synonym of *Sparisoma viride* (Bonnaterre) (male). The evidence, although not as strong as in the examples already cited, seems to warrant synonomizing these two forms. All *S. viride* examined were males, and all *S. abildgaardi* examined were females. Only immature stages of the *S. abildgaardi* red color phase were found, and only large, mature *S. viride* in their bright green phase were observed. During the breeding season, the adult red females of *S. abildgaardi* and the adult green males of *S. viride* were usually seen together in loose aggregations. Individuals of *S. viride* frequently chased individuals of *S. abildgaardi* but did not chase other parrot fishes. Also, it was noted by Longley (*2*) that *S. viride* has a spotted color phase similar to that of *S. abildgaardi*.

Brock and Yamaguchi (*3*) identified two species of Pacific Ocean parrot fishes as the male and female of each other. A similar finding has been described in the case of only one other species of parrot fish (*Sparisoma radians*), in the western North Atlantic Ocean (*2*).

These results further demonstrate the occurrence of sexual dimorphism in coral reef fishes and especially in the family Scaridae, which has been considered not to be sexually dimorphic, in general (*4, 5*).

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19 February 1957

Synthesis and Pharmacology of the Octapeptide Angiotonin

Arterial hypertension of renal origin seems to depend, at least during its initiation, on the liberation of a pressor substance from the kidneys. In 1939 a substance with the theoretically required properties was discovered simultaneously by Page and Helmer (*1*) and Braun-Menendez, Fasciolo, Leloir, and Munoz (*2*). It resulted from the action of a proteolytic enzyme, renin, which was extracted from kidneys, on a substrate synthesized in the liver and contained in plasma.

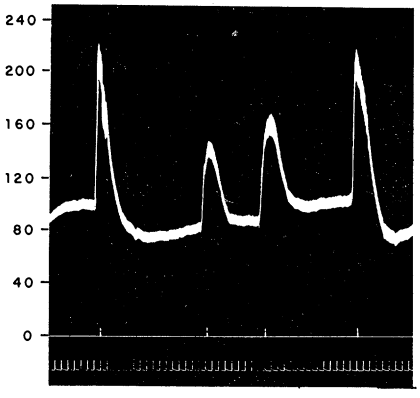

Fig. 1. Arterial pressure response in a dog under pentobarbital anesthesia and cardiovascular reactivity augmented with tetraethylammonium chloride. The marks on the abscissa indicate, left to right: (i) 5 μg of noradrenaline; (ii) natural angiotonin; (iii) synthetic angiotonin; (iv) 0.06 mg of serotonin.

More recently, Peart (*3*) isolated from the incubation mixture of renin (rabbit) and renin-substrate (beef) a pressor peptide containing ten amino acids (*4*) in the sequence L-asp, L-arg, L-val, L-tyr, L-val, L-his, L-pro, L-phe, L-his, L-leu (*5*). Skeggs *et al.* (*6*) obtained from the action of renin (hog) on its substrate (horse) a mixture of two vasoactive peptides, separable by countercurrent distribution, which they termed hypertensin I and II. They demonstrated that hypertensin I was converted into II by a chloride-activated enzyme occurring in plasma (*7*). Hypertensin I was shown to be a decapeptide with the sequence L-asp, L-arg, L-val, L-tyr, L-isoleu, L-his, L-pro, L-phe, L-his, L-leu; hypertension II was shown to be the corresponding octapeptide lacking the last two amino acids (*8*). Hypertensin I seemed to be inactive in the absence of converting enzyme (*7*).

In our work on the purification of angiotonin (hog renin, hog substrate), we were able to separate by countercurrent distribution an oxytocic-pressor principle from the dominantly pressor principle (*9*). The latter was transformed into the former by an enzyme present in whole, unhemolized blood, plasma, substrate fractions, hemolyzed red cells, and possibly urine. Distribution coefficients suggested a close relationship of the two principles with the two hypertensins of Skeggs *et al.* This was confirmed for the pressor principle, which we found to be identical with hypertensin I in amino acid composition and sequence.

On the basis of these data, it seemed very likely that the oxytocic-pressor principle was identical with hypertensin II. In order to determine the identity of these two principles, synthesis, based on the structure determined by Skeggs and coworkers for hypertensin II, was undertaken (*10*).

The four pure, crystalline dipeptides, cbz-β-Me-L-asp-NO_2-L-arg, cbz-L-val-L-tyr-Me, cbz-L-isoleu-L-his-Me, and L-pro-L-phe Me · HCl served as starting material. From the condensation of cbz-L-val-L-tyr-azide with L-isoleu-L-his-Me, a tetrapeptide was isolated. The carboxylic acid obtained from this tetrapeptide was condensed with L-pro-L-phe Me by the amide modification of the diethyl chlorophosphite method of Anderson and coworkers (*11*), to give the hexapeptide cbz-L-val-L-tyr-L-isoleu-L-his-L-pro-L-phe Me. The mixed anhydride formed from cbz-β-Me-L-asp-NO_2-L-arg-L-val-L-tyr-L-isoleu-L-his-L-pro-L-phe Me (mp, 182 to 185°C; $\alpha_D^{25} = -60.1$, $c = 1$ in methanol; $\alpha_D^{25} = -32.5$, $c = 1$ in dimethylformamide). After the removal of the protecting groups by hydrolysis and hydrogenolysis, a biologically active solution was obtained (20,000 units/mg of N). From this solution, a white powder was isolated containing a strongly pressor material and NaCl. After correction for NaCl, the activity was found to be 4000 units/mg of solid and by nitrogen determination as 22,000 units/mg of N (*9*). Pure natural angiotonin has an activity of 45,000 units/mg of N and 7800 units/mg of solid. Further purification of the synthetic angiotonin will be necessary before final comparison is justified. The material was also very active

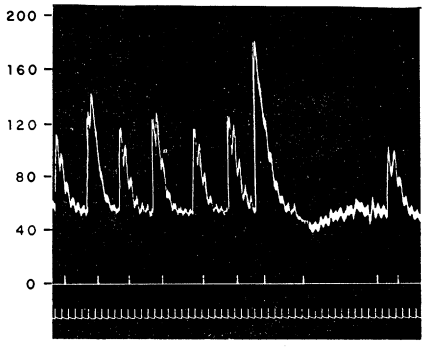

Fig. 2. Arterial pressure response in a pithed cat, comparing natural and synthetic angiotonin. The marks on the abscissa indicate, left to right: (i) 0.35 μg of solid synthetic angiotonin; (ii) 2 units of natural angiotonin; (iii) 0.41 μg of synthetic angiotonin; (iv) 1.6 units of natural angiotonin; (v) 0.41 μg of synthetic angiotonin; (vi) 0.47 μg of synthetic angiotonin; (vii) 5 μg of noradrenaline; (viii) 4 mg of benzodioxane per kilogram; (ix) 5 μg of noradrenaline; (x) 0.47 μg of synthetic angiotonin.

on an isolated rat's uterus. On two-dimensional paper chromatography, the material showed the expected eight amino acids in about equal quantities.

Comparison of the naturally occurring angiotonin with the synthetic showed that the form of the curve of arterial pressor rise in dogs, cats, and rats was identical with the same latent period as well. Neither had any significant effect on heart rate in vagotomized animals. Repeated injections produced no simple tachyphylaxis.

Augmentation of the response following injection of the ganglion blocking agents, tetraethylammonium chloride or hexamethonium chloride (*12*), occurred in large measure and equally with both the natural and synthetic substances. An example of such augmented response to noradrenaline, natural and synthetic angiotonin, and serotonin is shown in Fig. 1.

The responses in pithed cats were brisk and regular. A comparison of a "standard" sample of natural angiotonin and the synthetic is illustrated in Fig. 2. This experiment proves that the central nervous system is not necessary for the action of synthetic angiotonin.

The greatly enhanced response to noradrenaline (5 μg)was blocked by injection of benzodioxane. Both synthetic and natural angiotonin continued to elicit good rises in blood pressure, as did serotonin. This shows the site of action to be different from that of the usual pressor amines. The evidence obtained thus far makes it appear likely that our oxytocic-pressor principle from angiotonin is identical with hypertensin II.

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11 February 1957

Conversion of Diiodophenols to Side-Chain Analogs of Thyroxin

Interest in thyroxin analogs with side-chain variants was initiated by the early work of Harington (*1*) and subsequently renewed by Loeser and Trikojus (*2*), Frieden and Winzler (*3*), Barker *et al.* (*4*), Thibault and Pitt-Rivers (*5*) and Tomita and Lardy (*6*). Several summaries of these and other results on compounds with thyroxinlike activity have appeared (*4, 7*). Because of the great interest in side-chain analogs of thyroxin, we have been exploring several substituted diiodophenols as a source of corresponding diphenyl ether derivatives with appropriate side-chain functional groups. These condensation reactions are analogous to those suggested for 3,5-diiodo-L-tyrosine (DIT) by Harington (*1*), first shown by Ludwig and Von Mutzenbacher (*8*) and studied extensively by others (*9*).

An experimental basis for this approach was suggested when it was found that the biological activity of DIT and other substituted phenols increased with the age of the solution under test (*3, 10*). The percentage decrease in the total body length of toad tadpoles was used as a criterion for thyroxinlike activity as previously described (*3*) and in representative data summarized in Table 1. Each compound was dissolved in tap water, usually with the aid of dilute NaOH, and the solution was adjusted to *p*H 7.5 ± 0.3 with dilute HCl. Each experiment in Table 1 is the average of at least one duplicate of five animals per bowl incubated for 60 ± 20 hours at 29°C. Experiments 1 to 10 in Table 1 contained freshly prepared solutions of DIT (*11*). Aged DIT solutions (stored in brown bottles at 22°C at *p*H 7.5 for 10 months) were used in experiments 11 and 12. Thus freshly prepared DIT showed significant biological activity only in the absence of appreciable amounts of 2-thiouracil. The activity of DIT appeared to vary inversely with the thiouracil concentration. Another goitrogen, 2-mercaptoimidazole, similarly prevented DIT activity. As expected, aged solutions of DIT contain preformed thyroxin, and no effect of thiouracil was detected. Pitt-Rivers (*12*) has also noted the inhibition of the chemical conversion of DIT and its derivatives to the corresponding thyroxin compounds by thiouracil and other goitrogens.

Table 1 also presents data obtained from similar experiments with other single-ring compounds (*13*). All the substituted phenols tested showed thyroxinlike activity except 3-iodo-L-tyrosine (*14*) (experiments 13 to 15). The data in Table 1 include evidence for thyroxinlike activity for 3,5-diiodo-4-hydroxybenzoic acid (*15*) (experiments 16 to 18), 3,5-diiodo-4-hydroxyphenylacetic acid (*16*) (experiments 19 to 21), and 3,5-diiodo-4-hydroxyphenylpropionic acid (*17*) (experiments 22 to 24). In each case the biological activity could be prevented with thiouracil. The lesser activity of the benzoic acid compound (experiments 16 to 18) might be due to the relatively lower activity of its corresponding diphenyl ether, 4-(4′-hydroxy-3′,5′-diiodophenoxy)-3,5-diiodobenzoic acid (*3, 6*) (experiment 25) as compared with other diphenyl ethers, such as 4-(4′-hydroxy-3′,5′-diiodophenoxy)-3,5-diiodophenylpropionic acid (*18*) (experiment 26), L-thyroxin (*18*) (experiment 27), and L-triiodothyronine (*18*) (experiment 28). The addition of 3-iodotyrosine did not alter the response to DIT.

We have also studied the condensation reactions of substituted diiodophenols in an exclusively chemical system. Solutions (2 to 5 percent) of the 3,5-diiodo-4-hydroxy derivatives of benzoic, phenylacetic, and phenylpropionic acids were incubated under various conditions, and the reaction mixtures were

Table 1. Thyroxinlike activity of some substituted diiodophenols. The experimental conditions are summarized in the second paragraph of the text. The identity of the substituted diiodophenol involved in each experiment is given in the second and third paragraphs.

Expt. No.	Molarity of substituted diiodophenol	2-Thiouracil (%)	Decrease in length (%)
1	2.3×10^{-5}		12
2	5.8×10^{-5}		33
3	1.2×10^{-4}		48
4	2.3×10^{-4}		55
5	2.3×10^{-4}	0.0001	42
6	2.3×10^{-4}	0.0010	32
7	2.3×10^{-4}	0.010	25
8	2.3×10^{-4}	0.020	8
9	2.3×10^{-4}	0.005*	5
10	2.3×10^{-4}	0.010*	4
11	1.2×10^{-4}		56
12	1.2×10^{-4}	0.020	55
13	5.0×10^{-5}		3
14	1.0×10^{-4}		4
15	2.0×10^{-4}		4
16	1.3×10^{-3}		26
17	2.5×10^{-3}		42
18	2.5×10^{-3}	0.020	4
19	1.0×10^{-4}		44
20	2.0×10^{-4}		58
21	1.0×10^{-4}	0.020	3
22	4.0×10^{-5}		27
23	1.0×10^{-4}		54
24	1.0×10^{-4}	0.020	5
25	5.0×10^{-6}		41
26	1.0×10^{-7}		45
27	1.0×10^{-7}		31
28	1.0×10^{-7}		42

* The goitrogenic agent used in these two experiments was 2-mercaptoimidazole (*23*).