cells derived from the two distinct species. Between these two extremes there are many intermediate forms. The hybrids arising from the fusion of cells behave very much like sexually produced hybrids, even to the number of chromosomes in the hybrid cells.

DR. CAMPBELL'S studies of the "Embryo-sac of Pandanus" (*Ann. Bot.*, July, 1911) brings out the fact that at the time of fertilization of the egg there may be present as many as 64 antipodal nuclei, in addition to the usual egg apparatus at the micropylar end. This he regards as a primitive condition, or in other words as an older type of embryo-sac which has survived to the present.

THE new botanical periodical *Zeitschrift für Botanik* (Fischer, Jena), now in its third year, is proving to be a useful addition to the already long list of botanical journals. Its editors are Professors Jost (Strassburg), Oltmanns (Freiburg) and Solms-Laubach (Strassburg). Recent numbers contain papers as follows: "Contributions to our Knowledge of the Laminariaceae" (by Killian), "On the Development of Basidia in Uninucleate Mycelium of *Armillaria mellea*" (Kniep), "On the Reduction-division in the Zygotes of *Spirogyra*, and of the Significance of Synapsis" (Tröndle) in addition to many brief reviews, and classified titles of new literature.

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SPECIAL ARTICLES

PHOSPHORUS METABOLISM DURING EARLY CLEAVAGE OF THE ECHINODERM EGG[1]

IN his recent book on artificial parthenogenesis Professor Loeb[2] assumes that during cleavage of the ovum there is a progressive synthesis of nuclear material from constituents of or reserve substances in the cytoplasm. This assumption is based primarily on the observation of Boveri that following each cell-division there is a growth of the daughter-chromosomes in each resultant cell until their mass is approximately equal in each case to that of the original mother-chromosomes; in other words, the mass of nuclear material increases in a geometric ratio as cleavage progresses. The following observations are also brought forth by Loeb in support of this hypothesis of nuclein synthesis: "Miescher found that the lecithin content of the blood of the salmon was relatively high during spermatogenesis and mentioned the possibility that lecithin might furnish a building material for the nucleinic acid of the spermatozoon head. What is therefore true of the building of nucleinic acid in the spermatozoon is assuredly true also for the building of nucleinic acid in the egg. Hoppe-Seyler has mentioned the fact that all young, quickly growing tissues contain relatively large quantities of lecithin. This is especially true in the case of the ovum. The researches of Kossel have shown, on the other hand, that the yolk of hens' eggs contains no preformed nucleinic acid and the same has been shown to be true for the yolk of silkworms' eggs by Tichomirow. Since there is in the egg after fertilization a rapid synthesis of nuclear material at the expense of certain constituents of the protoplasm or of the yolk, since the latter is rich in lecithin which disappears during nuclein synthesis, it is allowable to suppose that lecithin supplies part of the material for the nucleinic acid."

It may be pointed out that this supposed use of lecithin is not consistent with the idea advanced by Overton,[3] Koch[4] and others that this substance among other lipoids plays an important rôle in the limiting membranes of many animal cells. It would seem indeed that as cleavage progresses more rather than

[1] I am indebted to the Commissioner of Fisheries, Hon. Geo. M. Bowers, for the facilities afforded; and to the director of the Beaufort Laboratory, Mr. H. D. Aller, for many personal courtesies extended to me during this work. Dr. E. P. Lyon has aided me greatly with suggestive criticism.

[2] Loeb, "Die chemische Entwicklungs-erregung des tierischen Eies," p. 18 ff. Berlin, 1909.

[3] Höber, "Physikalische Chemie der Zelle und der Gewebe," p. 114. Leipzig, 1902.

[4] Koch, *Zeit. f. Physiol. Chem.*, B. 63, S. 442. 1909.

less lecithin would be required unless one assumes that this substance functions in more than one way in the cell, which is, of course, quite possible. With these several possibilities open it seemed that some method of experimental attack could be used; that if the idea of nuclein synthesis during cleavage were true it should be possible to show as cell-division progresses a diminution of alcohol-soluble phosphorus with a corresponding increase of phosphorized material not digestible by pepsin.

Using the eggs of *Arbacia punctulata,* the phosphorus partition in the 2–4 cell stage was compared with that in early blastulæ. Two separate experiments are reported. The methods used were the same in each; so the only difference between them was that in Experiment II. a smaller mass of eggs was available —though the latter fertilized and developed a trifle better—than in Experiment I. The details follow.

After removal from the ovaries the eggs were washed several times with large quantities of filtered sea-water until they settled rapidly and left no material suspended in the supernatant liquid. They were then suspended in 2,000 c.c. of sea-water and fertilized with a small amount of sperm. The eggs were stirred and oxygen allowed to bubble through the suspension at short intervals. When the first cleavage occurred 1,000 c.c. were measured off and allowed to settle. The remaining eggs were allowed to divide for five hours from the time of fertilization, reaching then the early blastula stage. These eggs were not allowed to develop to the swimming stage because of the probable difficulty of separating them from the surrounding sea-water without causing cytolysis. 1,000 c.c. were taken and from this point were treated in precisely the same manner as the previous mass of eggs at first cleavage.

In each case—first cleavage and early blastulæ—after settling in sea-water, the latter was drawn off, using a centrifuge to concentrate the eggs as much as possible. It may be added here that previous trials had shown the practical impossibility of separating whole or cytolyzed eggs from the surrounding medium by filtration. Even if by special methods clear filtrates were obtained, the process of filtration was so prolonged that the material decomposed though thymol was added as a preservative. Resort was had, therefore, to a small hand centrifuge, which proved very satisfactory in separating the solid materials from the various liquids. Remarkably clear liquids were obtained in a very short time. During the time that undesirable chemical changes might take place the materials were kept on ice.

The eggs, freed now from sea-water as far as possible, were cytolyzed with successive small portions of distilled water (25–50 c.c.) until the combined filtrates in each case equaled 700 c.c. By this time the pigment was practically entirely removed and it was assumed that all water-soluble material had been taken out. The residual material was then extracted with successive 25 c.c. portions of boiling neutral ethyl alcohol (95 per cent. redistilled over NaOH) until the volume, including two final extractions with boiling absolute alcohol, equalled in each case 400 c.c.

Each of the residues after alcoholic extraction was dried at 60° C. and then digested for four days with 150 c.c. of pepsin-hydrochloric (2 grams of pepsin in 1,000 c.c. of .4 per cent. HCl). The peptic digests were then filtered and washed with cold water.

Phosphorus determinations were now made on (*a*) water-soluble material; (*b*) alcohol-soluble material; (*c*) the filtrates and (*d*) the residues from peptic digestion. Controls were also run on the pepsin solution and on the sperm used in each experiment. The material in each case was digested with 10 c.c. of concentrated sulphuric acid. The digestion was finished by the addition of a few drops of fuming nitric acid. The phosphorus was first precipitated by molybdic acid and weighed finally as magnesium pyrophosphate.[5] The data obtained are tabulated below. The figures represent milligrams of magnesium pyrophosphate.

[5] "Methods of Analysis," Bureau of Chemistry Bulletin 107 (revised), p. 2. 1907.

Material Analyzed	Experiment I.		Experiment II.	
	2-4 Cells	Blastulæ	2-4 Cells	Blastulæ
Water-soluble....	107.5	106.4	66.6	65.4
Alcohol-soluble...	18.1	18.6	11.9	12.3
Peptic filtrate.....	17.6	20.5	14.8	16.7
Peptic residue....	9.3	10.0	8.7	7.4

The results on the filtrates from peptic digestion are corrected in each case for the weight of magnesium pyrophosphate (9.0 mg.) in the pepsin solution used. In neither experiment was there any considerable phosphorus in the sperm used; and it was furthermore assumed that the larger part of the latter remained in the supernatant sea-water after the eggs had settled. It was practically impossible, however, to run control determinations on the supernatant sea-water because the large amount of salts in each case—over 30 grams —made it exceedingly difficult to carry out the preliminary digestion with sulphuric acid.

It will be seen that the relative phosphorus partition runs parallel in the two experiments; that there is not a significant difference in alcohol-soluble (lecithin?) phosphorus or phosphorus in the peptic residues (nuclein?) between the 2–4 cell stage and that of the early blastula. It seems justifiable, therefore, to conclude that *under the present experimental conditions there is during early cleavage of the echinoderm egg no evidence of a chemical synthesis of nuclear material from alcohol-soluble substances in the cytoplasm.*

These results, it is emphasized, are referable only to segmentations in an holoblastic egg prior to the possible ingestion of nuclein-containing food material from the sea-water. As to just what substances aid in the undoubted increase of total mass of morphologic nuclear substance—not to be confused with the specific substance, nuclein, which, as has just been shown, does not increase—as cleavage proceeds, there may here be mentioned only the possibility that the phenomena of streaming cause a morphologic or mechanical aggregate of chromatin-like material originally in the cytoplasm with nuclear chromatin during or following mitosis. A number of investigations on the distributed nucleus, quoted by Professor E. B. Wilson,[6] form an interesting commentary on the possibility just mentioned: "Balbiani, Gruber, Maupas and others have described various Infusoria (*Urostyla, Trachelocerca, Holosticha, Uroleptus*), as well as some rhizopods (*Pelomyxa*), in which the body contains very numerous minute chromatin granules of 'nuclei,' which Gruber showed to multiply by division. Balbiani long since showed that in *Urostyla* these bodies become concentrated toward the center of the cell at the time of division and Bergh demonstrated that they then fuse to form a macro-nucleus of the usual type that elongates, assumes a fibrillar structure and divides by fission. After division of the cell-body the macronucleus again fragments into minute, scattered granules, which in this case certainly represent a distributed nucleus. In the flagellate *Tetramitus* Calkins likewise finds numerous scattered chromatin granules, which at the time of division become aggregated into a single dividing mass; while in other forms the mass (nucleus) persists as such without (*Trachelomonas, Lagenella, Chilomonas*) or with (*Euglena, Synura*) a surrounding membrane."

Of significance also in this connection is Tennent's[7] observation that in eggs of *Arbacia* fertilized with *Moira* sperm when "the daughter nuclei are in the resting condition succeeding the first division, the cytoplasm contains many deeply staining rods. The nucleus at this time does not take the chromatin stain and appears like an empty vesicular structure. In eggs, of the same lot and on the same slides, in which the fibers of the second amphiaster have begun to form, the nucleus again takes the stain and shows the chromatic net, while the cytoplasm is seen to be free from the bodies described."

It seems possible to attack this problem of nuclein synthesis from another angle, namely, by comparing the ratio of purin nitrogen to

[6] Wilson, "The Cell in Development and Inheritance," p. 40. New York, 1902.

[7] Tennent, *Biological Bulletin*, Vol. 15, p. 127. 1908.

total nitrogen in eggs after first cleavage with that at the early blastula stage. Considerable material has been collected for further work along this line.

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Beaufort, N. C.,
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Observations on the Inheritance of Characters in Zea Mays Linn.[1]

In "Red Cuzco" and some other breeds of red maize, the red coloring matter is confined to the pericarp; being therefore a fruit character and not a seed character, it does not appear in the ear immediately resulting from a direct cross between a white female and a red male.

In a red dent breed under investigation, the red pigment occurs in the aleurone layer, and not in the pericarp. Being a seed-character, it is transmitted directly by the pollen grain to the ovule of a white breed. It behaves as a dominant to whiteness; where it meets yellowness in the same grain, it is more conspicuous than yellow. The writer has not met with a previous record of the occurrence of a red pigment of this character in the aleurone layer of the maize grain.

When this red dent is crossed with a white sugar breed the segregation, in the second generation, of the two pairs of characters redness *vs.* whiteness and starchiness *vs.* sugariness, is in approximately the following proportions:

Red	starchy	56.25	= 75%
	sugary	18.75	
White	starchy	18.75	= 25%
	sugary	6.25	

In other words:

Red Grains

Starchy grains	75%	of 75%	= 56.25%
Sugary grains	25%	of 75%	= 18.75%
			75.00%

White Grains

Starchy grains	75%	of 25%	= 18.75%
Sugary grains	25%	of 25%	= 6.25%
			25.00%

A single grain has been found on the ear studied, which distinctly shows the starchy character in one half and the sugar character in the other, a very unusual feature.

A study of row-numbers in maize-ears indicates that within certain limits the number of rows of grain on an ear is subject to fluctuating variation, which may perhaps be affected by season or food supply, or both. In more than thirty plants of Arcadia sugar-maize studied this year, each of which produced two ears on one stalk, the uppermost ear has had a different number of rows from that of the lower ear. On thirteen plants the largest number of rows occurred on the lower ear, while on eight plants the largest number was on the upper ear. In twelve plants of two ears the row-numbers were the same on both; in one case there were four more rows on one ear than on the other. Several plants of Hickory King, bearing two ears, have also produced different numbers of rows on the two ears.

The range of variation appears to be limited, however. A normally 8-rowed type ranges between 4 and 14 rows, while a normally 18-rowed type ranges between 12 and 24 rows.

The result of crossing an 8-row with an 18-row type of maize is to produce an intermediate type in the first generation, both 8-row and 18-row types practically disappear in the heterozygous form. The intermediate type bears mostly 10, 12 or 14 rows, the 12-row type greatly predominating. The experiment will be continued next year, to determine the proportion of the 8-row and 18-row types which reappear. The ears produced by the cross and the reciprocal cross are indistinguishable.

A white-cobbed breed crossed with a red-cobbed produces a red cob in the first filial generation, and so does the reciprocal cross.

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[1] Fuller details will shortly appear in the *Transactions of the Royal Society of South Africa.*