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The effect of a range of Triton non-ionic surfactants on rodent ovaries

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Cysts developing on rodent ovaries after the Triton series of non-ionic surfactants have been fed to or applied topically to the animals are subcapsular and associated with obstruction of the ostium. The ability of Triton X–100 to produce subcapsular cysts after being fed to animals has been confirmed but the mechanism by which it does so remains obscure.

We have previously reported the production of cysts in rodent ovaries after painting the non-ionic surfactant Triton X–100 on (Goldhammer & McManus, 1960) or feeding it to the animals (Goldhammer, McManus & Osborn, 1967). We have now determined the nature of the cysts and attempted to find out how they arise.

In 1960, Goldhammer & McManus reported on the toxicity of Triton X–100 when applied to various organs in the rat. We have also re-investigated these effects.

EXPERIMENTAL AND RESULTS

The dissecting microscope was used to differentiate between subcapsular and follicular cysts, to find the ostium and to assist with accurate injection of materials into the subcapsular space. Most cysts were found developing in the subcapsular space and were invariably associated with a blockage of the ostium.

Injection experiments. An Agla micrometer syringe was used to measure the volume of fluids injected. To assess the specificity of the response varying volumes of Triton X–100 undiluted and in solutions at different concentrations were injected into the subcapsular space (Table 1). The appearance of the ovary was noted after laparotomy 1 week later and the animals were killed at the times indicated in the Table. A slight accumulation of fluid was observed in some rats at the laparotomy 1 week after the subcapsular injection of undiluted Triton X–100 and subsequently there was a high incidence of subcapsular cysts in both mature and immature rats. There was a low incidence of cysts when Triton X–100 was injected in a dilute form.

The relative importance of the lipid or water solubility of the surfactants was investigated by injecting into the subcapsular space a range of Tritons, X–35, X–45, X–165 and N–100 (Table 2). All four Tritons are capable of producing cysts after subcapsular injection. The effects of xylol, liquid paraffin, distilled water and solutions of various concentrations of sodium chloride were also assessed (Table 3). Xylol obliterated the subcapsular space, liquid paraffin, water and dilute solutions of sodium chloride produced no visible changes and 3 ovaries in 14 rats showed peri-ovarian cysts after the subcapsular injection of 20% sodium chloride.

Table 1. *The effect of injection of Triton* X–100 *into the peri-ovarian space of rats.* There is a high incidence of subcapsular cysts when undiluted Triton X–100 was injected into the subcapsular spaces of mature and immature rats. There was a low incidence of cysts when Triton X–100 was injected in a dilute form.

No. of rats	Volume injected concentration (ml)	Time after injection that animal was killed	No. of subcapsular cysts found
3	0·01 undiluted	3 weeks	2
6 (1 ovary removed)	0·05 "	7 weeks	3
4	0·005 "	3 weeks	2
10 (3 weeks old)	0·005 "	3 weeks	7
3 (2 weeks old)	0·001 "	3 weeks	3
4	0·05 10%	8 weeks	1
6	0·25 1%	4 weeks	none
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Table 2. *The effect of the injection of various Triton surfactants into the peri-ovarian space of rats.* All of the water and lipid soluble Tritons are capable of producing subcapsular cysts after injection into the peri-ovarian space of rats.

No. of rats	Volume (ml)	Time (weeks)	No. of cysts
4	X–35 0·025	4	4
3	X–45 0·05	5	1
4	X–165 0·025	4	2
4	N–100 0·025	4	1

X–25 is water-insoluble, X–45 is water-dispersible, X–165 and N–100 are water soluble.

Table 3. *Effect of subcapsular injections of various substances on rat ovaries.* Xylol obliterated the subcapsular space, liquid paraffin, water and dilute solutions of NaCl produced no visible changes. Three ovaries in 14 rats showed subcapsular cysts after peri-ovarian injection of 20% NaCl

No. of rats	Volume (ml)	Time (weeks)	No. of cysts
7	Xylol 0·01	6	None*
6	Paraffin 0·01	6	None
6	Distilled water 0·05	5	None
14	NaCl (20%) 0·01	5	3
6	NaCl (0·9%) 0·05	5	None
3 (14 days old)	NaCl (20%) 0·001	3	None

* 3 ovaries shrunken, subcapsular space obliterated.

Two-week old animals responded to subcapsular injections of Triton X–100 and solutions of sodium chloride in the same way as mature animals.

Indian ink and carbon black experiments. A mixture of equal parts of Indian ink and either Triton X–100 0·1 ml (4 animals) or 0·9% sodium chloride (3 animals) were injected into the subcapsular space, the animals being killed after 3 (Triton)

and 4 weeks (saline). There were no cysts in the saline-treated ovaries and one on an untreated ovary of the Triton-treated animals. For the carbon black experiments the animals were given 0·02 ml of a suspension of carbon black in either Triton X–100 (5 animals) or saline (20%) (5 animals) and after killing at 6 weeks the treated animals had 3 cysts and the controls 4 cysts. These preparations leaked rapidly through the ostium and carbon particles could not subsequently be demonstrated in the subcapsular space or in cysts which had formed, though small quantities were observed in the surrounding tissues. In several experiments where no cysts were produced it was noted that the subcapsular space had been obliterated.

Feeding experiments. The experiment involving feeding of Triton X–100 to mice (Goldhammer & others, 1967) was repeated. 14 mice fed Triton X–100 at 0·3% v/w for 26 weeks had 10 cysts while 39 control mice had 1 cyst.

Goldhammer & McManus (1960) reported that the effects of surfactants varied according to the kind of tissue to which they were applied. In their experiments the amount of Triton X–100 applied was not measured, though it was applied to a known area in a known concentration. A calculation suggested that the amount applied might be in the toxic range for an intraperitoneal application and be unrelated to the site of application. The toxicity from the application of Triton X–100 to the livers of 10 rats, and kidneys of 4 rats in the dose range 0·01 to 0·08 ml was therefore compared with the toxicity of intraperitoneal injections in 28 rats. No gross difference in toxicity could be demonstrated. At autopsy, animals dying after intraperitoneal injection of Triton X–100 showed congestion of the peritoneum and ascites with lysed blood in the fluid, and there was gross haemolysis of blood taken from the heart. The dose of Triton X–100 that killed by intraperitoneal injection was much greater (50–100 fold) than that required intravenously. We concluded that much of the acute toxicity of the Triton was a consequence of the haemolysis.

Using azovan blue (2 mg/100 g) in rats and mice fed with Triton X–100 for varying periods of time, we have not observed any permeability changes which could be attributed to the Triton though permeability increases were observed in the genital tract and gonads of animals in oestrus.

DISCUSSION

We have demonstrated that the cysts are due to fluid distension of the subcapsular space and that this is invariably associated with obstruction of the ostium. These observations are in agreement with those of Alden (1942) who occluded the rat ostium with a ligature and showed that this led to the development of a peri-ovarian cyst. We surmise that the occasional cyst filled with blood stained fluid is the result of a small amount of bleeding following follicle rupture as we have only observed this in mature animals.

In the light of Alden's work, the mechanism of production of subcapsular cysts after painting or injection experiments seems fairly clear. The Triton series of surfactants are irritant when injected into tissues and will lead to damage to the peritoneal lining with increase in permeability, leakage of fibrin and some cell necrosis. Dr. R. Cummings (personal communication) has demonstrated in the guinea-pig a large increase in vascular permeability at the site of subcutaneous injections of the Triton series of surfactants even in high dilutions. Subcutaneous

injections of undiluted surfactants led to a zone of necrosis. Cummings could not show hypersensitivity to Tritons in experiments with guinea-pigs. He observed that water-soluble Tritons were more irritant that lipid-soluble Tritons. This confirms previous reports (Finnegan & Dienna, 1953).

The mechanism of cyst formation after the feeding experiments remains obscure. Denise Madill (personal communication) using intragastric injections of tritiated Triton X–100 has shown that little labelled material was excreted in the faeces and that most of the radioactivity was recovered from the urine within 24 h. It is possible that the absorbed Triton exerts an action on serous surfaces. As the ostium is narrow and the effects of blocking it are readily observable, this may be the only site at which the effects of the Triton are grossly apparent. Tritons might exert an effect by altering the vascular permeability of the region allowing a leakage of fibrin and hence blockage of the ostium. So far we have not been able to demonstrate any alteration in permeability after azovan blue injections. The only changes observed were those associated with oestrus when there was an increase in permeability in the ovary and uterus as evidenced by a leakage of blue dye into tissues.

REFERENCES

ALDEN, R. H. (1942). *Anat. Rec.*, **83**, 421–433.

FINNEGAN, J. K. & DIENNA, J. B. (1953). *Proc. Sci. Sect. Toilet Goods Ass.*, No. 20.

GOLDHAMMER, H. & MCMANUS, W. R. (1960). *Nature, Lond.*, **186**, 317–318.

GOLDHAMMER, H., MCMANUS, W. R. & OSBORN, R. A. (1967). *J. Pharm. Pharmac.*, **19**, 167–169.