Antithyroid Substances

1. THE MERCAPTOGLYOXALINES

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The high antithyroid activity of 2-mercaptoglyoxaline and its 4-ethyl and 4-propyl derivatives has been reported by Astwood, Bissell & Hughes (1945), and Jackman, Klenk, Fishburn, Tullar & Archer (1948). Moreover, 2-mercaptoglyoxaline has been shown by Stanley & Astwood (1947) to be among the most potent antithyroid compounds tested on man. Similar results, obtained by us with rats in an investigation of substances related to ergothioneine, led us to undertake a more systematic study of compounds possessing this ring system. The mercaptoglyoxaline structure (Fig. 1) seems to be particularly suitable

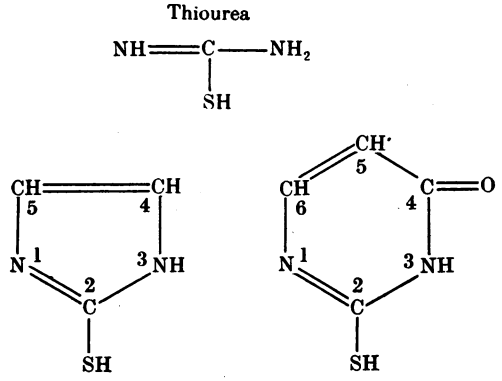

Fig. 1.

for the investigation of the effect of substituents on the antithyroid activity of thiol compounds because it offers a simpler ring system than that in the thiouracil series where there is a strongly polar carbonyl group.

Various workers have suggested that the antithyroid effect of thiol drugs is dependent on interference with an oxidative system in the thyroid gland responsible for the conversion of iodide to iodine, this interference being regarded as due to the reducing action of the thiol group. All the mercaptoglyoxalines showing appreciable activity have been found to react with iodine in the cold, and the oxidation of a number of thiols to the corresponding disulphides has been described by Biltz & Krebs (1912) and Balaban & King (1927; cf. also Miller, Roblin & Astwood, 1945). On this hypothesis, having in mind the benzenoid character of the glyoxaline ring, one would expect that an important factor influencing the potency of compounds of this type would be the ease of ionization or the lability of the hydrogen atom attached to the sulphur, and that such lability would be facilitated when electron-attracting groups were present or when the structure of the anion was such that it had a high resonance stability.

Such a hypothesis, however, is far from being adequate to account for all the facts. In the cases of aliphatic thiol compounds such as cysteine and glutathione, which are without antithyroid action, it may be that the great ease with which these undergo oxidation to disulphide forms does not allow them to reach the gland in a reduced state. Again, in the cases of 4:5-diphenyl-2-mercaptoglyoxaline (Astwood *et al.* 1945) and 2-mercaptonaphthiminazole (Bywater, McGinty & Jenesel, 1945) which have very low potency as compared with 2-mercaptobenziminazole, other factors must obviously operate. On the basis of the results described in this communication it is difficult to show any consistent correlation between the electronic character of the substituent groups and the antithyroid potency of the mercaptoglyoxalines investigated.

Of the various methods which have been used for the assay of antithyroid substances, those employing radioactive iodine (^{131}I) as a labelling agent for estimating interference with iodine uptake and concentration by the gland are probably the most convenient. Rats have been employed satisfactorily by several workers (Rawson, Tannheimer & Peacock, 1944; Franklin, Lerner & Chaikoff, 1944). Rawson, McGinty, Peacock, Merrill, Wilson & Lockhart (1948) demonstrated the blocking effect upon radioactive iodine uptake of antithyroid drugs administered over several days, whilst Larson, Keating, Peacock & Rawson (1945) similarly demonstrated the effectiveness in chicks of a single subcutaneous injection of 10 mg. of thiouracil. They further showed that the relation between the degree of inhibition and the logarithm of the dose of thiouracil was a linear function. McGinty, Rawson, Fluharty, Wilson, Riddell & Yee (1948) compared a number of anti-

thyroid drugs by giving rats or chicks a single dose by injection followed 1 hr. later by an injection of radioiodine. After 4 hr. the animals were killed and the uptake of isotope by their thyroid glands compared with that of controls. It was clearly established that within 8 hr. after administration, partial recovery of the gland from the action of the drug took place in all cases.

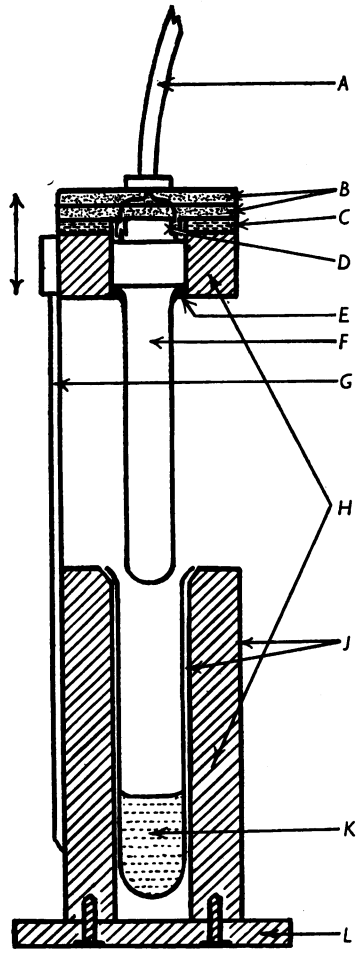

Fig. 2. Castle and mounting for Geiger-Müller immersion type counter. *A*, coaxial cable to scaling unit; *B*, 'Paxolin' caps; *C*, rubber washer; *D*, contacts; *E*, paraffin wax; *F*, Geiger-Müller counter; *G*, angle-brass guide; *H*, lead shielding; *J*, brass tubing; *K*, radioactive solution; *L*, removable lead base.

In the present work we have used a modification of the method of McGinty *et al.* (1948). This technique affords a rapid screening method by which promising drugs may be picked out for further study. It was necessary to ascertain whether or not the linear log. dose:response relationship exhibited by thiouracil was applicable also to the series of compounds under investigation, and for this purpose 2-mercaptoglyoxaline was selected as a representative compound for study.

METHODS

The synthesis of new compounds used in this investigation will be described elsewhere.

Test animals. Black and white hooded rats weighing approximately 50 g. were maintained for a minimum of 2 weeks, before testing, upon a batch of commercial cube diet known to be somewhat low in I_2 content (0·16–0·28 mg./kg.), but not so low as to produce goitrous animals. With diets of higher I_2 content, the uptake of ^{131}I was inconveniently low and less useful for the detection of compounds of low potency. Other batches of the same commercial diet have been shown to contain three to four times the above quantity of I_2, due, we suspect, to differences in the quality and origin of the fish-meal component. Animals were used in groups of five to eight for each assay; in a few instances where experiments have been carried out more than once, the results have been combined.

Dosing. A single dose of drug in aqueous solution was administered by stomach tube, in most cases at the level of 5 or 10 mg./kg. body wt.; when solubility difficulties were encountered, the Na salts or hydrochlorides were used.

Isotopic iodide. The stock of isotopic I_2 solution was diluted with 0·9% (w/v) NaCl to approximately 1·0 μc. of ^{131}I/ml. No addition of carrier iodide was made.

Assay technique. The isotope solution (0·5 ml.) was injected intraperitoneally into each rat 1 hr. after administration of the drug, and after a further 4 hr. the animals were killed by $CHCl_3$. The thyroid gland of each animal was dissected out, placed in a pyrex boiling tube containing 2 ml. of 2% (w/v) NaOH, dissolved by warming and diluted to about 15 ml. For counting, each solution was transferred to a single boiling tube suitably graduated for the relative dimensions of the tube and immersion counter, and made up to the mark (about 18 ml.) with distilled water. The lead 'castle' containing the counter is shown in Fig. 2. The solution (0·5 ml.) used for injection was similarly treated with NaOH, diluted and counted. The percentage uptakes of the dosed and undosed control animals were then compared. The results were expressed as the percentage depression of the mean uptake of the control animals, the significance of the results being evaluated statistically.

RESULTS AND DISCUSSION

The variation of ^{131}I uptake with time is illustrated by Fig. 3. Notwithstanding the larger uptake obtained over 24 hr., an interval of 4 hr. was selected for the single-dose experiments, since considerable lessening of drug action has been shown to occur within 8 hr. under similar circumstances by Larson *et al.* (1945). In experiments where drugs are continuously administered in the diet over several days, we prefer to use a 24 hr. period of ^{131}I uptake.

The dose-response curve of 2-mercaptoglyoxaline was found to be very similar to that of 2-thiouracil, although slightly more steep (Fig. 4). Both are typically S-shaped curves in which the central portion approximates to a straight line (cf. Larson

et al. 1945). It follows from this that valid comparisons of potency are best made between the limits of 20 and 80% inhibition of iodine uptake and also that comparisons with thiouracil should be drawn with caution.

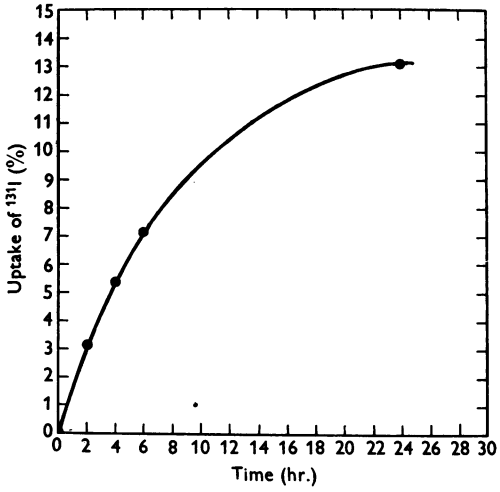

Fig. 3. Variation of ^{131}I uptake with time.

2-Thiolhistidine, its methyl betaine (ergothioneine), and 4-methyl-2-thiolhistidine in this test showed no significant activity. This result for ergothioneine is in agreement with that of Stanley & Astwood (1947) and Wilson & McGinty (1949) who were unable to confirm the finding of Lawson & Rimington (1947). Further work is proceeding with this substance.

For purposes of comparison we have included amongst the compounds examined potassium thiocyanate, thiourea, thiouracil and its 6-amino, 6-methyl, and 6-propyl derivatives. The results for these compounds, shown in Table 1, are in agreement with those reported by other workers using different methods of assay.

SUMMARY

A series of 2-mercaptoglyoxaline derivatives has been tested for antithyroid activity in rats using a single-dose technique combined with radioactive iodine uptake.

We wish to thank Glaxo Laboratories for their generosity in providing most of the rats used in the investigation, and the Medical Research Council Tracer Committee for the supply of radioactive iodide. We are indebted to Mr R. A. F. Bullerwell, Mr H. Heath, and Mr H. V. Morley, who prepared most of the compounds examined, and also to Sir Charles Harington, F.R.S., Burroughs Wellcome & Co., and the Lederle Laboratories for gifts of 2-thiolhistidine, ergothioneine and 6-aminothiouracil respectively. We are grateful to Miss B. E. Petchey for technical assistance and particularly to Prof. C. Rimington for continued interest and advice on the work. One of us (C.E.S.) acknowledges, with thanks, a grant from the Rockefeller Bequest to University College Hospital Medical School.

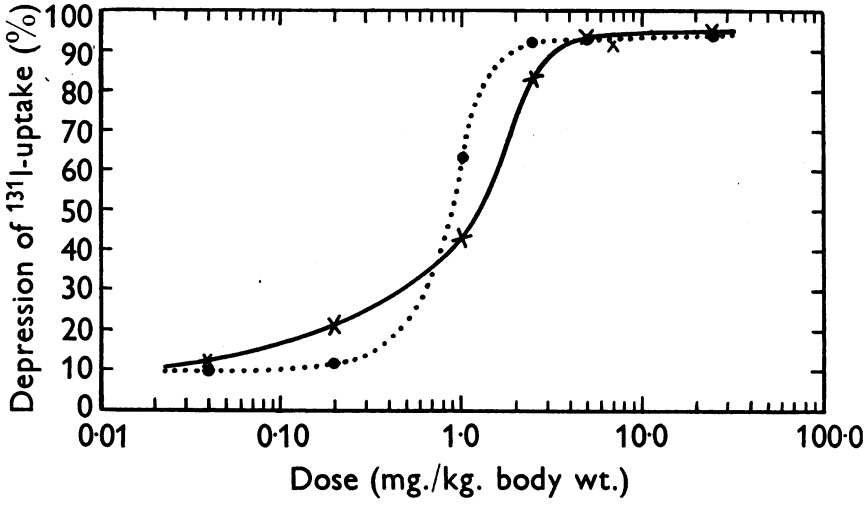

Fig. 4. Dose-response curves for 2-thiouracil (×—×—×) and 2-mercaptoglyoxaline (●···●···●). Single dose at 0 hr.; ^{131}I at 1 hr.; killed at 5 hr.

The mercaptoglyoxalines tested by the method described fall mainly into two groups, those in one group causing a depression of iodine uptake of under 30%, and those in the other of over 80% (Table 1). The parent compound and its 4-alkyl derivatives were all of high potency, although in this series, the introduction of the substituent alkyl group did not increase the activity as has been shown to occur with the thiouracils (Astwood *et al.* 1945).

Table 1. *Antithyroid activity of compounds as tested by the single injection technique in rats*

Drug	Dose (mg./kg.)	No. of rats dosed	Percentage depression of ^{131}I uptake	Significance of difference from control
2-Mercaptoglyoxaline	0·04	4	9·5	*C*
	0·20	5	11·7	*C*
	1·0	5	63·0	*A*
	2·5	6	92·4	*A*
	5	15	93·8	*A*
	25	11	94·7	*A*
4-Methyl-2-mercaptoglyoxaline	5	5	51·1	*B*
	6	6	61·1	*B*
4-Ethyl-2-mercaptoglyoxaline	5	4	87·7	*A*
	6·5	7	85·7	*A*
4-*n*-Propyl-2-mercaptoglyoxaline	5	5	86·8	*A*
4-*n*-Amyl-2-mercaptoglyoxaline	8·5	8	82·7	*A*
4:5-Dimethyl-2-mercaptoglyoxaline*	5	5	15·0	*C*
	6·5	7	−1·8	*C*
4-Methyl-5-ethyl-2-mercaptoglyoxaline	5	10	5·8	*C*
4-Ethyl-5-*n*-propyl-2-mercaptoglyoxaline	8·5	5	41·9	*A*
4-Phenyl-2-mercaptoglyoxaline	9	8	60·0	*A*
2-Mercaptobenziminazole	7·5	6	94·8	*A*
2-Mercapto-1′:2′-naphthiminazole	10	8	−21·8	*B*
3-Hydroxymethyl-2-mercaptoglyoxaline	5	5	94·2	*A*
	7	6	96·8	*A*
2-Benzylthioglyoxaline	10	5	26·4	*B*
4-Methyl-2-acetylmercaptoglyoxaline	8	6	57·7	*A*
4-Methyl-5-aldehydo-2-mercaptoglyoxaline	10	5	63·0	*A*
2-Mercaptoglyoxaline-4-carboxylic acid	7	7	9·7	*C*
4-Carbethoxy-2-mercaptoglyoxaline	8·5	7	70·5	*A*
4-Methyl-5-carbethoxy-2-mercaptoglyoxaline	5	5	2·6	*C*
4-*p*-Hydroxybenzyl-2-mercaptoglyoxaline	5	5	10·7	*C*
4-Methyl-5-*p*-hydroxybenzyl-2-mercaptoglyoxaline	5	4	12·2	*C*
4-($\alpha\beta\gamma\delta$-Tetrahydroxybutyl)-2-mercaptoglyoxaline	11	6	10·25	*C*
4-Aminomethyl-2-mercaptoglyoxaline	5	5	12·8	*C*
Product from action of formaldehyde on preceding compound	10	5	18·1	*B*
4-Carbethoxyamino-2-mercaptoglyoxaline	5	5	15·9	*B*
4-Dimethylaminomethyl-2-methylthioglyoxaline	10	5	7·9	*C*
3-Methyl-4-acetamido-2-mercaptoglyoxaline	5	5	15·9	*C*
4-Methyl-5-dimethylaminomethyl-2-mercaptoglyoxaline (acetate)	12	5	24·5	*B*
	50	5	27·7	*B*
3-Methyl-5-carbethoxy-4-amino-2-mercaptoglyoxaline	10	5	25·9	*C*
4-Methyl-5-bromo-2-mercaptoglyoxaline	10	6	15·1	*C*
2-Thiolhistidine	10	5	−7·5	*C*
4-Methyl-2-thiolhistidine	10	5	6·7	*C*
Ergothioneine (HCl, $2H_2O$)	0·04	5	6·3	*C*
	0·20	5	14·6	*C*
	1·0	16	8·9	*C*
	5	14	9·1	*C*
	15	5	7·1	*C*
	20	6	3·0	*C*
	25	16	−1·5	*C*
	75	6	14·8	*C*
2-Thiouracil	0·04	6	11·5	*C*
	0·20	6	21·0	*C*
	1·0	6	42·7	*B*
	2·5	6	83·5	*A*
	5	6	93·5	*A*
	7	5	91·4	*A*
	25	6	95·3	*A*
6-Methyl-2-thiouracil	7	6	92·6	*A*
6-*n*-Propyl-2-thiouracil	8·5	6	96·3	*A*
6-Aminothiouracil	7	10	−21·6	*B*
2-Thiohydantoin	6	6	89·8	*A*
1-Benzyl-2-thiohydantoin	10	5	88·2	*A*
Thiourea	4	5	41·0	*B*
Potassium thiocyanate	5	6	11·3	*C*

* This compound was also administered over a period of 9 days at a level 0·25% in the diet and showed a cumulative effect resulting in a depression of ^{131}I uptake of 43·0%.

The significance of differences in ^{131}I uptake between treated and control animals was calculated using Student's *t* test

$$\left[t=\frac{\text{Difference between } ^{131}\text{I uptake of dosed and control animals}}{\text{Estimated standard deviation}}\sqrt{\left(\frac{N_1N_2}{N_1+N_2}\right)}\right].$$

The level of significance is indicated in the last column of the table as follows: $P<0{\cdot}001$, *A*; $0{\cdot}1>P>0{\cdot}001$, *B*; $P>0{\cdot}1$, *C*.

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Studies in Congenital Porphyria

1. INCORPORATION OF ^{15}N INTO COPROPORPHYRIN, UROPORPHYRIN AND HIPPURIC ACID

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Like other rare inborn errors of metabolism, congenital porphyria is of considerable genetic interest and might be expected to give valuable information about normal metabolism in man. Congenital porphyria has been the subject of a large number of investigations, but the reason why large amounts of highly carboxylated porphyrins of series I are formed and excreted has so far not been elucidated. Shemin & Rittenberg (1946) have shown that glycine is the specific precursor of at least some and probably all the nitrogen atoms of the protoporphyrin in circulating haemoglobin. Later work of Wittenberg & Shemin (1949) and Muir & Neuberger (1949) has shown that at least one of the nitrogen atoms of rings I and II and one of rings III and IV, respectively, are derived from glycine. It is therefore to be expected that, if the porphyrins of series I and III have part of their metabolic pathway in common, glycine should also be the precursor of the nitrogen atoms of coproporphyrin and uroporphyrin I. Owing to the fact that the labelled newly formed haem is greatly diluted with pre-existing unlabelled haem, the ^{15}N content of the newly formed protoporphyrin present in haemoglobin can only be calculated by a mathematical analysis (Shemin & Rittenberg, 1946). In a porphyric, on the other hand, we may assume that excreted porphyrins are not diluted to any large extent by material formed prior to the date of administration of labelled glycine and we might thus expect to get an approximate but direct measure of the ^{15}N content of newly formed porphyrins.

The present paper deals with the incorporation of ^{15}N into the porphyrins, into the haem of circulating haemoglobin and into the urinary hippuric acid after feeding with labelled glycine a patient suffering from congenital porphyria. We have also investigated the incorporation of ^{15}N into the haem of the circulating haemoglobin of a normal person given a similar amount of labelled glycine.

Some of the results reported here were the subject of a preliminary communication (Gray & Neuberger, 1949).

EXPERIMENTAL

General

Experiments with a porphyric

The subject was a 32-year-old male, G.L. (60 kg. body weight), who had suffered from porphyria since birth. His clinical history up till the age of 9 has been described by Mackay & Garrod (1926). Since then, he has continually excreted mahogany-coloured urine and the skin condition has progressed each summer. As a result of secondary infection he has now lost an eye, both pinnae of the ears and all the terminal phalanges of his hands. The skin of the exposed parts is a mass of scar tissue intermingled with patches of deeply pigmented skin. The patient was given labelled glycine in two separate experiments.