SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE EFFECT OF ULTRA-VIOLET IN PRODUCING FUSION OF EGGS OF CHAETOPTERUS

DURING the summer of 1926 at the Marine Biological Laboratory, Woods Hole, Massachusetts, while repeating some experiments on the effects of ultra-violet radiation on eggs of *Chaetopterus* before insemination, in order to preserve series of them for cytological study, I noticed during the cleavage stages a tendency of the eggs to fuse. Such fused eggs developed as far as the trochophore stage, as many as fifteen eggs frequently taking part in the fusion. This result is best obtained by an exposure of sixty to seventy seconds, the eggs being 21.5 cm distant from a Cooper-Hewitt mercury vapor arc lamp. Since the effect of ultra-violet radiation at lower exposures, namely, thirty, forty and fifty seconds, produces profound alterations of the cortex, as I shall show in a forthcoming paper, I assume that the fusion due to longer exposures is likewise attributable to changes in the cortex. For example, in observations made on eggs inseminated after radiation, on August 12, I found the next day innumerable fused eggs. Some fused masses were made up of twelve to fifteen eggs. Subsequently, it was learned that fusion is more readily brought about in dishes containing closely crowded eggs, and in dishes with few eggs in which the eggs are brought into close proximity. The fusion therefore, is primarily the result of radiation, and not of overcrowding, since of two equal lots of eggs from the same female—one lot inseminated with and one without previous exposure to the ultra-violet rays and suspended in equal volumes of sea-water—fusion takes place only in the exposed lot. Eggs centrifuged before insemination, that show while living a gray cap which after fixation with solutions containing osmic acid proves to be a disk of oil drops, behave in the same way.

Ultra-violet radiation has another interesting effect on eggs of *Chaetopterus*. In the swimming stage, the single trochophores show the apical tuft of long cilia displaced sometimes as much as 90°. In the majority of cases, however, this displacement amounts to about 75°. This result seems to indicate a change in the original organization of the egg. While it is true that ultra-violet radiation induces some eggs to differentiate without cleavage, the types here described show fairly normal cleavage, especially after the shorter exposures.

I might point out also that at times normal fertilized eggs of *Chaetopterus* used during these observations failed to form a yolk lobe. This is invariably a sign that the eggs are not in optimum condition.

DEPARTMENT OF ZOOLOGY, HOWARD UNIVERSITY
E. E. JUST

A SIMPLE METHOD FOR EXPERIMENTAL PARTHENOGENESIS

HYPERTONIC sea-water is undoubtedly the simplest method for the experimental initiation of development. Hypertonicity is brought about by the addition of concentrated salt solution to the sea-water. The salts most commonly used are sodium, potassium and magnesium chloride. In practice, one makes up a 2.5 M solution of the first or second, or a 1.25 M solution of the third, varying proportions of the molar solution and sea-water being employed.

A very simple method for experimental parthenogenesis is as follows. To clean filtered sea-water, crystals of Na_2SO_4 are added to excess. After the supernatant sea-water has become perfectly clear, it is decanted. Eggs exposed to this sea-water plus Na_2SO_4 for thirty to sixty minutes show a high per cent. of cleavage and plutei on return to normal sea-water. While in the solution, the eggs show separated membranes. A nicer method is to take the supernatant sea-water after the addition of the Na_2SO_4 in excess and add it to sea-water in varying proportions. For example, one may set up dishes in a series, the first of which contains the Na_2SO_4 sea-water solution alone; the second, nine parts of the solution plus one part of normal sea-water; the third, eight and two parts respectively; the fourth seven and three, etc. To each dish equal amounts of eggs from the same female are added. Eggs are then transferred at fifteen-minute intervals during a period of two hours. In this way one may take into account any variations in response of eggs during a given season to exposure to hypertonic sea-water.

E. E. JUST

THE AMOUNT OF OSMIC ACID IN FIXING SOLUTIONS NECESSARY TO BLACKEN FAT

FIXING agents containing osmic acid are generally conceded to be the best for cytoplasmic fixations. There are several difficulties that militate against successful results with such agents. Preparations which the worker oftentimes describes as "over-osmicated" stain very poorly with Heidenhain's iron hematoxylin. Since this stain is so generally employed by cytologists fixatives containing osmic acid are less frequently used than others. Bouin's, for example, is many times preferred. Some of the difficulties in the way of successful results with osmic acid in combination with

other chemicals may be removed. One of the chief obstacles to success is the amount of osmic acid employed.

In the first place, most of the osmic acid on the market is of inferior grade and, what is perhaps just as important, not always of the weight labeled. Best results are obtained with the best quality of the acid. If a given tube supposed to contain one gram of osmic acid actually contains more, then what the worker makes up as a 2 per cent. solution is obviously of greater strength. Working with some of these inferior grades of osmic acid, tubes of which frequently contain more than one gram, I have found that I could get very satisfactory results by using as little as 0.75 cc of osmic acid in Fleming or Meves solution. Indeed, in some cases, the amount of acid used was less than half this amount. The cytoplasm is very well fixed, and the staining with iron hematoxylin leaves little to be desired. Such small amounts of osmic acid have been successfully employed in the fixation of most diverse types of cells. The criterion for the amount of osmic acid necessary for good cytoplasmic fixations is that which will blacken the oil drops in the centrifuged uninseminated egg of *Arbacia*. One need simply to prepare a solution of 1 gram of the acid in 50 cc of distilled water, then take 4 or 3.5 cc of this with chromic and acetic acids as used in Fleming or Meves solution, and prepare also other solutions using instead 2, 1.5 and 0.75 cc of osmic acid. Eggs after centrifuging are placed in these solutions for thirty to sixty minutes, after which they are washed in several changes of tap or distilled water and examined under the microscope. If the disk of oil drops, the so-called gray cap in the living egg, is blackened in that solution containing the least amount of osmic acid, this constitutes sufficient evidence that enough of the acid has been used. After the use of these solutions containing small amount of osmic acid, the cells are perfectly preserved, both as regards nuclear and cytoplasmic structures. After iron hematoxylin, the cytoplasm is a clear pale blue, mitochondria are stained dark blue and the chromosomes after breakdown of the nucleus are stained black. Results with this method are infinitely superior to those obtained by the so-called weaker Fleming. That is to say, in my experience at least, it is better to alter the amount of osmic acid alone, leaving the chromic and acetic acids in the proportions originally given by Fleming and Meves.

E. E. JUST

SPECIAL ARTICLES

VARIATION IN THE PERCENTAGE OF PROTEIN IN THE GRAIN OF A SINGLE WHEAT PLANT

THAT a plant may vary markedly in composition is common knowledge to all engaged in plant investigations. Causes and significance of such variations, particularly of the percentage of given proteins in wheat, have been the subject of much study, because of the relation this property of grain has to the quality of bread. As information was desired on the probable range of variation in the percentages of protein in the grain of single wheat plants, experiments were performed that were designed to obtain marked differences in this character of wheat. Some of the data which were obtained are given in the following table. The values given are those of the percentages of protein in the grain of different heads on the same plant. They were chosen as representatives of the lowest and the highest protein grain grown on an individual plant, but are not to be considered as the lowest or highest values that possibly could have been obtained.

TABLE I

VARIATION IN THE PERCENTAGE OF PROTEIN IN THE GRAIN OF DIFFERENT STALKS OF THE SAME WHEAT PLANT

Variety	Percentage of protein		Difference	
	Low	High	Actual	Percentage
Bunyip	13.6	17.6	4.0	30
Cedar	12.3	18.6	6.3	57
Dart's Imperial	10.9	11.1	.2	2
Early Baart	10.4	12.2	1.8	17
Fulcaster	8.2	11.4	3.2	39
Hard Federation	11.8	17.3	6.5	55
Sonora	6.4	14.0	7.6	119
White Australian	10.2	13.0	2.8	27

Variation in the percentage of protein in wheat is directly related to that of the supply of nitrogen available to the plants at different growth periods—the later in growth a given supply is absorbed the higher the protein content of the grain. Thus an essential feature of the experiment was that of providing conditions whereby a given supply of nitrogen would become available late in the growing period of some stalks and early in case of others. This required that each plant have two distinct crops of stalks, one that arose early in its life and the other late. The requirement was obtained by planting seed in soil deficient in nitrogen in order to restrict stalk formation of the early growth period to one culm per plant. But later in growth—ninety days after planting—an ap-