strated mark a step forward in the culture of mosquito tissues *in vitro*. I thank Dr. W. Trager of the Rockefeller Institute for his advice, and Mrs. Ch. Horak for her help.

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J. PELEG

Israel Institute for Biological Research,
Ness-Ziona.

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Inability of Metabolizable Oils to Mimic Corpus Allatum Hormone Stimulation of Ovarian Development in Mosquitoes

THERE has been considerable experimental evidence that the juvenile hormone, secreted by the corpus allatum, is responsible for the maintenance of the larval condition in immature insects and the initiation of ovarian development in the adult^{1,2}.

Recently, Larsen and Bodenstein³ were able to show that a crude oil extract of cockroaches prepared after the method of Williams⁴ induced ovarian development in anautogenous mosquitoes. Most of these extracts proved to be exceedingly toxic. Some of the mosquitoes died a few hours after injection, while others lived for several days. The extract initiated ovarian development in non-blood fed anautogenous *Aedes aegypti* and *Culex pipiens*. Because of the toxicity of the preparation only a few insects survived to show this effect, but the experiments demonstrated that the activating principle is not species specific. The ovarian development of anautogenous mosquitoes provides a sensitive and simple method for the bioassay of the juvenile hormone.

These experiments of Larsen and Bodenstein³ have recently been criticized by Gilbert and Schneiderman^{5,6}. The major criticisms were that "no evidence was offered to show that the extract prevented the larval tissue of any insect from assuming its adult aspect", and they also suggested that there was no hormone effect present in the extract at all but that ovarian development in the mosquito was induced by some nutritive factor in the oil. Gilbert⁶, quoting unpublished data of Harman and Schneiderman, claims that "The possible nutritive role of the insect oil itself cannot be excluded. Indeed, injections of any metabolizable oil like peanut oil or insect oil will stimulate the ovarian development of pseudo-allatectomized milkweed bugs". Yoeli and Mer⁷ claim that the lipid-rich stroma of red blood cells contains some constituent necessary for the development of eggs in *Anopheles*. Gilbert and Schneiderman⁵ infer that the experiments of Yoeli and Mer are further proof that ovarian development in the mosquitoes was a result of oil nutrition rather than a hormone.

Experiments have now been completed which show there is no gonadotrophic factor in peanut oil or hormone-free insect oil. Refined peanut oil U.S.P. (Magnus, Mobe and Reynard) was injected into 85 female mosquitoes (Table 1). The mortality was probably due to injection-trauma rather than any toxic effect of the peanut oil. After six days all the females were killed and none of them showed any ovarian development beyond the 24-48 h diapause stage of all unfed female mosquitoes. When the mosquitoes were killed the drop of peanut oil was observed intact in the haemocoel and had no apparent effect on any of the mosquito tissues.

There remained the possibility that a hormone-free cockroach extract oil might be more nutritious than peanut oil and thus be capable of inducing ovarian development. To eliminate any possibility of a nutritious oil inducing ovarian development a hormone-free cockroach

extract oil was prepared after the manner of Williams⁴, as Larsen and Bodenstein³ prepared their extract in previous experiments. Adult cockroaches less than 12 h after moulting and before the tanning of the cuticle were used to prepare this extract. This stage in the cockroach life-cycle was chosen to prepare a hormone-free extract based on the work of Bodenstein and Sprague⁸ in which they were able to show that shortly before metamorphosis and up to 12 h after the last moult there is little or no allatum hormone present in the organism. If any allatum hormone is present at this time it must be in very small amounts because the colleterial gland assay of Bodenstein and Sprague⁸ in newly metamorphosed adults was essentially negative.

The cockroach extract oil was injected into 55 adult female mosquitoes at least 24 h after emergence. As can be seen in Table 1, none of the mosquitoes showed any ovarian development at the end of six days. There was a fairly high mortality, which coincides with earlier work³ in which we pointed out that the crude cockroach extract was toxic, and only a few mosquitoes survived.

Table 1. *Aedes aegypti* INJECTED WITH PEANUT OIL AND HORMONE-FREE COCKROACH EXTRACT OIL FROM NEWLY METAMORPHOSED COCKROACHES, *Periplaneta americana*

(The cockroach oil extract was made from animals within 12 h of the last moult before the cuticle had tanned or hardened)

	No. mosquitoes injected	No. mosquitoes survived	% mortality	No. mosquitoes showing ovarian development 6 days after injection
Peanut oil	85	74	13	0
Cockroach extract oil	55	43	22	0

Gilbert and Schneiderman^{5,6} referred to the work of Yoeli and Mer in attempting to substantiate their contention that "some metabolizable oil" was responsible for ovarian development. Blood is a good nutritional substrate for the formation of yolk material; but by a series of ligation, decapitation, extirpation and implantation experiments it was shown⁹ that egg development in mosquitoes is under hormonal control. The triggering mechanism for the initiation of ovarian development appears to be gut distension. Day¹⁰ showed that when washed erythrocytes were fed to mosquitoes, being particulate in nature, they did not enter the diverticula but went to the mid-gut where they could form a coagulum and initiate ovarian development. The same is true in the experiments of Yoeli and Mer⁷. Hormone secretion necessary for ovarian development in mosquitoes is triggered, probably by gut distension, but the number of eggs developed is undoubtedly related to the amount of nutrient available in the mid-gut. Ovarian development in mosquitoes will not be initiated by injections into the haemocoel of whole blood, peanut oil, or cockroach extract oil which is free of hormone.

There is adequate experimental evidence that allatum hormone is present and is actively secreted in cockroaches^{8,11}.

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JOSEPH R. LARSEN

Department of Entomology,
University of Illinois,
Urbana.

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