The Effect of Compounds Allied to Resorcinol upon the Uptake of Radioactive Iodine (^{131}I) by the Thyroid of the Rat

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Bull & Fraser (1950) observed the induction of myxoedema and goitre by the prolonged application of resorcinol ointment to varicose ulcers, subsidence of the myxoedema on withdrawal of the ointment, and lowering of the thyroid function on its re-application. The antithyroid action of resorcinol in rats was established by Doniach & Fraser (1950), who found that subcutaneous injection of the drug considerably lowered the thyroid iodine uptake in a manner comparable with that of methylthiouracil. The experiments reported below were undertaken in sequel to this work, in an attempt to determine whether this antithyroid activity is specific to recorcinol, or whether it is exhibited by related compounds.

EXPERIMENTAL

Materials. A number of mono- and poly-hydric phenols and their derivatives were selected from compounds commercially available, purity being checked by melting point determinations. These agreed to within 5° with the figures quoted in the literature, except in the cases of 2:7-dihydroxynaphthalene (185°), gallic acid (240°) and *p*-hydroxybenzoic acid (220°), where the melting points experimentally found are given in parentheses.

Hydroxyquinol and its triacetate were synthesized according to the method of Thiele (1898). The former melted correctly at 140°, the latter at 91·5°, 5° below the figure quoted by Vliet (1941).

Methods. The method used for assay of antithyroid activity in the rat was similar to that described by Doniach & Fraser (1950). The compounds were tested in small groups, each group with its own controls, on a total of thirty-five experimental days spread over a period of 1 year. Since the uptake of iodine by the thyroid of the rat varies with environmental temperature, diet and size of the animal, we concentrated on standardizing these conditions for each experimental day rather than for the whole period, and only made direct comparisons of iodine uptake within any one group of controls and treated rats. In most experiments, female albinos of average weight 150 g. were used, four for each compound and four controls.

The compounds were administered by subcutaneous injections of 1 ml. of solution in water (or, where necessitated by reason of insolubility, in 1:5 ethanol-water mixture), mostly at two dose levels, 0·25 and 0·1 m-mole/rat. This was equivalent to 180 and 70 mg./kg./rat in the case of resorcinol. In one experiment 0·06 m-mole of compound per rat and in another 0·125 m-mole were used. In some cases 2 ml. of solvent were needed, and in a few cases suspensions instead of solutions had to be used.

Controls received a subcutaneous injection of ethanol-water mixture in those few groups where it was used as a solvent for the drugs. To provide an additional standard for comparison a group of four rats injected with resorcinol was included on most experimental days.

Ten to twenty minutes after receiving the compound each rat was given an intraperitoneal injection of 1 ml. of distilled water containing 10 μc. of carrier-free radioactive ^{131}I, of half-life 8 days, in the form of NaI. The controls were similarly injected and all rats were killed about 2 hr. later. The short time of exposure to the compounds of 2·5 hr. was chosen purposely, because of the known rapid metabolism and excretion of the phenols. Owing to difficulties in exact timing, the treated rats were killed at an average 2 hr. 5 min., and the controls (usually dealt with last) 2 hr. 10 min., after receiving ^{131}I.

The thyroids were removed attached to the trachea, and each was digested overnight at 60° in a 10 ml. screw-capped bottle containing 5 ml. ethanolic NaOH (5 g. NaOH (A.R.), 50 ml. distilled water, ethanol to 100 ml.). After cooling, 5 ml. water was added to each bottle, the mixed contents of which were poured into a liquid counter, type M. 6 (CV 2145) (Twentieth Century Electronics) and the radioactivity was determined. The rate of count varied from about 500 to 5000/min. All the samples from any one experimental group were counted in one session. For the calculation of means and standard errors the results were finally recorded to the nearest 10 counts/min. Comparisons were then made between the figures of the treated and the control rats in each group.

RESULTS

The experimental findings, of which one detailed example is given in Table 1, are summarized in Tables 3–6. It is seen from Table 1 that the variation in iodine uptake in similarly treated rats is sufficiently marked to mask any possible correspondence with animal weight. The average time of exposure of the treated animals to the radioactive iodine in this case was 2 hr. 10 min. and for the controls 2 hr. 21 min. That this small difference in time did not significantly influence ^{131}I uptake is shown by the fact that the count rate in one rat treated with the inactive (i.e. non-antithyroid) compound, 2:3-dihydroxynaphthalene, exceeded three of the control figures. Similar individual overlaps occurred in all experiments which included inactive drugs. A factor which may well have lowered the mean count rate in the treated animals is the likely non-specific toxic reaction of phenols in all tissues including the thyroid.

Table 1. *The effect of various compounds on* ^{131}I *uptake by the thyroid gland. Sample experiment*

(Dose, 0·1 m-mole/rat, all female albinos.)

Treatment	Rat weight (g.)	Time (min.) Between injection of compound and of ^{131}I	Time (min.) Between injection of ^{131}I and killing	Counts × 10^{-1}/min. Individual values	Counts × 10^{-1}/min. Mean + S.E.	Thyroid uptake of ^{131}I (as % of controls)	P*	Specific antithyroid activity
Thymol	170	14	121	202	272±44	63	0·05–0·02	Nil
	175			337				
	180			357				
	160			190				
2:3-Dihydroxynaphthalene	170	19	127	186	308±61	72	>0·05	Nil
	170			319				
	170			255				
	160			470				
Resorcinol	160	18	131	62	47±7·8	11	<0·001	Marked
	160			33				
	170			59				
	160			34				
p-Cresol	165	17	136	240	252±25	59	0·01–0·001	Slight
	155			191				
	155			264				
	175			311				
2:4-Dihydroxybenzoic acid	150	15	135	83	103±16	24	<0·001	Marked
	160			144				
	165			112				
	170			73				
Pyrogallol	160	14	140	198	232±30	54	0·01–0·001	Slight
	170			196				
	165			212				
	165			322				
Controls	175	0	141	470	429±31	100	—	—
	170			339				
	170			465				
	175			444				

* P = the probability that the difference between the means of the control and treated groups occurred by chance. This figure was derived from Student's t test.

The significance of difference between the count rates of each group and those of the controls was calculated by Student's t test, and the probability that such a difference was due to chance was recorded in terms of probability P derived from Fisher & Yates (1948). For convenience of tabulation we have converted the ratios between the mean ^{131}I uptakes of treated and control rats into approximate percentages.

It is seen in Table 1 that resorcinol produced a reduction in iodine uptake to about 11% of the control figure, followed in order of activity by 2:4-dihydroxybenzoic acid which reduced uptake to about 24%. The figures for P are influenced by degree of scatter, as well as by lowering of thyroid uptake, and this scatter tends to push results to the inactive side. Since, however, we must take into consideration the non-specific toxicity which may lower the thyroid uptake of all treated animals, we have only accepted as antithyroid those drugs which cause a reduction in iodine uptake to 50% or less with a $P<0·05$. In most cases where the means show a twofold or greater reduction, P was 0·01 or less. We have not regarded as antithyroid those drugs which show a significant ($P=0·05$–$0·02$), but only slight, reduction in iodine uptake, e.g. thymol in Table 1. We have also considered the results of repeated experiments and the effect of differing concentrations of the compounds (Tables 3–5), in the final assessment of antithyroid activity (Table 6).

Toxic effects. Most of the compounds, both active and inactive, proved toxic when given at a dose of 0·25 m-mole/rat. The toxic effects, which were either absent or minimal in the 0·1 m-mole dose, were manifested as an intense shivering lasting in the worst cases (e.g. catechol) up to 1 hr. followed by 15–30 min. lethargy, or as a lethargy lasting up to 1 hr. from the outset. All the dihydroxynaphthalenes used in these experiments, at a dose of 0·25 m-mole/rat, produced a deep narcosis lasting up to 2 hr., the animals remaining warm during the whole period. In the strength of 0·1 m-mole/rat

Table 2. *Effect of narcosis and shock on thyroid iodine uptake*

Treatment	Experiment no. 33: Thyroid iodine uptake (as % of controls)	33: *P*	34: Thyroid iodine uptake (as % of controls)	34: *P*	35: Thyroid iodine uptake (as % of controls)	35: *P*
Resorcinol (0·1 m-mole/rat)	—	—	14	<0·001	—	—
Controls	100	—	100	—	100	—
$MgSO_4.7H_2O$ (0·75 mg./100 g.)	92	>0·05	—	—	—	—
Nembutal anaesthesia	—	—	81	>0·05	—	—
$MgSO_4$ + ATP	58	0·01–0·001	—	—	40·5	<0·001
Ether anaesthesia	—	—	60	0·05–0·02	—	—

Table 3. *Effect on thyroid iodine uptake of monohydric phenols and certain derivatives*

(All experiments were on groups of four rats unless otherwise stated.)

Name of compound	m-moles/rat	Thyroid uptake of ^{131}I (%)	*P*
Phenol	0·25	34	0·01–0·001
	0·25	40	>0·05
	0·25	45	0·05–0·02 (8 rats)
	0·25	53	0·01–0·001 (8 rats)
	0·1	>100	>0·05
o-Cresol	0·25	52	0·02–0·01
	0·25	54	0·05–0·02
	0·1	67	>0·05
	0·1	>100	>0·05
m-Cresol	0·25	84	>0·05
	0·25	80	>0·05
	0·1	>100	>0·05
p-Cresol	0·25	52	0·05–0·02
	0·25	64	>0·05
	0·25	49	0·05–0·02
	0·1	59	0·01–0·001
	0·1	>100	>0·05
Thymol	0·25	55	0·02–0·01
	0·25	72	>0·05
	0·1	63	0·05–0·02
α-Naphthol	0·25	84	>0·05
β-Naphthol	0·25	94	>0·05
o-Hydroxybenzoic acid	0·25	74	>0·05
m-Hydroxybenzoic acid	0·25	88	>0·05
	0·25	89	>0·05
p-Hydroxybenzoic acid	0·25	81	>0·05
Saligenin	0·25	53	0·01–0·001
	0·25	44	0·02–0·01
	0·1	>100	>0·05

none of the compounds produced more than 15–30 min. slight lethargy, or very mild shivering detectable only by palpation.

Experiments designed to investigate the effect of 2 hr. deep narcosis and shock upon thyroid iodine uptake were carried out.

The iodine uptake was measured by the same method used for testing the effect of the phenol derivatives. The results are presented in Table 2. It will be seen that nembutal produced no significant lowering of iodine uptake and ether anaesthesia a reduction to 60% of that of the controls. Two groups of rats were shocked by the combined injections of magnesium sulphate and adenosinetriphosphate, as described by Green & Stoner (1944). The magnesium sulphate (75 mg./100 g. body weight) was given subcutaneously. The adenosinetriphosphate (ATP) was given intraperitoneally in

Table 4. *Effect on thyroid iodine uptake of resorcinol and certain derivatives*

(All experiments were in groups of four rats unless otherwise stated.)

Name of compound	m-moles/rat	Thyroid uptake of ^{131}I (%)	*P*
Resorcinol	0·25	14	Range of 8–23% (mean of 9 exps.)
	0·125	23	0·01–0·001 (2 rats)
	0·1	24	Range of 11–45% (mean of 11 exps.)
	0·06	40	0·05–0·02
Orcinol (5-methylresorcinol)	0·25	15	<0·001
	0·1	23	0·01–0·001
	0·06	46	0·05–0·02
2-Methylresorcinol	0·25	11	<0·001 (3 rats)
	0·1	11	<0·001
	0·06	54	0·05–0·02
4-Chlororesorcinol	0·25	13	0·01–0·001 (2 rats)
	0·1	25	<0·001
	0·06	41	0·02–0·01
4-Hexylresorcinol	0·25	51	0·01–0·001
	0·25	22	0·01–0·001
	0·1	80	>0·05
Resorcinol monacetate	0·25	13	<0·001
	0·1	16	0·01–0·001
	0·06	59	>0·05
Resorcinol diacetate	0·25	13	<0·001
	0·1	23	0·05–0·02
Resorcinol monomethyl ether	0·25	32	0·02–0·01
	0·25	71	>0·05
	0·1	64	0·05–0·02
Resorcinol dimethyl ether	0·25	97	>0·05
2:4-Dihydroxybenzoic acid	0·25	23	<0·001
	0·25	19	<0·001
	0·1	63	>0·05
	0·1	51	0·05–0·02
	0·1	24	<0·001
2:4-Dihydroxybenzaldehyde	0·25	13	0·05–0·02
Naphthylresorcinol (1:3-Dihydroxynaphthalene)	0·25	18	<0·001
	0·25	16	<0·001
	0·25	9	<0·001
	0·1	20	<0·001

divided doses totalling 20 mg./100 g. rat in Exp. 33 and 82·5 mg./100 g. rat in Exp. 35. The former produced a severe toxic state lasting 1 hr., the latter a severe toxic state lasting the full 2 hr. supervening between commencement of the experiment and killing of the animals. The rats were cold and extremely lethargic. The thyroid iodine uptake was significantly lowered in both groups: to 58% in Exp. 33 and 40·5% in Exp. 35; but by no means so effectively as the reduction to 14% produced by the non-toxic (0·1 m-mole) dose of resorcinol in Exp. 34. It would appear from these results that deep narcosis or a non-specific toxic state are very unlikely to produce more than two- to threefold lowering of thyroid iodine uptake within the conditions of our experimental method. However, it is probable that the marked narcotic effects observed with the dihydroxynaphthalenes contributed significantly to the lowered thyroid uptake found.

DISCUSSION

Tables 3–5 show that all the most active compounds except 2:7-dihydroxynaphthalene, are, or may be considered as, resorcinol derivatives. Resorcinol itself is strongly active: its isomers, quinol and catechol, are inactive. The activity of the resorcinol and hydroxyquinol acetates is probably due to hydrolysis in the body. The non-activity of the methyl ethers supports this conclusion, since one would not expect them to be so hydrolysed. The substitution of a chlorine atom in the 4-position or

Table 5. *Effect on thyroid iodine uptake of polyhydric phenols and certain derivatives*

Name of compound	m-moles/rat	Thyroid uptake of ^{131}I (%)	*P*
Resorcinol	0·25	14	Range of 8–23% (mean of 9 exps.)
	0·125	23	0·01–0·001 (2 rats)
	0·1	24	Range of 11–45% (mean of 11 exps.)
	0·06	40	0·05–0·02
Catechol	0·25	53	>0·05 (3 rats)
	0·125	>100	>0·05 (6 rats)
	0·1	>100	>0·05
Quinol	0·25	61	0·05–0·02
	0·25	61	0·05–0·02
	0·25	70	>0·05
	0·125	>100	>0·05
	0·1	>100	>0·05
1:3-Dihydroxynaphthalene	0·25	18	<0·001
	0·25	16	<0·001
	0·25	9	<0·001
	0·1	20	<0·001
2:3-Dihydroxynaphthalene	0·25	17	<0·001
	0·25	25	0·01–0·001
	0·25	56	0·01–0·001
	0·1	43	<0·001
	0·1	58	0·01–0·001
	0·1	72	>0·05
1:5-Dihydroxynaphthalene	0·25	40	0·01–0·001
	0·25	53	0·01–0·001
	0·1	70	>0·05
2:7-Dihydroxynaphthalene	0·25	18	0·01–0·001
	0·25	18	<0·001
	0·1	17	<0·001
Pyrogallol	0·25	14	<0·001
	0·25	48	0·02–0·01
	0·1	83	>0·05
	0·1	67	0·05–0·02
	0·1	54	0·01–0·001
	0·1	56	0·02–0·01
Phloroglucinol	0·25	16	<0·001
	0·25	25	<0·001
	0·1	11	<0·001
	0·1	15	<0·001
	0·1	33	<0·001
Hydroxyquinol	0·25	17	<0·001
	0·1	22	<0·001
	0·1	15	0·05–0·02
Hydroxyquinol triacetate	0·1	28	0·05–0·02
2:4-Dihydroxybenzoic acid	0·25	23	0·01–0·001
	0·1	63	>0·05
2:5-Dihydroxybenzoic acid	0·25	>100	>0·05
	0·1	91	>0·05
Gallic acid	0·25	77	>0·05
	0·1	93	>0·05
	0·1	72	>0·05

a methyl group in the 2- or 5-position does not diminish the antithyroid activity of resorcinol, nor does the presence of a carboxyl group: 2:4-dihydroxybenzoic acid was comparable to resorcinol in its activity, whilst its 2:5-isomer proved inactive.

The interpretation of the results becomes more debatable when a third hydroxyl group is present as a substituent in the ring. The strongly marked activity of phloroglucinol is to be expected, since, for our purpose, it can be regarded as a resorcinol derivative. Its isomer pyrogallol, which may be regarded as a hydroxy derivative of either catechol

Table 6. *Summary of antithyroid effects of compounds tested*

Dose (m-mole)	Group 1. Active. Uptake 11–34% of controls	Group 2. Slightly active. Uptake 40–58% of controls	Group 3. Inactive. Uptake over 63% of controls
0·25	2-Methylresorcinol Resorcinol monacetate Resorcinol diacetate 4-Chlororesorcinol 2:4-Dihydroxybenzaldehyde Resorcinol (1:3-dihydroxybenzene) 1:3-Dihydroxynaphthalene Orcinol (5-methylresorcinol) Hydroxyquinol (1:3:4-trihydroxybenzene) 2:7-Dihydroxynaphthalene Phloroglucinol (1:3:5-trihydroxybenzene) 2:4-Dihydroxybenzoic acid Pyrogallol (1:2:3-trihydroxybenzene) 4-Hexylresorcinol	2:3-Dihydroxynaphthalene Phenol 1:5-Dihydroxynaphthalene Saligenin (*o*-hydroxybenzyl alcohol) Resorcinol monomethyl ether *o*-Cresol Catechol (1:2-dihydroxybenzene) *p*-Cresol	Quinol (1:4-dihydroxybenzene) Thymol (5-methyl-2-*iso*propylphenol) *o*-Hydroxybenzoic acid Gallic acid *p*-Hydroxybenzoic acid *m*-Cresol α-Naphthol *m*-Hydroxybenzoic acid β-Naphthol Resorcinol dimethyl ether 2:5-Dihydroxybenzoic acid
0·1	2-Methylresorcinol Resorcinol monacetate 2:7-Dihydroxynaphthalene Hydroxyquinol 1:3-Dihydroxynaphthalene Phloroglucinol Resorcinol diacetate Orcinol Resorcinol 4-Chlororesorcinol Hydroxyquinol triacetate 2:4-Dihydroxybenzaldehyde	2:4-Dihydroxybenzoic acid 2:3-Dihydroxynaphthalene	Thymol Resorcinol monomethyl ether Pyrogallol 1:5-Dihydroxynaphthalene *p*-Cresol 4-Hexylresorcinol Gallic acid 2:5-Dihydroxybenzoic acid Saligenin Phenol *m*-Cresol Catechol Quinol

A few compounds found inactive at the 0·25 m-mole concentration were, for that reason, not tested at the 0·1 m-mole concentration.

or resorcinol, is markedly less antithyroid than resorcinol. On the other hand, the antithyroid action of hydroxyquinol is strongly marked, indicating that it acts as a resorcinol derivative. The total inactivity of gallic acid helps to confirm the contrast found between pyrogallol and phloroglucinol.

In order to summarize the findings, we expressed each mean count rate on each experimental day as a percentage of the mean control figure on that day. When more than one test had been made of a given compound in the same concentration the percentages found on different occasions were averaged. These final percentages (Table 6) fell into three groups. Group 1 showed a reduction in iodine uptake to 11–34%, group 2 a reduction to 40–58%, and group 3 no significant reduction.

The compounds are arranged for each dose level and in each column in order of diminishing activity. Thus, with the 0·25 m-mole dose, 2-methylresorcinol produced an 11% and 4-hexylresorcinol a 34% uptake. This method of treatment of the results is of value as a means of expressing definite trends, though it does not give an accurate presentation.

Those compounds which showed slight activity at 0·25 m-mole and were inactive at 0·1 m-mole have been regarded as not proven to be antithyroid. Of the two compounds which showed slight activity at the 0·1 m-mole dose level, 2:4-dihydroxybenzoic acid is a resorcinol derivative and exhibited marked antithyroid activity at the higher concentration. The other compound, 2:3-dihydroxynaphthalene, showed a feeble antithyroid activity which was only slightly enhanced at the higher concentration.

The results strongly favour the conclusion that this form of antithyroid action arises from the presence of two hydroxyl groups meta-substituted to each other in a benzene ring. The 2:7-dihydroxynaphthalene forms the only major exception to this conclusion. 4-Hexylresorcinol was inactive at the lower concentration.

Possible modes of action of resorcinol derivatives. Evidence was put forward previously (Doniach & Fraser, 1950) that resorcinol interfered in the thyroid with the organic binding of iodide and did not lower the concentration of inorganic iodide. In this it simulates the action of thiouracil rather than that of thiocyanate.

In the opinion of Pitt-Rivers (1950) 'the antithyroid action of any compound may be expressed as a function of its reducing power and preferential reactivity with iodine, which inhibits the formation of thyroxine...'. It may be doubted, however, whether this hypothesis can fully account for all the facts established in the present investigation, since, among the compounds which we tested, there is no correlation between the antithyroid action and reducing power. Aromatic compounds containing hydroxyl groups oriented ortho or para with respect to each other (such as catechol, quinol, pyrogallol and hydroxyquinol) are stronger reducing agents *in vitro* than the meta isomers, yet in these experiments most of them have shown no distinctive antithyroid effect.

Differences in antithyroid potency may reflect differential concentration in the thyroid rather than differences in avidity for iodine. Garton & Williams (1949) studied the excretion of quinol, catechol and resorcinol in rabbits, and found that the first two compounds are almost entirely conjugated when excreted, only 2% of the catechol and a trace of quinol being isolated free in the urine. On the other hand, as much as 11–12% of resorcinol was excreted free. This quantitative difference may mean that the resorcinol-treated rats might have a higher blood level of the phenol than rats treated with catechol or quinol.

Dr W. E. Knox, of the National Institute for Medical Research, Mill Hill, brought to our notice a significant correlation between our results and those of Elliott (1932) who catalysed the oxidation of a series of phenols by hydrogen peroxide and milk peroxidase. The compounds tested included phenol, the cresols, catechol, quinol, resorcinol, pyrogallol, gallic acid and β-naphthol. Resorcinol alone was not oxidized by milk peroxidase, the activity of which it inhibited.

Elliott's findings, taken together with Dempsey's (1944) histochemical demonstration of a thyroid peroxidase and the demonstration of such a peroxidase *in vivo* by De Robertis & Grasso (1946), are compatible with the existence of a specific peroxidase of the thyroid gland, similar to milk peroxidase in that it is inhibited by resorcinol, but not by other phenols.

SUMMARY

1. The effect of a representative selection of phenolic substances on thyroid iodine uptake in the rat was studied, using radioactive iodine.

2. With one exception, all the compounds found to be strongly antithyroid could be regarded as derivatives of resorcinol.

3. Possible modes of action of these derivatives on the thyroid are discussed.

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The Activity of Enzymic and Acidic Digests of Insulin

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In attempting to determine the nature of insulin and to obtain an active grouping or adjunct from it, many degradations have been carried out using proteolytic enzymes and acids. These studies were summarized by Hill & Howitt (1936) and by Jensen (1938). Proteolytic enzymes were favoured for this work as the mild conditions of their use and their specific action allowed more chance of preserving an active grouping.

The older work is unanimous in declaring that the activity of insulin is readily destroyed by impure pepsin, trypsin and papain, but in general only whole digests were examined for activity. Fisher & Scott (1934), however, examined the activity of the trichloroacetic acid precipitates of a crystalline

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