In allen Versuchen über Abbau der Angiotensine wurden die Amide (H und D) am raschesten inaktiviert. An zweiter Stelle bezüglich Empfindlichkeit gegen abbauende Fermente stand α-*L*-Asp¹-Angiotensin II (A). Von Rattenserum wurden die beiden Verbindungen B und C langsamer als die α-Verbindungen, untereinander jedoch mit ungefähr gleicher Geschwindigkeit zerstört. In Humanplasma fanden sich die gleichen Differenzen zwischen α- und β-Verbindungen. Im Rattennierenextrakt war ein deutlicher Unterschied hinsichtlich Inaktivierung nur zwischen Angiotensin-Amid (H) und den freien Säuren nachweisbar, nicht hingegen zwischen α- (A) und β-*L*-Asp¹-Angiotensin II (B) oder der entsprechenden *D,L*-Verbindung (C) (siehe Figur 2 und 3 sowie Tabelle).

Eine unterschiedliche Bindung an Eiweiss beim Koagulieren während des Kochens wurde ausgeschlossen. Unmittelbar vor Beendigung der Inkubation entnommene Proben wiesen die gleiche pressorische Aktivität auf wie das Überstehende nach der Ausfällung. Veränderungen der Inkubationstemperatur bei konstantem pH und konstanter Bebrütungszeit ergaben im Rattenserum die gleichen Unterschiede in der Abbaugeschwindigkeit zwischen den einzelnen Angiotensinen wie bei Variationen der Inkubationszeit. Bei Veränderungen des pH fand sich im Rattenserum der stärkste Abbau bei pH 7,4 mit einer grossen Differenz zwischen α- und β-Angiotensinen. Unter pH 5 verlief die Zerstörung viel langsamer und der Unterschied zwischen den Substanzen verschwand. Nachweis des Abbaus in Rattenserum mittels Chromatographie[5] ergab für alle Angiotensine einen gemeinsamen Abbauweg mit Freisetzung von Aminosäuren. Bei den beiden α-Angiotensinen (A und H) fand sich zusätzlich eine Zerstörung des Moleküls vom Aminoende her, die bei den β-Verbindungen (B und C) fehlte. Nähere Angaben hierzu bleiben einer ausführlichen Arbeit vorbehalten.

«Angiotensinase»-Empfindlichkeit in Humanfrischplasma (Heparinzusatz). Angaben wie in Figur 2 und 3

Präparat	2 min	4 min	10 min	30 min	120 min
H	50%	41%	13%	—	—
A	—	—	42%	23%	11%
B	—	—	63%	51%	38%
C	—	—	81%	78%	57%

Zusammenfassend ergibt sich somit, dass zwischen Intensität und Dauer der pressorischen Wirkung einerseits und der Zerstörung der Angiotensine im Plasma andererseits ein deutlicher Zusammenhang besteht: Die β-Asp¹-Angiotensine sind am Blutdruck doppelt so wirksam wie die α-Asp¹-Angiotensin-II-Verbindungen und sind bei Inkubation in Rattenserum oder Humanplasma gegen abbauende Fermente («Angiotensinase») resistenter. Daraus kann geschlossen werden, dass eine Aminopeptidase wesentlich am Abbau von Angiotensin II im Plasma beteiligt ist. Bei Inkubation in Rattennierenextrakt besteht hingegen bezüglich Abbaugeschwindigkeit vorwiegend eine Differenz zwischen dem Amid α-Asp¹(NH_2)-Angiotensin II (H) und den untereinander ungefähr gleichen restlichen Substanzen, die langsamer inaktiviert werden.

Summary. β-Asp¹-Angiotensin II had a more pronounced action on the blood pressure of nephrectomized rats than the corresponding α-compound. β-Asp¹-Angiotensins were more slowly destroyed by rat serum, rat kidney homogenate, and human plasma than α-compounds, especially α-Asp¹-Angiotensin II amide.

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Forschungslaboratorien der CIBA Aktiengesellschaft, Pharmazeutische Abteilung, Basel (Schweiz), 6. August 1962.

[5] Herrn Dr. B. RINIKER danken wir für die chromatographischen Untersuchungen.

Studies on the Histopathological Changes Induced by DDT in the Liver, Kidney, and Intestine of certain Fishes

CAMERON and CHENG[1] stated that at present nothing is known about the action of DDT beyond the fact that it interferes in some way with the normal function of the nervous system. The study of histopathological changes in the tissues of fishes exposed to DDT is of great economic importance. Hence the author proposes to study the changes caused by DDT in the liver, kidney and intestine of fishes. The microscopic changes occur predominately in the liver of fishes, and consequently these have been described in greater detail.

Technique. The tissues were fixed in 10% formaline, Zenker-Helly, Bouin's fluid and Carnoy fixatives. The sections were cut at 6–8 μ and stained with Mallory triple, haematoxylin and eosin stains. No histopathological changes in the tissues similar to those of experimental fishes were noted in the fishes of the controlled experiments.

Observations. The pathological changes caused by DDT on different fishes and tissues are described below.

1. *The liver.* The histopathological changes in the liver include damage of liver cells, vacuolar degeneration of the cytoplasm of the cells, localised necrosis and parenchymatous degeneration of cells and hypertrophy of hepatic cells.

(a) *Ophiocephalus punctatus.* The hypertrophy of hepatic cells, necrosis and margination of cells were observed from DDT infected liver (Figure 2). The liver damage was indicated by a scattered distribution of parenchymatous cells. The principal lesion was the vacuolar degeneration of the cytoplasm of the cells. The hepatic cells were moderately vacuolated, the vacuoles being either small or large, and in certain cells these may be single or multiples. The central hepatic cells stained less deeply than the peripheral ones. The centralobular hypertrophy was well marked. In severely affected liver, the centre of the cells appeared empty. The cells of the periphery were more dense. There was slight displacement of the nucleus from its original central position. The lesion occurred more often in the central area than the peripheral area.

[1] G. R. CAMERON and K. K. CHENG, Brit. med. J. *2*, 819 (1951).

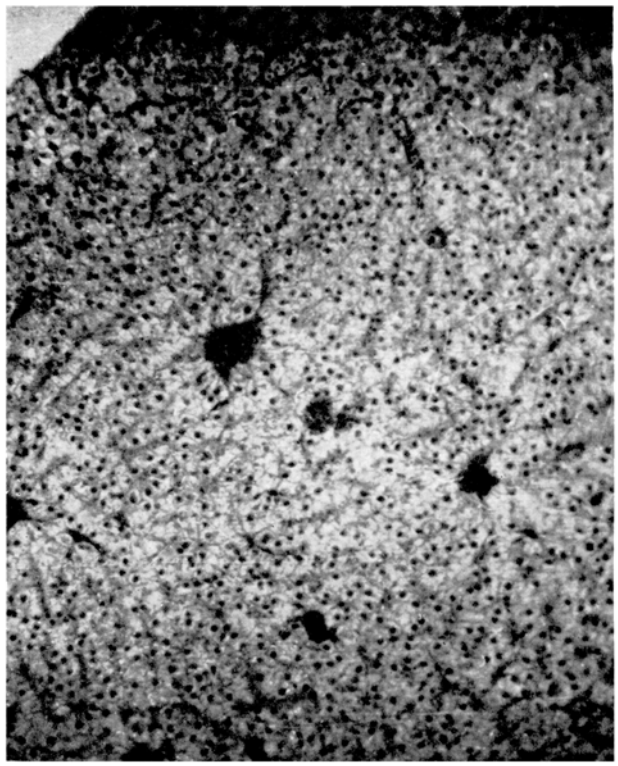

Fig. 1. Photomicrograph of T.S. of normal fish liver, cells showing the usual pattern of cells size and cytoplasmic granules (control slide × 130).

The combination of hypertrophy and margination is typical of chlorinated hydrocarbon poisoning. No such changes were observed at very low dosage (5 p.p.m.), in such cases the only alteration being a parenchymatous degeneration of the liver cells. The degree of these alterations varied with dosages. At higher dosage (50 p.p.m.) the hepatic cells alterations were more marked, but at lower dosage these histopathological changes were at their minimum.

(b) *Heteropneustes fossilis*. Histopathological changes varied with the concentration of dosage. The affected liver cells were scattered when the changes were more severe (Figure 3). A few hepatic cells in the central area had slightly atrophied. Changes in parenchymatous cells consisted of margination and hypertrophy. Localised necrosis of the liver cells was also noticed. The lesion occurred more often in the central than in peripheral area. The main lesion was the degeneration of liver cells. The central hepatic cells stained less deeply than the peripheral ones. The clumping of the hepatic cells was also noticed at a few places. There was slight displacement of the nucleus from its original central place. The peripheral cells showed considerable variations in size, a large number of them having become shrunken. The hepatic cells were not moderately vacuolated. At higher dosages (60 p.p.m.) margination was characteristic and the alteration of hepatic cells was more marked. Such a change did not occur at very low dosage (10 p.p.m.), the only changes being the degeneration of the parenchymatous cells.

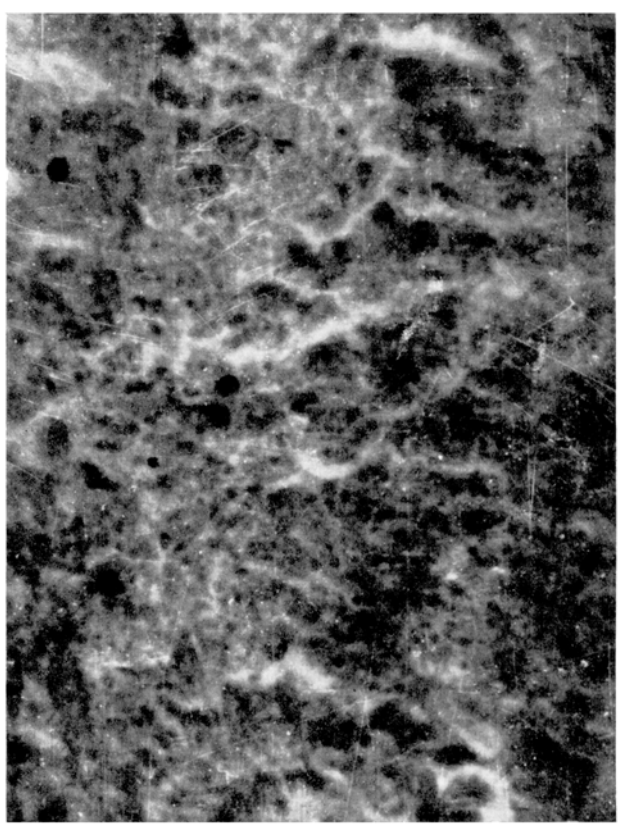

Fig. 2. Photomicrograph of T.S. of infected liver from DDT of *Ophiocephalus punctatus* showing liver cells with moderate degree of margination and hypertrophy (× 600).

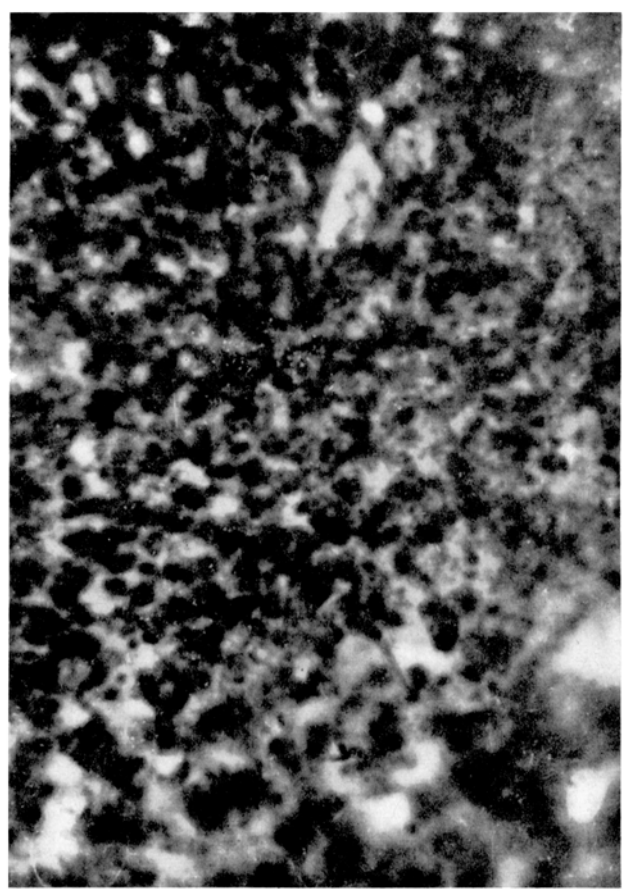

Fig. 3. Photomicrograph of T.S. of infected liver from DDT of *Heteropneustes fossilis* showing scattered distribution of parenchymatose cells (× 600).

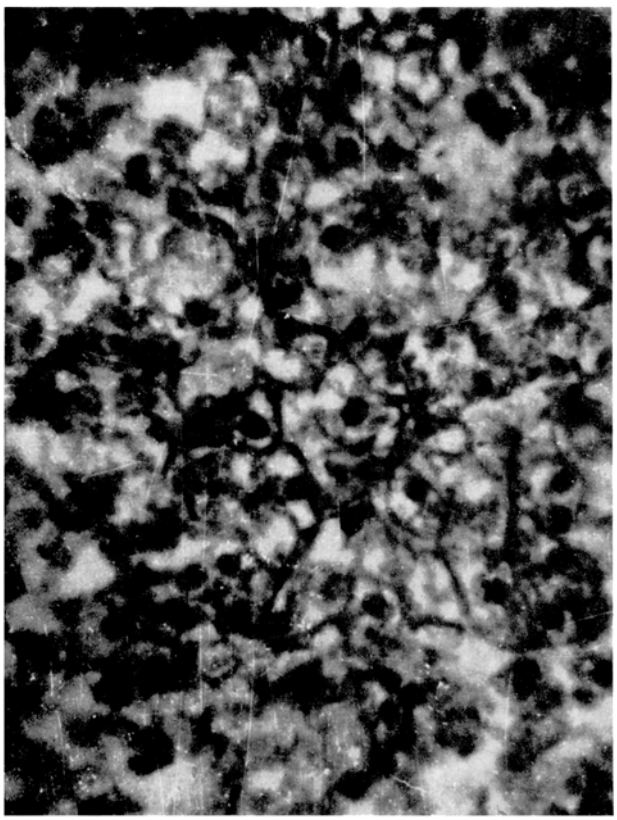

Fig. 4. Photomicrograph of T. S. of infected liver from DDT of *Trichogaster fasciatus* showing vacuolated liver cells and hypertrophy of cells (×600).

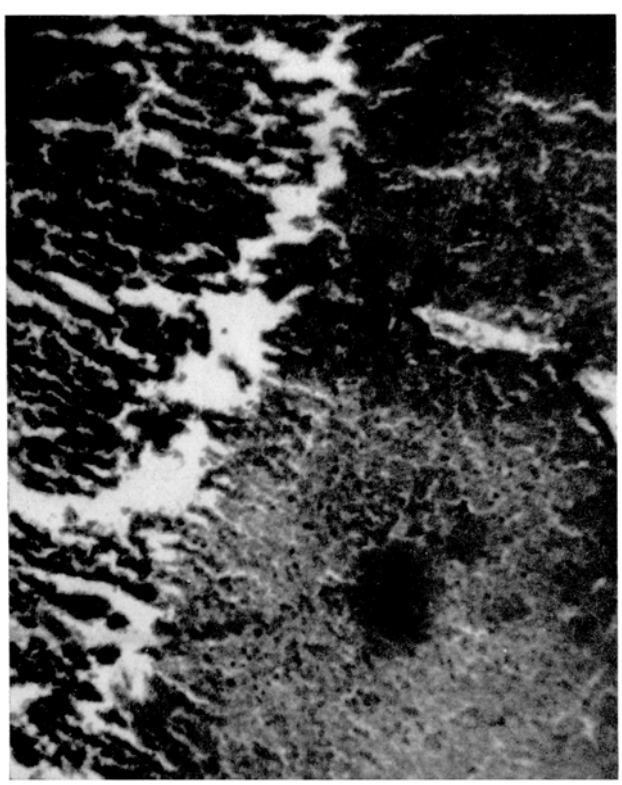

Fig. 5. Photomicrograph of T.S. of infected liver from DDT of *Barbus stigma* showing necrosis and hypertrophy (× 130).

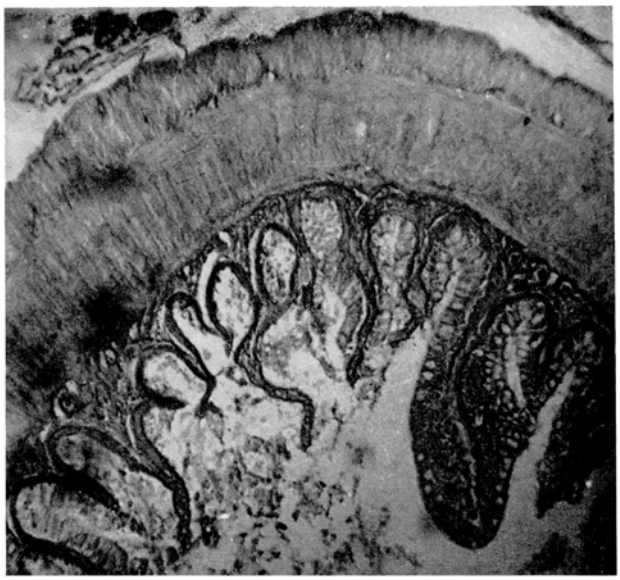

Fig. 6. Photomicrograph of T.S. of infected intestine from DDT of *Ophiocephalus punctatus* showing degeneration of epithelium lining (× 100).

Fig. 7. Photomicrograph of T.S. of infected kidney from DDT of *Ophiocephalus punctatus* showing degeneration of renal tubules (× 100).

(c) *Trichogaster fasciatus*. A moderate degeneration in parenchymatous cells was noticed. The liver damage was indicated by the scattered distribution of parenchymatous cells all over the organ. The main lesion was the vacuolar degeneration in the cytoplasm of cells. In severely affected liver, the centre of the cells appeared empty. The cells of the periphery were more dense and stained more deeply than those at the centre of the liver. The damage occurs more often in the central area of the organ. Localised necrosis and hypertrophy of cells were perceptible. The cells were moderately vacuolated. The combination of hypertrophy and vacuolation was well marked. At a few places the cells appear to form compact aggregations. The polygonal shape of cells was deformed (Figure 4). The histopathological changes are more

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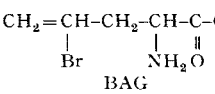
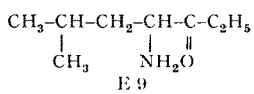
The Michaelis Constants of Catheptic Activity in Xenopus Tail Tissue after *in vivo* Treatment with Two Leucine Analogues[1]

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

[1] With the aid of a grant from the Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung. – Author's present address: Institut fur experimentelle Gerontologie, Nonnenweg 7, Basel (Switzerland).

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