concentration was 0·1%; concentrations up to 0·5% were tolerated but little consumed. Four experiments were conducted over a period of eight months.

Experiment I. Triton X100 was administered as a 0·1% solution (v/v) in drinking water to 47 female and 12 male mice over 8 months. The animals were 4–6 weeks old at the beginning of the experiment. Vaginal smears were taken from about two thirds of the females at least 3 times a week over the entire experimental period.

Experiment II. 49 female and 12 male mice were maintained on a diet of standard mouse cubes containing 0·3% (v/w) of Triton X-100 from the age of 4–5 weeks for 8 months. Vaginal smears were taken from all the females as above.

No gross alterations of the oestrous cycle and no microscopic changes in the testes of the treated animals were observed over the 8 month period.

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The ovaries showed enlarged "cysts", at least four to five times the size of the whole ovary. The incidence of this cystic condition was 23·4% and 40·8% for the "water" and "diet" experiments respectively compared with 20·8% in a control group of 98 animals ($P = 0{\cdot}15$ N.S.).

From each group ("diet" "water" and control) 18 females, chosen at random equal numbers being in oestrus, were killed at the end of the eight month period and ovarian sections made. These were cut 4μ thick at constant depth and were stained with haematoxylin-eosin. Follicles and corpora lutea at least 400 μ in diameter were counted in serial sections taking equal numbers of sections for each group. As tested by analysis of variance the differences were not statistically significant.

Examination of uterine sections from treated "diet" animals revealed pronounced cystic changes in this organ. In three treated "diet" mice and one treated "water" mouse, one ovary showed a haematoma-like gross enlargement not encountered in control animals.

Experiment III. 20 female mice were ovariectomized at 10 weeks. After three days, they were fed a diet of mouse cubes containing 0·3% of Triton X-100 (v/w) for 8 months to find if the surfactant possessed oestrogenic activity. Vaginal smears were taken either daily or four times a week. Four animals died in the first 4 months and of the remaining 16 two showed intermittent vaginal cornification over the last two months of the experiment. When the two animals were killed the uteri were swollen and hyperaemic and histological examination showed no remnant of ovarian tissue. The vaginal smears in the other 14 animals were always negative. These animals had atrophic uteri.

Experiment IV. This was to assess the effect of Triton X-100 upon fertility. Eighty 6-months old female mice were randomly allocated to 3 groups.

One group (30 mice) was fed on Triton X-100 (0·3% v/w) for 4 months and then transferred to control diet for the remaining 4 months. A second group (30 mice) received the same treatment in reverse order. The third group (20 mice) acted as a control. After 8 months the 3 groups were mated with 14 males which had been kept on a normal diet. During mating 4 females were caged with one male: the males were circulated among the groups of females. All females were fertile but cannibalism prevented comparison of litter size. In a subsequent experiment each female was housed individually and no difference was observed between the average litter size of the treated and the control animals.

Discussion

The phenomenon needing explanation is the increased incidence of cysts in the ovaries and uteri, occurring after prolonged feeding with Triton X-100.

It is assumed that some of the ingested surfactant reached the ovary. There is evidence that some surfactants of "Span" and "Tween" type are largely excreted unchanged (Treon, 1965) but we are unaware of any reports on the metabolism of the "Triton" type of surfactant.

Assuming that the effects seen are not due to impurities, we consider that any tenable explanation of them must include an action of Triton X-100 on living membranes. It is well known that cell membranes exercise selective permeability. Nissim (1964) and Hart & Nissim (1964) have suggested that the movement of glucose across intestinal cells is regulated by certain protein receptor sites, some situated on the membrane, some intracellular. They considered that the cationic surfactant cetrimide reacted with receptor proteins to alter the rate of glucose entry into the cells. It is possible that Triton X-100 may exert one or more of the following actions upon cell membranes: a reduction of surface tension at the membrane interface; an action upon receptor sites or pores; an alteration of lipid constitutents of the membrane structure (Booij, 1962). Various workers have postulated that hormones exert part or the whole of their effects, by influencing the activity of enzyme systems or by altering physical properties of limiting membranes (Smith & Williams, 1965). It could be that the Triton X-100 rendered ovarian cells more responsive to pituitary gonadotrophins. It is significant that the phenomenon took a long time to appear and that only some of the treated animals showed a response.

Acknowledgements. We wish to thank Robert Bryce & Co., Sydney, for donation of some of the surfactant materials used in this work and Mr. H. Lahoud, Mr. N. Watson and Mr. P. Harris, of the School of Wool Technology for their help.

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