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ANTITHYROID ACTIVITY OF THIOHYDANTOINS

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The antithyroid activity of 2-thiohydantoin and some derivatives has been measured in rats, after a single dose and, also, after daily administration for 6 weeks. 2-Thiohydantoin and its 5-alkyl derivatives from 5-methyl to 5-sec-butyl- showed considerable antithyroid activity at a dose of 0.05 m.mole/kg. by mouth, but 5-*n*-hexyl-2-thiohydantoin had insignificant activity at this dose level. The presence of polar groups in the 5-substituent was associated with reduction or complete loss of activity. By the single-dose technique, 2-thiohydantoin was more potent than its 5-alkyl derivatives. The opposite result was obtained by other methods of assessment after 42 days of treatment. Some of the possible factors involved are discussed especially in relation to the difficulty of comparing results obtained by the different methods of assessment. The acute toxicity of 2-thiohydantoin and of its 5-methyl and 5-ethyl derivatives is reported. No toxic effects were found after daily administration of several 2-thiohydantoins at a dose of 50 mg./kg. for 6 weeks.

The biological activity of thiohydantoins, unlike that of hydantoins, has not received much attention in the past. Lewis (1913) reported some acute toxicity studies. Astwood, Bissell, and Hughes (1945), Jackman, Klenk, Fishburn, Tullar, and Archer (1948) and Searle, Lawson, and Morley (1951) included some thiohydantoin derivatives in the large number of compounds which they examined for antithyroid activity. Thiohydantoin is very similar structurally to thiouracil and mercaptoglyoxaline (Fig. 1) and certain of its

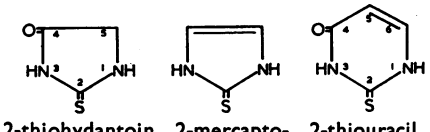

FIG. 1.

derivatives, like the parent compound, were found to have antithyroid activity. However, no systematic study of this action of thiohydantoins has been reported. 2-Thiohydantoin, sixteen 5-substituted derivatives, 1-methyl-2-thiohydantoin, and thiohydantoic acid, have been tested for antithyroid activity in rats. This activity has been measured in various ways and compared with that of 2-thiouracil. The relationship of structure and activity is discussed with reference to previous work on derivatives of 2-thiouracil and 2-mercaptoglyoxaline.

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METHODS

Preparation of Compounds.—All the compounds tested have been described previously. References to the methods of preparation of 2-thiohydantoins are listed here: 5-carboxymethyl- (Johnson and Guest, 1912), 5-carbamoylmethyl- and 5-indolylmethyl- (Swan, 1952), 5-*p*-hydroxybenzyl- (Taschner, 1951), 1-methyl- and 5-(4′-aminobutyl)- (Elmore and Ogle, 1957), 5-methylsulphonylethyl (Elmore, Ogle, and Toseland, 1956), and the remainder were prepared by the method of Jackman *et al.* (1948). Thiohydantoic acid (*N*-thiocarbamoylglycine) was prepared by the method of Elmore, Toseland, and Tyrrell (1955). All compounds were chromatographically pure (Elmore and Ogle, 1957).

Suppression of ^{131}I Uptake.—This was measured in young rats (about 6 weeks old) following the technique described by McGinty, Rawson, Fluharty, Wilson, Riddell, and Yee (1948). A similar method was used by Searle, Lawson, and Hemmings (1950). Each substance was given in aqueous solution by stomach tube, in a dose of 0.05 m.mole/kg. body weight. All were relatively insoluble and some were given as solutions of the Na salts. Groups of 5 to 7 rats were used to test each substance and as controls, which received water. One hour after dosing, all rats were given an intraperitoneal injection of radioiodide

(approximately 3 μc. of ^{131}I in 1.0 ml. H_2O, without added carrier). The rats were killed with chloroform after a further 4 hr. The thyroid glands were removed immediately and dissolved overnight in 2.0 ml. of 2% NaOH in an incubator at 37°. The volumes were then made up to 10 ml. and counted in a Veall (1948) liquid counter. The ^{131}I content was expressed as % of the injected dose.

Antithyroid Activity after Prolonged Administration of Test Substances.—Young rats (about 6 weeks old) were matched for weight and kept in individual cages. They were allowed water and commercial cube diet *ad libitum*. Thyroid activity was assessed by three criteria, apart from ^{131}I uptake described above.

Weight Gain.—Body weight was measured every third day, after voidance of urine and faeces.

Thyroid Weight.—The thyroid glands were dissected out after death and weighed on microscope slides.

Thyroid Histology. — Thyroid hyperplasia was assessed histologically by a mid-section through one lobe. Assessment was performed using the same criteria and scoring system as used by Astwood (1943).

Results

Antithyroid Activity Following Single Dose of Drug.—The mean depression of ^{131}I content of the thyroid glands for each substance tested is expressed as % of the control glands (Table I). 2-Thiohydantoin is very active, but thiohydantoic acid, which is prepared from it by mild alkaline hydrolysis, is without activity. 1-Methyl-2-thiohydantoin is similar to the parent compound. All the other compounds are 5-substituted thiohydantoins. High potency remains with ascending 5-alkyl substitution, until there is a sudden loss of activity with 5-*n*-hexyl thiohydantoin. Ring substitution, illustrated by the 5-benzyl derivative, still gives high activity, but this disappears with 5-*p*-hydroxy-benzyl derivative. This, and succeeding compounds in Table I, show low activity or are inactive. They contain polar groups in the side-chain. 5-*p*-Hydroxy-benzyl-2-thiohydantoin is still inactive when given by intraperitoneal injection, suggesting that inactivity is not due to lack of absorption from the intestine.

Increasing the dose shows that some slight antithyroid activity of a thiohydantoin containing a polar substituent can be demonstrated. 5-*p*-Hydroxy-benzyl-2-thiohydantoin produces 48% depression of 4 hr. ^{131}I uptake, when the dose is increased tenfold, namely to 0.5 m.mole/kg.

Comparative Antithyroid Activity by Single Dose Technique. — Dose/response relationships for several thiohydantoins were studied and compared with that for 2-thiouracil. At least five doses were used for each compound and % depression of 4 hr. ^{131}I uptake calculated by comparison with the mean uptake in a control group of rats. Regression slopes of the dose/response curves were calculated and relative potency of the substances was compared at the level of 70% depression of ^{131}I uptake (Table II and Figs. 2 and 3). The dose/response curves were reduced to linearity within the ranges of observations, by using logarithmic scales. The horizontal logarithmic scale is $\log_{10}$ (dose in mg.) and the vertical logarithmic scale is $\log_{10}\left(1/1-\frac{\%\text{ depression}}{100}\right)$, or in terms of observation, $\log_{10}$ (mean control uptake/mean trial uptake). Regression slopes were then calculated by least squares.

TABLE I

ANTITHYROID ACTIVITY OF VARIOUS THIOHYDANTOINS IN RATS AS TESTED BY A SINGLE ORAL DOSE OF 0·05 M.MOLE/KG.

The significance of differences in ^{131}I uptake between treated and control animals was calculated using Student's *t* test. The level of significance is indicated in the second column of the table, as follows: $^{**}P<0.01$; $^{*}0.05>P>0.01$; N.S. $P>0.05$.

Compound	Mean Depression of ^{131}I Content of Thyroid as % of Control Group	No. of Rats Treated	No. of Controls
2-Thiohydantoin	88**	5	5
Thiohydantoic acid ..	−26 N.S.	6	6
1-Methyl- ,, ..	79**	5	5
5-Methyl- ,, ..	82**	5	5
5-Ethyl- ,, ..	80**	6	5
5-*n*-Propyl- ,, ..	75**	6	5
5-*iso*-Propyl- ,, ..	79**	5	5
5-*n*-Butyl- ,, ..	82**	6	6
5-*iso*-Butyl- ,, ..	86**	6	6
5-*sec*-Butyl- ,, ..	86**	5	5
5-*n*-Hexyl- ,, ..	18 N.S.	6	6
5-Benzyl- ,, ..	90**	6	6
5-*p*-Hydroxybenzyl- ,, ..	−18 N.S.	7	5
5-Carbamoylmethyl- ,, ..	3 ,,	7	6
5-Carboxymethyl- ,, ..	−24 ,,	6	5
5-Methylthioethyl- ,, ..	31*	5	5
5-Methylsulphonylethyl- ,, ..	−24 N.S.	6	6
Indolylmethyl- ,, ..	33*	7	7
5-(4′-Aminobutyl)-hydrochloride ,, ..	18 N.S.	6	7

TABLE II

REGRESSION SLOPES OF DOSE/RESPONSE CURVES FOR THIOURACIL AND VARIOUS THIOHYDANTOINS

Compound	Regression Slope (Units in Mean Log Response/Unit of Log Dose) ± S.E.		Common Slope	Dose in mg. for 70% Depression of ^{131}I Uptake (Accurate to −30, +50%, See Text)
2-Thiouracil ..	0·764	0·080		0·93
2-Thiohydantoin ..	0·741	0·072	0·735	1·20
5-Benzyl- ,, ..	0·699	0·084		2·10
5-Methyl- ,, ..	0·457	0·115	0·455	3·00
5-*n*-Propyl- ,, ..	0·452	0·068		2·70
5-*iso*-Butyl- ,, ..	0·619	0·085		3·00
1-Methyl- ,, ..	0·351	0·080		1·50

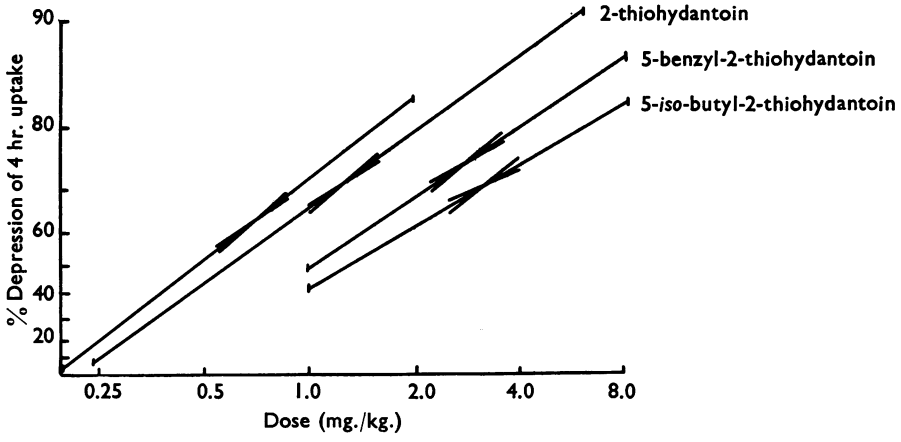

FIG. 2.—Calculated regression lines from dose/response curves for 2-thiouracil, 2-thiohydantoin, 5-benzyl-2-thiohydantoin, and 5-*iso*-butyl-2-thiohydantoin. Horizontal logarithmic scale is $\log_{10}$ (dose in mg.). Vertical logarithmic scale is $\log_{10}$ (mean control uptake/mean trial uptake). ±S.E. of regression line is indicated for each substance. The left-hand regression line refers to 2-thiouracil.

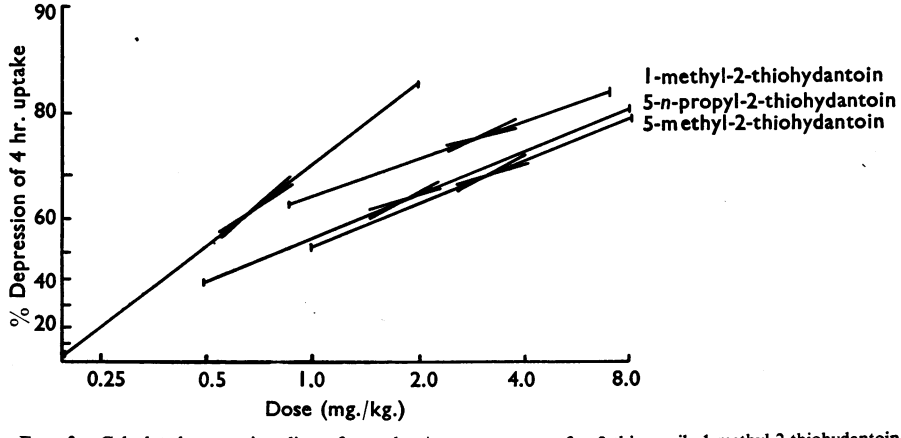

FIG. 3.—Calculated regression lines from dose/response curves for 2-thiouracil, 1-methyl-2-thiohydantoin, 5-*n*-propyl-2-thiohydantoin, and 5-methyl-2-thiohydantoin. Horizontal logarithmic scale is $\log_{10}$ (dose in mg.). Vertical logarithmic scale is $\log_{10}$ (mean control uptake/mean trial uptake). ±S.E. of regression line is indicated for each substance. The left-hand regression line refers to 2-thiouracil.

An analysis of variance, corresponding to a goodness of fit test and a test of parallelism for the regression lines, is shown in Table III. The means of groups of rats given the same dose did not deviate from the regression line more than would be expected from the within group variations; there was thus no indication either of non-linearity of regression or of some uncontrolled experimental error affecting all rats in a dose group, and the deviations gave no indication of statistical significance (F=0.89).

The regression lines deviated significantly from a common regression slope (F=3.41). Thus relative potency cannot be compared from these

TABLE III

ANALYSIS OF VARIANCE TO TEST FOR A COMMON REGRESSION SLOPE OF DOSE/RESPONSE CURVES AND GOODNESS OF FIT OF REGRESSION LINES

The double asterisk indicates significant at the 1% level.

	Degrees of Freedom	Mean Square	F Criteria
Deviations of regression lines from parallelism	6	1,240	−F criterion =3·41**
Deviations of dose level group means from regression lines ..	21	287	−F criterion =0·89 N.S.
Variations within dose level groups	220	364	

slopes, apart from comparison at a given % depression. However, common regression slopes could be ascribed to two groups of substances, as within these groups there was no significant deviation from parallelism. Relative potency can be assessed without qualification within the group 2-thiouracil, 2-thiohydantoin, and 5-benzyl-2-thiohydantoin, and between 5-methyl-2-thiohydantoin and 5-*n*-propyl-2-thiohydantoin, but not between these groups or the other two thiohydantoins tested (see Table II). 2-Thiouracil and 2-thiohydantoin have almost equal potency, and the latter is nearly twice as potent as its 5-benzyl derivative. Interpretation of the flatter slopes of 5-methyl, 5-*n*-propyl-, 5-*iso*-butyl-2-thiohydantoin and 1-methyl-2-thiohydantoin is difficult and is discussed later. All the substances are compared at one particular % depression. It is difficult to give precise standard errors for the doses giving 70% depression, as the mean control uptake for each dose/response curve is based on observations from 7 to 10 rats. Unfortunately, there is significant variation in controls measured at different times, probably due to variations in iodine content of diet, but limits of error probably range from −30% to +50%. When the substances are compared in this way, 2-thiohydantoin is the most potent, and substitution of methyl, *n*-propyl or *iso*-butyl in the 5-position reduces activity about threefold.

Duration of Activity.—The durations of action of 2-thiohydantoin and 5-methyl-2-thiohydantoin are compared with 2-thiouracil (Table IV). 2-Thiohydantoin and its 5-methyl derivative produced greater depression of iodide uptake than 2-thiouracil, when uptake was measured from 1 to 5 hr. after the dose. These thiohydantoins were calculated to be more potent than 2-thiouracil from the dose/response curves. However, when the interval between dosing and giving ^{131}I was lengthened to 4, 8 and 12 hr. this effect of the thiohydantoins became less than 2-thiouracil. This is suggestive evidence that the duration of action of these thiohydantoins was shorter than 2-thiouracil.

Effects of Prolonged Administration.—The effect of various thiohydantoins given daily for 42 days was studied by observing changes in growth rate, thyroid weight, histological changes in thyroid and 24 hr. ^{131}I uptake (Table V). 2-Thiohydantoin and four 5-alkyl derivatives were tested. The other three compounds, 5-benzyl, 5-methylthioethyl- and 5-carboxymethyl-2-thiohydantoin,

TABLE IV

THE DEPRESSION OF ^{131}I UPTAKE PRODUCED BY 2-THIOHYDANTOIN, 5-METHYL-2-THIOHYDANTOIN, AND 2-THIOURACIL. ^{131}I GIVEN 1 HR., 4 HR., 8 HR., AND 12 HR. AFTER THE DRUG AND UPTAKE MEASURED 4 HR. LATER

† Indicates a dose estimated to produce equal depression of ^{131}I uptake at 1 hr.

Compound	Oral Dose† (mg./kg.)	% Depression of ^{131}I Uptake 1 to 5 hr. after Dose	% Depression of ^{131}I Uptake 4 to 8 hr. after Dose	% Depression of ^{131}I Uptake 8 to 12 hr. after Dose	% Depression of ^{131}I Uptake 12 to 16 hr. after Dose
2-Thiohydantoin ..	0·3	88	85	48	21
5-Methyl- ,, ..	0·4	82	73	42	15
2-Thiouracil	0·1	72	67	53	35

TABLE V

EFFECT OF VARIOUS THIOHYDANTOINS ON GROWTH RATE, THYROID WEIGHT AND HISTOLOGY AND 24 HR. ^{131}I UPTAKE IN RATS GIVEN EITHER 50 MG./KG. OR 10 MG./KG. DAILY BY SUBCUTANEOUS INJECTION FOR 42 DAYS

The significance of differences in daily weight gain, thyroid weight and ^{131}I uptake between treated and control animals was calculated using Student's *t* test. The level of significance is indicated as in Table I.

Compound	No. of Rats Dosed		Mean Daily Weight Gain as % of Mean Daily Weight Gain of 8 Control Rats		Mean Increase in Thyroid Weight Compared with Mean Thyroid Weight (mg./100 g./Rat) of 8 Control Rats		Histological Changes of Hyperplasia (2 Rats in Each Group) Classified According to Astwood		Mean Depression (2 Rats in Each Group) of 24 hr. ^{131}I Content of Thyroid as % of Mean of 4 Control Rats	
	50 mg./kg.	10 mg./kg.	50 mg./kg.	10 mg./kg.	50 mg./kg.	10 mg./kg.	50 mg./kg.	10 mg./kg.	50 mg./kg.	10 mg./kg.
2-Thiohydantoin ..	4	4	95 N.S.	96 N.S.	3·4*	2·1*	+++	++	72**	68**
5-Methyl- ,, ..	4	4	76*	93 ,,	3·2**	0·8*	+++	+	63**	58**
5-*n*-Propyl- ,, ..	4	4	86 N.S.	100 ,,	9·0**	2·0**	++++	++	69**	37*
5-*iso*-Propyl- ,, ..	4	4	71**	103 ,,	19·8**	7·8**	++++	+++	86**	45*
5-*iso*-Butyl- ,, ..	4	4	84 N.S.	105 ,,	8·0**	1·6*	++++	+	36**	18 N.S.
5-Benzyl- ,, ..	4	4	56**	89 ,,	27·2**	8·2**	++++	+++	83**	64**
5-Methyl-thioethyl- ,, ..	4	4	97 N.S.	99 ,,	1·5*	1·0*	+	+	47*	35*
5-Carboxy-methyl- ,, ..	4	4	95 ,,	108 ,,	0·0 N.S.	0·1 N.S.	−	−	−3 N.S.	−21 N.S

were chosen to represent an aromatic derivative, and polar compounds. The substances were given by subcutaneous injection at two dose levels, 10 mg./kg. and 50 mg./kg. The smaller of these doses was approximately twice the dose of 0.05 m.mole/kg. used in the single-dose technique. Control rats were given distilled H_2O subcutaneously. Depression of daily weight gain was only found with the 5-methyl, 5-*iso*-propyl, and 5-benzyl derivatives at the level of 50 mg./kg. and this was associated with changes in thyroid weight, histology, and 24 hr. ^{131}I uptake. As judged by these criteria, each substance produced uniform and consistent effects. The one striking exception was 2-thiohydantoin, which had been found, from the dose/response curves to single injections, to be the most potent of the thiohydantoins tested. After 6 weeks of administration there was no depression of weight gain, and only moderate increase of thyroid weight, compared with its 5-methyl *iso*-propyl and benzyl derivatives. However, it still produced pronounced depression of 24 hr. ^{131}I uptake at the end of the 42-day period of administration. The degree of histological change produced by 2-thiohydantoin seemed to correlate with increase of thyroid weight, as it appeared to do for all other compounds tested. Also striking were the differences in effects produced by the two propyl derivatives. 5-Methylthioethyl-2-thiohydantoin was weakly active when tested by the single dose technique and remained so with this method. The compound with the polar carboxyl group, 5-carboxymethyl-2-thiohydantoin, had no activity by either method.

Apart from the effects described above, all the rats remained well and active during administration of these compounds. Liver, kidneys, adrenal glands and testes showed no change in gross weight and no histological changes compared with those from control rats at the end of the 6 weeks period.

Acute Toxicity. — The acute toxicity was measured by LD50 values for three thiohydantoins given by stomach tube as suspensions in 1% methyl cellulose (Table VI). These toxicity studies were followed for 72 hr., but all deaths occurred within 12 hr. Because of the extreme insolubility of these compounds, LD50 values for injection routes could not be measured. Doses of 200 mg./kg., which were the highest that could be conveniently injected subcutaneously in solution, produced no deaths.

TABLE VI

ACUTE TOXICITY OF THIOHYDANTOINS IN YOUNG RATS ABOUT 6 WEEKS OLD

LD50 values (mg./kg. body weight).

Compound	Dose Given Orally as Suspension in 1% Methyl Cellulose
2-Thiohydantoin ..	420
5-Methyl- ,, ..	1,050
5-Propyl- ,, ..	980

DISCUSSION

Several different methods are available for the assessment of antithyroid activity, and for the study of the relation between structure and activity. Such methods fall roughly into two groups. The substance to be tested is given by mouth, or by injection, or in the diet for some days, and antithyroid activity is assessed by the degree of hyperplasia on histological examination (Astwood, 1943), increase in thyroid weight (Astwood *et al.*, 1945; Jensen and Kjerulf-Jensen, 1945), decrease in thyroidal iodine concentration (Bywater, McGinty, and Jenesel, 1945). A combination of these measurements has also been used (Astwood *et al.*, 1945; Seifter and Ehrich, 1948). Alternatively, antithyroid activity is measured after a single dose of the test substance, using uptake of radioiodine (Larson, Keating, Peacock, and Rawson, 1945). Many compounds were tested in this way by McGinty *et al.* (1948) and later by Searle *et al.* (1950). These observers used experimental animals, and thyroidal radioiodide was measured on dissected thyroids. Stanley and Astwood (1947), using *in vivo* counting and single-dose technique, have assayed drugs for antithyroid activity in man. However, there has been little work on the correlation of results obtained using single or repeated doses and there is a general assumption that comparable results may be obtained by either method (Searle *et al.*, 1950). The results of the present investigation on thiohydantoins do not support this assumption. Using the single-dose technique, dose/response curves indicate that 2-thiohydantoin is almost equipotent with 2-thiouracil, and that 5-methyl, 5-propyl, 5-butyl and 5-benzyl derivatives of 2-thiohydantoin are less active than the parent substance. On the other hand, after daily administration and on assessing the response by increase in thyroid weight and hyperplasia histologically, 2-thiohydantoin appears to be much less active than these derivatives. These results resemble those found by Astwood *et al.* (1945) for 6-substituted derivatives of 2-thiouracil. Propyl and benzyl derivatives were about ten times as potent, and among the alkyl substituted compounds, peak activity was found with the propyl-derivative.

It was also found that activity was greatly diminished in 6-*n*-hexyl-2-thiouracil, and it is of interest that 5-*n*-hexyl-2-thiohydantoin has no activity on acute testing in the present study. This striking relationship between activity and number of carbon atoms on the side-chain of 6-substituted thiouracils was confirmed by McGinty and Bywater (1945a and b), Vanderlann and Bissell (1946) and Anderson, Halverstadt, Miller, and Roblin (1945). The relationship for alkyl substitution in the 5-position of 2-thiouracil is not so apparent (Astwood *et al.*, 1945 ; Anderson *et al.*, 1945).

Much work has also been done with derivatives of 2-mercaptoglyoxaline, which is structurally very similar to 2-thiohydantoin. Searle *et al.* (1950), using the single-dose technique, did not find any increase of activity with 4-alkyl substitution on 2-mercaptoglyoxaline, and they compared this with the results obtained by Astwood *et al.* (1945) on chronic testing. Searle *et al.* (1950) do not appear to have studied dose/response curves with all their 4-alkyl derivatives. It is possible that varying rates of metabolism or excretion may account for differences found between single-dose testing and more chronic administration of compounds. However, no great difference was found between 2-thiohydantoin and its 5-methyl derivative when the rate of return of thyroid activity to normal was measured, and the activity of both of these was of shorter duration than that of 2-thiouracil. Lawson, Rimington, and Searle (1951) noted that the duration of action of 2-mercaptoglyoxalines was apparently less than that of 2-thiouracil. This problem of destruction and excretion requires further study. Jackman *et al.* (1948) studied some 4-substituted mercaptoglyoxalines and 5-substituted thiohydantoins, but related their activity to 2-thiouracil and not to the parent compounds. In agreement with the present results, they found no change in activity with varying numbers of carbon atoms in the 5-alkyl side chain of 2-thiohydantoins. Activity is severely diminished by the presence of a polar substituent at $C_{(5)}$ and in the 2-thiohydantoin ring. This is clearly illustrated by a comparison of the antithyroid properties of 5-benzyl and 5-*p*-hydroxybenzyl-2-thiohydantoin. The largest effect arises from groups such as $-NH_3^+$ $-CO.O^-$ on the one hand and $-CO.NH_2$ $-OH$ on the other. Compounds containing any of these substituents might be bound by plasma proteins, the former by electrovalent linkages and the latter by hydrogen-bonds. Such binding would prevent the drug from reaching the thyroid in a significant concentration. A group such as $-SCH_3$ could be bound rather weakly by an ion-dipole interaction, and it is noticeable that 5-methylthioethyl-2-thiohydantoin has a weak but significant antithyroid activity. Alternatively, interaction between drug and plasma proteins may not be important, and the result may be a true representation of activity within the thyroid. Whatever the explanation may be, a similar correlation between chemical structure and antithyroid properties has been observed with 6-substituted-2-thiouracils (Astwood, 1943 ; Astwood *et al.*, 1945 ; McGinty and Bywater, 1945a and b ; Vanderlann and Bissell, 1946 ; Miller, Dessert, and Anderson, 1948 ; Horner, Kimmig, and Schreiner, 1952), and substituted 2-mercaptoglyoxalines (Astwood, 1943 ; Astwood *et al.*, 1945 ; Searle *et al.*, 1950, 1951).

All the thiohydantoins tested in the present work contain the thioureido group, and this is also common to thiouracils and mercaptoglyoxalines, which have been studied by others. The mechanism of action of compounds containing this group is still not certain, but it is likely that some enzyme system catalysing oxidation of iodide to iodine in the thyroid is blocked (Astwood, 1954). It is therefore disturbing to find significant deviations from parallelism of dose/response curves for different thiohydantoins. The flatter slopes for 5-*n*-propyl, 5-methyl, 5-*iso*-butyl and 1-methyl-2-thiohydantoin may be due to the observations being taken higher up the shoulder of a response curve with a lower limiting % depression. This can occur with enzyme inhibitors of the partially competitive type (Dixon and Webb, 1958). Similar behaviour has also been noted by Astwood (1945) for 2-thiouracil and its derivatives, and he concluded that quantitative comparisons could only be made at some selected level of response. Similar treatment is required for the thiohydantoins and they have been compared here at the level of 70% depression of ^{131}I uptake. The central portion of the dose/response curve using this method approximates to a straight line. Larson *et al.* (1945) and Searle *et al.* (1950) suggest that comparison of potency should be made between the limits of 20% and 80%. Differences of slope for dose/response curves may be a reflexion of differences of inhibitor constant for the various drugs and the enzyme concerned.

The acute toxicity of 2-thiohydantoin was much greater than either its 5-methyl or 5-ethyl derivative. This confirms the toxicity studies on 2-thiohydantoin and its 5-methyl derivative in rabbits reported by Lewis (1913). No toxic effects

were noted from any of the thiohydantoins studied after daily subcutaneous administration of 50 mg./kg. for six weeks. Diminished growth rate, which occurred with a few 2-thiohydantoins, correlated with degree of antithyroid activity, and this delayed growth is probably due to considerable reduction in thyroxine secretion as suggested by Lawson *et al.* (1951). Convulsions, which were reported by Searle *et al.* (1951) to occur with administration of 1-benzoyl-2-thiohydantoin, at a lower daily dose than used in this work, were not observed with any thiohydantoin given.

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