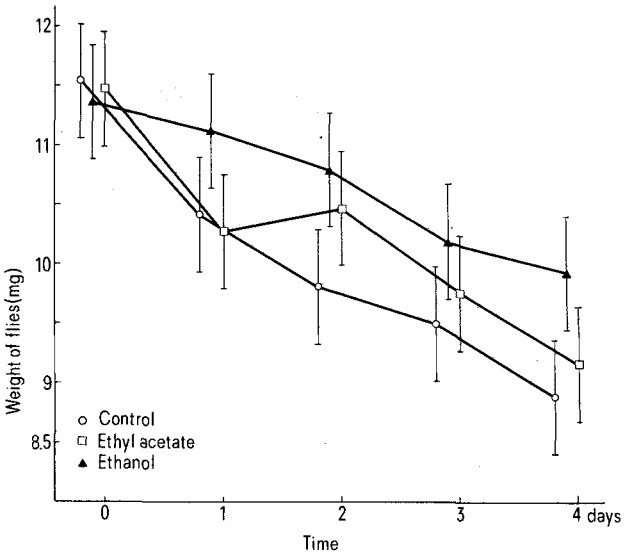

Figure 2. Body weights of sample of 10 flies (mg) of each of days 0-4. Ethanol (△), ethyl acetate (□), control (○). Vertical lines are 95% confidence intervals calculated from residual errors of ANOVA. Each point is the mean of 12 measurements.

males. The average weight of a sample of 10 females was 11.25 ± 0.14 g as compared with 9.38 ± 0.14 g for males. But the sexes did not respond differently to different chemical treatments, as indicated by the statistically non-significant sex x chemical treatment interactions in all experiments.

It seems as if the response of adult flies to ethanol and ethyl acetate vapor depended on the net balance between the toxicity of the chemical and its benefit as an energy source. That benefit must be evaluated according to the physiological need of the fly. The young or starving fly does not have a large energy reserve, and therefore can utilize the chemical vapor as an emergency energy source.

The well fed fly does not need such benefit, hence the toxicity effect dominated the response. Even though the dual toxic and beneficial effects of ethyl acetate and ethanol vapor were demonstrated, the experimental unit was very stressful. However, the intrinsic stresses, e.g. starvation and desiccation, were similar for all treatments.

Using the same system, 8 other esters (all acetates from propyl to amyl, both n- and i-, ethyl propionate and ethyl n-butyrate) also were studied. Except for amyl acetate, young flies were able to utilize these esters to increase longevity[12]. Genetic studies of polymorphic esterases are seriously circumvented by the lack of information about the natural substrate[10,13]. Atmospheric short chain esters offer an opportunity to study possible differences between esterase allozymes. Using young *D. buzzatii* flies, different esterase phenotypes responded differently to some esters (n- and i-propyl acetate, n- and i-butyl acetate and ethyl propionate)[12].

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Large scale isolation of zonae pellucidae from ovarian oocytes of mice

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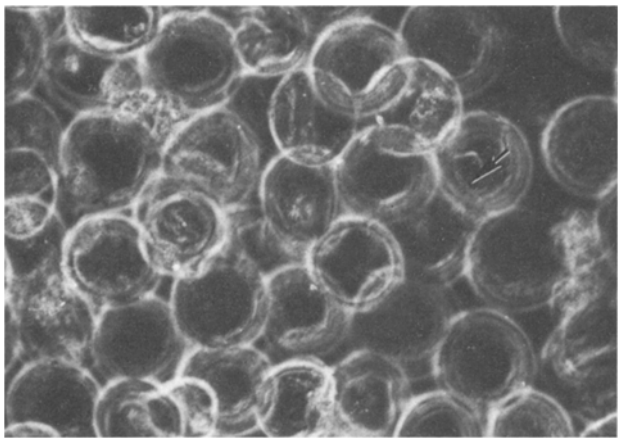
Summary. A large scale method for the isolation of zonae pellucidae (ZP) from mouse ovaries is described. It involves the squashing of the ovaries on a screen (70 × 70 M mesh) and sedimentation of the material in a discontinuous gradient. It is possible to obtain free ZP in useful quantities in about 1 h's work.

Several methods for large scale isolation of zonae pellucidae (ZP) from large mammals have been reported, for example pig ovaries[1-3] and cow ovaries[3,4]. However, no large-scale method for the isolation of ZP from mice have been reported (Dunbar, personal communication). The procedure reported by Bleil et al.[5] cannot be considered a large-scale one (it yields about 50 ZP per h of work).

In this communication a simple method for the isolation of large amounts of entirely free ZP from mice ovarian oocytes is described. It consists of the squashing of the ovaries on a wire screen, followed by washing off the squashed material in a phosphate buffered saline solution (PBSS). After 2 centrifugation steps, the material is deposited on the top of a discontinuous gradient, composed of 2 layers. After 20-30 min sedimentation, free ZP are recovered from the upper half of the intermediate 8% sucrose layer.

Materials and methods. 18-25-day-old white mice were sacrificed by cervical dislocation. The ovaries were removed and freed from the Fallopian tubes and retroperitoneal fat cushion, washed in a phosphate buffered saline solution (PBSS), (NaCl 7‰; pH: 7.2), maintained at 4 °C, and then placed on a piece (2.5 × 2.5 cm) of wire screen, (stainless steel, 70 × 70 M mesh).

Ovaries were squashed with a spatula and the lower face of the mesh was washed in a culture dish containing PBSS in



Dark field picture of part of a sample of isolated zonae pellucidae suspended in a liquid medium. Concentric circles in several units correspond to the various planes of phocus (arrow).

order to collect the components set free by disruption of the ovaries; intact follicles, naked oocytes, empty ZP and follicle cells. After this step the sample was centrifuged, washed in PBSS and centrifuged again at 200 rpm, (2 min each), in order to separate the population of follicle cells.
The sample was then resuspended in 2 ml of PBSS and gently deposited on the top of a discontinuous gradient composed of 2 layers. The one at the bottom (A, 3 cm high) is a 20% sucrose in PBSS and the intermediate layer (B, 6 cm high) is a phenol red colored PBSS + 8% sucrose.
Differential sedimentation of the various components according to their mass occurs in about 20–30 min. After this period the upper half of the intermediate layer (B) was pipetted out and centrifuged briefly. The supernatant was discarded and the pellet placed in a culture dish to check the quality of the sample at the light microscope level.
Some of the samples were fixed in 2.5% glutaraldehyde in phosphate buffer, (pH: 7.2) for 1 h at 4 °C and postfixed in 1% buffered OsO_4 followed by embedding in Durcupan ACM (Fluka). Sections were made in a Sorvall MT2 ultramicrotome, stained in uranyl acetate and examined in a Siemens Elmiskop 1.

Results and discussion. Using this method, which is a modification of the one already published for the isolation of ovarian follicles[6] and ovarian oocytes of mice[7], it is possible to obtain 15–25 entirely free ZP per ovary. The total number of ZP depends only on the number of ovaries used in each squashing experiment. The time required after the ovaries are placed on the mesh until the ZP are free and completely isolated from other ovarian components is no longer than 1 h.
In these experiments most of the free ZP were found in the upper half of the intermediate layer (B), well preserved oocytes were found in the lower half of the same layer and intact follicles were found in layer (A). Only a part of the free follicle cells were found in the top layer (original sample).
Examination of the samples at the light and electron microscope level showed that cytoplasmic components were absent from isolated ZP. The figure included in this paper illustrate at the light microscope level, the appearance of ZP in a fresh sample.
Collection of ZP from small mammals by the method proposed here offers the following advantages; ZP can be easily obtained in desirable quantities; the procedure is not time consuming and does not require special equipment and reagents; careful handling of the samples yields ZP almost entirely free of contaminants from ooplasm or from follicle cells, and other ovarian cell types can be simultaneously obtained for other studies.

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Control of growth in the rat thyroid – an example of specific desensitization to trophic hormone stimulation

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Summary. Rat thyroid follicular cells desensitized to the mitogenic action of TSH by prolonged stimulation retain the capacity to respond to the mitogenic stimulus of wounding. The implications for thyroid growth control and neoplasia are discussed.

Prolonged growth stimulation leads in many tissues to tumor formation[1-3]. The dramatic initial response to trophic hormone however is usually short-lived[4-6] and a period of quiescence follows before tumors develop. The thyroid, with its easily-manipulated trophic stimulus – thyrotrophin (TSH) – provides an excellent model for studying this phenomenon. Sustained elevation of serum TSH leads to a limited period of thyroid growth[7,8] followed by a 'plateau' before tumors appear[9]. We have shown[8] that the lack of a continued growth response of the normal follicular cell to TSH is due to a decline in mitotic activity rather than to an increase in cell death rate. We have also shown[8] that this occurs despite a sustained increase in follicular cell function, thus demonstrating that there is an uncoupling of the growth and functional responses to TSH during prolonged stimulation.
To test the specificity of this loss of mitotic responsiveness, we have now investigated the effect of an alternative stimulus – wounding – on these chronically TSH-stimulated thyroids.
Materials and methods. Seven male Wistar rats aged 12 weeks were treated with the goitrogen aminotriazole