Distribution of Protease Activity in the Blastula and Early Gastrula of *Discoglossus pictus*

Recent investigations[1] have demonstrated in the early gastrula of *Amblystoma* a gradient of dipeptidase activity with a maximum in the blastoporal region. On the other hand, studies with labelled amino acids[2] have shown a higher rate of incorporation in the blastoporal lip than in any other region. These facts suggest that this region must be the site of a higher turnover of proteins. Therefore it seemed interesting to investigate the distribution of protease activity in the different territories of the blastula and early gastrula.

It should be mentioned here that determinations of protease activity on whole embryos[3] have shown a decreasing rate of activity from fertilization to the neurula stage.

Females and males of *Discoglossus pictus* were injected with 400 I.U. (400 I.U./1 mg) of Gonadotrop Schwang kindly supplied by the Firma CIBA.

Fertilized eggs were usually obtained 20 h after the injection and were kept at room temperature until they reached the desired stage.

Blastulae at stage 9 of SHUMWAY[4], i.e. late blastulae, and gastrulae at stage 10, i.e. accumulation of pigment at the site of the blastoporal lip, were used. The embryos were dissected in concentrated HOLTFRETER's solution, according to Figures 1 and 2. Blastulae were dissected in half, along the equator, thus obtaining an animal and a vegetal half. Early gastrulae were dissected as in Figure 2*a*, the two lateral regions being discarded. The yolk mass of the central sector was discarded with two cuts, as indicated in Figure 2*b*, and the dorsal part was then divided into two regions: region 1 which comprises presumptive chorda-mesoderm and presumptive neurectoderm; and region 2, comprising only presumptive epidermis.

For each experiment, 30 embryos were dissected. The explants were rinsed in cold 0·65% NaCl solution in phosphate buffer 0·01 *M*, pH 7·4, and finally homogenized in 0·4 cm³ of the same solution.

Protease activity has been measured on the whole homogenate, according to DUSPIVA[5]. Preliminary experiments showed that the optimum for the protease activity of this material is at pH 4·9; hence all our assays were run at this pH.

Activity has been expressed as micromoles of Tyrosin released *per* 1 mg of N (total or cytoplasmic, as will be discussed presently), after 2 h of incubation at 30°C.

The problem of the reference in the calculation of enzymatic activity is a serious one in the case of Amphibian embryos. The unequal distribution of yolk between animal and vegetal halves, is likely to give rise to misleading results, should the enzymatic activity be calculated on the basis of total N. This question has been thoroughly discussed by GREGG and LØVTRUP[1], who have suggested to use as a reference the 'cytoplasmic Nitrogen', i.e. the Nitrogen which is extractable in the above-mentioned NaCl solution, which in fact appears to leave unbroken both yolk platelets and pigment granules, which can be removed by low-speed centrifugation (1700 × *g* for 15 min). In the present experiments, both

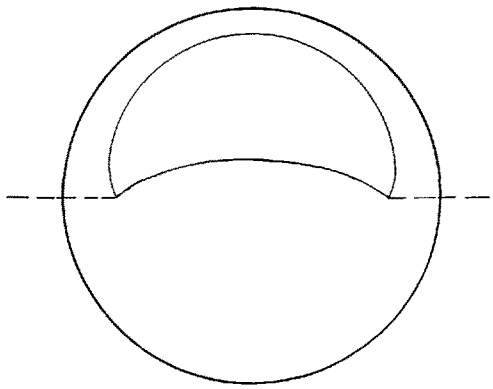

Figure 1

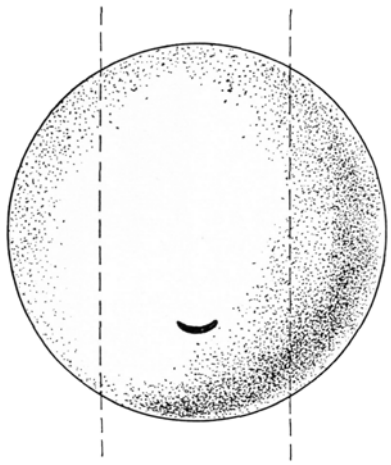

Figure 2*a*

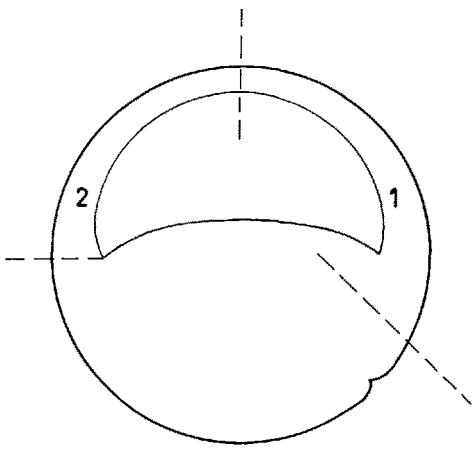

Figure 2*b*

rotal (TN) and extractable (EN) nitrogen were used as a teference in each set of experiments. Nitrogen was determined by direct Nesslerization after combustion.

Blastula. Our determinations have shown that in the blastula of *Discoglossus* the EN has the following distri-

[1] J. R. GREGG and S. LØVTRUP, C. r. Trav. Lab. Carlsberg, Sér. Ch. *27*, 307 (1949).

[2] F. FRIEDBERG and R. M. EAKIN, J. exp. Zool. *110*, 33 (1949). – J. L. SIRLIN, Exper. *11*, 112 (1955). – J. L. SIRLIN and C. H. WADDINGTON, Nature *174*, 309 (1954).

[3] E. URBANI and L. DE CESARI GOROMALDI, Ric. scient. *24*, 2364 (1954).

[4] W. SHUMWAY, Anat. Rec. *78*, 139 (1940).

[5] F. DUSPIVA, Protoplasma *32*, 261 (1939).

bution: 51·4 ± 2·2% in the animal half and 35·6 ± 4·5% in the vegetal half.

Accordingly, if protease activity in these two regions is calculated on the basis of TN, a slightly higher activity is found in the animal half: 58·1 ± 3·06% of the total activity or 0·57 ± 0·024 μM of Tyrosin (per 1 mg N) for the animal half as compared with 0·40 ± 0·026 μM of Tyrosin in the ventral half.

On the basis of EN, on the other hand, it is found that the activity is more or less equally distributed between the two regions, being in the animal half 48·1 ± 6·4% of the total activity or 1·00 ± 0·12 μM of Tyrosin as compared with 1·20 ± 0·15 μM of Tyrosin in the vegetal half.

Gastrula. The Figures given by GREGG and LØVTRUP[1] indicate that there is no difference in yolk content between region 1 and 2 of the early gastrula. Our determinations have entirely confirmed this. On the other hand the difference in protease activity between these two regions is striking, being considerably higher in 1 than in 2, irrespective of the reference being TN or EN (Table).

Protease activity in regions 1 and 2 of the early gastrula of *Discoglossus pictus*. Activity in micromoles of Tyrosin/1 mg N/2 h incubation at 30°C.

Experiment	Activity *per* TN		Activity *per* EN	
	Region 1	Region 2	Region 1	Region 2
1/3	0·70	0·14	1·57	0·31
12/3	0·33	0·23	0·48	0·31
19/3	0·64	0·26	–	–
22/3	0·39	0·27	–	–
15/5	0·95	0·22	1·25	0·83
29/5	0·86	0·56	1·29	0·95

These results, although of preliminary character, appear to be in line with the assumption that the presumptive chorda-mesodem, together with the presumptive neurectoderm of the early gastrula are the site of a higher renewal of proteins.

The activities we have observed are, however, rather low; which has prevented us, thus far, from carrying on the investigation on smaller explants, i.e. on less heterogeneous presumptive regions.

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Riassunto

È stata determinata l'attività proteasica nelle differenti regioni della blastula e della gastrula di *Discoglossus pictus*. Nella blastula non esiste una differenza di attività fra metà animale e vegetativa, quando si prenda come riferimento l'azoto citoplasmatico. Nella giovane gastrula il territorio presuntivo della corda e del sistema nervoso mostra una attività più elevata della epidermide presuntiva.

On the Biological Function of Cathepsin in Tail Tissue of *Xenopus* Larvae

In conclusion from earlier experiments on catheptic activity in larval tails of *Xenopus* during normal and chemically suppressed regeneration, the hypothesis was put forward that cathepsin might be concerned mainly with protein degradation[1]. However, in literature which refers to embryonic development[2] or to regeneration[3], both synthetic and catabolic functions are assigned to cathepsin. Obviously breakdown of proteins occurs during early phases of regeneration and in embryos, when yolk is utilised, but always concomitantly with formation of new tissues. Hence it appears rather difficult to draw definite conclusions on the predominating function of the cathepsin system.

During development, the tail of *Anuran* larvae shows a clear sequence of morphogenetic events in which growth is followed by tissue resorption. At the chemical level, this would involve first mainly synthesis and later breakdown of tissue proteins. Hence a comparison of catheptic activity during these phases would permit more precise conclusions on its functional significance.

Xenopus larvae were selected after hatching and raised in small groups at 20°C under optimal feeding conditions. At the onset of metamorphosis (i.e. eruption of the fore limbs[4]) the larvae were transferred individually into glass-distilled water and kept without food, until they were used. For stageing, the anaesthetised larvae (MS 222 1:6000) were measured ventrally. 'Body length' is the distance from the upper jaw to the tail tip, and 'tail length' is defined by the distance from the caudal intersection between the hind limb and the tail to the tail tip. These readings are accurate to within ± 0·1 mm.

Catheptic activity was determined according to the method of DUSPIVA[5], which uses casein-urea as substrate. Since details of this procedure are described elsewhere[1], only the modifications for the present experiments are mentioned:

a) Homogenates of tails, amputated 1 mm behind the anus, were prepared in glass-distilled water at low temperature (+ 2°C).

b) Catheptic activity was expressed in terms of an arbitrarily fixed '*Tail Unit*' by using a standard curve which relates extent of splitting to homogenate (i.e. cathepsin) concentration. These data were based on corresponding quantities of total nitrogen, determined according to the micromethod of BOELL and SHEN[6]. Assays for catheptic activity ($\bar{x}$) and total nitrogen ($\bar{y}$) were done in triplicates of the same homogenate. The standard error for the specific activity ($\bar{x}/\bar{y}$) was calculated according to the following formula[7]:

$$s_{\bar{x}/\bar{y}} = \pm \frac{1}{\bar{y}} \cdot \sqrt{s^2_{\bar{x}} + \left(\frac{\bar{x}}{\bar{y}}\right)^2 \cdot s^2_{\bar{y}}}.$$

Growth and resorption of the larval tail. Body and tail lengths increase at linear rates which fall slightly before

[1] P. K. JENSEN, F. E. LEHMANN, and R. WEBER, Helv. physiol. Acta *14*, 188 (1956).

[2] S. LØVTRUP, C. r. Lab. Carlsberg, sér. chim. *29*, 261 (1955). – E. URBANI, Exper. *11*, 209 (1955).

[3] J. NEEDHAM, *Biochemistry and Morphogenesis* (Cambridge Univ. Press, 1950).

[4] P. GASCHE, Helv. physiol. Acta *2*, 607 (1944).

[5] F. DUSPIVA, Protoplasma *32*, 211 (1939).

[6] E. J. BOELL and S. C. SHEN, Exper. Cell. Res. *7*, 147 (1954).

[7] I am indebted to Prof. S. ROSIN (Bern) who was kind enough to work out this formula.