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Development of the Meninges in the Chick.

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Harvey and Burr^{1, 2} have presented evidence that in the salamander, the neural crest was a necessary requisite for the formation of the leptomeninx. We have continued the study by means of chorio-allantoic grafts in the chick. Two series of operations were performed by Dr. Van Campenhout. In the first, the prosencephalon of 50-hour chicks was transplanted adjacent to an allantoic vessel of a 9-day host and allowed to develop from 7 to 9 days. In the second, an entire segment of the thorax or abdomen from a 50- to 98-hour chick was transplanted on the chorio-allantoic membrane of 9-day hosts. These also were permitted to develop for a period of approximately 9 days. Our knowledge of the distribution of the neural crest cells indicates that at the time of operation no such cells have as yet reached the region of the forebrain. It could then be transplanted devoid of neural crest investiture. On the other hand, during the period immediately following the 50-hour incubation period, the neural crest has begun its migration about the spinal cord in the thoracic and abdominal segments. Thus we have the nervous system transplanted without neural crest in the first series and with neural crest in the second. Since all the other conditions were as nearly constant as possible any difference in the structure of the meninges could be attributed to the presence or absence of the neural crest.

The material from the above experimental procedure was prepared for microscopic study using chiefly the Masson stain. A critical examination shows that there is a marked difference in the membranous investment of the nervous system in the 2 series. In series one, without neural crest, the nervous system is surrounded by a series of fibrous laminae of obvious mesenchymal origin. These laminae are in direct contact with the external limiting membrane of the forebrain. All the cellular elements lie outside the connective tissue fibrils. The blood vessels lying adjacent to the nervous system also lie outside this primitive membrane. When blood vessels penetrate the neural wall the connective tissue fibrils tend to pene-

¹ Harvey, S. C., and Burr, H. S., *Proc. Soc. Exp. Biol. and Med.*, 1924, **22**, 52.

² Harvey, S. C., and Burr, H. S., *Arch. Neur. and Psych.*, 1926, **15**, 545.

trate beneath the limiting membrane for a short distance. No evidences of fluid spaces could be found about the nervous system. The ventricles were closed systems filled with blood cells and detritus. As a result of this series of transplantations a portion of the nervous system is more or less completely surrounded by a condensation of mesenchyme which has many of the characteristics of a primitive dura.

When, on the other hand, a portion of the nervous system was transplanted together with the neural crest, the relations are quite different. The same condensation of mesenchyme and the beginning of a fibrous sheet occurs as in the first series of transplanted material. But, between the mesodermal sheet and the nervous system, there can now be seen a single or double layer of cells with oval nuclei and scanty cytoplasm tangentially arranged. These occupy the space between the outer mesenchyme and the external limiting membrane of the spinal cord. Through the same region runs a vascular network, branches of which enter the nervous system accompanied by cellular elements. No fluid spaces could be identified with any certainty. It seems reasonable to conclude that the neural crest contributes very largely, if not entirely, to the formation of a thin investing membrane between the mesenchyme and the nervous system. Since blood vessels are included in it and since it lies between a relatively thick membranous sheet and the nervous system, it may be assumed to be a leptomeninx. This does not mean that mesenchymal elements are excluded from participation in the formation of the pia-arachnoid. It does indicate quite clearly, that in the absence of neural crest the pia-arachnoid fails to develop. Further experiments to determine the relative proportion of the neural crest and mesenchyme in the leptomeninx are in progress.

Flexner³ made parallel experiments on *Amblystoma* and following Weed,⁴ concluded that the presence or absence of neural crest produced no difference in the meningeal surroundings of the neural tube and that any difference which might result was due to the presence or absence of cerebral spinal fluid surrounding the nervous system. It is no part of our hypothesis that the cerebrospinal fluid is an unimportant factor in the differentiation of the meninges. Weed and his associates indicated quite clearly that the effusion of cerebrospinal fluid into the immediate surroundings of the ner-

³ Flexner, L. B., *Contributions to Embryology*, 1929, No. 110, Carnegie Inst., Wash., Pub. 394.

⁴ Weed, L. H., *Contributions to Embryology*, 1917, No. 14, Carnegie Inst., Wash., Pub. 225.

vous system is responsible for the delaminations occurring in the meninges. We may accept that as an established fact. But that fact does not preclude the possibility that the material which delaminates as a result of the activities of the cerebrospinal fluid is derived from other sources than mesenchyme. On the contrary, the experiments we have presented indicate that the neural crest and the mesenchyme aided by the presence of the cerebrospinal fluid form the investing membranes of the central nervous system.

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Action of *Ilex Opaca* on the Heart.

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Ilex opaca or American holly is an evergreen tree growing throughout the Atlantic section of the United States. The leaves and fruit are the parts of the plant that have been used in medicine; in domestic practice alterative properties have been assigned to it and by eclectic practitioners it has been used as an anti-intermittent, febrifuge, tonic and diaphoretic. The "Black Drink" by which the Indians "cleansed" themselves by drinking sufficient to cause vomiting was an aqueous extract prepared from the leaves of the holly. The dried leaves of certain species of *ilex* are used by the inhabitants of Paraguay to prepare a stimulating beverage.

The present experiments were undertaken to determine the effects of extracts of the above fruit on the amphibian heart. Male frogs (*Rana pipiens*) were used. A cannula was tied in the inferior vena cava and the heart arranged for perfusion by the Symes' method, with Ringer's solution in one bottle and Ringer's solution plus the drug in the other. The movements of the lever were recorded on a smoked drum. The extract was prepared by macerating the dried fruit in number 20 powder with 70% alcohol for 4 days and filtering. When required for use the alcohol was evaporated off and the thick syrupy remains added to frog Ringer's solution.

Perfusion in a proportion of one part of the crude drug in 750 parts of Ringer's solution resulted in an increase in the amplitude of the beat. Although the diastole of the heart was increased to some extent the main effect was a more complete shortening of the