ON THE EXPERIMENTAL PRODUCTION OF EDEMA IN LARVAL AND ADULT ANURA.

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Edematous frog larvæ are among the commonest types of abnormalities which can be produced by experimental means. When ova of the two- or four-celled stage are placed in solutions of potassium cyanide, acetone, butyric acid, or ethyl alcohol for from 12 to 24 hours, and then subsequently developed in tap water, an edematous condition of the body frequently, though not always, results. Hertwig[1] has produced such larvæ by exposing early cleavage stages to the influence of radium. An edematous condition of the body also occasionally results when ova are allowed to develop in direct sunlight in the laboratory and may even be met with among the controls. The life of such larvæ is relatively short.

The edematous frog larvæ thus far observed by the writer can be grouped into two main types: Type 1, in which, with the exception of their general edematous condition, the frog larvæ appear externally to be otherwise normally developed; Type 2, in which some marked abnormality, especially of the head region and vascular system, usually accompanies an edematous condition of the body.

All the larvæ referred to in this paper were in the stage at which the pronephros acts as the functional kidney and in which the mesonephros is either not at all developed, or is represented only by its earliest anlage.

An examination of serial sections of a large number of both types of edematous frog larvæ shows that in all cases, and without exception, there is a marked deficiency evident in the development of certain specified tubules in the pronephros. Reconstructions after the method of Born clearly indicate that this deficiency is associated with the

[1] Hertwig, O., *Arch. mikr. Anat., 2te Abt.*, 1911, lxxvii, 1 (see Tables II and III for examples of edematous frog larvæ).

tubules which normally constitute the greater portion of the kidney and which occupy a medial and ventral position. In some larvæ none of these particular tubules had even been developed. In all other cases, in which only their partial development had occurred, the few tubules present were always greatly hypertrophied and were often lined by an exceedingly thin-layered epithelium which closely resembled endothelium in appearance.

The less extensive tubular complex which normally occupies a dorsolateral position in the pronephros and into which the nephrostomal canals directly open was, however, invariably found to be developed to a considerable degree in all the edematous larvæ examined. It was lined by a thick-layered epithelium which is the only epithelium of the pronephros in the writer's experience, in both normal and edematous frog or toad larvæ, that has thus far been observed to absorb colloidal acid dyes. In all cases this dorsolateral tubular complex was found to be much hypertrophied and often bladder-like in appearance and, together with the three nephrostomal canals which open into it, was occasionally found to constitute the only portion of the kidney that had been formed. In such extreme cases the pronephric duct was either a solid structure or was not connected with the kidney at all.

Associated with this general defect in kidney development the lymph sinuses of the body, especially those of the head region, were often found to be greatly distended with fluid. In larvæ of Type 1 in which the edema is generalized, no defect in the development of any organs of the body was evident except that of the pronephric tubules referred to. In some cases (Type 2) the anterior lymph hearts were absent, and other prominent defects in the vascular system met with which need not be mentioned here. The pericardial and cœlomic cavities were often greatly expanded and blebs or blisters of various sizes were occasionally formed under the epidermis. All these cavities were filled with fluid, as could easily be determined in larvæ which had been placed in solutions of colloidal acid dyes.

We know that water and certain colloidal acid dyes[2] are continu-

[2] 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.

ously absorbed through the integument in both larval and adult frogs and toads when the latter are allowed to remain in such media, and that the excess of fluid taken into the body in these cases is normally eliminated by the kidneys. Since the water taken into the body by absorption through the integument must necessarily reach the kidneys by way of the vascular system, the possibility suggests itself that the edematous condition of the frog larvæ referred to may be due to the deficiency observed in the development of certain specified tubules of the kidney, or, in other words, to a block in the normal kidney function. In consequence of this an excess of water which is constantly being taken into the body through the integument is retained in the body, since it can no longer be eliminated by the kidneys. That this is actually the case, as far as these particular frog larvæ are concerned, seems to be borne out by experiments on adult frogs and toads in which an edematous condition of the body can be produced by ligation of the ureters.

The distal ends of the ureters in the frog and toad lie in the dorsal wall of the cloaca and open dorsally into the latter independently of the bladder, which opens on the ventral wall of the cloaca. It is therefore not a difficult matter to ligate the ureters by means of artery forceps and small metal clamps, or to pass a ligature around the ureters which can be tied over the urostyle. In such cases neither the bladder nor the outlet of the intestinal canal through the cloaca is interfered with.

Among 53 ligation experiments thus far made by the writer, chiefly on adult toads, the six mentioned in Table I illustrate the range of results obtained. In all cases the toads[3] were kept in water for a considerable time and weighed at intervals before applying the ligature. This was done in order to establish a normal balance, under such conditions, between the absorption of water through the integument and its elimination from the body by the kidneys.

The first figure in each of the columns of Table I (Experiments 1 to 6) indicates the weight of the toad in gm. at the time the ureters were ligated and before the toad was again placed in water. The succeeding figures in each column indicate the observations made at different intervals of time.

[3] *Bufo lentiginosus* Shaw.

TABLE I.

Time.	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5	Experiment 6
hrs.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
0	22.8	30.5	30.9	45.9	53.9	49.1
0.50		32.7				
0.52				51.5		
1.10					54.5	
1.35		34.2				
1.40	23.75			54.5		
2.20				57.0	55.4	
2.43	24.8					
3.35			34.0			
5.05	26.9					
5.45				64.4 (40%)		
6.10	27.6					
6.55						55.9
7.45	28.7					
11.55	31.2					
16.55		50.8 (66.5%)				
17.55					60.3	
19.00					60.9	
20.00					61.7	
20.50					62.7	
23.25			58.6 (89.6%) Removed ligature.			
23.30					68.4	
23.40	37.5 (64.4%)					
24.05					70.5 (30%)	
25.25			47.5			52.2
25.55			43.9			
28.35			37.9			56.1
29.55			35.2			
30.50			32.9			
31.55			30.9			
48.40						68.4 (39.3%)

These experiments should not be confused with the well known experiments of Overton[4] who ligated the cloacal opening but not the ureters. When only the cloacal opening is ligated, the flow of urine through the ureters into the cloaca and bladder is not interrupted. The urine in such cases, as stated by Overton, will fill up the bladder and then back up into the intestinal canal. When the ligature is removed, this urine can be readily expressed from the body through the cloaca. On the other hand, as we shall see in the following experiments, after removal of the ligature from the ureters, no fluid can ordinarily be immediately expressed from the cloaca as, in this case, the excess of fluid contained in the body has been deposited in the tissues and body cavity, and not in the bladder and intestinal canal. The six experiments referred to in Table I were made during the month of August.

Experiment 1.—When killed the toad was in a comatose condition and had gained 64.4 per cent in weight in 23 hours and 40 minutes. On removal of the silk ligature no fluid was passed from the cloaca. A clear fluid containing albumin and weighing 6.4 gm. was removed from the subcutaneous lymph sinuses. A clear fluid weighing 4.9 gm. which threw down a precipitate in nitric acid that disappeared when acetic acid was added, was also removed from the body cavity. An excess of fluid amounting to 3.4 gm. still remained in the tissues after removal of the fluid from the lymph sinuses and body cavity. The lungs were inflated and intensely congested. The blood vessels, especially those of the abdomen, integument, and kidneys were also greatly congested.

Experiment 2.—When killed the toad was partially comatose and had gained 66.5 per cent in weight in 16 hours and 55 minutes. No fluid could be expressed from the cloaca on removal of the small metal clamp with which the ureters had been ligated. A bloody fluid weighing 11.6 gm. was removed from the subcutaneous lymph sinuses and 3.2 gm. of fluid of a similar character were found in the body cavity, leaving an excess of fluid amounting to 5.5 gm. in the tissues of the body after removal of fluid from the lymph sinuses and body cavity. The condition of the lungs and blood vessels was essentially the same as in Experiment 1.

Experiment 3.—In this case the dorsal wall of the cloaca was tightly clamped with an artery forceps, the teeth of which undoubtedly cut deeply into the tissues. After being placed in water the toad gained 89.6 per cent in weight in

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[4] Overton, E., *Verhandl. physik.-med. Ges. Würzburg*, 1904, xxxvi.

the course of 23 hours and 25 minutes, at the end of which time the forceps was removed. The toad was in a comatose condition and a few drops of fluid could be expressed from the cloaca. The toad was not killed, and at the end of 8 hours and 30 minutes, after removal of forceps, had regained its normal weight and was very active.

Experiment 4.—When killed the toad had gained 40 per cent in weight in the course of 5 hours and 45 minutes and was still quite active. In ligating the ureters with a silk ligature which passed under the ureters and over the urostyle, a large blood vessel was punctured. The subcutaneous lymph sinuses of the body contained 7.1 gm. of a clear fluid which coagulated on exposure to air, and the body cavity 4.3 gm. of a fluid in which considerable blood was present. The excess of fluid remaining in the tissues after removal of fluid from the lymph sinuses and body cavity amounted to 7.1 gm. The lungs, kidneys, and subcutaneous blood vessels were intensely congested.

Experiment 5.—In this instance the ureters were compressed by a small metal clamp, the jaws of which pressed tightly upon, but did not cut into the tissues. When the toad was killed it was very weak and had gained only 30 per cent in weight in the course of 24 hours and 5 minutes. On removal of clamp no fluid could be expressed from the cloaca. About 7.7 gm. of a slightly bloody fluid were removed from the subcutaneous lymph sinuses and only 1 gm. of a clear fluid containing albumin was found in the body cavity. The excess of fluid still remaining in the tissues after its removal from the lymph sinuses and body cavity amounted to 7.9 gm. The lungs were greatly expanded and intensely congested. Marked signs of congestion were observed on the abdomen, especially in the region of the pelvis.

Experiment 6.—This illustrates an instance in which a gradual increase in body weight is followed by a slight decrease, and then succeeded by a gradual and progressive increase in body weight which develops into an intense form of generalized edema. In this particular case an artery forceps was used in which the teeth had been filed down so that they would not cut into the tissues, and the pressure exerted on the ureters was also relatively slight. When the toad was killed it had gained only 39.7 per cent in weight in the course of 48 hours and 40 minutes. The slow progress of the edema was probably due to an incomplete ligation of the ureters. On removal of forceps about 2.1 gm. of semifluid excreta were expressed from the cloaca. The fluid removed from the subcutaneous lymph sinuses was clear, coagulated on exposure to air, and amounted to 4.2 gm. The fluid present in the body cavity was extremely bloody and also weighed 4.2 gm. The excess of fluid still remaining in the tissues after its removal from the lymph sinuses and body cavity amounted to 8.8 gm. The lungs, kidneys, left oviduct, mesenteries, and subcutaneous blood vessels were intensely congested.

The toad was weighed twenty-one times in the course of this experiment. Only the more critical data, however, are mentioned in Table I.

The experiments clearly show when the ureters are completely ligated and the toad is placed in water, that an increase in body weight immediately takes place which gradually progresses into an intense form of generalized edema. An analysis of the conditions described need not be given here, further than that the progress of the edema appears to be related to the extent and character of the abrasion produced in the tissues at the point of ligation, as well as upon the state of congestion of the tissues which results from ligation.

On the basis of these observations it does not appear difficult to explain the edematous condition of the frog larvæ mentioned above.

We know that the glomeruli of the pronephros are not directly connected with the renal tubules, as is the case in the meso- and metanephros, but project into the body cavity independently of these tubules in the vicinity of the nephrostomes. The filtrate of the pronephric glomeruli must necessarily, therefore, pass into the body cavity before entering the pronephric tubules by way of the nephrostomes. If, on account of a deficiency in their development, the tubules of the pronephros should be unable to take care of all the glomerular filtrate, or to secrete fluid obtained from the pronephric veins, an excess of fluid which could not be eliminated by the kidneys would necessarily remain in the body cavity and tissues and, in the course of time, would increase in amount so that a typical edematous condition of the body would finally result. Such I believe to be an explanation of the edematous condition observed in these frog larvæ.

Further significance of the above observations lies in the fact that they do not appear to bear out Fischer's[5] view that the cause of edema, in the anura at least, resides in the tissues, and is due to an overproduction or accumulation of acids within the body which causes them to take up water. It has also been pointed out by various authors[6] that this view is untenable on account of the limited variation of the hydrogen ion concentration in the body.

[5] Fischer, M. H., Œdema and nephritis, New York, 2nd edition, 1915.

[6] Henderson, L. J., and Cohn, E. J., *J. Am. Chem. Soc.*, 1918, xl, 857. Crozier, W. J., *J. Biol. Chem.*, 1916, xxiv, 255, 443; 1918, xxxv, 455.