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Absorption and Excretion of Pheniodol Labelled with Radioactive Iodine

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Pheniodol (α -phenyl- β -(4-hydroxy-3:5-diiodo-phenyl)-propionic acid) was introduced under the name 'Biliselectan' as a peroral cholecystographic agent by Dohrn & Diedrich (1940). After early clinical trials in Germany by Kleiber (1940), Grunke & Finger (1940), Lauer-Schmaltz (1940), Naumann (1941) and Rittweger (1941) and in Britain by Kemp (1943), and more extensive ones in America by Einsel & Einsel (1943), Marshall (1943), Wasch (1943), Dannenberg (1944), Bryan & Pedersen (1944), Hefke (1944), Ochsner (1944), Paul, Pohle & Benson (1944), Vaughan & Eichwald (1944), Unfug (1946) and Brewer (1947), it has been accepted (Harper, 1949) as the cholecystographic agent of choice. It is intended to include it in the Addendum (1951) to the British Pharmacopoeia, 1948.

The general pharmacology of pheniodol in animals has been discussed by Junkmann (1941), Modell (1942) and Howard (1948). However, except in Junkmann's paper, almost no information on the absorption and excretion of pheniodol by animals has been published. Junkmann's paper appeared when World War II was well under way; it is very difficult of access in this country and it may therefore be summarized here, especially as it has been quoted successively by several authors who do not all appear to have seen it. Junkmann, who was primarily interested in comparing pheniodol with tetraiodo-phenolphthalein, measured the iodine content of bile from biliary fistulas in two rabbits and examined the urinary excretion rate for rats and two men. He showed that most of the iodine in the urine was present as pheniodol by acidifying the urine, extracting into ether, evaporating the ether and recrystallizing the residue from benzene. The product was identified as pheniodol by its melting point and by formation of an insoluble calcium salt. A few distribution results have been mentioned by Howard (1948) and Salter, Karandikar & Block (1949).

The lack of metabolic data may be due to the difficulty of estimating organically bound iodine in biological fluids. The radioactive isotope labelling technique, however, is well suited to metabolic experiments, and we have used the method to study the absorption and excretion of pheniodol by rats and cats. Pheniodol labelled with radioactive iodine (^{131}I) was prepared by iodinating α -phenyl- β -4-hydroxyphenylpropionic acid with radioactive iodine.

Pheniodol labelled with ^{131}I .

In our first set of experiments, single doses of labelled pheniodol were given orally, subcutaneously or intravenously to rats, and the excretion of radioactive iodine was studied over a 3-day period. Single doses of the labelled compound were then injected intravenously or intraduodenally into anaesthetized cats, and the iodine blood levels and urinary and biliary excretions were followed over a 6 hr. period. By using a butanol-extraction procedure the proportion of radioactive iodine present in organic combination as pheniodol could be measured. The general experimental procedures were similar to those used for labelled thyroxine (Clayton, Free, Page, Somers & Woollett, 1950).

EXPERIMENTAL

Preparation of labelled pheniodol

A solution containing ^{131}I (1 mc.) in the form of iodide was transferred to a centrifuge tube containing NaI (10 mg.) and the volume of the solution was made up to 1 ml. Ether (1 ml.) and 1.0 ml. of 0.3% H_2O_2 followed by 4 drops of N-HCl were then added. The contents of the tube were

mixed by means of a capillary pipette at intervals during 2 hr. The ether layer was separated and the colourless aqueous layer washed with a second 1.0 ml. portion of ether. The combined ether extracts were added to a solution of 0.7 g. of α -phenyl- β -4-hydroxyphenylpropionic acid in 60 ml. of 8% (w/v) aqueous ethylamine solution and the mixture was stirred for 15 min. The theoretical quantity (6.3 ml.) of 1.9N- I_2 in excess NaI was added dropwise during 5 min. and the stirring continued for 1 hr. 60 ml. of $CHCl_3$ were added; on acidification with HCl, the product passed into the $CHCl_3$ layer. This layer was washed once with 10% (w/v) $Na_2S_2O_3$ to remove free iodine and twice with water and concentrated to 15 ml.; 30 ml. of light petroleum (b.p. 100–120°) were added and the radioactive product (1.2 g.) crystallized: it melted at 157–160° (cf. 159–160°, Baker & Sansbury, 1943) and had a specific activity of about 250 μ c./g. The purity of the product was confirmed polarographically.

Polarography of pheniodol

When examined under the conditions that we used for thyroxine (Borrows, Hems & Page, 1949), pheniodol gave a characteristic polarogram with a step having the half-wave potential, -1.47 V. (measured against the saturated calomel

Fig. 1. Polarogram for 1.9×10^{-3} M-pheniodol in 20% (v/v) isopropanolic solution containing 1.0% (w/v) of tetramethylammonium bromide and 2.65% (w/v) of Na_2CO_3 . The dropping mercury electrode at a pressure of 55.5 cm. of mercury and on open circuit in 0.1N-KCl at 25° had a drop time of 3.13 sec.; the weight of mercury dropping/sec. was 1.82 mg.

electrode). Since α -phenyl- β -4-hydroxyphenylpropionic acid does not give a polarographic step, the technique provides a simple method for finding out whether iodination has gone to completion. A typical polarogram for pheniodol is shown in Fig. 1. The following polarographic procedure was used: about 10 mg. of the labelled product were dissolved in a mixture of 2.5 ml. of 2.0N- Na_2CO_3 , 2.0 ml. of redistilled isopropanol and 1.0 ml. of 10% (w/v) tetramethylammonium bromide and the mixture made up to 10 ml. with distilled water. A known portion was transferred to the cell of a photographic recording polarograph (Cambridge Instrument Co.) and, after bubbling with O_2 -free N_2 for 10 min., examined

over the potential range -0.8 to -1.8 V. The height of the step at -1.5 V. gave a measure of the amount of pheniodol in the solution.

By using the polarographic technique for analysis, the partition coefficient of pheniodol between *n*-butanol and 1.0N- Na_2CO_3 was found to be 2 and between *n*-butanol and 1.0N-HCl about 10. This observation suggested that a modification of the extraction procedure of Blau (1935) for thyroxine could be used to separate pheniodol from inorganic iodide, from which in this way it was demonstrated that our product was essentially free.

Animal experiments

Rat experiments. The excretion and breakdown of pheniodol by rats were first studied. Paired Wistar strain rats of the same age and weight (70–100 g.) were placed in metabolism cages in which the urine and faeces could be collected separately. The animals were maintained on a stock diet consisting of a paste prepared from wholemeal flour, dried milk and dried yeast, mixed with water. Groups of two rats were given single oral doses of a suspension containing 50 mg. or single subcutaneous injections of an aqueous suspension containing 60 mg. or single intravenous injections of an aqueous solution containing 74 mg. of labelled pheniodol/kg. of body weight. The suspensions contained 3.0% (w/v) of pheniodol with 1.0% (w/v) of tragacanth as suspending agent and the solutions 1.2% (w/v) of pheniodol in dilute Na_2CO_3 solution (pH 9.0).

The urine and faeces were collected every 24 hr. for a period of 3 days and examined for total and butanol-soluble radioactive iodine. At the end of each collection period the metabolism cages, funnels and separators were washed down with a 'carrier' solution consisting of 0.4N-NaOH containing 0.004% of pheniodol and 0.004% of NaI. The final washings were carried out with distilled water. All the washings were added to the urine samples and the total volumes were recorded. At the end of the third day the rats were killed and the thyroid glands, livers, kidneys, intestines and carcasses examined for residual radioactive iodine.

Cat experiments. Experiments were undertaken to determine the proportion of ^{131}I appearing in the bile of cats. The operational procedure was as follows. After anaesthetization with ether, followed by tracheotomy, the femoral vein was exposed and cannulated. A chloralose solution (0.08 g./kg. of body weight) was then introduced intravenously to maintain anaesthesia during the experiment. For collection of urine the abdomen was opened and the urethra cannulated, thus allowing the bladder to be completely emptied at each collection. The common bile duct was cannulated so that bile could be collected continuously, the gall-bladder being isolated by ligation of the cystic duct: 10 ml. samples of blood were obtained from the carotid artery at hourly intervals.

The anaesthetized cats were given either single intravenous injections of a solution containing 50 mg. or single intraduodenal injections of a suspension containing 80 mg. of labelled pheniodol/kg. body weight. Samples of blood, bile and urine were collected every hour for a period of 6 hr. and examined for total and butanol-soluble radioactive iodine. At the end of the experiments, the cats were killed and the thyroid glands, liver, stomach, kidneys and small and large intestines were removed and examined for radioactive iodine.

Preparation of samples for the Geiger-Müller counter

Urine, blood and bile. A preliminary experiment showed that some chemical breakdown takes place when pheniodol is refluxed with 2N-NaOH. Samples of urine, blood and bile to be examined for pheniodol were therefore treated with an equal volume of 2N-NaOH containing as 'carrier' 0.01 % of unlabelled pheniodol and 0.01 % of NaI, and the mixture was divided into two portions. The first portion was tested for total radioactivity by digesting on a steam bath for 4 hr., adjusting its pH value with 35 % (v/v) HNO₃ to about 9.0 and then transferring a 10 ml. portion to a jacketed Geiger-Müller counter (cf. Veall, 1948). The number of disintegrations/min. was measured on a Dynatron scaling unit. The background count was about 10/min.

The second portion was tested for labelled pheniodol by a butanol extraction procedure. The sample was acidified to pH 3.5 with 2N-H₂SO₄ and extracted in turn with an equal and with a half volume of *n*-butanol. Portions of 10 ml. of the combined butanol extracts and of the aqueous layers were transferred to a jacketed Geiger-Müller counter for measurement of their radioactivity.

Faeces. Samples of 5 g. of faeces were digested on a steam bath for 4 hr. with 20 ml. of 2N-NaOH containing as 'carrier' 0.01 % of pheniodol and 0.01 % of NaI. The residual solid matter was centrifuged off and digested for 4 hr. with a 10 ml. portion of the NaOH 'carrier' mixture. A third extraction was performed and the pH of the combined alkaline extracts was adjusted to about 9.0 with 35 % (v/v) HNO₃. The radioactivity of a 10 ml. portion was measured in a jacketed Geiger-Müller counter.

Stomach, intestines, kidney, liver and thyroid glands. Samples of stomach, intestine, kidney, liver and thyroid gland were digested by a modification of the procedure of Howarth (1949). The organ was minced and a 5 g. portion

(or if less than 5 g. in weight, the whole organ) was refluxed for 4 hr. with 10 ml. of 2N-LiOH in 20 % (v/v) ethanol containing as 'carrier' 0.01 % of pheniodol and 0.01 % of NaI. The residual solid matter was removed by centrifuging and the radioactivity of the supernatant liquid, after adjustment to pH 9.0, was measured in a jacketed counter.

RESULTS

Rat experiments

The excretion of radioactive iodine by rats was studied during a 3-day period following a single oral, subcutaneous, or intravenous dose of either a solution, or a suspension of labelled pheniodol. The excretion and final distribution results listed in Tables 1 and 2 are mean values obtained from at least two experiments.

Within 3 days of an oral dose, about 50 % of the radioactive iodine administered was excreted in the faeces and 25 % in the urine. About 90 % of the iodine in the faeces and 78 % of that in the urine was present in organic combination as pheniodol. Subcutaneous and intravenous doses of pheniodol were also rapidly absorbed, about 40 % of a subcutaneous and 50 % of an intravenous dose being excreted in the faeces and 20 % of a subcutaneous and 30 % of an intravenous dose in the urine. The relative proportions of pheniodol-iodine were similar to those after oral dosage. The lower recoveries in the subcutaneous experiment were probably due to unabsorbed pheniodol remaining at the injection site.

Table 1. Excretion of ¹³¹I by pairs of rats after a single dose of labelled pheniodol

Sample	Time of collection (hr.)	¹³¹ I as percentage of ¹³¹ I in dose		
		Group A, oral dose of suspension containing 50 mg. of labelled pheniodol/kg. of body wt.	Group B, subcutaneous injection of suspension containing 60 mg. of labelled pheniodol/kg. of body wt.	Group C, intravenous injection of solution containing 74 mg. of labelled pheniodol/kg. of body wt.
Urine	0-24	15.5	11.8	24.4
	24-48	6.5	5.9	6.3
	48-72	2.9	1.8	2.5
Faeces	0-24	24.3	16.6	23.8
	24-48	19.3	14.3	21.0
	48-72	5.3	7.0	7.7

Table 2. Distribution of ¹³¹I in pairs of rats 3 days after a single dose of labelled pheniodol

Organ or fluid	¹³¹ I found in organ or fluid as percentage of ¹³¹ I in dose		
	Group A, oral dose of suspension containing 50 mg. of labelled pheniodol/kg. of body wt.	Group B, subcutaneous injection of suspension containing 60 mg. of labelled pheniodol/kg. of body wt.	Group C, intravenous injection of solution containing 74 mg. of labelled pheniodol/kg. of body wt.
Thyroid gland	0.24	0.15	0.12
Liver	0.45	0.38	0.30
Kidney	0.10	0.06	0.09
Intestine	6.3	2.1	2.8
Urine (total)	24.9	19.5	33.2
Faeces (total)	48.9	37.9	52.5

Cat experiments

Anaesthetized cats were injected with labelled pheniodol either intravenously or intraduodenally; the blood levels of radioactive iodine and the biliary and urinary excretion rates were measured during a 6 hr. period. The mean results for two experiments by each injection route are given in Table 3. The final distribution of the radioactive iodine is recorded in Table 4. The percentage of the dose present in the blood has been calculated on the assumption that the blood volume (in ml.) is equivalent to 6.5 % of the body weight in g. (cf. Dukes, 1947).

The data listed in Table 3 indicate that a high biliary excretion rate is obtained after both intravenous and intraduodenal administration, a maximum value being reached about 2 hr. after an intravenous and 1 hr. after an intraduodenal injection. At least 85 % of the radioactive iodine

secreted in the bile and 65 % of that in the urine is present in organic combination as pheniodol.

An additional intraduodenal experiment was undertaken on a pregnant cat. The excretion rate and distribution of radioactive iodine were similar to those for normal cats, except that less than 0.1 % of the radioactive iodine administered was found in the two foetuses 6 hr. after injection, indicating that pheniodol does not pass the placental barrier.

DISCUSSION

Relatively little quantitative information on the distribution and excretion of pheniodol has been published. The original German work indicated that pheniodol is readily absorbed from the gastro-intestinal tract through the portal circulation and is excreted in part through the liver, the iodine remaining in organic combination as it passes through the body.

Table 3. Metabolism of ¹³¹I by cats after dosage with labelled pheniodol

(— Means 'not determined'.)

Method of administration and quantity administered	Time of collection (hr.)	Percentage of ¹³¹ I in dose in blood*	Percentage of ¹³¹ I in dose secreted in bile	Organically bound ¹³¹ I as percentage of total ¹³¹ I in bile	Percentage of ¹³¹ I in dose excreted in urine	Organically bound ¹³¹ I as percentage of total ¹³¹ I in urine
Intraduodenal injection (80 mg. labelled pheniodol/kg.)	0.5	7.1	—	—	—	—
	0.1	3.4	9.3	84	2.2	69
	1-2	2.7	8.0	82	2.1	66
	2-3	3.3	9.5	83	2.0	—
	3-4	2.4	4.9	84	1.2	60
	4-5	2.4	2.5	82	0.8	68
Intravenous injection (50 mg. labelled pheniodol/kg.)	5-6	2.2	2.2	85	0.4	66
	0.25	24.9	—	—	—	—
	0.5	16.7	—	—	—	—
	0.1	16.1	3.1	82	0.10	65
	1-2	13.7	5.1	84	9.7	67
	2-3	11.6	3.5	89	7.1	—
	3-4	8.8	3.1	87	7.2	—
	4-5	7.1	3.4	—	5.0	—
	5-6	5.7	1.3	—	4.1	—

* Calculated on the assumption that the blood volume (in ml.) was equivalent to 6.5 % of the animal body weight (in g.).

Table 4. Distribution of ¹³¹I in organs of a cat 6 hr. after dosage with labelled thyroxine

(— Means 'not determined'.)

¹³¹I found in organ or fluid as percentage of ¹³¹I administered by

Organ or fluid	¹³¹ I found in organ or fluid as percentage of ¹³¹ I administered by	
	Intraduodenal injection of 80 mg. of labelled pheniodol/kg.	Intravenous injection of 50 mg. of labelled pheniodol/kg.
Total urine (0-6 hr.)	8.7	33.2
Total bile (0-6 hr.)	36.0	19.5
Blood*	2.4	5.7
Thyroid gland	—	0.013
Liver	4.2	7.2
Kidney	0.34	0.77
Stomach	0.59	0.59
Small intestine and contents	7.0	3.3
Large intestine and contents	0.13	0.31

* Calculated on the assumption that the blood volume (in ml.) was equivalent to 6.5 % of the animal body weight (in g.).

Junkmann (1941) reported that rabbits after an oral dose of 0.25 g. of pheniodol/kg. of body weight excreted 63 % of the dose in the urine during the first 2 days. During the same period 58 % of a dose of 1.0 g./kg. was excreted. Men excreted 50 % of an oral dose in the urine during the first 24 hr. and 61–83 % in the first 72 hr. In our rat experiments much smaller doses (60 mg./kg. of body weight) were used, and only 25 % of the dose was excreted in the urine and 50 % in the faeces. We have confirmed Junkmann's observation that the bulk of the iodine excreted in the urine appears in organic combination as pheniodol. The proportion of unchanged compound excreted in the urine is very much greater than we observed for thyroxine (Clayton *et al.* 1950); since the pheniodol dose in the rat experiments was sixty times larger than the thyroxine dose and in the cat experiments over one thousand times larger, the differences are not unexpected. The final distribution of radioactive iodine in rats (Table 2) is largely what was anticipated. The amount of iodine appearing in the thyroid gland is insignificant and probably arises from slight decomposition of circulating pheniodol.

Although pheniodol has been used as a cholecystographic agent for 10 years, no data for its biliary excretion rate have been published so far. Junkmann (1941) measured the iodine content of bile from biliary fistula in two rabbits, 5 and 24 hr. after single oral doses of 1.0 g. of pheniodol/kg. of body weight. Howard (1948) undertook similar experiments on six dogs and two cats, 1–7 days after single oral doses of 150–400 mg./kg. Both investigators used larger doses than we did and their results cannot be compared with ours. Salter *et al.* (1949) compared the distribution of pheniodol with that of

3:5-diiodotyrosine after intraperitoneal injection into litter-mate rats. Their preliminary results for the distribution of pheniodol in liver, kidney, blood, muscle, spleen and thyroid were reported graphically, but unfortunately their results also are not suitable for comparison with our data.

SUMMARY

1. Single doses of pheniodol labelled with radioactive iodine have been administered orally, subcutaneously and intravenously to rats, and the excretion of radioactive iodine has been studied over a 3-day period. After oral dosage about 50 % of the administered radioactive iodine was excreted in the faeces and 25 % in the urine. After subcutaneous injection 40 % of the dose appeared in the faeces and 20 % in the urine. After intravenous injection 50 % of the dose appeared in the faeces and 30 % in the urine.

2. A single dose of labelled pheniodol was injected intravenously and intraduodenally into anaesthetized cats, and the radioactive iodine blood levels and biliary and urinary excretion rates were measured over a 6 hr. period. After an intravenous injection 20 % of the dose was excreted in bile and 33 % in the urine; after an intraduodenal injection 36 % appeared in the bile and 9 % in the urine.

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