units that may be present in isozymes at positions 3 to 7. Preliminary studies on heat lability of embryonic isozymes indicate that when treated with 50°C for 30 minutes, isozymes at positions 5,6,7 were heat labile, but those at positions 3,2,1,0^1, and 0^2 were still enzymatically active. This is further evidence that more than one structural gene codes for subunits that form the isozymes at positions 3 to 7. The three extracted variant types are considered to be regulatory variants that control the rate and/or time of subunit synthesis by structural genes, similar to the lactate dehydrogenase variant studied in mouse erythrocytes by Shows and Ruddle (1968).

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Bairati, A. and M.E. Perotti, University of Milan, Italy. Occurrence of a compact plug in the genital duct of D. females after mating.

Some experiments have been performed to control the previously reported assumption (1) that the ejaculatory bulb secretion is injected with sperms into the female genital duct during mating. Females (10 for each interval) have been separated from males at various intervals from the beginning of mating. Their genital apparatus has been dissected in saline isotonic solution and observed with dissection, phase contrast and electron microscopes.

The following results have been obtained: 1) during the first 5 minutes from the beginning of mating no material is observed in the female genital duct. 2) between 5 and 7 minutes a compact plug appears filling the uterus lumen. It is cylindrical and made up of a homogeneous, thick and translucent substance. Before the appearance of the plug no sperms are present in the uterus and at about 7 minutes only few sperms have been observed in the most caudal portion of the female genital duct. 3) at 10 minutes, the mass acquires its largest size and many sperms appear within the uterus beyond the plug. Furthermore, some sperms are observed beating between plug surface and uterus walls. 4) at 12 minutes a very large number of sperms is assembled in the cephalic portion of the uterus. Some sperms are present also in the ventral receptacle. Within 14 minutes the sperms fill the receptacle and the spermathecae. 5) the plug is visible in the uterus since 5-7 minutes up to 6 hours - 6 hours and 30 minutes from the beginning of mating and disappears after the first egg has been laid.

Histochemical stainings demonstrated that both the bulb secretion and the plug inside the uterus possess the same staining properties, viz.:i) they stain with Sudan III and Sudan Black. ii) they reduce and osmium tetroxide solution, acquiring a deep dark coloring. iii) the material can be extracted and staining prevented when the material is treated with fat-dissolving solutions. iv) PAS staining is not positive. The foregoing findings further substantiate the assumption that the plug which is found inside the uterus after mating is formed by the secretion produced by the ejaculatory bulb. As to the nature of such a secretion, it may be assumed to consist mostly of fatty material; in point of fact, in view of the viscosity and compactness of the secretion, the latter may be presumed to be of a waxy nature. As to the functional interpretation of the plug, its homegeneousness and compactness would suggest a mechanical kind of function in the first place. If the plug were formed at the end of the mating, after the sperms have been introduced, the most obvious supposition would be that of the plug acting as an obstacle to the outflow of the sperms. As, however, it is found before sperms are introduced, its function is likely to be that of a factor favoring the travel of the sperms from the vagina to the spermathecae and to the seminal receptable. The fact should be remembered that Drosophila sperms are very long cells endowed with a spiral motion. A likely assumption is that the waxy plug works as a central axis which aids the sperm progress, forcing the sperms to swim between the surface of the plug and the walls of the uterus. Besides, by causing the uterus to dilate, the plug helps the sperms to reach the opening of the storage organs. The foregoing hypothesis is backed by observations performed with electron microscopy on uteri of females that had been separated 10 minutes after the beginning of mating. The electron microscope pictures demonstrated that bundles of sperms were located between the uterus walls and a homogeneous granular mass which fills the central portion of the uterus cavity. As far as the chemical function of the plug is concerned, no data are available at present that may either substantiate or rule out the

possibility of its containing such substances as may increase sperm motility or affect some unknown activity either of the sperms or of the female reproductive organs.

At any rate, the waxy plug may be regarded as a fertility factor. As a decrease in ejaculatory bulb secretion has been observed following repeated matings (1), variations in fertility rates may be caused not only by a reduction in accessory gland secretion (2) but also by inadequate activity of the ejaculatory bulb.

Finally, it must be definitely said that, on the strength of all the findings reported, the plug which is found in the uterus after a mating has simply nothing to do with the fluid secretion which Patterson (3) and other workers have reportedly noticed inside the genital duct of the Drosophila genus as a reaction to insemination. The fact must not be overlooked, indeed, that the plug is present after 5 to 8 minutes since mating beings, before any sperm is present and before any reaction is exhibited by the female genital duct's mucosa - and, more important still, the fact should be remembered that the plug is formed by the ejaculatory bulb secretion. This does not mean that a reaction to insemination may not occur, as noted particularly with interspecies matings, but merely that the waxy plug should not be regarded as the product of such a reaction. At this stage, two different assumptions should be investigated: either the waxy plug is the only material contained within the female genital duct of D. melanogaster besides the sperm after mating, or, together with it, the duct also contains the fluid secretion produced by reaction to insemination. Should the first hypothesis be verified, the plug and fluid secretion would be one and the same thing, and the actual existence of a secretory activity primed by insemination would then call for further investigation. As reaction to insemination is generally regarded as an effective selection mechanism in interspecies matings in the Drosophila genus, the finding we have just reported would seem to acquire a general biological and genetical significance as well as to warrant further, more systematic, investigations.

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Gateff, E. and H. A. Schneiderman. Case Western Reserve University, Cleveland, Ohio. Long term preservation of imaginal disc cell lines at low temperature.

When lines of imaginal disc cells with novel developmental capacities arise in the course of in vivo culture (Hadorn, 1965) one wants to maintain them for further study. To do this involves repeated subculturing in adults at intervals of one or two weeks. The time intervals can be lengthened to a month by implanting the tissue fragments into adults of D. virilis which are larger. But as more and more novel lines arise the investigator is forced to destroy certain lines because of the difficulties of keeping them continuously subcultured. We have modified a preservation technique originally designed to preserve bacterial cultures at low temperatures (Bouroncle, 1965).

The preservsng medium is a solution of 75% Drosophila Ringer's, 15% calf serum and 10% dimethylsulfoxide. One ml. of this solution is placed in a sterile ampoule. The adult abdomen containing the fragment of tissue to be preserved is separated from the thorax and cut open at the posterior tip. This leaves the abdomen open at both ends. The abdomen containing the imaginal disc fragment is placed in the vial which is then sealed in a flame and placed in a dry-ice-acetone bath at -80°C and then into a -78°C deepfreeze.

When the tissues are needed, the ampoule is thawed in a 40°C waterbath and then cut open. The abdomen is washed three times in Ringer's and the implanted tissues may now be used. These frozen tissues retain the capacity to grow when cultured in adult abdomens and to differentiate when implanted into larvae. The longest time tissues were kept at low temperatures was three and one-half months. When thawed, both the frozen implanted tissues and the organs of the frozen adult host abdomens appeared normal.

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