

ON THE EXPERIMENTAL PRODUCTION OF EDEMA BY NEPHRECTOMY.

By W. W. SWINGLE.

(From the Laboratory of Comparative Anatomy, Princeton University, Princeton.)

(Received for publication, March 27, 1919.)

The question of the causation of edema is one that has interested clinical pathologists and other investigators for many years. Though much has been done to clear up certain phases of the problem, it has still remained in doubt to what extent, if any, a generalized edema of the body may be due to an inefficiency of the kidney function. Recently, however, the work of McClure¹ has shed considerable light upon this obscure problem.

McClure has shown that the edematous condition frequently met with in young frog larvæ is invariably associated with a deficiency in the development of the head kidney (pronephros). Furthermore, he has been able to produce an edematous state of the body at will in adult frogs and toads by ligation of the ureters. His experiments clearly show that a generalized edema of the body may result in Anura from a block in the kidney function.

The writer concluded that it might be of interest in this connection to extirpate the pronephros or head kidney of very young frog larvæ, shortly before the period when this organ becomes functional. According to the viewpoint of McClure edema should result in such cases. The results justified expectations, as the following experiments show.

Several egg masses of *Rana sylvatica* LeConte were collected and brought to the laboratory to develop. Evidently the eggs had been laid early in the morning of the preceding day, for most of them were in early segmentation stages (8 to 16 cells). The larvæ hatched within a few days and were apparently normal in every respect.

¹ McClure, C. F. W., On the experimental production of edema in larval and adult Anura, *J. Gen. Physiol.*, 1918-19, i, 261.

Larvæ of 5.5 to 7 mm. in total length are in the proper developmental stage for successful extirpation of the pronephros and Wolffian ducts. Slightly older larvæ (7 to 8 mm.) give fair results but are not so easily handled as the younger stages. All extirpations were performed under the binocular microscope with a blunt dissecting needle and a razor-edged, spear-head needle. The larvæ were narcotized with chloretone.

The glandular portion of the pronephros in larvæ of 5.5 to 7 mm. in length shows very clearly as a small rounded elevation or nodule, just caudal to the gills, on either side of the head. This elevation represents the position of the coiled pronephric tubule² with its three nephrostomes which open into the cœlomic cavity. The Wolffian ducts extend caudad and lie just ventral and medial to the edge of the heavy band of musculature or myotomes. In favorable specimens, with good illumination, the exact location of the Wolffian duct is represented by a very faint line in the position just described. The ducts in the later stages open dorsally into the cloaca. The operation of removing the pronephros or its duct is simple and the wound closes within an hour or two.

Total Nephrectomy.

The glandular portion of the pronephros was removed on both sides of the body from 110 larvæ. In many of the smaller larvæ of the group, *i.e.* in those measuring 5.5 mm., it was observed that the Wolffian ducts had not yet opened to the exterior. The larvæ, appropriately controlled, were left in well aerated water over night. On the following morning, examination showed all the larger 7 mm. larvæ to be edematous. The edema in its incipient stages appeared chiefly as a swelling of the submaxillary lymph sinus.

20 hours after the operation, 16 larvæ showed marked edema. 5 hours later 36 were edematous; at the end of 36 hours, 97 larvæ showed a typical edema. The few larvæ which remained normal

² Field, H. M., The development of the pronephros and segmental duct in amphibia, *Bull. Mus. Comp. Zool.*, 1891, xxi, 201. Huber, G. C., On the morphology of the renal tubules of vertebrates, *Anat. Rec.*, 1917, xiii, 305.

showed upon microscopic examination a turgor of the lymph spaces, and finally after several hours more (the longest 48) all larvæ were edematous. The mortality was very slight up to 36 hours. After this time, the extremely edematous larvæ usually burst, unless killed and preserved in fixing fluids. Rupture of the body wall usually occurred at the point of operation.

Several greatly swollen larvæ were observed to burst while being examined. These were at once placed in separate containers and carefully watched. Several hours later these same larvæ were again observed to be edematous. They were then left over night. Two were found dead the next morning. The remaining two had burst again during the night; they recovered but again became edematous and were finally killed.

Partial Nephrectomy.

In 50 larvæ the glandular portion only of the right or left pronephros was extirpated. The larvæ averaged 6.5 mm. in length. At the end of 24 hours, only three larvæ showed indications of edema and, in all three, the edema was confined chiefly to the side from which the pronephros had been removed. All the larvæ showed swelling and hypertrophy of the remaining uninjured pronephros.

38 hours after the operation half the larvæ of the culture was edematous. In the early stages, the swelling was almost always on the side from which the kidney had been removed. Dissection of the pronephros remaining showed the tubular complex to be much hypertrophied and distended with fluid. A comparison of the pronephros of such larvæ with those from normal controls of the same age showed a difference in size, though we should expect it in such cases.

The onset of edema in the larvæ of this group was very much slower and less marked than in any other culture of the larvæ. In fact, fully one-half showed only slight edema, which was confined chiefly to a slight turgor of the submaxillary lymph sinus. 50 hours after the operation ten larvæ were counted which showed no edema whatever, and no indication of rupture of the body wall. It is clear that the mesonephros of these larvæ was beginning to function.

Removal of Portion of Wolffian Ducts.

A series of 62 larvæ was operated upon; the nature of the experiment varied from that just described in that, instead of extirpating the glandular portion of the pronephros, sections of its duct, on either side, were cut out just cephalad to the cloacal opening. Larvæ of 7 mm. in length were used in this experiment.

20 hours after the operation, examination of the larvæ showed great swelling of the glandular part of the pronephros. The swelling protruded as large knots of edematous tissue on either side of the head region. Edema appeared in most of the larvæ a few hours later. A curious condition now resulted. In addition to the marked turgor of the head region, and to the two swellings on either side of the body, representing the hypertrophied pronephros, there was a large edematous swelling on either side of the larva in the posterior part of the body at the place where the cut ends of the Wolffian ducts ended blindly in the tissues. Evidently the pronephros was functioning, but the fluid was being forced into the tissues of the posterior end of the body.

An operation of a somewhat different nature from those recorded above was also attempted. Twenty-five larvæ, measuring 7 mm. in length, were used. The Wolffian ducts on either side were severed, just cephalad to the cloacal opening, and dissected out anteriorly well up toward the glandular portion of the pronephros. The ducts were permitted to hang outside the larvæ through the incision made in the body wall. Considerable mortality resulted, no doubt due to the severity of the operation and consequent destruction of such important vascular organs as the posterior cardinals and dorsal aorta. Two-thirds of the larvæ died during the first 24 hours, and at the end of 36 hours only three remained alive. When examined, they showed practically no edema, except a slight turgor of the head lymph sinuses and swelling of the glandular portion of the kidney. The Wolffian ducts still hung outside the body and were apparently functioning as excretory ducts, despite their abnormal position. The incision in the body wall at the point where the ducts protruded had not healed at this time. Complete healing of the wound would no doubt have pinched off the ducts or else have closed them by pressure. All

the larvæ died, and it was impossible to keep them alive long enough to see whether the wound would have healed, leaving the ducts functional.

It was observed during the course of the experiments that the onset of the edema appears to be coincident with the opening of the Wolffian ducts into the cloaca. It is at about this time also that the yolk mass begins to undergo rapid resorption. Apparently water is not rapidly absorbed through the integument until the yolk mass undergoes considerable diminution.

In this connection, it was suggested by Professor McClure that the onset of edema in the nephrectomized larvæ should coincide with the initiation in the embryonic body of a continuous lymph flow through the lymphatics, between the periphery and the veins, since the attainment of this condition presents the first opportunity for water, absorbed through the skin and taken up by the lymphatics, to reach the kidneys by way of the lymphatic and blood vascular systems. This condition is established normally in frog and toad larvæ, according to McClure,³ at about the time when the intestine has differentiated out of the yolk mass, and the yolk has undergone resorption. I feel sure that the onset of edema occurred at about this time in the nephrectomized frog larvæ.

The extirpation experiments recorded here show that any malfunctioning of the kidney, any block in its excretory canals, or complete loss of function, as for instance in nephrectomy, leads to an excessive accumulation of fluids in the lymph sinuses, tissues, and body cavity. They therefore confirm in a striking manner the results obtained by McClure and, as far as the Anura are concerned, it can now be regarded as an established fact that edema may arise from a block in kidney function.

I wish to acknowledge my indebtedness to Professor McClure for many helpful suggestions and for the interest he has shown in the work.

³ McClure, C. F. W., On the behavior of *Bufo* and *Rana* toward colloidal dyes of the acid azo group, *Memoirs of The Wistar Institute of Anatomy and Biology*, No. 8, Philadelphia, 1918, 48; On the provisional arrangement of the embryonic lymphatic system, *Anat. Rec.*, 1915, ix, 281.

Addendum.—After this article was in press, the work of Miss Ruth B. Howland⁴ came to my attention. The results obtained by Miss Howland on the embryos of *Amblystoma* are essentially similar to those described above by me for the frog. It is an agreeable duty for me to acknowledge, however, the full priority of Miss Howland's results.

⁴ Howland, R. B., On the effect of removal of the pronephros of the amphibian embryo, *Proc. Nat. Acad. Sc.*, 1916, ii, 231.