lysine (VII) (75% yield; m.p. 143–148°; $[\alpha]_D^{20}$ −67°, c 0.5, EtOH; $E_{1,9}=0.53$ Leu. *Anal.* Calcd. for $C_{41}H_{66}O_{15}N_8 \cdot H_2O$: C 52.99; H 7.39; N 12.06. Found: C 52.98; H 7.28; N 12.06). Condensation of VII with IV in pyridine by DCCI[7] afforded N-CTB-pyroglutamyl-alanyl-O$^\beta$-t·Bu-aspartyl-prolyl-asparaginyl-N$^\varepsilon$-CTB-lysyl-phenylalanyl-tyrosyl-glycyl-leucyl-methioninamide (VIII) (35% yield; m.p. 155–158° dec.; $[\alpha]_D^{20}$ −59°, c 0.3, EtOH. *Anal.* Calcd. for $C_{72}H_{108}O_{20}N_{14}S \cdot H_2O$: C 56.15; H 7.21; N 12.74. Found: C 55.80; H 7.34; N 12.51) which was treated with HCl/TFA for 2 h and subjected to counter-current distribution first in *n*·butanol/EtOH/AcOH/H_2O 5:1:1:8 (40 transfers, tubes 18–30) and subsequently in H_2O/*n*·butanol/pyridine/0.1 *N* aqueous ammonia 80/34/20/1 (40 transfers, tubes 22–32) to give pyroglutamyl-alanyl-aspartyl-prolyl-asparaginyl-lysyl-phenyl-alanyl-tyrosyl-glycyl-leucyl-methioninamide, which for solubility reasons was transformed into its trifluoroacetate (IX) (40% yield; m.p. 180° dec.; $E_{1,9}=0.4$ Glu; $E_{1,9}=0.33$ Leu; $[\alpha]_D^{20}$ −56°, c 0.2 in EtOH; λ_{max} 278 mμ, $\varepsilon=1780$. *Anal.* Calcd. for $C_{58}H_{84}O_{16}N_{14}S$. $CF_3COOH \cdot 2H_2O$: C 50.90; H 6.35; N 13.85. Found: C 51.01; H 6.25; N 13.53). IX was found to be homogeneous, and showed amino acid composition, electrophoretic mobility, behaviour towards trypsin and chimotrypsin[8] and the same biological properties as natural physalaemin, thus confirming the formula deduced from degradative experiments[2,9,10].

Riassunto. Per condensazione fra fenilalanil-tirosil-glicil-leucil-metioninamide e N-carbo-ter-butossi-piroglutammil-alanil-β-ter-butil-aspartil-prolil-asparaginil-N$^\varepsilon$-carbo-ter-butossi-lisina in presenza di dicicloesilcarbodiimmide si è ottenuto l'endecapeptide VIII che per trattamento con una soluzione di HCl in acido trifluoroacetico ha fornito la piroglutammil-alanil-aspartil-prolil-asparaginil-lisil-fenilalanil-tirosil-glicil-leucil-metioninamide identica, per proprietà chimiche, fisiche e biologiche alla physalaemina naturale.

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O. GOFFREDO, and R. DE CASTIGLIONE

Laboratori Ricerche Chimiche Farmitalia, Milano (Italy), May 29, 1964.

[7] More straightforward approaches to the synthesis of physalaemin having been rejected owing to the impossibility of securing crystalline intermediates, we had to take the risk of a partial racemization of the lysine residue during the condensation. We felt confident we would be able to eliminate the unwanted isomer by crystallization and we think we have succeeded, since trypsine completely splits the lysine-phenylalanine bond of our synthetic physalaemin sample.

[8] We are indebted to Dr. A. ANASTASI for these assays.

[9] We hope to be able to report in the near future the characteristic and the biological activities of a number of fragments of physalaemin and of several synthetic peptides embodying the features of both eledoisin and physalaemin.

[10] We express our appreciation to Dr. B. CAMERINO, Director of these Laboratories, for his interest in the work.

Selective Destruction in Testes Induced by Fluoroacetamide

During research carried out in order to find out substances causing hyperplasia of the epithelium of small bile-ducts, we have seen a peculiar destructive action of fluoroacetamide (FAA) on the testicular germinal epithelium.

The FAA was given orally, added to diet in the proportion of 50 mg/kg of food, to male rats weighing from 150–160 g. They were killed by exsanguination after 30, 64 and 90 days of treatment. At the end of the experimental period the body weight was increased on the average by 88% on the initial values.

In the necroscopic examination the testes revealed a notably reduced volume and such a flaccid consistence as if they were emptied of the greater part of their contents. The Table shows their weight in mg/100 g of body weight. The histological examination carried out on organs fixed in Bouin's fluid and stained by haematoxylin and eosin showed a gradual disappearance of the testicular germinal epithelium, from the most mature cells to the spermatogonia.

The tubules of the testes of the animals after 64 days of treatment were almost completely lacking in the seminal cells; only some spermatogonia and the Sertoli's cells were apparently undamaged as well as the interstitial cells. During the evolution of the regressive damage which causes the destruction of the testicular germinal epithelium, peculiar giant cells appear, perhaps by fusion

Days of treatment	Average weight ± S.E.M.
Controls (8)	0.97 ± 0.05
30 days (8)	0.55 ± 0.07
64 days (9)	0.32 ± 0.03
90 days (10)	0.30 ± 0.08

In parentheses the number of experiments performed.

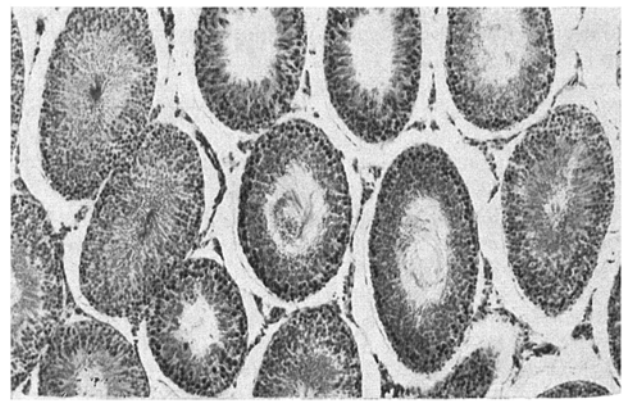

Fig. 1. Testis of normal rat (× 230).

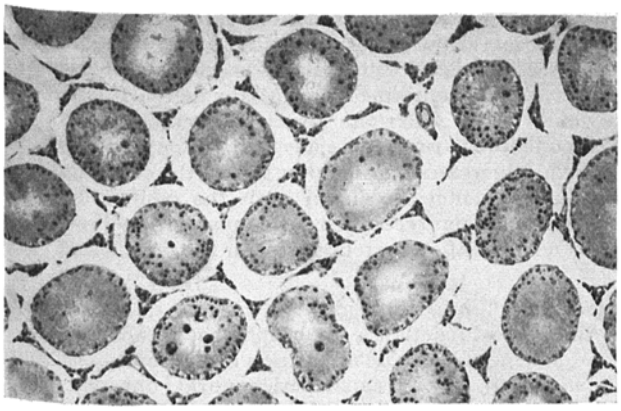

Fig. 2. Testis of rat after 30 days of treatment. The photograph shows a clear-cut decrease of the germinal epithelium and numerous giant cells as well as a marked atrophy of the seminal tubules (× 230).

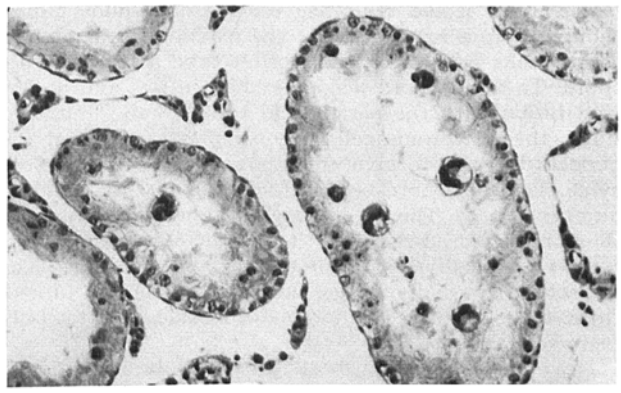

Fig. 3. Testis of rat after 30 days of treatment. A higher magnification of another section of the testicle of the same animal shows numerous pseudo giant cells (× 585).

of degenerating spermatids. The behaviour of the intestinal mucosa, in which a normal number of mitoses was seen, shows that the FAA acts selectively on the testicular germinal epithelium and that its action is not strictly related to rapid cellular multiplication as is the case with radiomimetic substances. In the liver no notable proliferative phenomena were observed in the small bile-ducts. On the other hand, in our experiments the FAA acts firstly on the most mature elements of the germinal epithelium and not on the cells where the mitoses are more numerous. Thus, the FAA differs from other experimental conditions (radiation, etc.) which produce testicular atrophy, firstly damaging the germinative cells (spermatogonia). On the basis of those results it is clear that FAA produces a testicular atrophy by a selective action on the seminal epithelium.

Further study on the mechanism of action of this substance is currently under way.

Riassunto. Gli autori descrivono le lesioni del testicolo del ratto osservate nel corso di sperimentazioni con la fluoroacetamide. Tali lesioni consistono in alterazioni regressive interessanti elettivamente la linea seminale.

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Istituto di Farmacologia, Università di Pisa (Italy), April 21, 1964.

Hypochord in the Anurans

The hypochord in the anurans develops, according to some authors, below and parallel with the notochord[1] as a fibrous structure and later becomes connected with the perichordal tube by the fibrous tissue[2]. Shumway and Adamstone[3] opine that it develops from the lower surface of the chorda plate and its functions are unknown.

The present investigation deals with the analysis of the ontogeny of the hypochord in an anura (*Rana tigrina*) and its role in the development of the vertebral column with special reference to the localizations of alkaline phosphatase in the process. Fertilized eggs of *R. tigrina* from the midgastrula to the opercular stage (Shumway[4] stages 11 to 25), tadpoles at various stages of development and some adults were fixed in Zenker's fluid for histological study; for alkaline phosphatase, Gomori's modified technique[5] was followed.

Observations. The hypochord is formed out of endoderm when the notochord is already differentiated with covering sheaths, and the perichordal tube is formed round the notochord with an aggregation of mesenchymatous cells (stages 11 and 12). In the notochordal portion, the reaction for alkaline phosphatase is intense. In the hypochord, the reaction is less but greater than that of the perichordal tube region. Later (stages 13 to 15), the perichordal tube becomes well formed round the vacuolated notochord and the hypochord gradually separates from the endoderm to abut against the perichordal tube. The hypochord cells show two types of reaction: (a) cells in contact with the hypodermis show intense reaction and (b) cells in contact with the perichordal tube show less reaction. The mesenchyme cells round the perichordal tube and hypochord also show less reaction. The hypochord now starts vacuolations with less reaction for alkaline phosphatase (stages 16 and 17) and on its outer surface a layer similar to the elastic layer of the notochord is formed. The localizations for alkaline phosphatase become diminished in the perichordal region. In the successive stages of development (stages 18 to 25), the notochord becomes vacu-

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[2] W. G. Ridewood, Anat. Anaz. *13*, 359 (1897).
[3] W. Shumway and F. B. Adamstone, *Introduction to Vertebrate Embryology* (John Wiley & Sons, Inc., New York 1958), p. 111.
[4] W. Shumway and F. B. Adamstone, *Introduction to Vertebrate Embryology* (John Wiley & Sons, Inc., New York 1958), p. 118.
[5] G. Gomori, *Microscopic Histochemistry* (Chicago University Press, 1952).