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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15. Recrystallized Eastman Kodak product. An interesting variation in the response of toad tadpoles was noted. Toad tadpoles collected at Los Angeles, Calif., in 1948 gave good metamorphic responses to this compound. Toad tadpoles collected at Tallahassee, Fla., in 1953 gave only poor responses to the compound.
16. Obtained by iodinating 4-hydroxyphenylacetic acid by the method of Block and Powell (*11*).
17. Prepared by an unpublished method of H. M. Walborsky, J. Eagle McRae, and E. Frieden.
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4 February 1957

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-

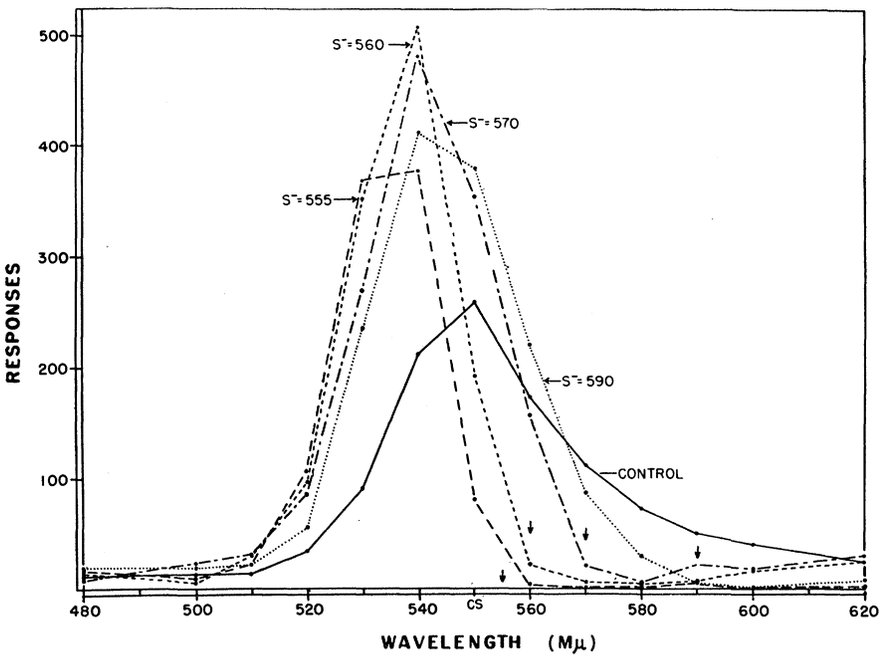

Fig. 1. Mean generalization gradients of a control group and four discrimination groups, identified by the respective values of the negative stimulus (S^-). Arrows indicate the positions of the negative stimuli.

terion of discrimination was a negatively accelerated decreasing function of stimulus difference.

Two generalization tests were administered to each bird on successive days. Each test consisted of 130 0.5-minute presentations of various wavelengths. Thirteen test wavelengths were used—480, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, and 620 mμ—and each was given ten times. Each block of the 13 stimuli was arranged in a different random order. The control group was tested after 5 days of training, and the discrimination animals were tested after they had reached the criterion of discrimination.

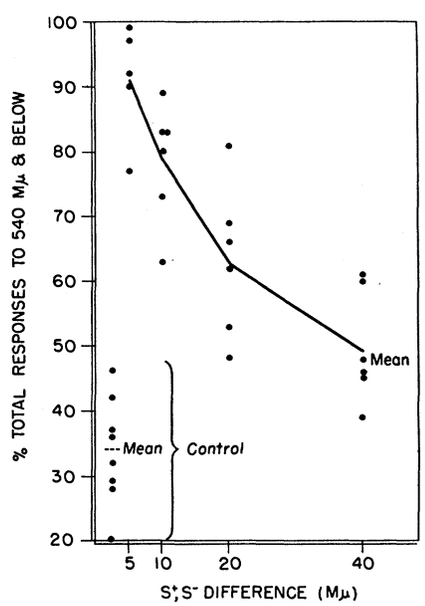

Fig. 2. Percentage of responses to wavelengths shorter than the positive stimulus, as a function of stimulus difference.

The mean generalization functions for all groups are shown in Fig. 1. These gradients were constructed by plotting the total number of responses to each stimulus against the wavelength of that stimulus. The postdiscrimination gradients, while they show many of the characteristics of the generalization curve obtained after simple conditioning, differ from the control in two ways. The postdiscrimination gradients are higher than the control curve, and they appear to be displaced along the abscissa in the direction of shorter wavelength. Only five of the birds in the four discrimination groups showed maximum responding at 550 mμ, and these five gradients evidenced truncation.

Both the right sides and left sides of the postdiscrimination gradients are ordered without inversion in relationship to the various values of the negative stimulus. The truncation of the curves for the groups trained with 555 and 590 mμ as negative stimuli is considered to be a consequence simply of the failure to include in the test series wavelengths near the inferred peaks of these functions, the phenomenon of displacement not having been anticipated. The displacement of the gradients for the discrimination groups may be illustrated by plotting for each subject the percentage of its responses which were emitted to 540 mμ and shorter wavelengths (Fig. 2).

The wavelength loci of the individual gradients which were truncated were estimated by a method of graphical linear extrapolation. The lines connecting the last two values on either side of the truncation were extended to their intersection and the value below the intersection on the wavelength axis was read off. These values, considered as differences from the positive stimulus, along with the similar values from those gradients that showed clear peaks when plotted against stimulus difference, show a clear relationship comparable to that in Fig. 2. The differences in "peak shift" among groups are significant beyond the 1-percent level of confidence in terms of an analysis of variance.

The differences in height between the experimental and control curves (Fig. 1), contrary to first impression, do not signify a correlated difference in total area under the curves. No significant differences between the means of groups were found for the total number of responses emitted during the two tests.

It may be observed that these results do not support the notion that discrimination training weakens behavior to the negative stimulus *and nothing more* (*4*). The evidence suggests that the major result of discrimination training is to bring a large proportion of the responses available in extinction under the control of another range of stimuli, those which do not ordinarily gain control of the response as the result of simple conditioning without differential reinforcement.

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3. In order to obtain the maximum brightness level, the output of the monochromator was not adjusted for luminosity. The luminosity function, obtained by means of heterochromatic matches with a MacBeth illuminometer, is closely approximated by the tungsten emission curve multiplied by the human photopic visibility function.
4. A paper describing the theoretical significance of these findings is in preparation.

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24 January 1957