pronounced in fishes which are subject to higher dosage of DDT (30 p.p.m.). Such changes did not occur at very low dosages (3 p.p.m.).

(d) *Barbus stigma*. The infected hepatic cells were scattered in distribution. Necrosis of liver cells, parenchymatous degeneration and hypertrophy of hepatic cells were well marked. The vacuolation was not so much marked as in the case of the other fishes. The vacuolar degeneration of cytoplasm of liver cells was also less marked. The cells of the periphery were more dense. The lesins were more marked in the central than in peripheral area (30 p.p.m.). At places the cells had disappeared completely creating vacuity in the organ (Figure 5).

2. *The intestine*. The common pathological finding was the degeneration of the lining of the epithelium. Here and there the mucous membrane shows ruptures. In the circular and longitudinal muscles, a few vacuoles could be detected. In *Ophiocephalus punctatus* (Figure 6) and *Heteropneustes fossilis* there was a greater degeneration of the lining of epithelium than the marked damage of the mucosa layer. In mucosa and submucosa layers the vacuolation was more marked than that of the epithelium. A few villi along with the mucosa disappeared altogether. Here and there the goblet cells also disappear completely from the villi. The histopathological changes were more marked in *Ophiocephalus punctatus* and *Heteropneustes fossilis* than in *Barbus stigma* and *Trichogaster fasciatus*.

3. *The kidney*. A moderate degeneration of the epithelium and the loss of parenchymatous cells of the renal tubules was noticed. The microscopic changes were less pronounced in the kidney than those in the liver, the kidney being affected to a lesser extent. These changes were more pronounced in the kidney of *Ophiocephalus punctatus* (Figure 7) as compared with *Barbus stigma*, *Heteropneustes fossilis* and *Trichogaster fasciatus*.

Zusammenfassung. Es wurde der Einfluss von DDT auf verschiedene Organe von Fischen untersucht. Bei niederen Dosen (3 p.p.m.) wurden zelluläre Läsionen und Leberhypertrophie beobachtet und bei einer Dosis von 50 p.p.m. trat Nekrose ein. Symptome von Atrophie in der Niere wurden beobachtet, ebenso wurde die Darmschleimhaut beeinflusst.

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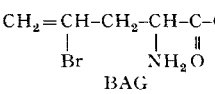
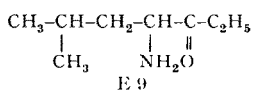
The Michaelis Constants of Catheptic Activity in Xenopus Tail Tissue after *in vivo* Treatment with Two Leucine Analogues[1]

The two structural analogues of leucine, γ-bromoallylglycine[2] (BAG) and 4-amino-6-methylheptanone-3[3] (aminoketone E 9), have recently been shown to have similar inhibitory effects on the development of the explanted chick embryo[4].



BAG



E 9

In embryos treated with either substance, the inhibited organ primordia showed a considerable increase in specific catheptic activity. E 9 has been shown to inhibit tail regeneration in Xenopus larvae with a concurrent increase in activity of cathepsins[5,6] and acid phosphatase[7]. An increase in proteolytic activity was also observed in tail tissue of unamputated larvae kept in the solution of the analogue, but not after direct incubation of tail tissue on casein in the presence of E 9[6]. It appeared therefore that we were dealing with an effect caused by a 'metabolic' action of the analogue on the enzyme-forming system or some pathway involving regulation of enzyme action, and not by a direct action of the analogue on the kinetic properties of the enzyme itself. In order to elucidate this point further, the effect of *in vivo* treatment with E 9 and BAG on the affinity of the cathepsins for the casein substrate (as measured by the Michaelis-Menten constant) was determined. If our idea was correct, no effect of *in vivo* treatment with E 9 on this constant was to be expected.

Experimental. Larvae of *Xenopus laevis*, 30–34 mm long, from the same batch of eggs, were kept in solutions of the analogues and in distilled water. For each experiment, 15 larvae of identical size were selected, and 5 each put into 200 ml water or analogue solution. The concentrations of analogue used are given in the Table I. After 5 days, during which the larvae were not fed, the entire tails were amputated, dried briefly on filter paper, pooled and weighed. They were then homogenized with enough 0.25 *M* sucrose (containing 0.001 *M* EDTA) to give 10% homogenates. Homogenization was carried out in a Teflon homogenizer cooled in ice. 8.8 µl aliquots were incubated on 60 µl casein solution, made up in 34% urea with McIlvaine buffer at pH 5.0. The final casein concentrations in the incubation mixture were 0%, 0.24%, 0.48%, 0.72%, and 0.96%. After $2^1/_2$ h at 38°C, the reaction was terminated by the addition of 100 µl 10% trichloroacetic acid, and the tubes kept over night in ice. After centrifugation, 100 µl of the supernatant were taken and the Folin-Ciocalteu reaction carried out (reading at 760 mµ). The results were plotted according to the modified equation of LINEWEAVER and BURK (see DIXON and WEBB[8]):

$$\frac{s}{v} = \frac{1}{V} \cdot s + \frac{Km}{V}$$

s = substrate concn.
v = reaction rate at s
V = maximal reaction rate
Km = Michaelis constant

were s/v is plotted against s. The intercept of the straight-line plot with the abscissa gives $-Km$. The regression

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lines were calculated for each series of points by standard statistical procedure, and the values of s calculated for $s/v = 0$. This is more accurate than the usual method of graphic extrapolation.

Tab. I. Michaelis constants of catheptic activity in Xenopus tail tissue after 5 days *in vivo* treatment with leucine analogues. Casein substrate

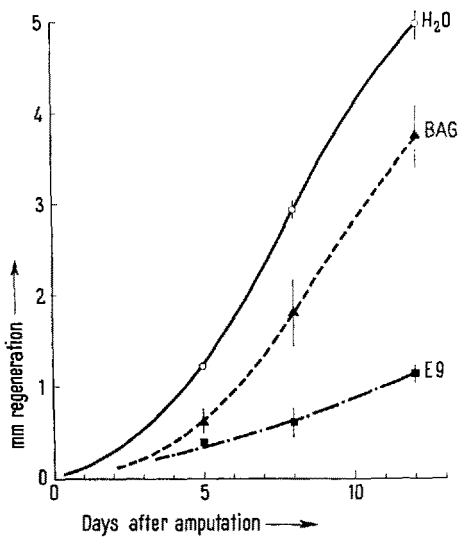
Experiment No.	Larvae in water	Larvae in E9		Larvae in BAG	
	Km	*Km*	Concentration $M \cdot 10^{-3}$	*Km*	Concentration $M \cdot 10^{-3}$
1	0.247%	0.275%	1.74	—	—
2	0.360%	0.387%	2.0	0.470%	0.5
3	0.270%	0.344%	2.0	0.415%	1.0
4	0.254%	0.281%	3.0	0.474%	2.0
Average	0.283%	0.322%		0.453%	
standard error	±0.026	±0.027		±0.019	

Significance of differences (t-test):

Water–E9:	$p > 0.3$	not significant
Water–BAG:	$p < 0.01$	significant
E9 –BAG:	$p = 0.015$	significant

Tab. II. Relative reaction rates of cathepsins on 1% casein after *in vivo* treatment with leucine analogues. The values are calculated as ΔE_{760} per h per mg fresh weight and given in % of the values for the untreated larvae in each experiment

Experiment No.	Larvae in water	Larvae in E9		Larvae in BAG	
			Concentration $M \cdot 10^{-3}$		Concentration $M \cdot 10^{-3}$
1	100	114	1.74	—	—
2	100	144	2.0	118	0.5
3	100	158	2.0	97	1.0
4	100	135	3.0	103	2.0



Regeneration of the amputated tail tip of Xenopus larvae kept in water (control regenerates), $2 \cdot 10^{-3} M$ BAG and $3 \cdot 10^{-3} M$ E9. Length of regenerates in mm. The points give the averages of 5 regenerates, and the vertical lines the standard error.

Results. The experimental values found for *Km* after E 9 treatment are not significantly different from the control values (larvae kept in water) (Table I). They seem to confirm the idea that this analogue does not affect the affinity between enzyme and substrate. Yet, as Table II shows, there is a considerable increase in catheptic activity (per unit fresh weight) in the E 9-treated tissue. We can therefore conclude that, since the relationship of enzyme to substrate has not been changed, any increase in enzymatic activity observed must result from an increase in actual enzyme content of the tissue. The possibility that the analogue had released the enzyme from a structurally bound inactive state can be disregarded, since under our conditions of assay the structures in question (the lysosomes), if they existed in our material, must have been totally ruptured in all homogenates.

In contrast, BAG significantly increased the value of *Km* (Table I), while not affecting the reaction rate (Table II). This is also in contrast to its effect on the chick embryo, where specific catheptic activity was increased up to 250%[4]. An increase in *Km* means a lowered affinity of the enzyme for the substrate, and BAG might therefore interfere directly at the level of the active sites of the enzyme. The possibility must be kept in mind that BAG might selectively inhibit one more of the enzymes that make up the complex called cathepsin. The lack of effect on the overall reaction rate is due to the sufficient excess of substrate present.

The morphological effects of E 9 and BAG on the regeneration of the amputated tail tip of Xenopus larvae is shown in the Figure. The length of the regenerates from the line of amputation along the chorda is given in mm (see LEHMANN[9] for details of the procedure). E 9 at $3 \cdot 10^{-3} M$ inhibits regeneration to about 80%, whereas BAG at $2 \cdot 10^{-3} M$ (both concentrations are the highest that can be used on Xenopus) gives less than 30% inhibition after 12 days. This again is in contrast to the effects of these analogues on the chick embryo, where BAG was more effective than E 9[4]. Here (as with the chick embryo[4]) there seems to be a correlation between the effect on catheptic activity and the extent of growth inhibition: the analogue giving higher proteolytic activity is also the stronger inhibitor. This again would seem to confirm the mechanism formulated by HAHN and HERRMANN[4], and already tentatively suggested by HAHN and LEHMANN[6]: that inhibition of protein accumulation can be obtained by increasing proteolysis without affecting protein synthesis.

The values for *Km* in normal, untreated tail tissue found in these experiments are considerably lower than both the value of 0.74% found by HAHN and HERRMANN on chick embryo homogenates[4] and that of ca. 1% found by BENZ and LEHMANN on rat liver, using nitrocasein as substrate[10]. Whereas this latter figure is probably due to the nature of the substrate used[11], the former result was obtained under the same experimental conditions as used in the present investigation on Xenopus. It can therefore tentatively be assumed that we are faced here with a species-specific difference in enzymatic characteristics.

Zusammenfassung. Die Michaelis-Konstante *Km* der katheptischen Aktivität im Schwanzgewebe von Xenopuslarven wird durch das stark regenerationshemmende

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leucinanaloge Aminoketon E 9 nicht beeinflusst, obwohl nach 5 Tagen *invivo*-Behandlung der Larven mit $3 \cdot 10^{-3} M$ E 9 ein starker Anstieg der spezifischen Kathepsinaktivität im Schwanzgewebe zu beobachten ist. Das leucinanaloge γ-Bromallylglycin (BAG) hemmt die Regeneration wenig, erhöht jedoch die *Km* deutlich. Es wird postuliert, dass E 9 eine Zunahme der Kathepsine im Schwanzgewebe bewirkt, ohne die kinetischen Eigenschaften dieser Enzyme zu beeinflussen, während BAG an den aktiven Zentren der Kathepsine wirkt und die Affinität zum Substrat herabsetzt.

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Electroretinographic Responses to Polarized Light in the Wolf-Spider *Arctosa variana* C. L. Kock[1]

The ability to make correct astronomical orientation, even in the shade, displayed by the wolf-spider *Arctosa variana* C.L.K. (Lycosidae), and the possibility of modifying consistently the escape direction by interposing and rotating a polaroid sheet between the spider and the sky, led PAPI[2] to conclude that this spider uses the plane of vibration of polarized light for orientation. A similar conclusion was reached by GOERNER[3] on the basis of oriented reactions of *Agelena labyrinthica* (Clerck) (Agelenidae).

The aim of the present experiments was to determine whether the electroretinogram (ERG) of *Arctosa variana* would show a differential response to light polarized in different planes.

This spider, like most others, has four pairs of simple eyes. The anterior median eyes (AM) differ from the other three pairs (indicated as anterior lateral, Al; posterior median, PM; posterior lateral, PL), both in their embryological origin and structure. Adult and subadult males and females were used. Under light chloroform anaesthesia, the animals were secured by means of adhesive tape to a small board, and all the eyes but the one under investigation were covered with a lightproof mixture of shellac and lamp black. A silver–silver chloride electrode (diameter approx. 100 μ) was inserted through a small hole in the cephalothorax near the eye under investigation; an 'indifferent' electrode was placed in contact with the abdomen. Conventional DC and AC amplifying and recording systems were used. A uniform source of non-polarized light, obtained by passing the light of a projector lamp down a bundle of glass fibres (American Optical Co.), was focused on the eye by means of a microscope objective. The axis of the light beam coincided as nearly as possible with the optical axis of the eye. A polaroid filter could be inserted between the microscope lens and the eye. Neutral filters could also be inserted in the path of the rays to control the intensity of stimulation. Flashes of light of durations from 10 msec up to several seconds could be delivered to the preparation by means of an electromagnetically operated camera shutter. Routinely, flashes of 100 msec were used at a repetition rate of 0.1/sec. In each experiment only one eye was investigated. At the end of each experiment the completeness of the covering of the other eyes was checked.

The following results were obtained: The EEG consists of a negative wave which has a latency of about 15 msec, rises to a maximum in about 120 msec, and then slowly declines in amplitude. At the end of stimulations lasting up to 250 msec, the potential falls to the baseline. For stimuli of longer durations, a positive overshoot appears at the end and becomes more prominent as the duration is increased. The amplitude of the response is linearly proportional to the logarithm of the stimulus intensity for any given duration. It seems likely that the response represents the sum of the generator potentials produced by the retinal receptor cells. This hypothesis is supported by the investigations performed by AUTRUM[4] on the electrical responses to light of several invertebrate species.

When *AM or PM eyes* are stimulated by a beam of *linearly polarized light*, the EEG shows a consistent and at times conspicuous difference in amplitude depending on the plane of polarized light. When the $\vec{e}$ vector of the beam lies in a plane normal to the ventral plane of the animal, the EEG is at a minimum. When the polaroid filter is rotated clockwise from this position, the response reaches a maximum at 90° and has intermediate amplitudes at 45° and 135°. The increase above the minimum may be as much as 100%. So far it has not been possible to find evidence of a similar phenomenon in the *AL and PL eyes*, although some minor and inconsistent differences in response have been observed.

These results are in agreement with the conclusion of PAPI that this animal uses polarized light for orientation and supports the hypothesis that the mechanism of analysis lies within the eye.

It remains to be determined whether these results can be explained by some physical characteristic of the cornea or optical apparatus of the eye, or by some property inherent in the retinal cells themselves.

Zusammenfassung. Registrierung der Elektroretinogramme jedes Auges der Wolfspinne *Arctosa variana* C.L.K. nach Reizung mit polarisiertem Licht. Bei Reizung der vorderen und hinteren Mittelaugen erhält man während der Polarizatordrehung Minima und Maxima. Der Effekt unterbleibt an den vorderen und hinteren Seitenaugen.

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Istituto di Fisiologia Umana e di Biologia Generale dell'Università di Pisa (Italy), July 16, 1962.

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