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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V. D'AMELIO and M. P. CEAS

Laboratory of Comparative Anatomy, The University of Palermo, Italy, December 27, 1956.

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

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

the first signs of metamorphosis appear (Fig. 1). In the present group of larvae, this happened at an age of 41 (1 larva), 46 (2 larvae) and 47 days (1 larva) respectively,

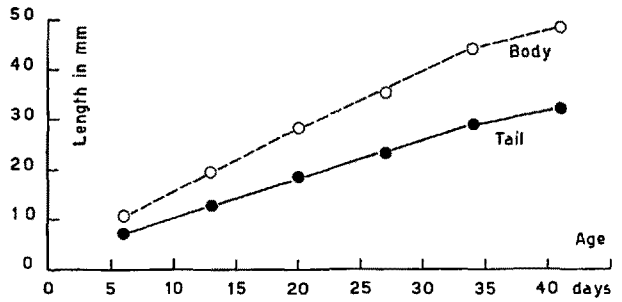

Fig. 1. — *Growth curves for body and tail lengths of Xenopus larvae.* Each point represents the mean of 4 larvae raised at 20°C.

at a mean tail length of 32·3 mm. In contrast to the rather extended growth period, tail resorption occupies a few days only (Fig. 2). The maximum tail length (L_{max}) is attained at the beginning of metamorphosis.

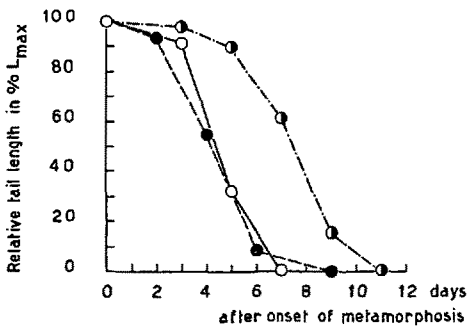

Fig. 2. — *Reduction of tail length during metamorphosis.* Each curve shows the tail reduction for one larve at 20°C.

Actually, the rate of tail resorption as judged from the decreasing tail length is not constant and extends over varying lengths of time. In all cases, after a slow initial reduction, resorption sets in at a great speed, thus producing a decrease in tail length of about 70% within only four days.

Since at a given temperature growth rate and the beginning of metamorphosis depend upon the state of nutrition[4], tail length should provide a more reliable reference than age for larval stages.

Catheptic activity during growth and resorption of tails. There is a greater variation in specific activity within a given batch of larvae than between larvae from different batches, but in relation to tail length a general trend of the curve is evident (Fig. 3). If all larvae up to 29 mm tail length are considered, the regression line indicates a falling specific activity of cathepsin in relation to increasing tail length. On the other hand, the regression line, referring to the larvae $>$ 29 mm tail length up to the onset of metamorphosis, demonstrates an increasing trend of the specific activity in pre-metamorphosis larvae. From the t-test, the difference between the mean specific activities for larvae with tail lengths $>$ 15–29 mm and $>$ 29–L_{max} was found to be highly significant ($1‰ > P > 1\%$), thus demonstrating a higher level of catheptic activity in tail tissue of pre-metamorphosis larvae.

During metamorphosis, when the larval tail is resorbed by autolysis, the specific activity shows an almost exponential increase (Fig. 4). In the last resorption

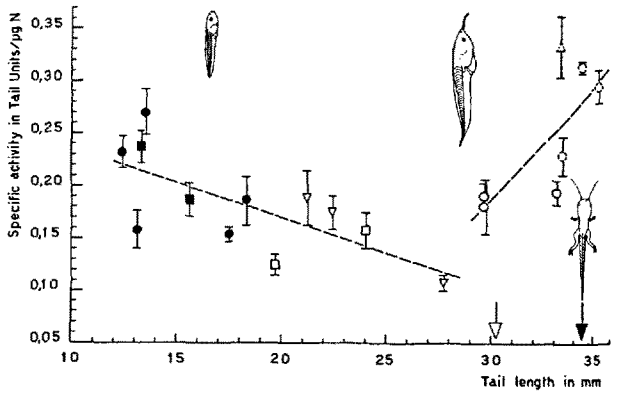

Fig. 3. — *Specific catheptic activity during tail growth.* Larvae from different batches are indicated by special signs, white ones referring to individual and black ones representing 3 larvae. Signs with broken lines indicate larvae at the beginning of metamorphosis. The black arrow marks the mean maximum length and the white one gives the minimum length of tails, observed in 34 larvae with erupted fore-limbs. The vertical lines represent the standard error of the specific activity.

stages, the activity is about 22 times higher than at the beginning of metamorphosis.

These experiments show: (1) that *tail growth* (i.e. formation of tissue proteins) is characterised by a *decrease in catheptic activity* per unit total nitrogen; (2) that this

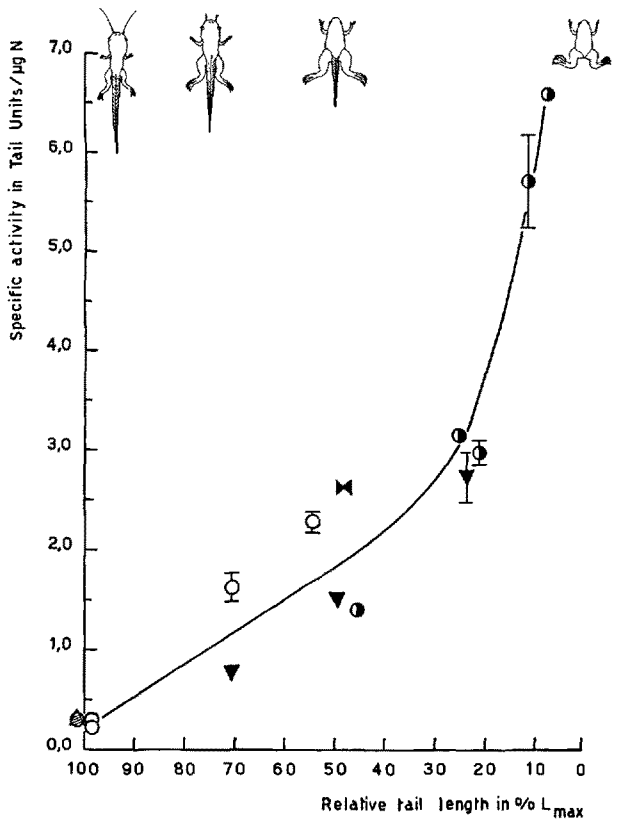

Fig. 4. — *Specific catheptic activity during tail resorption.* All signs refer to individual larvae; their maximum tail length (L_{max}) was fixed as 100%. The standard error is only indicated in those cases where it exceeds the diameter of the sign.

trend is, however, *changed* in *pre-metamorphosis larvae*, when the tails are still growing and as yet no signs of tissue resorption are detectable; (3) that, concomitantly with progressive *tail resorption* (metamorphosis), a spectacular *increase* in *catheptic activity* takes place. Hence, there is good evidence to assign a predominantly *catabolic function* to the *cathepsin system* in *tail tissue* of *Xenopus* larvae. It is not yet known which factors control catheptic activity, but preliminary results, obtained by paper chromatography of methanol extracts of tails, revealed remarkably different patterns of amino acids and peptides in growing and resorption stages. Finally, the high level of activity observed in pre-metamorphosis larvae could reflect a physiological change in the tissue. It may have some relation to the onset of 'competence'[3] in the tail tissue, i.e. its reactivity to metamorphosis stimuli. This hypothesis, however, remains to be confirmed by further experiments.

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R. WEBER

Zoological Institute, University of Bern (Switzerland) December 21, 1956.

Zusammenfassung

Die Bestimmung der Kathepsinaktivität an wachsenden und in Rückbildung begriffenen Schwänzen von *Xenopus*larven mittels der von DUSPIVA[5] ausgearbeiteten Casein-Harnstoffmethode führte zur Feststellung, dass dem Kathepsinsystem eine vorwiegend proteolytische Funktion zukommt.

Anoxygene Reaktivierung der Zellvermehrung nach anoxybiotisch hervorgerufener Zytostase (Anabiose)

Aerob ausschliesslich atmende Zellen (Atmungszellen) sind ausserstande, sich ohne die Mitwirkung von Sauerstoff fortzupflanzen[1]. Aerob gärende Zellen dagegen (Gärungszellen) bleiben im O_2-freien Substrat noch über eine Reihe von Passagen partiell vermehrungsfähig, verlieren aber von Generation zu Generation sukzessiv an Proliferationskraft, bis sie gänzlich zu wachsen aufhören (Anabiose)[2]. Dass es *nicht* die Gärung ist, welche die Energie für das partielle Wachstum in der Anoxybiose liefert, geht experimentell daraus hervor, dass die anabiotischen Zellen unbeeinträchtigt weitergären[3], wobei auch nicht ein Substratwechsel (Entfernung der Gärungshemmstoffe, erneute Zuführung von Akzessorien) die Zytostase aufzuheben vermag. Erst wenn dem System wieder Sauerstoff zugeleitet wird, tritt reversibel Zellvermehrung ein und später sogar, nach kontinuierlicher O_2-Einwirkung, partielle Proliferation unter anaeroben Bedingungen.

Der in der Anoxybiose diminutiv verlaufende Wachstumsprozess der Gärungszellen und die oxydative Reaktivierbarkeit ihrer anaeroben Proliferationsfähigkeit nach eingetretener Anabiose sind nicht anders zu erklären als durch das Vorhandensein eines anoxygenen Energiepotentials, dessen respiratorische Entstehung und Akkumulation, vom anabiotischen Zellstadium ausgehend, sich auch tatsächlich auf chromoanalytischem Wege nachweisen lässt[4]. Evidenter noch tritt – mit der Testschärfe des von uns ausgearbeiteten histochemischen M-T-Verfahrens[5] – das anoxygene Energiepotential bei bestimmten zytoplasmatischen Reaktionen des mikrobiellen Phosphatwechsels in Erscheinung[6].

Auf der experimentellen Suche nach dem Energiereservestoff – der in der Anoxybiose synthetische Zellreaktionen (endergonisch) ermöglicht, im Verlaufe der Diminutionszüchtung aufgezehrt und bei darauffolgender Oxybiose erneut gebildet wird – leiteten wir die entsprechenden Isolierungsmassnahmen in der Weise ein, dass wir aus den normalen Gärungs- und Atmungszellen nach bekannter Methode[7] Zellkochsäfte herstellten. Diese liessen wir alsdann, unter Wahrung strengster (doppelt gesicherter) anaerober Versuchsbedingungen, auf anabiotisches Zellmaterial in vermehrungsfähiger Aussaat einwirken.

In den als Beleg angeführten Fällen (Tabelle) erfolgte die Bereitung der Zellkochsäfte aus *Saccharomyces carlsbergensis* Rasse U, *Torulopsis* Stamm S und *Candida Mycoderma* (REESS). Zur Kontrolle wurden in jeder Gruppe Proben mit Malzwürze (10%ig) vorgenommen. Als Testzellen dienten gleichfalls die oben angegebenen drei Hefearten, die erstere bis zur Anabiose diminutiv gezüchtet, die beiden folgenden in desoxygenem Zustand verwendet. Sämtliche Zellkochsäfte gelangten im Verschnitt 1:1 mit 15%iger Vorderwürze zum anoxybiotischen Ansatz. Die kritischen Proliferationsprüfungen führten wir – unter galvanometrischer O_2-Spurenkontrolle nach TÖDT[8] – in einer hermetisch abgeschlossenen Anoxybiose-Apparatur[9] durch. Während der zwischenzeitlichen Bebrütungsstadien waren die Kultivierungskolben in gasdicht isolierten, mit Reinstickstoff beschickten Sammelbehältern abgestellt. Das entscheidende *generative* Ergebnis, um das es sich vorliegend zunächst handelt, zeichnet sich beim Vergleich der charakteristischen Proliferationsdaten (Tabelle) zwischen den einzelnen Gruppen und innerhalb jeder Zellkategorie mit aller Deutlichkeit ab.

Wertet man in umstehender Tabelle die erste Gruppe (*Sacch. carlsbergensis* Rasse U) zunächst für sich allein aus, so leitet sich – auf Grund der experimentellen Endvermehrungszahlen – der Nachweis daraus ab, dass im Zellkochsaft der Gärungs- und Atmungszellen ein thermostabiler Reserveenergiestoff enthalten ist, der unter anoxygenen Bedingungen die Zellvermehrung nach eingetretener Anabiose zu reaktivieren oder, biodynamisch ausgedrückt, die respiratorische Funktion der ergonischen Reaktionskoppelung zu substituieren vermag. Im

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