the part on which it is to rest is given a thin uniform coating of melted beeswax, then the new part is added, pressed down and held in position for a short time until the wax hardens. After the model is thus built up the exposed surfaces are covered with a thin coating of wax, which on hardening smooths the surface and tends to prevent injury from excess moisture.

One objection to reconstructions made entirely of wax is that they are very apt to become distorted when exposed to the variable temperature of an ordinary room. Models constructed as described above are not so easily affected, since the beeswax between the strawboard sections is protected. Such models are also lighter, easier to handle, and not so liable to injury when packed and shipped as are the wax ones. In this respect they differ little from those made of blotting paper. The greatest advantage over both the wax and blotting paper methods is the rapidity with which a reconstruction can be made, without the sacrifice of accuracy. The fact that the blanks are easily preserved makes possible their use at a later time in checking over the details of the model, which often is of great assistance.

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## INEXPENSIVE LANTERN SLIDES FOR TEXT OR TABULAR DATA

IT is often desirable to project text, mathematical formula or tabular data upon a screen for purposes of class instruction or during the presentation of a paper at a scientific meeting. Use of a lantern saves the time of the instructor which would be necessary to inscribe the data on a blackboard and also makes for greater accuracy and legibility. The obvious drawback to the use of the lantern lies in the cost of photographed lantern slides.

I have found that very satisfactory slides may be prepared by making use of the ordinary "Dermatype" stencil sheets such as are used for mimeograph work. The data are cut on this stencil by the bare type of a typewriter so as to occupy an area on the stencil measuring approximately 7 x 8.5 cm. By the use of elite type, rather extensive tables can be printed within this space. A portion of the sheet, with the data in the center, measuring 8.5 x 10 cm, is then cut from the stencil by a sharp knife or a safety razor blade and mounted between two thin sheets of glass of the same dimension. Black paper tape is later used to bind the edges of the glass.

Such a lantern slide projects light figures or letters on a light blue background, the data being plainly visible even in a fairly well-lighted room.

Such lantern slides are permanent and can be prepared at a cost for materials of approximately five cents each.

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# SPECIAL ARTICLES

## SPECIFICITY IN FERTILIZATION

THE results of experiments that I completed while working as a research associate of the Carnegie Institution of Washington, at the Misaki Marine Biological Station of Tokyo Imperial University, add to our knowledge of the factors controlling specificity in fertilization.

The methods devised made it possible to eliminate cortical block to activation in every cross-activation attempted, and to obtain better than 90 per cent. early development in all cross-activated material. This percentage of cross-activation is unusually high, but the especial significance of the results lies in the fact that of thirteen cross-activations made, eight were from four pairs of reciprocal crosses. It is well known that the facility with which a cross-activation is made in one direction is no indication of the degree of success which may be anticipated with the reciprocal cross.

All the work was done with echinoids. The preliminary studies of species fertilization revealed the fact that the eggs of *Heliocidaris tuberculata* did not form a separated fertilization membrane, and that the eggs of *Temnopleurus toreumaticus*, shortly after insemination, contracted strongly within the fertilization membrane, not to resume their normal rounded form until just prior to the first division.

We think of developmental reactions in terms of eggs. Ordinarily, we endeavor to give the egg such treatment that it will follow its own normal course of development. These experiments followed a different plan. I decided to inseminate the *Heliocidaris* egg with *Temnopleurus* sperm and then attempt to make this egg show the reactions that are normally shown by the *Temnopleurus* egg following species activation, namely, to form a separated fertilization membrane and to contract strongly a few minutes after activation.

To state the matter in another way: After some comparative study I took as a working hypothesis the idea that sperms differ in degree, at least, in their effect on the egg. The effect produced by a spermatozoön on the egg of its own species may be called, for convenience, its natural effect. The work done by a spermatozoön in producing the natural effect is an indication of the natural potency of the spermatozoön.

Proceeding on the idea of using sperms as units of an *activating substance,* the plan of supplementing the natural potency of a spermatozoön by increasing or by diminishing its task was tried. A spermatozoön was regarded as having a definite amount of energy, an amount which might be sufficient, or too much, or not enough to produce the effect desired, and which, therefore, may be used as it is, or its task made more difficult or easier, as the occasion demands. In other words, it was decided to grade the spermatozoa to different degrees of activation by indirect means. What really was done was to grade eggs to the natural potency of the spermatozoön.

Seven cross-activations of the egg of *Heliocidaris tuberculata* were made. In no two of these was the necessary treatment of the egg the same. *Heliocidaris* eggs accept species activation readily in unmodified sea water; they must be treated with solutions that are regarded as increasing their permeability before they can be activated by, *e.g., Temnopleurus* sperms; while they must be treated with solutions that are regarded as decreasing their permeability, before they can be activated by, *e.g., Astriclypeus* sperms.

*Heliocidaris* and *Temnopleurus* are both regular echinoids. They belong to different families of the same sub-order. A cross between them is therefore of inter-family width. *Astriclypeus manni* is a Clypeastroid. A cross between *Astriclypeus* and *Heliocidaris* is therefore inter-ordinal.

HELIOCIDARIS ♀ × TEMNOPLEURUS ♂

For this cross-activation the following method was used:

(1) Eggs inseminated in 100 cc sea water + 2 cc 2½ M NaCl, and transferred to

(2) 100 cc sea water + 6 cc 2½ M NaCl. The eggs separated membranes in this solution. Transferred to

(3) 82 cc sea water + 18 cc 2½ M NaCl. This solution produced a strong contraction of all eggs which had formed membranes. Transferred to

(4) Sea water. 90 per cent. of these eggs underwent regular cleavage.

Step (2) was necessary for the removal of internal block to the fusion of the germ nuclei.

HELIOCIDARIS ♀ × ASTRICLYPEUS ♂

For this activation the eggs were washed in sea water and placed in

(1) ⅝ M $CaCl_2$ for 3 minutes; then transferred to

(2) 800 cc sea water + 5 cc n/10 NaOH + *Astriclypeus* sperms; after 3 minutes transferred to

(3) Sea water.

All the eggs formed perfect membranes and segmented regularly. ⅝ M $BaCl_2$ and ⅝ M $SrCl_2$ were used in place of $CaCl_2$ and gave the same results. Segmentation did not take place unless the eggs were transferred to a considerable volume of sea water, as indicated, following the treatment with $CaCl_2$, $BaCl_2$ or $SrCl_2$.

The control in both cases consisted of eggs receiving the respective treatments described above, but with insemination omitted. Without insemination, no membranes were separated and cleavage did not take place. The methods were not interchangeable. *Heliocidaris* eggs were not activable by *Temnopleurus* sperms following the treatment with $CaCl_2$, nor were they activable by *Astriclypeus* sperms following any treatment with NaOH or NaCl, or any combination of these that was tried. On the other hand, *Heliocidaris* eggs which have simply stood in sea water for four hours will, when inseminated with *Astriclypeus* sperms, give as high as 95 per cent. regular segmentation.

These two examples represent the two types of treatment which were found to be necessary in order to obtain cross-activation of the eggs of *Heliocidaris* by the various sperms used. In five of the seven cross-activations of this egg which were made, a preliminary treatment of the egg with NaOH or NaCl or a combination of NaOH and NaCl, a treatment which may be looked upon as increasing the permeability of the egg, was necessary. In the two remaining cross-activations it was necessary to give the egg treatment with solutions of the divalent salts of the alkaline earths, which are regarded as decreasing the permeability of the egg.

Having determined that the sperms used in these activations may be divided into two groups on the egg, it is of interest to find whether this grouping on a physico-chemical basis can be related to any other determinable differences. The only other evident difference is the difference in size of eggs of the different species used. *Astriclypeus* and *Peronella* sperms, the two which form the smaller group, are from species having large eggs. *Peronella* eggs are from .3–.4 mm in diameter. The other group contains sperms from species having eggs that are comparable in size to those of *Arbacia, Strongylocentrotus purpuratus, Lytechinus variegatus,* etc. The idea of natural potency and the relative mass, or the relative surface of the egg to be activated therefore suggests itself. Any discussion of this idea, at this time, would be little more than speculation. There is simply the evidence that *Astriclypeus* and *Peronella* sperms produce too great an increase of permeability when used with small eggs, for activation to be successful.

Six cross-activations of eggs were made with the sperms of *Heliocidaris.* In every instance the only treatment found to be necessary was insemination in sea water to which NaOH or NaOH and NaCl had been added. If this treatment increased the perme-

ability of the eggs, there were differences of degree of increase necessary. *Heliocidaris* sperms seem to be low in ability to increase permeability. The fact that *Heliocidaris* eggs, when species fertilized, form no separated membranes, together with the fact that when *Heliocidaris* sperms are used as cross-activators the eggs must be graded to the natural potency of the sperms by being given increased permeability, support such a conclusion.

The most striking features of the results, when they are viewed as a whole, is their emphasis of the fact of the specificity of the spermatozoön in the activation of the egg of its own species. The idea of the specificity of the spermatozoön in fertilization is one whose truth has been assumed, rather than established. My working hypothesis, that sperms differ in degree, at least, in their effect on the egg, has been established as a fact by the evidence of the experiments. The sperms of each species were found to have a characteristic natural effect. The eggs of different species had to be graded to the natural potency of the sperms.

Somewhat subsidiary to the larger aspect, but closely related to it, is the suggestion as to the nature of sensitization of the egg to activation. Loeb, Robertson, A. R. Moore and others have used $BaCl_2$, $CaCl_2$ and $SrCl_2$ to sentitize sea urchin eggs to activation by blood serum, oöcytin, foreign sperms, etc. Is sensitization more than a process of grading permeability?

At first sight there seems to be lack of harmony between my conclusions and those of Professor Loeb, stated in "The Organism as a Whole," "that the specificity which allows the sperm to enter an egg is a surface effect which can be increased or diminished by an increase or diminution in the concentration of OH as well as of Ca" (p. 77), and "that if the concentration of calcium was increased it was not necessary to add as much NaOH" (p. 74). If we refer to Loeb's fuller consideration of this subject, *Archiv f. Entw-Mech.*, Vol. 40, 1914, p. 318, the matter assumes a different aspect. Loeb found that the addition of calcium led to an increase in the number of eggs which formed membranes, but to a decrease in the number which developed. Insemination following straight NaOH treatment gave 90 per cent. membranes and 60 per cent. development. Insemination following $CaCl_2$ + NaOH treatment gave 100 per cent. membranes and 8 per cent. development. If the eggs were given a preliminary treatment with HCl for the purpose of removing the chorion-like jelly layer of the egg, and then inseminated in sea water + $CaCl_2$ + NaOH, about 80 per cent. membrane formation and development followed (*loc. cit.*, pp. 319–20). The use of HCl introduced a new factor, a factor which is not negligible, which was not considered in its relation to other reactions.

But it is on the basis of comparison of Loeb's observations on the results following insemination after straight NaOH and straight $CaCl_2$ + NaOH treatment, with my own, that the explanation of the differences seems possible. It seems to be a question as to whether the membrane has been formed as a result of activation by a spermatozoön or for another reason. Loeb found that the addition of $CaCl_2$ led to an increase in the number of eggs which formed membranes, but that few of the eggs developed. In my observations *Astriclypeus* eggs, *without insemination,* formed membranes upon treatment with $CaCl_2$; while *Heliocidaris* eggs, treated with $CaCl_2$, formed membranes only after the eggs were inseminated. In one case the membrane was formed by the action of the $CaCl_2$ solution alone, in the other it was the result of activation by a spermatozoön. It is possible that Loeb was dealing with similar material.

This raises the question as to whether development, in the material with which I have been working, was parthenogenetic or was the result of fertilization. The controls showed that development occurred only when the eggs had been inseminated. It is safe to say that activation was effected by sperms. I was able to remove internal block, when it occurred, by secondary treatment with NaCl. In the eggs of some species, it was possible to see the fusion of egg and sperm nuclei; in other eggs such an observation was impossible. The eggs were activated by sperms, but whether true fertilization or false fertilization and a subsequent elimination of paternal chromatin occurred is a question that can be answered only by cytological examination of fixed material.[1]

There is constantly increasing evidence supporting the belief that NaCl increases the permeability of protoplasm, while divalent salts, such as $BaCl_2$, $CaCl_2$ or $SrCl_2$, decrease its permeability. It is therefore logical to conclude that with one group of sperms activation occurred when the permeability of the egg was increased, and with the other when its permeability was decreased.

Permeability to what? Let it be clear that I mean permeability to water, ions and salts, and not penetrability to sperms. Given the intimate contact that is afforded by agglutination, penetrability is understandable. R. S. Lillie, McClendon and others have shown that fertilization is accompanied by an increased permeability of the egg. The present results indicate that one of the factors conditioning the fertilization reaction is a specific degree of permeability.

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[1] The material which the author collected for an investigation of this kind was destroyed in Yokohama on September 1, in the fire following the earthquake.