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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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*).

Table 2. Paper chromatography of substituted diiodophenols and some corresponding diphenyl ethers.

Compound	R_f*	Color of spot†
3,5-Diiodo-L-tryosine	0.27	pink
N-Acetyl-3,5-diiodo-L-tyrosine	0.36	pink
3-Nitro-L-tyrosine	0.15	yellow
3-Iodo-L-tyrosine	0.27	pink
3,5-Diiodo-4-hydroxybenzoic acid	0.16	yellow
4-(4′-Hydroxy-3′,5′-diiodophenoxy)-3,5-diiodobenzoic acid	0.71	purple
3,5-Diiodo-4-hydroxyphenylacetic acid	0.30	pink
4-(4′-Hydroxy-3′,5′-diiodophenoxy)-3,5-diiodophenylacetic acid	0.66	purple
3,5-Diiodo-4-hydroxyphenylpropionic acid	0.30	pink
4-(4′-Hydroxy-3′,5′-diiodophenoxy)-3,5-diiodophenylpropionic acid	0.64	purple

* R_f was determined in solvent of the following composition: *n*-butanol, 40 parts; NH_4OH, 15 parts; ethanol, 5 parts.
† The developing reagent for these spots was the diazotized sulfanilamide reagent prepared as described by Bolling *et al.* (*24*).

analyzed by paper chromatography. The systems described in Table 2 were used for the separation and the detection of the respective diphenyl ethers from the appropriate single-ring compound. It is of interest to note that the diphenylether compounds consistently gave purple test spots, while the single-ring compounds produced pink and yellow colors with diazotized sulfanilamide. Both the 3,5-diiodo-4-hydroxyphenylacetic and 3,5-diiodo-4-hydroxyphenylpropionic acids gave small yields of the corresponding acetic and propionic acid analogs of thyroxin when they were incubated at *p*H 7.5 at 37°C for 5 to 15 days. Occasionally, unknown compounds were detected in the incubation mixtures. No condensation product was detected from the incubation of 3,5-diiodo-4-hydroxybenzoic acid. Saul and Trikojus (*19*) have reported the condensation of 3,5-diiodo-4-hydroxyphenyllactic acid to its corresponding diphenyl ether.

A successful attempt was made to isolate 4-(4′-hydroxy-3′,5′-diiodophenoxy)-3,5-diiodophenylpropionic acid. An insoluble barium salt of this acid was isolated from an incubation mixture. It was triturated with dilute HCl and recrystallized several times from an equal mixture of alcohol and water. A small yield (no more than 1 percent) of the acid, which melted at 214° to 215°C, was obtained. The sample appeared to be identical with an authentic sample (*20*) of 4-(4′-hydroxy-3′,5′-diiodophenoxy)-3,5-diiodophenylpropionic acid as determined by mixed melting point, mixed paper chromatography, and infrared spectrum. This avenue of synthesis may be of value in the preparation of the acetic acid analog of thyroxin, a compound suggested as an important metabolite of the thyroid hormone (*21*).

In this article we have reported the conversion of certain substituted diiodophenols to the corresponding diphenylether derivative with the expected thyroxinlike activity. These condensation reactions may serve as simple models for the study of the mechanism of condensation of DIT to thyroxin. The *in vivo* biological activity of substituted diiodophenols with acid side chains has also been noted. The presence of a goitrogenic agent prevented the tadpole response. It is, therefore, probable that the biological activity of these single ring compounds is due to their conversion to the corresponding biologically active dephenyl ether. However, it cannot yet be ascertained whether the condensation reaction occurred in the tadpole incubation medium or in the organism (*22*).

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Discrimination Training Effect on Stimulus Generalization Gradient for Spectrum Stimuli

It has been shown (*1*) that after the training of a pigeon to peck at a key illuminated by monochromatic light, a test for stimulus generalization during extinction with other wavelengths reveals an orderly relationship between wavelength and rate of responding. The experiment described in this report (*2*) was designed to determine how the generalization gradient is affected by explicit discrimination training.

An automatic Skinner box contained a translucent key illuminated by a diffraction grating monochromator (Bausch and Lomb model 33-86-40, with incandescent source, *3*). Thirty-two pigeons were trained to peck the key in an otherwise dark box with a stimulus light of 550-mμ wavelength (band width, 16.5 mμ). Food reward was given on a 1-minute variable-interval schedule. Five daily sessions were divided into 30 1-minute work intervals separated by 10-second "blackouts" during which no visual stimulus was present.

The birds were then divided into five groups. Eight birds, which were not given further training, were used to furnish a control generalization gradient. Four other groups of six were given discrimination training in which the positive stimulus was 550 mμ. The negative stimuli for the various groups were 555, 560, 570, and 590 mμ, respectively. The positive and negative stimuli were presented successively in random order for 1-minute intervals, separated by 10-second dark periods. Responses to the positive stimulus were rewarded according to the previous variable-interval schedule, while responses to the negative stimulus were never rewarded. Discrimination training was continued until a criterion of five successive minutes of no responding to the negative stimulus was met. The time required to meet the cri-