The ratio of the initial fast rate to the slow rate at the later stage of hydrolysis is equal to roughly about ten times the estimated ratio of the intensity of the ninhydrin spots on paper chromatograms of this preparation. This suggests that the mono-substituted cysteine derivative is much less susceptible to hydrolysis by the C—S-lyase than is the disubstituted derivative.

Fig. 3 shows the influence of substrate concentration on the rate of hydrolysis of djenkolic acid (A), S-ethyl-L-cysteine (B), and L-lanthionine sulfoxide (C). It will be noted that the Michaelis constants K_M vary considerably less than the values for V, the maximum rate when the enzyme is saturated with substrate. Let us assume as a first approximation, that K_M is a measure of the traditional steady-state constant $(k_{-1} + k_2)/k_1$ according to the usual formation $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E +$ product, and that V is a measure of the magnitude of k_2. If K_M remains constant while k_2 increases, then k_1 (the velocity constant for combination of enzyme with substrate) must also increase. This analysis corroborates the previous conclusion that the susceptibility to hydrolysis as function of the S-substituent depends upon the absence of structures in this substituent which would tend to interfere with hydrogen bond formation between the pyridoxal phosphate moiety of the enzyme and the substrate [2]. Such interference would tend to decrease the rate of formation of enzyme-substrate complex (k_1) and also decrease the rate of decomposition of the complex (k_2). This would tend to maintain K_M at a constant value, as was found experimentally.

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Received November 7, 1960.

Preparation and Properties of Human Thyroglobulin

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The preparation and properties of thyroglobulin of animal origin has been described by several investigators [1-5]. Some chemical and physico-chemical studies on human thyroglobulin will be described briefly in the following.

The thyroid glands were frozen, sliced and extracted with 0.15 M NaCl for about 20 h at 5°C. The mixture was then centrifuged and the sediment discarded. Neutralized, saturated ammonium sulphate was added to the supernatant at a final saturation of 33 %. The sediment was removed by centrifugation and additional ammonium sulphate was added to the supernatant at a final saturation of 50 %. The sediment was collected by centrifugation and dissolved in distilled water. The solution was dialyzed against distilled water, and the dialysate was treated with ammonium sulphate as described above. The protein fraction precipitated between 33 and 50 % salt saturation was collected and investigated chemically and physico-chemically.

The iodine content of the material isolated from the thyroid glands was determined according to Kendall [6]. Nitrogen was determined by the Kjeldahl method. Ultracentrifugal studies were performed using the Spinco analytical ultracentrifuge working at 59 780 rpm. The protein concentration in the samples used for the ultracentrifugal analyses was 1 % and the total salt concentration in these samples was 0.17 M (0.15 M NaCl and 0.02 M phosphate, pH 7.0).

The main part of the iodine of the thyroid extracts was recovered in the protein fraction precipitated between 33 and 50 % saturation of ammonium sulphate. Table 1 gives the results of the chemical analyses carried out on the proteinaceous material thus obtained. The values for iodine and nitrogen content agree with those reported previously for animal thyroglobulin [2,3].

Table 2 shows the results of the ultracentrifugal analyses. It can be seen that the main high molecular weight component of the analyzed solutions has a sedimentation constant ($S_{20,\,aq}$) of about 17 *S*. Similar

Table 1. Chemical composition of preparations of human thyroglobulin.

Preparation	Iodine content, % of dry weight	Nitrogen content, % of dry weight
I *	0.440	14.9
II **	0.324	14.5

* Preparation from thyroid tissue surgically removed from a case of nontoxic goiter.

** Preparation from normal thyroid glands obtained at autopsy.

Table 2. Ultracentrifugal analyses of preparations of human thyroglobulin.

Sedimentation const. ($S_{20, aq}$), of high molecular weight components in preparation		Amounts of components, expressed as per cent of total high molecular weight material in prep.	
Prep. I (*cf.* Table 1)	Prep. II	Prep. I	Prep. II
~ 7 *S*	6.9 *S*	3	3
11.6 "	11.7 "	11	6
17.8 "	17.1 "	81	86
23 "	26 "	5	5

values have been reported for thyroglobulin isolated from hog [4,7] and calf [5].

It therefore seems very probable that the main, high molecular weight component in the extracts studied by us represents human thyroglobulin.

The data given above suggest that the chemical and physico-chemical properties of human thyroglobulin are similar to those of thyroglobulin of animal origin.

We wish to thank Dr. A. Ehrenberg for carrying out the ultracentrifugal analyses.

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Received November 11, 1960.

2,2′-Diphenyl-3,3′-diindolylmethane

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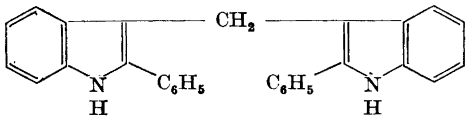
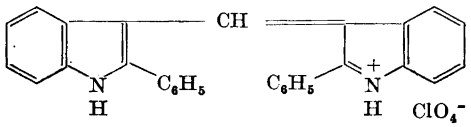
In a recent paper [1] on the chemistry of 3-hydroxymethylindoles we reported on their self-condensation to 3,3′-diindolylmethanes on boiling with water or aqueous sodium hydroxide. An exception was 3-hydroxymethyl-2-phenylindole which yielded 2-phenylindole on refluxing with 10 % sodium hydroxide. The expected product, 2,2′-diphenyl-3,3′-diindolylmethane (I), was described by Dahlbom and Misiorny [2] who claimed to have obtained this compound from 2-phenylindole and formaldehyde. However, the melting point which



I



II

they reported (184—185°) for this substance seemed to be unusually low, since all the other known 3,3′-diindolylmethanes melt considerably above the corresponding indoles, as indicated in Table 1.

This led us to suggest that the compound, m.p. 184—185°, was not authentic 2,2′-

Table 1. Melting points of substituted indoles and 3,3′-diindolylmethanes. °C.

Substituent	Indole	3,3′-Diindolylmethane
None	52—53	167—168
1-Methyl	below 0	112.5—113
2-Methyl	62	237—238
1,2-Dimethyl	56	161.5—162.5
1-Methyl-2-phenyl	100—101	185—186
2-Phenyl	187—188	184—185