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An unexpected effect of arachis oil: increased peroxidase activity in murine submandibular gland

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An unexpected effect of arachis oil was found during work on the effect of oestradiol-17β on peroxidase activity in the submandibular gland of the mouse. This was studied because an increasing number of mammalian peroxidases (EC 1.11.1.7) appear to be oestrogen-responsive including the salivary enzyme in man (Cockle 1980). Arachis oil is widely used to dissolve a variety of steroid hormones for administration experiments. The stimulation of peroxidase activity should now be added to the other hormone-like biological properties of arachis oil.

Materials and methods

Female mice (To) were reared in the Specific Pathogen Free Unit at the Clinical Research Centre. Immature mice for administration experiments were used at 3–4 weeks of age.

All reagents were of analytical quality or of the highest purity available. 2,2'Azino-di (3-ethylbenzothiazoline-6-sulphonic acid), ABTS, was obtained from Boehringer U.K., Lewes, East Sussex. Arachis oil was prepared by the Pharmacy, Northwick Park Hospital. All other reagents were obtained from British Drug Houses, Poole, Dorset.

Treatment of mice. Immature mice were administered arachis oil or oestradiol-17β (80 μg kg^{-1}) dissolved either in arachis oil or in 3% (v/v) ethanol in 0·9% NaCl (saline) by subcutaneous injection. Mice were then killed by cervical dislocation at various times after treatment, and submandibular glands and uteri were removed and weighed.

Treatment of submandibular salivary glands. Glands were generally analysed immediately, but if storage could not be avoided, the intact glands were kept at −20 °C for up to 2 weeks. Peroxidase activity is stable in gland homogenates for up to 2 weeks at −20 °C, but unstable after storage at 4 °C for 24 h.

On the day of analysis, the salivary glands were washed free from blood in 10 mmol $litre^{-1}$ Tris-HCl pH 7·2, and then homogenized for 20 s in fresh buffer using an Ultra-turax homogenizer to give a 2·5% (w/v) homogenate. The homogenate was centrifuged at 1500 *g* for 5 min, and the supernatant containing about 95% of the peroxidase activity in the whole homogenate, was retained for the peroxidase assay.

The assay was performed, using the chromogen 2,2'-azino-di(3-ethyl-benzothiazoline-6-sulphonic acid), ABTS, by the method of Shindler et al (1976) with the modification that the reaction was carried out at 25 °C in a final volume of 3 ml. The final assay medium after addition of the sample aliquot, contained 1 mmol $litre^{-1}$ ABTS, 0·1 mmol $litre^{-1}$ H_2O_2 in 0·1 mol $litre^{-1}$ sodium acetate pH 4·4. The enzyme reaction was initiated by addition of 30 μl of salivary gland extract, and the formation of product was monitored continuously by measurement of the increase in absorbance at 412 nm in glass cuvettes of 1 cm path length. The enzyme activity was proportional to the protein concentration throughout the range used. The absorbance of one μmole of the ABTS oxidation product per ml of assay medium is 32·4 at 412 nm (Shindler et al 1976). Enzyme activity has been expressed in units g^{-1} of total protein. One 'unit' of activity is defined as the amount of enzyme required to form one μmole of product min^{-1} under the conditions of the assays. Total protein concentrations were determined by the method of Miller (1959).

Results

Salivary peroxidase activities in the submandibular glands of immature mice were determined at 2 hourly intervals throughout the day. There was no significant change in the mean activities using a one-way analysis of variance ($P > 0{\cdot}05$), and in subsequent experiments animals were killed within the period 08.30 to 18.30 h. In contrast, salivary peroxidase in man has previously been shown to vary three-fold throughout the day (Cockle 1980).

In the initial experiments on the effects of oestradiol-17β, mice in the control group received arachis oil and were killed 18 h after treatment; a time interval when a significant response to oestrogen was observed in rat uterine peroxidase (Lyttle & De Sombre 1977). After arachis oil alone there was an increase in peroxidase activity but not submandibular gland weight.

Another experiment (Table 1) was designed, firstly to confirm the effect of arachis oil on peroxidase activity in submandibular salivary glands, and, secondly, to distinguish possible effects of oestradiol-17β from those of oil. Immature, female mice were divided randomly into three groups each of which received subcutaneous injections as follows: the 'control' group received a mock injection, the 'oil' group received arachis oil alone, and the 'oestradiol in oil' group received oestradiol-17β (80 μg kg^{-1}) in arachis oil. The number of mice in each group was 20 to enable changes in activity to be resolved, and mice were killed 24 h after injection.

Both arachis oil and oestradiol-17β in oil caused a significant increase in submandibular peroxidase activity

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Table 1. Effects of arachis oil and oestradiol-17β in arachis oil on submandibular gland peroxidase activity. Mice were randomly selected into three groups (n = number in group). All mice were killed 24 h after treatment. A Student's t-test was used to test the significance of the results: control/oil, t (37) = 2·667 $P < 0·02$; control/oestradiol in oil, t (38) = 4·302 $P < 0·001$; oil/oestradiol in oil, t (37) = 1·612 $P > 0·1$.

Group	n	Mean (s.d.) peroxidase activity (units g^{-1} protein)
Control	20	118 (29·6)
Oil	19	143 (28·9)
Oestradiol in oil	20	158 (29·2)

when compared with the effect of mock injection which had no effect on weight or peroxidase activity.

However, the effect of arachis oil was not significantly different from the effect of oestradiol-17β in arachis oil.

The possibility remained that an 'oestradiol effect' may not be detectable in the presence of an 'arachis oil effect'. For this reason, a second vehicle containing 3% (v/v) ethanol in the saline was chosen for the final administration experiment. No response in salivary peroxidase activity was observed up to 42 h after treatment even after increasing the dose of hormone to 160 μg kg^{-1}.

Discussion

Arachis oil caused a significant increase in salivary gland peroxidase activity which should be added to the other biological effects of this widely used vehicle. Both sesame and arachis oil have been reported to have definite androgenic effects in that they promote comb growth in young chicks (McCartney et al 1977). Arachis oil consists largely of polyunsaturated triglycerides (26% linoleic acid esters) which are unlikely to be capable of interaction with steroid receptors. However, in addition, the oil contains readily detectable quantities of sterols and cyclic triterpenoids (Carlisle & Ellis 1968), which may have endocrine properties. Since arachis oil did not cause an increase in uterine weight in mice, the oil was not 'classically' oestrogenic. However, this does not exclude the possibility that components of the oil have some other oestrogen-like properties in addition to the stimulation of peroxidase activity. Increased peroxidase activity has been suggested as a marker of oestrogenic activity (see Cockle 1980). However, we have been unable to increase activity in submandibular gland with the classical oestrogen, oestradiol-17β. The phrase 'oestrogen-like' may nevertheless still be useful. For example, arachis oil contains β-sitosterol which possesses some juvenile-like hormone activity and affects moulting in locusts (Carlisle & Ellis 1968).

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