been described in *Fundulus* eggs by Trinkaus (*6*). Removal of the blastocoel roof in this form has no effect upon further epiboly of the remaining peripheral portion of the blastoderm. Trinkaus has provided evidence for the role of the contractile tension developed by the surface gel layer which "pulls the blastoderm down over the yolk." The operation of a similar mechanism in the hen's egg is doubtful in view of the following observations. Small defects created in the path of the advancing blastoderm demonstrate that growth is not limited to one direction. In addition to downward spread, the blastoderm, after bypassing the defect, is capable of both lateral and upward growth. Furthermore, since cauterization at site 3 does not cause an immediate cessation of growth (as would be expected if contractile tension were pulling the blastoderm down) and, furthermore, has no effect upon growth until the blastoderm margin actually touches the damaged region, it is clear that contractile tension does not influence blastodermal growth in the hen's egg. Recent evidence (*7*) indicates that the role of the surface contractile tension in *Fundulus* epiboly is not a major one.

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On the Nature of the So-Called Background Material in Estrogen Fractions of Extracts Prepared from Hydrolyzed Urine[1]

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In 1948 Friedgood, Garst, and Haagen-Smit (*1*) reported an essentially new micromethod for the extraction and partition of crystalline estrone, estradiol, and estriol, and for their quantitative assay by ultraviolet spectrophotometry. In the course of applying this method to the extraction of the natural estrogens from hydrolyzed urine, to their subsequent separation from urinary phenols and neutral steroids, and to their partition from one another, it was found that the so-called background material (*2–8*) interfered significantly (*9–11*).

We have tried various methods for the separation of the "background" material from the urinary estrogens: as, for example, modification of the procedure for the steam distillation of the phenolic fraction, various washes of the extracts, changes in the solvents for equilibration, and variations of the pH at which the extractions were done. Although some of these attempts produced considerable reduction in the amount of the interfering ultraviolet-absorbing material in the final extracts, some interference persisted. At that point in the investigation a number of observations from a variety of experiments indicated that the "background" material might consist of a phenolic steroid or steroids which developed a quinone structure during the extraction procedure. A fundamental analysis done on a partially purified sample lent further support to this interpretation of the data (Table 1). A large part of this steroidlike mixture has been found to exhibit physical and chemical properties similar to those of estriol when studied in the Craig distributor and by rubber chromatography according to the method of Nyc, Maron, Garst, and Friedgood (*12*). Moreover, comparison of the ultraviolet spectrum of this steroidlike material with that of a synthetic estrogen derivative (*13*) indicates a possible structural relationship of the steroidlike material to the natural estrogens (Fig. 1).

TABLE 1

FUNDAMENTAL ANALYSIS OF STEROIDLIKE MATERIAL: COMPARISON WITH VALUES FOR THEORETICAL ESTRIOL DERIVATIVES

	Carbon (%)	Hydrogen (%)
Steroidlike material	71.5	7.6
Theoretical estriol catechol	71.1	7.9
Theoretical estriol quinone	71.6	7.3

This steroidlike material is found consistently in the urine of both males and females; and it is increased in amount about threefold during pregnancy. The excretion values in nonpregnancy urines are rather constant; they are of the order of 3–4 mg/24-hr sample.

A further study of this steroidlike material is now in progress in order to achieve its complete identification, as well as its separation from the natural urinary estrogens.

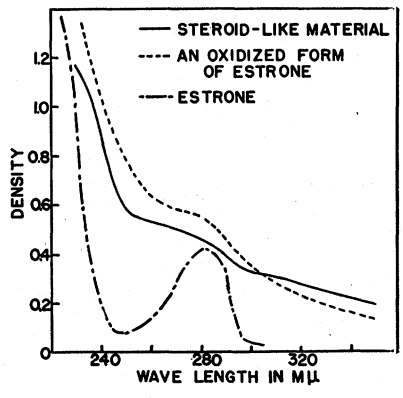

FIG. 1. Comparison of the ultraviolet absorption of the steroidlike material with that of an oxidized form of estrone. The latter was produced by exposing a mixture of estrone and 10% of its weight of riboflavin to 100 ft-c of light for 3 days.

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Inbred Strains of *Culex* Mosquitoes

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Many studies are in progress using mosquitoes as laboratory animals, some of the most prominent of which are studies of various insecticides and the resistance of mosquito strains to them. The results of these experiments have varied widely for a number of reasons. One important source of the varying results can probably be found in the inherent genetic differences in the stocks used.

The genetic experiments using *Culex* mosquitoes in this laboratory have indicated that wild-caught strains of *Culex pipiens* and *C. quinquefasciatus* vary widely in many respects. Ordinary laboratory-bred strains also show a wide range of expression of phenotypic characteristics. In the course of our experiments it has been necessary to develop inbred strains of these two species, with the ultimate aim of as high a degree of homozygosity as possible.

One stock of *C. quinquefasciatus* maintained in this laboratory was originally obtained from Don W. Micks at Galveston, Texas, and had been maintained by him for several generations as a laboratory stock. A wild-caught strain of *C. pipiens,* from Champaign, Ill., is at present in use.

These stocks have been carefully inbred according to the plan described below. All stocks were begun from single egg rafts. The larvae from each raft were raised separately, and the pupae transferred to a new cage. Males and females from the same egg raft were allowed to mate, the egg rafts were collected, and the larvae raised in separate pans. From this F_1, one egg raft was selected, the adults were allowed to emerge and mate, and F_2 rafts collected. Thus each generation is the result of brother-and-sister mating, and each succeeding generation (F_1, F_2, F_3, F_4, etc.) is derived from a single egg raft, raised in a new cage. There is no backcrossing and no outcrossing, even to members of the same original stock, and thus the inbreeding is as strict as possible in animals with bisexual reproduction.

The *C. quinquefasciatus* stock is at present in the F_{20} generation, and the *C. pipiens* stock in the F_{12}. Following the method given by Wright (*1*) the *pipiens* stock in the F_{12} is calculated to be more than 95% homozygous, and the *quinquefasciatus* in the F_{20} should be about 99% homozygous. Sixteen generations of brother-sister matings will result in about 98% homozygosity. By way of contrast, one technique commonly used in mass cultures of these mosquitoes has been to isolate all F_1 rafts, then isolate the F_2, and so forth. This method is little more than random mating and, assuming that other factors (mutation, selection, etc.) are negligible, the population should be just as heterozygous in the nth generation as it was in the first. It is therefore possible that many laboratory colonies, as well as wild-caught strains, have considerably different genetic backgrounds. Other characteristics of these stocks, defined in terms of the biological properties which they possess are as follows:

C. pipiens

a) Stenogamic: Mating and egg deposition take place in cages $30 \times 30 \times 30$ cm. Although most strains of *C. pipiens* (not *molestus* or *autogenicus*) are eurygamic, we have never experienced any difficulty in getting this strain to mate in cages of this size. If, of course, eurygamy is defined on the basis of mating on the wing rather than on the basis of cage size, the term is a matter of definition. This strain has never been observed to mate at rest, but will mate in flight. It is a striking coincidence that of several strains of *C. pipiens* which have been collected in the vicinity of Champaign, none has ever been found which would not breed in cages of this size if other rearing conditions were right.

b) Anautogenous: A blood meal is required before egg deposition. As far as we know, we have never had an autogenous egg raft. Repeated isolations of males and females, supplied with water, moistened prunes, raisins, apple slices, or sugar solutions, have never resulted in a single egg raft. On the other hand, egg deposition has always been associated with a previous blood meal.

c) Ornithophily: Although this strain will bite man—infrequently, and only when no bird blood is available—its definite preference is for birds. We normally use pigeons, but the strain has also fed on chickens. Individual females seem to differ in their biting habits. Some will avidly feed on the pigeon no matter at what time it is introduced into the cage; others prefer to feed in the evening.

d) Modified heterodynamy: Heterodynamy is a term which has been applied to those strains which undergo a diapause, or temporary cessation of reproductive function, especially during the winter months. Few laboratories have succeeded in rearing *C. pipiens* on a year-round basis, owing principally to this factor. Our strain seems to possess the genetic basis for this character, but its manifestation is modified, we believe, by the use of continuous light in the