THIS book, while professing by title to deal with the general question of fertilization and parthenogenesis, is concerned with a greater field. It might better perhaps be spoken of as a critique of the elementary phenomena of development, notably the events of fertilization and first cleavage. The author is personally familiar with this subject, having done a great deal of original work in the field himself. In spite of this the presentation of the material and the interpretation given by the various investigators is to a large degree unprejudiced by the author's own researches.

While one might expect that the early chapters would be devoted primarily to the interpretation of the phenomena of the fertilization, the physiology of the gametes, et cetera, the author considers first what he terms the physiology of segmentation. A clear presentation follows not only of the numerous theories of cell division but also of the initial period in cell division which precedes first cleavage and follows fertilization itself. In this part of the book, as in the chapters which follow, the author deals with not only the evidence derived from physical and chemical methods of investigation, but also that from cytology. Here, as has been mentioned above, the author's first-hand knowledge of the material makes the review decidedly valuable.

A special chapter is devoted to the events which take place between the time of activation and segmentation. Here Dalcq considers not only the events of normal fertilization, viz., the cortical and internal changes in the egg, the migration of the pronuclei, etc., but also the questions of polyspermy and parthenogenesis. The author seems to distinguish between fertilization and activation, a consideration of which follows.

One can not but feel that the chapter on activation is too brief, though some of the discussions which might be looked for here appear in the final chapter of the book. The author presents here a table which contains a summary of the agents and forms used in experiments on artificial parthenogenesis, which is very useful. The cytological pictures presented accompanying and following activation are treated in a separate chapter, as are also what the author terms the purely physiological results of activation. In one of the later chapters of the book which is devoted to the maturation of the gametes, Professor Dalcq has attempted the correlation of the cytological data with the evidence obtained by other methods. The author concludes the book with a chapter on the meaning of activation, in which he gives briefly the various theories which have been presented to explain the phenomena of fertilization.

Not since the publication of "The Problems of Fertilization" by F. R. Lillie has there appeared a book devoted to this subject. The actual space in the present volume concerned with the fertilization question is not as great as the title would lead one to expect. This fault is, I believe, a fortunate one, for while a great deal has been done on the fertilization reaction in the interim, the phenomena involved are so intimately connected with the events preparatory to the first cleavage that the two are difficult to separate and find themselves easily associated in the same book. The analysis of the material is fairly complete, though some aspects have been treated in a rather brief manner.

Unfortunately, a few errors in the names of scientific investigators (which it is to be hoped will be eliminated in the next edition), have crept into both text and bibliography.

The book has a great many figures and tables, some of the latter being taken from original works while others have been compiled in a most useful way by the author. The book has appended a bibliographical list of some thousand titles. Unfortunately, as is the case with other volumes of this series, there is no index.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE DETERMINATION OF NITRATES AND NITROGEN

IN determining nitrates in highly organic soils by the ordinary method of shaking with some material such as cupric hydroxide, which takes out the color by absorption and occlusion, considerable variation occurred in checks, probably due to interfering substances and the occlusion of nitrates. In determining total nitrogen a fresh sample was required. It was also found that the regular Kjeldahl method was burdensome where the digestion was excessively long, as is the case on most samples high in organic matter. This is especially true of samples of plant tissues.

The idea of distilling off the nitrate nitrogen from a concentrated sulfuric acid solution into an oxidizing solution and catching the other nitrogen as ammonium sulfate, which then could be oxidized to nitric acid and distilled off suggested itself. In order to do this it is necessary to destroy the organic matter and keep the nitrogen oxidized to the high valency state without decomposition during distillation. Chloric acid

is very vigorous in destroying organic matter and sodium chlorate was chosen as the oxidizing agent. As a result of tests on several entirely different samples it was found that the nitrates did not vary greatly between checks and ran slightly higher than by the ordinary method and that the total nitrogen checked well with the regular Kjeldahl method, taking much less time. At the same time a clear solution of the inorganic elements, contaminated only by sodium sulfate and sulfuric acid, was obtained, the hydrochloric acid formed being decomposed to free chlorine and driven off.

PROCEDURE FOR NITRATE NITROGEN

The sample should be air dry and ground to pass at least a twenty-mesh sieve. Place one gram of plant or animal tissue or ten grams of mineral soils into a dry five hundred cc Kjeldahl flask. Add twenty-five cc of cold 75 per cent. sulfuric acid and connect to an ordinary glass-water-cooled condenser by means of a glass-tube connection so that some air cooling takes place. Extend the tube several inches through the rubber stoppers used, in order to prevent fumes from readily acting on the rubber.

Catch the distillate in a five hundred cc Erlenmeyer flask containing fifty cc of a chlorine dioxide solution. This is prepared by bubbling chlorine dioxide from a generator into water until the water takes on a bright yellow color and green fumes form above the surface. The chlorine dioxide is generated by dropping concentrated hydrochloric acid on about fifteen to twenty grams of potassium chlorate in twenty-five cc cold water. The chlorine dioxide should pass through glass as it oxidizes rubber. The generator must be kept cold and not too much hydrochloric acid added at once. Warming to 70° or above may cause an explosion. With ordinary amounts of hydrochloric acid, however, the explosion usually does no harm.

The gases given off from the digestion mixture are made to bubble through the chlorine dioxide solution by means of a bent glass rod connected to the condenser and inserted in the yellow liquid in the tilted Erlenmeyer. At the same time chlorine dioxide gas is bubbled through the solution by means of another bent glass rod from the generator. The chlorine dioxide should be bubbled in fast enough to keep the solution yellow and greenish fumes in the air above the solution.

Gradually heat the sample, regulating the flame so that too rapid bubbling does not occur. Care should be taken not to let the solution draw back by suddenly lowering or withdrawing the flame. Distil until all water is over and the sides of the Kjeldahl begin to be washed down by the condensing of sulfuric acid fumes (five to fifteen minutes). Disconnect the flask. Set aside the residue to cool for the determination of nitrogen other than nitrate nitrogen. Wash out the condenser several times with a wash bottle into the distillate.

Boil the distillate until it has remained colorless a few minutes. Add a bit of litmus and just neutralize with strong sodium hydroxide. Push the litmus, which should be blue, to the top of the flask with a glass rod. Hold the litmus with the rod and wash them both with water. Remove the litmus. Evaporate the solution to dryness, avoiding spattering. Determine nitrates by the regular phenoldisulfonic method, using five to ten cc of the acid and sodium hydroxide as the base.

DETERMINATION OF AMMONIA, AMINO AND COMPLEX ORGANIC NITROGEN

To the original black digested solution of the sample which is now cool, add twenty-five cc water. Cool to a point where the hand can be held on the flask without discomfort. Add five grams of fine potassium chlorate or sodium chlorate if potassium is to be determined. Leave standing until the solution is a clear red and most of the foaming has ceased. Gentle heating with shaking may be necessary to start the reaction, but do not heat above 70° C. The sample is usually ready to distill in five to fifteen minutes. The sample may be left standing over night, which results in almost complete oxidation before distillation. Wash down the sides with fifty cc water and connect to the condenser arrangement as set up for nitrates. Too rapid heating with much excess chlorate may cause an explosion, but as a rule very little of the chlorate is left and in the dilute solution the explosion is harmless.

As soon as green fumes cease, distil vigorously into an Erlenmeyer. If the distillate tends to get colorless and does not remain a strong yellow color, bubble chlorine dioxide gas in the solution as in the nitrate determination. Continue the distillation until the water is distilled off and a nearly colorless sulfuric acid solution is left. This is told by the rise in boiling point and the condensing back of acid on the neck of the flask. Disconnect the digestion flask and wash out the condenser into the Erlenmeyer flask. Treat just as was done with the distillate of nitrate nitrogen. If much nitrogen is expected an aliquot should be taken or the original sample made quite small.

The digestion residue is now colorless and contains the other inorganic elements. By dilution and nearly complete neutralization of the sulfuric acid with sodium hydroxide, a clear, almost neutral, solution of the remaining inorganic elements will be obtained and aliquots may be taken for the determination of the elements desired by the standard methods in clear

solution, with the exception of sodium, the halogens and sulfur.

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THE NEPHELOMETER IN MYCOLOGY

THE use of the nephelometer in the biological sciences is not new. It has been used to measure the growth of bacteria in nutrient solutions and has been adapted to the determination of protein, using sulfosalicylic acid as a precipitating agent. A method of determining nitrogen as ammonia, using a modified Nessler's reagent, has been worked out for extremely dilute solutions of ammonia or ammonium salts.

It occurred to the writer that a water culture of a fungus such as oidium could be determined by this method, provided the hyphae were broken up sufficiently. A culture of oidium in a dilute nutrient solution was shaken up in a shaking machine for various lengths of time and with the addition of different acids and bases. Attempts to use glass beads and clean sand as aids in the breaking up process failed because of the colloidal silica formed by the rubbing together of the particles.

Best results were obtained by using a glass-stoppered, wide-mouthed bottle approximately four times as high as the diameter and fitted inside with a cone-shaped roll of nichrome gauze. This cone should be tall enough to have the tip pressed upon by the glass stopper and thus prevent its shaking about. The bottle should be not over one third or one fourth full of the culture and should be shaken rapidly for at least twenty minutes. The culture being dashed back and forth through the wire gauze at an angle is torn to pieces small enough to stay in suspension for a considerable time. The culture should be neutral as even a slightly acid solution attacks the wire under these conditions and a colored solution results which is useless for a nephelometric determination. It may be found desirable to remove the gauze cone and add sufficient concentrated sulfuric acid to make a 10 per cent. solution and again shake for five minutes. The increased specific gravity of such a solution will help to keep the particles from settling out. Every effort must be made to keep any turbidity other than that due to the fungus from developing. If any calcium was used in the nutrient solution the addition of sulfuric acid will cause a very objectionable precipitate. In any case a part of the sample should be filtered and the filtrate run as a blank.

If it is merely desired to compare various solutions, one of them may be used as a standard, or a suitable standard can be made by adding one drop of saturated silver sulfate solution to 200 cc of distilled water and adding just enough dilute HCl to completely precipitate the silver. This solution darkens after a time but is good for several readings.

By observing the precaution necessary to successful determinations with a nephelometer the writer has found that the readings are proportional to the amount of growth present in the solution.

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AN ACCURATE METHOD OF TAKING READINGS ON EXCEEDINGLY LOW ROCK DIPS

IN regions of nearly horizontal bedded rocks it is practically impossible to make accurate dip readings with a Brunton compass where the dip is less than fifty feet per mile. It is however often essential in deciphering low structures to get dependable results quickly in the field. The procedure described below was found to meet this requirement very satisfactorily.

Tie a long nail on one end of 100 feet of fish-line. Drive this nail into the bed of rock of which the dip is to be read. Stretch the fish-line across the rock face. Usually a 50-foot exposure or a 25-foot exposure can be had. Such lengths facilitate calculation. Mark the fish-line at 25, 50 and 75-foot intervals by tying on pieces of red yarn or silk thread. Have a small line-level such as is commonly used by carpenters placed over the center of the line and raise the line until the bubble reads level. Then with a carpenter's 6-foot folding rule, note the dip and make the calculation.

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SPECIAL ARTICLES

THE ACTION OF GLUTATHIONE AND HEMOGLOBIN ON THE GROWTH OF FIBROBLASTS IN VITRO

FOR some time it has been known that fibroblasts and epithelial cells proliferate indefinitely in vitro in a medium composed of equal parts of plasma and embryo juice.[1] If the factors responsible for the unlimited growth of tissues in such a medium could be

[1] A. Carrel, "Artificial Activation of the Growth in Vitro of Connective Tissue," *J. Exp. Med.*, 17: 14, 1913; "Tissue Culture and Cell Physiology," *Physiological Review*, 4: 1, 1924; "Les Milieux Nutritifs et leur Mode d'Emploi dans la Culture des Tissues," *C. R. de la Soc. de Biol.*, 96: 603, 1927; Fisher, A., "A Three Months Old Strain of Epithelium," *J. Exp. Med.*, 35: 367, 1922; A. Carrel and A. H. Ebeling, "The Multiplication of Fibroblasts in Vitro," *J. Exp. Med.*, 34: 317, 1921; "Survival and Growth of Fibroblasts in Vitro," *J. Exp. Med.*, 38: 487, 1923.