Regional Differences in Catheptic Activity in *Xenopus laevis* embryos

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INTRODUCTION

That the intracellular proteolytic enzymes known collectively as ‘cathepsins’ may have a special function in newly forming tissues was first suggested by the findings of Orechowitsch, Bromley, & Kusmina (1935). These authors observed that in certain amphibians regenerating tail-tissue had a much higher catheptic activity than the normal, non-regenerating tail. Their findings have been confirmed and extended recently in work on *Xenopus laevis* larvae, where it has been shown (Jensen, Lehmann, & Weber, 1956) that a peak in catheptic activity is reached in the regenerating tail-tip on about the 7th day after amputation. A further finding (Deuchar, Weber, & Lehmann, 1957) was that the early regenerate had a much higher catheptic activity relative to total nitrogen than had the stump-tissue immediately adjacent to it. The cathepsins seemed, therefore, to have the highest activity in the region of most active protein synthesis. But although Fruton et al. (1953) and Izumiya & Fruton (1956) have shown that under certain conditions *in vitro* a cathepsin may reverse its proteolytic action and catalyse synthetic steps, there is as yet no definite evidence that cathepsins ever catalyse protein synthesis *in vivo*. It remains possible, however, that some raw materials for the synthesis of new tissue proteins are provided by a prior proteolysis in which cathepsins play a part. Preliminary studies (Deuchar, Weber, & Lehmann, 1957) indicated that there was a rise in free amino acid concentration immediately after the rise in catheptic activity in the regenerating tail. But it was not proved that these amino acid increases resulted directly from protein breakdown.

During the early development of sea-urchin embryos, there are rapid fluctuations in free amino acid concentration (Kavanau, 1954) suggesting that the balance between rates of protein breakdown and synthesis is constantly changing. This is not surprising in an embryo that has no external source of nitrogen and therefore synthesizes much of its new protein during development at the expense of storage material such as the yolk. Associated with the breakdown of yolk and other proteins, one might expect also to observe a high

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catheptic activity in intra-embryonic tissues of most anamniote vertebrates, just as there is in the yolk sac of the chick embryo (Borger & Peters, 1933; Mystkowski, 1936). The evidence so far is scanty, however. Løvtrup (1955), Urbani (1955), and Vecchioli (1956) found only low catheptic activities in whole amphibian embryos, compared with larval stages. Some evidence of regional differences in the embryo is reported by d’Amelio & Ceas (1957), who find that in the early gastrula of Discoglossus pictus the dorsal lip has a higher catheptic activity than ventral tissue.

The dorsal parts of Xenopus embryos show, per unit dry weight, higher concentrations of free amino acids than ventral parts (Deuchar, 1956). Since this suggests that the relative rate of protein breakdown may be highest in dorsal parts, the catheptic activities of these same embryonic parts have been compared in the present work. The comparisons have been made first in absolute terms, then per unit dry weight (including fat) in order to compare them with the amino acid concentrations. But since the unequal distribution of yolk in dorsal and ventral parts greatly influences dry weight data (cf. Gregg & Løvtrup, 1950), the number of cells—and hence the catheptic activity per cell—has been estimated for each embryonic part as an alternative way of expressing the results. The catheptic activities of yolk and supernatant have also been compared after centrifugation of homogenates, as a possible clue to how closely linked the cathepsins are with yolk breakdown. Finally, an attempt has been made to characterize the cathepsins of these embryos more precisely by testing their reaction to cysteine, an activator of the mammalian cathepsins II and III (Bergmann & Fruton, 1941).

MATERIAL AND METHODS

Preparation of samples. All glassware used in these experiments had been sterilized, and the salines and distilled water were autoclaved. Xenopus embryos were demembranated and dissected in Holtfreter’s saline. Early gastrulae (stage 10 of Nieuwkoop & Faber, 1956) were divided into two parts: dorsal lip and remainder. Late gastrulae, early neurulae, late neurulae, and tail-bud stages (Nieuwkoop & Faber’s stages 12½, 14–15, 19, and 25–26) were divided into dorsal and ventral portions by horizontal cuts opening the gut along its length. Gut-roof endoderm was therefore included in the dorsal part with axial mesoderm and neural tissue, while the ventral part included gut-floor endoderm and all the lateroventral mesoderm and epidermis. The embryonic parts were collected in groups of from 3 to 40 according to the experiment, transferred to glass-distilled water, then homogenized in the minimum of glass-distilled water in a ground-glass homogenizer on an ice-bath. Any material sticking to the homogenizer rod was washed into the tube with further distilled water, and at the same time the homogenate was adjusted to a convenient volume. For all volume adjustments and the taking of aliquots of homogenate or reagents,
Carlsberg-type constriction pipettes (Linderstrøm-Lang & Holter, 1933) with siliconed inner surfaces were used. As few as 3 embryonic parts homogenized in 100 μl. could serve as a sample for determining catheptic activity, but when dry weights were also to be estimated, larger samples (40 parts) were necessary.

**Determination of catheptic activity.** The method was based on that of Duspiva (1939), and has been described more fully by Deuchar, Weber, & Lehmann (1957). Determinations were made on duplicate aliquots of homogenate and these duplicates did not differ more than 2 per cent. in activity. The reaction mixture consisted of 33.3 μl. of homogenate with 112 μl. of a 1:1 mixture of 2 per cent. casein with phosphate-citrate-ammonia buffer solution (Duspiva, 1939). The pH of the mixture was 4.5 (preliminary experiments having shown that the catheptic activity was maximal at this pH). For convenience, a long incubation time of 18 hours at 38°C. was used. (A serum-broth test for bacterial infection, carried out on one set of samples at the end of the incubation period, proved negative.) 1,000 μl. of cold 5 per cent. trichloro-acetic acid (TCA) was then added to all tubes and they were left for at least 2 hours in the refrigerator to complete the precipitation of undissolved casein. After centrifugation at 3,500 r.p.m. for 25 minutes, a 1,000 μl. aliquot of each supernatant was taken. To this, 1,000 μl. of 13.5 per cent. sodium carbonate solution was added, followed by 1,000 μl. of Folin-Ciocalteu reagent, diluted 1/5. After 1/2 hour the extinction value was read in a Hilger Uvispek spectrophotometer at 780 mμ, using distilled water as reference blank. Reagent blanks (casein/buffer with an aliquot of distilled water instead of homogenate) were incubated with each experimental series, and their mean extinction subtracted from all experimental values. In some experiments casein and homogenate blanks were also set up. These contained exactly the same mixture as experimental samples but had TCA added to them at once, and were not incubated. Since these blanks gave extinction values that were only 5 per cent. of the final experimental values, no extra correction was introduced except when homogenates of widely differing material were being compared.

**Expression of enzyme activity.** A test with known dilutions of a standard homogenate of *Xenopus* late gastrulae showed that under the present assay conditions the extinction values at 780 mμ were not proportional to homogenate concentration except over a very narrow range of dry weight (Text-fig. 1A). Therefore for quantitative comparisons between different embryonic tissues and stages it was necessary to convert the spectrophotometer readings into other units that bore some proportionate relation to enzyme activity. The possibility that after so long an incubation as 18 hours the enzyme reaction would be inhibited by the accumulated products of casein hydrolysis or by other substances had also to be allowed for. The time-curve for a standard homogenate of late gastrulae (Text-fig. 2A) shows, in fact, a flattening as early as 8 hours, and tends towards an asymptote at the final extinction value of 0.52. Now when this terminal value 0.52 is substituted for \( a \) in the formula relating extinction
to time for a first-order reaction (viz. \( kt = \log e \frac{a}{a-x} \), where \( x \) = the extinction value at any time \( t \)) then a plot of \( \log e \frac{a}{a-x} \) against time for the same standard homogenate (Text-fig. 2B) is approximately linear up to 18 hours. The reaction thus behaves as though it were first-order up to this time and it would then be

**Text-fig. 1.** A: Extinction values at 780 m\( \mu \) plotted against dry weight, for a standard homogenate of late gastrulae. B: \( \log e \frac{a}{a-x} \) plotted against dry weight for the same homogenate \((a = 0.52; x = \text{extinction value})\).

expected that a plot of \( \log e \frac{a}{a-x} \) against homogenate concentration \((a = 0.52\) as above; \( x = \text{extinction at any given concentration}\)) would also be linear. Text-fig. 1B shows this to be true over a wide range for the data of Text-fig. 1. Making the assumptions that these findings hold for all homogenates, the value \( \log e \frac{a}{a-x} \) can be used as an 'activity value', i.e. a proportionate measure of enzyme activity. Since the time-curves for homogenates of a number of different
TEXT-FIG. 2. A: Extinction values at 780 m\(\mu\) plotted against time, for a standard homogenate of late gastrulae. B: \(\log_e \frac{a}{a-x}\) plotted against time, for the same homogenate during the first 30 hours \((a = 0.52; x = \text{extinction value})\).

TEXT-FIG. 3. Extinction values plotted against time, for different embryonic stages and parts. (Data up to 30 hours only.)
embryonic stages and parts (Text-fig. 3) appear to tend towards the same asymptote at extinction 0.52, and since all the graphs of Text-fig. 4 are reasonably linear, the above assumption seems justified. All spectrophotometer readings (x) obtained in the experiments have therefore been converted into activity values by means of the same formula, and are quoted as such in the tables and figures.

Cell counts. The procedure of Sze (1955) was followed. Five embryonic parts were collected in 60 µl. of 1 per cent. citric acid in a microtube (capacity 300 µl.). After half an hour 60 µl. of 1 per cent. orcein in 45 per cent. (v./v.) acetic acid was added. The tissues were then broken up into free cells and nuclei with a glass rod fitting the inner dimensions of the tube. Care was taken to retrieve cells adhering either to the rod or to the walls of the tube, so that only negligible losses occurred. The number of cells and free nuclei was counted in 5 samples of the dispersion on a haemocytometer slide. At least 12 dispersions of each embryonic part were counted in the same way, and hence the mean numbers of cells per part were calculated.

Centrifugation of yolk. Whole neurulae were washed and homogenized in 0.28 M sucrose solution, buffered to pH 7 with M/15 phosphate buffer (Deuchar, 1953). After taking aliquots of the whole homogenate for determination of catheptic activity, the remainder was centrifuged at 3,500 r.p.m. on a semimicro angle centrifuge (Baird & Tatlock Ltd., London), for 1½ minutes. This was the minimum time required to throw down a compact mass of yolk. The slightly cloudy supernatant was removed, and the yolk mass was washed twice by stirring it with 25 times its volume of buffered sucrose solution and recentrifuging. Aliquots of supernatant and yolk were used separately for assays of catheptic activity. (The volumes of original homogenate, supernatant, and yolk were adjusted so that each 33.3 µl. aliquot would be equivalent to the contents of one embryo.)

Dry weights. Known volumes of the homogenate remainders, after removal of aliquots for cathepsin assays, were transferred to weighing bottles and weighed on a balance accurate to 0.1 mg., after drying for 2 hours at 100°C. The dry weight per aliquot was then calculated.

EXPERIMENTAL RESULTS

CATHEPTIC ACTIVITY IN WHOLE EMBRYOS AND PARTS OF EMBRYOS COMPARED IN ABSOLUTE TERMS

A homogenate aliquot equivalent to one whole embryo or one part was used for each assay of catheptic activity. The results (means of 5 determinations on whole embryos of each stage, and 10 determinations on each embryonic part) are given in Table 1.

WHOLE EMBRYOS. Late gastrulae showed a slightly higher catheptic activity than early gastrulae, and early neurulae a higher activity than late gastrulae (P < 0.01 for both these differences). Late neurulae had, on the other hand, a
lower catheptic activity than early neurulae \((P < 0.001)\), then tail-bud stages showed again a slight increase in activity \((P < 0.05)\).

*Parts of embryos.* At all stages ventral parts showed a higher catheptic activity than dorsal parts. The difference was greatest in the early gastrula, and less marked but still significant in neurulae \((P < 0.01)\) for the dorsoventral differences in the late neurulae and tail-bud stages, and for all other stages \(P < 0.001\).

*Catheptic activity per unit dry weight, compared between dorsal and ventral parts of the embryo*

Each of the comparisons was made between homogenates of dorsal or ventral tissues over a series of different dilutions. Glass-distilled water was used as diluent, and homogenates of ventral parts were diluted 3 times as much as those of dorsal parts in order to obtain comparable ranges of dry weight in the two series. The results, plotted as graphs of activity values against dry weight (Text-fig. 4), show clearly that at each embryonic stage the activity per unit dry weight is highest in the dorsal part. Each pair of curves represents the results of only one experiment, but at least two repeat experiments were made for each stage, and the activity was always significantly higher per unit dry weight in the dorsal parts \((P < 0.05)\) for early gastrulae; \(P < 0.01\) for all other stages).

*Catheptic activities per cell*

The mean numbers of cells, estimated from counts of 12–14 samples of each embryonic part, are given in Table 1. As it was not possible to count cells in aliquots of the same homogenates used for cathepsin assays, only the mean cell numbers and mean activities could be used for estimates of the catheptic activity per cell. The resulting data (right-hand column of Table) are therefore only approximations since their variability is unknown. However, it is clear that except in the early gastrula the cell numbers are always highest in the dorsal part of the embryo \((P < 0.001)\), whereas the absolute catheptic activity, as already remarked, is always highest in the ventral part. Hence there can be no doubt whatever of the much higher catheptic activity *per cell* in ventral parts of the embryo.

*Distribution of catheptic activity between yolk and yolk-free parts of the cells*

Since the original volumes of homogenate, supernatant, and washed yolk platelets had been adjusted (see Methods) so that each \(33.3 \mu l\) aliquot was the equivalent of 1 early neurula's total contents, non-yolk, contents, or yolk contents, the proportionate distribution of catheptic activity between yolk and other cell constituents is clear from a direct comparison of the activity units (bottom two rows of Table 2). It should be mentioned that haemocytometer counts showed the supernatant aliquots to contain, on an average, 500 small yolk platelets per \(\mu l\), as compared with 70,000 platelets of all sizes per \(\mu l\) in the precipitate aliquots. The good agreement between the data for different samples
Text-fig. 4. Catheptic activity plotted against dry weight, for dorsal and ventral parts of embryos.
indicates that the centrifuging procedure, although very brief, was accurately reproducible. Reasonable agreement between yolk platelet counts on the various samples confirmed this. As Table 2 shows, there is clearly a much higher activity

### Table 1

*Catheptic activity and numbers of cells in whole embryos and dorsal and ventral parts*

(For number of determinations per mean see text)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Region</th>
<th>Catheptic activity*</th>
<th>Number of cells†</th>
<th>Catheptic activity per cell (× 10⁵)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early gastrula</td>
<td>Whole</td>
<td>2.445 ± 0.075</td>
<td>30,700 ± 2,000</td>
<td>7.964</td>
</tr>
<tr>
<td></td>
<td>Dorsal lip</td>
<td>0.455 ± 0.030</td>
<td>16,100 ± 1,200</td>
<td>2.826</td>
</tr>
<tr>
<td></td>
<td>Ventral part</td>
<td>1.820 ± 0.069</td>
<td>14,600 ± 900</td>
<td>12.466</td>
</tr>
<tr>
<td>Late gastrula</td>
<td>Whole</td>
<td>2.828 ± 0.076</td>
<td>52,600 ± 1,700</td>
<td>5.376</td>
</tr>
<tr>
<td></td>
<td>Dorsal half</td>
<td>0.663 ± 0.051</td>
<td>32,400 ± 1,200</td>
<td>2.046</td>
</tr>
<tr>
<td></td>
<td>Ventral half</td>
<td>1.731 ± 0.035</td>
<td>20,200 ± 900</td>
<td>8.569</td>
</tr>
<tr>
<td>Early neurula</td>
<td>Whole</td>
<td>3.518 ± 0.095</td>
<td>181,000 ± 12,800</td>
<td>1.944</td>
</tr>
<tr>
<td></td>
<td>Dorsal half</td>
<td>1.082 ± 0.067</td>
<td>112,100 ± 8,000</td>
<td>0.965</td>
</tr>
<tr>
<td></td>
<td>Ventral half</td>
<td>1.577 ± 0.091</td>
<td>69,000 ± 5,100</td>
<td>2.286</td>
</tr>
<tr>
<td>Late neurula</td>
<td>Whole</td>
<td>2.740 ± 0.093</td>
<td>184,300 ± 12,000</td>
<td>1.487</td>
</tr>
<tr>
<td></td>
<td>Dorsal half</td>
<td>1.128 ± 0.083</td>
<td>118,400 ± 7,900</td>
<td>0.953</td>
</tr>
<tr>
<td></td>
<td>Ventral half</td>
<td>1.406 ± 0.108</td>
<td>65,900 ± 4,300</td>
<td>2.134</td>
</tr>
<tr>
<td>Tail-bud stage</td>
<td>Whole</td>
<td>3.334 ± 0.223</td>
<td>207,900 ± 13,700</td>
<td>1.604</td>
</tr>
<tr>
<td></td>
<td>Dorsal half</td>
<td>0.908 ± 0.060</td>
<td>136,400 ± 9,330</td>
<td>0.666</td>
</tr>
<tr>
<td></td>
<td>Ventral half</td>
<td>1.849 ± 0.244</td>
<td>55,000 ± 6,700</td>
<td>3.362</td>
</tr>
</tbody>
</table>

* Values for whole embryos obtained by a different set of assays from those on parts of embryos.
† Figures for whole embryos by summing individual data for parts of embryos.

### Table 2

*Distribution of catheptic activity between cell contents*

(A–E are separate homogenates, each equivalent to one whole early neurula)

<table>
<thead>
<tr>
<th>Activity</th>
<th>Mean and standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.776 ± 0.031</td>
</tr>
<tr>
<td>B</td>
<td>1.800 ± 0.031</td>
</tr>
<tr>
<td>C</td>
<td>1.776 ± 0.031</td>
</tr>
<tr>
<td>D</td>
<td>1.835 ± 0.031</td>
</tr>
<tr>
<td>E</td>
<td>1.721 ± 0.031</td>
</tr>
</tbody>
</table>

in the supernatant than in the yolk (P < 0.001), but the activity remaining bound to the washed yolk is nevertheless considerable (about 40 per cent. of the activity in the supernatant). The data on whole homogenates are not comparable with those for early neurulae in Table 1 since they refer to different batches of eggs, but a preliminary experiment had shown that there was no significant difference
between the catheptic activities of comparable embryos homogenized in distilled water and in buffered sucrose.

Effects of cysteine on catheptic activity

11.3 µl. aliquots of 0.033 M solution of cysteine-hydrochloride in Duspiva buffer were added to 33.3 µl. aliquots of homogenates of whole gastrulae immediately before incubating with casein BUFFER solution. Hence the final concentration of cysteine-hydrochloride in the incubation mixture was 0.0024 M. The buffer solution had to be readjusted to produce the optimal pH of 4.5 as in all other incubations. Control aliquots of the same homogenates had 11.3 µl. of pure buffer solution, without cysteine, added to them. Blanks (distilled water instead of homogenate) both with and without cysteine were also incubated. The catheptic activity was much higher in the samples with added cysteine than in the controls. (Mean activity per homogenate sample was 0.982 ± 0.050 without cysteine; 2.070 ± 0.150 with cysteine added; P < 0.01 for the difference.) The incubation time in this experiment was 4 hours.

DISCUSSION

Although Lövtrup (1955), Urbani (1955), and Vecchioli (1956) found only low and either constant or slightly decreasing catheptic activity in successive pre-tail-bud stages of whole amphibian embryos, the results presented here (Table 1) show that significant increases do occur. Since these are small in terms of extinction values, they may not have been detected by the earlier authors merely because their assays were adjusted to sensitivities suitable for the much higher catheptic activity in larvae. In the present results the increases of catheptic activity during gastrulation and neurulation are specially interesting since this is the time when the developmental fates of the embryonic tissues become determined and, in newt embryos, some new tissue-specific antigens appear (Clayton, 1953). So the rise in catheptic activity may have some relation to qualitative changes in the proteins. Since the only evidence we have on the function of cathepsins in *Xenopus* (Weber, 1957) suggests that they are proteolytic, they are probably best thought of in the embryo as mobilizing raw materials for synthesis, by the breakdown of pre-existing protein such as the yolk.

A striking feature of the regional differences in catheptic activity per unit dry weight is how closely they correspond (Text-fig. 5) to regional differences in free amino acid concentration (Deuchar, 1956). The late gastrula is the only exception, with a higher catheptic activity in its dorsal part, while there is no predominance of free amino acid here until the early neurula stage. This is reminiscent of the sequence of events observed in regenerating tail-tips of *Xenopus* larvae (Deuchar, Weber, & Lehmann, 1957), where high catheptic activity preceded increases in free amino acid. It may again be postulated (see Introduction) that the free amino acid increases result from proteolysis by cathepsins, but further evidence is needed to establish this.
Unfortunately it was not possible to measure concentrations of free amino acid per embryonic part or per cell, for comparison with the absolute catheptic activities and activities per cell. But since all these ventral embryonic parts have at least twice the dry weight (including fat) of the corresponding dorsal parts, it was clear from earlier data that the total free amino acid per ventral part must, like the catheptic activity, be much higher than per dorsal part. A small additional experiment (results summarized in Table 3) has confirmed this estimate.

### Table 3

**Concentration (expressed as extinctions at 510 mμ after ninhydrin reaction; Benz, 1957) of total free amino acid in dorsal and ventral parts of embryos**

<table>
<thead>
<tr>
<th>Stage of Development</th>
<th>Total Free Amino Acids Per mg. Dry Wt.</th>
<th>Catheptic Activity Per mg. Dry Wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early gastrula</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
</tr>
<tr>
<td>Late gastrula</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
</tr>
<tr>
<td>Early neurula</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
</tr>
<tr>
<td>Late neurula</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
</tr>
<tr>
<td>Tail-bud stage</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
</tr>
</tbody>
</table>

**Text-fig. 5.** Diagrammatic comparison of amino acid concentrations and catheptic activity per unit dry weight, in dorsal and ventral parts of embryos. Black areas: significantly higher values than white areas of the same embryo.
It follows that the free amino acid concentrations per cell are also, like the catheptic activity per cell, highest in the ventral parts of the embryos. This close parallel between the distributions of catheptic activity and free amino acid, whichever of these three different ways the data are expressed, lends support to the idea that cathepsins produce free amino acids in the embryo.

One remarkable general feature of the results is the complete inversion of dorsal/ventral differences according to whether they are expressed per unit dry weight or per cell and per part. Gregg & Lovtrup (1950) and Pickford (1943) have already demonstrated that quantitative comparisons between parts of embryos are greatly affected by the yolk content. They preferred to refer their data to 'non-yolk nitrogen' or 'extractable nitrogen' instead of to dry weight or total nitrogen, and other authors (cf. d'Amelio & Ceas, 1957) have followed suit. The idea underlying this preference is that the yolk not only contributes heavily to dry weight and total nitrogen, but is also relatively inert enzymatically. But this last contention can no longer be held, at least with respect to proteolytic enzymes. Harris (1946), Barth & Barth (1954), and Flickinger (1956) have demonstrated phosphoprotein breakdown in amphibian yolk, free of other cell constituents. In recent electron-microscope and solubility studies Gross & Gilbert (1956) have gone even further in demonstrating the complexity of yolk platelets, and speak of them as 'organelles'. Finally, in the present work the centrifugation experiments have shown considerable catheptic activity in the yolk fraction. The reference basis chosen for expressing quantitative data must depend on the particular viewpoint of each author. Here, while the data referred to dry weight show that dorsal parts of *Xenopus* embryos have the highest proportion of catheptic activity to total organic material, the data per part emphasize, in contrast, that there is, in sum, more catheptic activity in ventral parts. Finally, the per cell basis has been used because embryonic cells are undoubtedly important functional units, capable of much independent behaviour and differentiation. It has been specially interesting to demonstrate that ventral tissues, which are so often placed at the bottom of gradients in amphibian embryos that one has come almost to believe that they are in some way inferior, have in fact a higher catheptic activity per cell than dorsal tissues. A further interesting point emerging from the counts of cells is that the total cell numbers per embryo agree very closely with those in corresponding stages of *Rana pipiens* (Sze, 1953).

It is possible that the distribution of catheptic activity is related to the absolute numbers of yolk platelets in the cells and to the relative need for yolk breakdown. In chick embryos (Borger & Peters, 1933; Mystkowski, 1936; Emanuelsson, 1955) cathepsins are concentrated mainly in the yolk-sac, which is the chief site of yolk breakdown. In amphibians, however, the times and places of greatest yolk breakdown are not clearly known. It has been held (Bragg, 1939; Konopacki & Konopacka, 1926) that no appreciable breakdown of yolk platelets occurs until the late neurula stage, and that it then takes place mainly in the dorsal
tissues, but this view has never been supported by any clear quantitative observations. On the other hand, Daniel & Yarwood (1939) observed histological signs of yolk-breakdown as early as the zygote stage in newts. Moreover, recent electron-microscope studies (Yamada, pers. comm.; Bellairs, 1958) have shown in preneurula stages of newt and chick embryos some ultrastructural changes in the yolk platelets that may be the beginnings of their breakdown. It seems probable, then, that at the stages in which the catheptic activity has been studied here, some degree of yolk breakdown is taking place. The high proportion of catheptic activity remaining in the yolk fraction, even after thorough washing, suggests that in *Xenopus* the cathepsins are concerned in this process of yolk utilization. Some component of the catheptic system may even be an integral part of the yolk platelet.

It is known (Barth & Barth, 1954; Flickinger, 1956) that a phosphoprotein phosphatase plays an important part in yolk breakdown in *Rana* eggs, and it seems reasonable to believe that cathepsins act in conjunction with this enzyme. Casein, which is itself a phosphoprotein with many biochemical properties similar to vitellin, the predominant protein of yolk (Needham, 1931), is readily split by standard phosphatase preparations only after prior treatment with protease (Schmidt & Thannhauser, 1943). One might therefore suggest that yolk behaves like casein and that yolk breakdown takes place in two steps: first, a proteolysis carried out by cathepsins, and, secondly, a splitting off of phosphate by phosphoprotein phosphatase.

**SUMMARY**

1. The total catheptic activity per embryo in *X. laevis* increases during gastrulation and early neurulation, decreases slightly during late neurulation, then increases again at the early tail-bud stage.

2. When dorsal and ventral parts of gastrulae, neurulae, and tail-bud stages are compared, the catheptic activity per unit dry weight is highest in dorsal parts, but the absolute activity per part, and the activity per cell, are highest in ventral parts of the embryos.

3. The catheptic activity is increased in the presence of 0·0024 M cysteine-hydrochloride.

4. The yolk platelets, separated from other cell-constituents by centrifuging, carry a considerable proportion of the catheptic activity, though not as much as the supernatant fraction.

5. The possibilities that the catheptic activity gives rise to increased concentrations of free amino acids and that it assists in yolk breakdown are discussed.

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