Research Notes.

Experiments on the Development of the Chick and Duck Embryos cultivated *in Vitro*.

THE latest contribution to our knowledge of the potentialities of an organizer-region of the blastoderm of chick is a paper by C. H. Waddington[1] who during the course of his studies has reached most interesting conclusions as regards the rôle played by the primitive gut, primitive streak and the influence on each other of the germinal layers. The previous workers who adopted the method of isolation, were mainly concerned with the study of self-differentiation of tissues of Fowl embryo which they transplanted to the chorio-allantoic membrane and also by cultivating them *in vitro*. Waddington used the latter method in his study of the developmental mechanics of the early chick and duck blastoderm, especially during the stages between the laying of the egg and the appearance of the head process. The first set of experiments deals with the normal *in vitro* development of the chick and duck and the determination of the location of the organ-forming materials in the early stages of the blastoderm. The explanted embryo of the chick which remains alive *in vitro* for 2 or 3 days, reaches the critical period when the circulatory-organs appear and possibly due to a maladjustment of the beating of the heart and the blood formation in the area vasculosa, the embryo dies. The rates of growth and of differentiation are always slower *in vitro* than *in vivo* and the difference, according to Waddington, is nearly represented by the ratio 1 : 1.5. In his studies on the differentiation of portions of blastoderm, he shows that if a cut is made through the primitive pit or just in front of it, before the appearance of the head process, no embryonic organs are developed in the anterior regions, except a small quantity of neural tissue; if, however, the cut is made just behind the primitive pit, a head and also a thin tail are formed, the latter bearing a medullary plate, somites and a notochord. In the first experiment, chiefly where the blastoderm is cut through the primitive pit, the half behind the cut develops all the axial structures except part of the head. If the embryo is cut a little behind, and well behind as far back as the middle of the primitive streak, it is noticed

[1] *Phil. Trans. Roy. Soc.*, Series B, 221, 1932.

from Waddington's data, that while in the former case, at least medullary folds arise, in the latter there is no trace of embryonic organs. These experiments of Waddington do not confirm the results of authors like T. E. Hunt, B. H. Willier and M. E. Rawles who in employing the chorio-allantoic technique, found that the hinder region of the primitive streak is not capable of self-differentiation in the absence of Hensen's node. In the second set of experiments designed to test the organizing influence of endoderm in the development of the embryo there were two distinct lines of investigations. Isolated endoderm and epiblast were cultivated for purpose of determining the rôle they played in organ formation. If the endoderm is removed in middle or late primitive streak stages of blastoderms the explanted epiblast is found to possess the power of differentiating a neural plate, somites and notochord though somewhat disturbed from their normal relations. The formation of the notochord, which is usually referred to as endodermal in origin, is to be accounted for by the results of recent work in Vertebrate Embryology according to which, the notochord material originally resides in the epiblast and later becomes invaginated at the primitive pit. The explanted endoderm has not given any definite results, though it was noticed that the yolk endoderm cells from the margin of the disc grew centripetally so as to cover the entire area below the area pellucida. Experimentally, on account of great distortions taking place in the explants and the formation of blisters, it is not possible to ascertain the precise age at which the germinal layers acquire the power of self-differentiation and directional influence. Again for the purpose of determining the nature and extent of mutual influence of epiblast and endoderm, Waddington separated them and readjusted them so that the longitudinal axis of the former was at right angles to that of the latter. The results of the experiment naturally will depend on the amount of healing of the tissues and on the extent of the organic contact between them. In successful preparations, positive results have been obtained. It is found that if the operation is performed on a blastoderm in which the primitive streak is not yet fully grown, the direction of its growth is influenced by

the position of the endoderm, *i.e.*, anterior part of the primitive streak, and of the resulting neural folds is bent towards the anterior end of the endoderm while the posterior end of the primitive streak is deflected away from this pole. Waddington points out that the definite deflection of the primitive streak and the neural folds may not be a secondary phenomenon due to the mechanical properties of the tissues, but should be explained on the hypothesis that as in the formative stages of the primitive streak, its axis is determined by the growing endoderm below, this influence of the anterior end of the endoderm is continued to be exerted even under experimental conditions. The endoderm, chiefly at the growing points, has therefore a direct influence on the axial relations of the primitive streak and also those of the neural folds. The continuity of the neural plate without interruption or lesion points to the fact that the neural material is derived only from presumptive medullary material and not from other sources.

The next series of experiments deal with the determination of the nature and scope of the organizing influence of the primitive streak, firstly by testing the inductive capacity of the entire structure and then that of isolated pieces. When two specimens of blastoderm, the underlying endodermal layer having been removed, are opposed so that their ventral faces are in contact and the primitive streaks do not touch their whole length, definite evidence is obtained that the growing primitive streak can induce the formation of a medullary plate in an epiblast with which it is in contact. Waddington further evidences in support of the view that the induced medullary plate is not formed from presumptive neural material which has been prevented from moving to its normal place, by the removal of the endoderm, but the induction is due both to the form building movements and the qualitative differentiation of the cells. To the interesting inquiry whether a medullary plate in these experiments can be induced at any angle to the normal neural plate of the same epiblast, the answer is furnished that they can be induced in any orientation making an angle of 150 degrees. The grafting experiments devised for the purpose of testing the inducing capacity of the primitive streak, either whole or in fragments taken from

definite regions, shows that practically all the grafts give rise to a certain amount of mesoderm and as regards the neural tissue, the anterior portions of the primitive streak frequently and the posterior rarely, and the middle pieces sometimes do form. In these experiments, fragments of primitive streak were introduced between the endoderm and epiblast of a blastoderm chiefly through a hole made in the endoderm. If pieces of the primitive streak were cultivated separately they are observed to differentiate to neural tissue, somatic mesoderm, and the injured part of the primitive streak regenerates. If in the process of regeneration the epiblast were considered as a whole, these two organ forming movements, the superficial centripetal and the deeper centrifugal, leading to the adjustment of the tissues, and the capacity for the induction of tissues is possessed by the primitive streak and the labile determination of the presumptive tissues is dependent on the tissue forming movements.

In summarising this piece of extraordinarily interesting work, it was found necessary to follow clearly the different types of experiment in the order in which they have been described and also the language of the author as far as possible in order to sustain the interest of scientific readers.

The Innervation of the Heart of the Crustacea.

[J. S. Alexandrewicz, *Quarl. Journ. Micros. Sci.*, **75**, No. 298, June 1932.]

IN the heart of the Decaped Crustacea three systems of nervous elements can be distinguished: (a) a local system of neurons distributed in the heart itself supplying impulses necessary for the regular contractions of the muscles of the heart; (b) a system of fibres running from the Central nervous system to the heart, inhibitory and acceleratory in function; (c) a system of nerves supplying the valves of the heart holding their muscle fibres in contraction during the diastolic period of the heart. There are also nerves supplying the muscles of the pericardium which are evidently sensory in function.

The Origin and Migration of the Primordial Germ-cells of Sphenodon Punctatus.

[Margaret Tribe & F. W R. Brambell. *Quart Journ. Micros. Sci.*, **75**, No. 298, June 1932.]

THE primordial germ-cells of Sphenodon originate in the yolk-sac endoderm and are

differentiated very early. They are very large in size and are filled with yolk-granules. They migrate through the yolk-sac endoderm and mesoderm by their own amœbeid movements entering the embryo by way of the venous blood stream or by migration through the extra-embryonal endoderm and splanchnic mesoderm. They thus reach the germinal ridges, lose their yolk content and enter the prophase of the heterotypic division.

Sex Reversal and Experimental Production of Neutral Tassels in Zea mays.

[John H. Schaffner, *Bot. Gaz.*, **90**, 3, 1930.]

THE fact of sex reversal has been established for many species of plants, the reversal being brought about commonly in one direction as readily as in the other, *i.e.*, from male to female and from female to male. In the transition from one sex to the other, a physiological state of zero or neutral point must be passed through. This transition neutral condition is interesting on account of the characters developed in the tissue. For example, *monœcious or diœcious species* frequently develop bisporangiate flowers on the neutral zone, others develop sex mosaics, rudimentary flowers or peculiar vegetative structures. The author concludes that by simple control of environmental conditions vestigial tassels can be produced in Zea mays at will, the natural photoperiodic gradient extending from August to November, producing sometimes 40% or more of neutral tassels in a lot. The following general types of tassels can be developed by proper photoperiodicity, staminate, carpellate and neuters and four sex mosaics—staminate neuters, carpellate neuters, staminate-carpellate and *staminate-carpellate-neuters. With the de-* creasing photoperiodicity, femaleness is only expressed at the base of the tassel, the top being neuter and the middle region staminate. With the increasing photoperiodicity, femaleness may also be expressed at the top of the tassel and its branches, or sometimes forming sex mosaics. Even pure lines react to photoperiodicity in the same way as commercial heterozygous varieties giving rise to complete sex reversals and all possible types of sex mosaics. Male expression can be suppressed completely in the monœcious Zea mays when it is grown in a decreasing

photoperiodic environment of suitable length. The specific sex condition developed is independent of any balance of sex determining or sex producing genes. It is obvious that genetic experiments involving sex conditions are of no value unless the reactions obtained are interpreted in the light of ecological conditions present.

Nature and Development of the Tracheids of the Ophioglossaceæ.

[Gasper A. Luharidge, *Bot. Gaz.*, **98**, 2, 1932.]

THE object of the study has been the tracing of the development of the tracheids of the Ophioglossaceæ, since tracheids are unusual for ferns, in that they bear on their walls distinct bordered pits resembling those characteristic of many of the higher plants, instead of scalariform markings characteristic of Filicales. All the three genera of Ophioglossaceæ are considered. Fresh material was fixed in Jeffrey's corrosive sublimate and picric acid, alcohol-formalin, and alcohol-formalin-acetic acid. All material was treated with 10% Hydrofluoric acid for a week to remove traces of silica. Erythrosin and crystal violet, safranin with crystal violet and hæmatoxylin (Ehrlich's and Heidenhain's) were used. Combination of foast-green and safranin gave excellent differentiation of the three regions of the wall. The terminal buds in most species of Ophioglossaceæ consist of a series of three to five leaves, one of which reaches maturity each year. The rate of differentiation and maturation of tracheids is rapid and takes place just behind the apical region of the stem, the transition region being very short. The differentiation and maturation of the leaf-trace is very slow, this being attributed to the rapid growth of the leaf and the consequent extension of the region over which differentiation takes place, whereas the stem, being less conservative, crowds the transition region to a short space. Lignification and maturation of tracheids begin in the leaf as early as four years before the leaf reaches maturity. The secondary thickening differs from that of the Spermatophytes in being made up of cellulose and the tertiary thickening of lignin, which covers the secondary thickening and forms bordered pits.