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The excretion and metabolism of oral ^{14}C-pyridostigmine in the rat

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1. Pyridostigmine labelled with carbon-14 in the methyl group of the quaternary nitrogen has been used to investigate the excretion and metabolism of the drug after administration of single doses (500 μg) to the rat by stomach tube.

2. Pyridostigmine is slowly excreted in the urine; the maximum excretion occurs between 1–3 hr after administration. In 24 hr 42% of the dose is excreted in urine and 38.4% is present in faeces and intestinal contents.

3. The peak concentration of radioactivity in liver and blood occurs about 2 hr after administration.

4. About 75% of the radioactivity in urine is present as unchanged pyridostigmine, the remainder as metabolite.

5. The results are compared with those previously obtained after oral administration of neostigmine.

6. It is concluded that after oral administration the absorption of pyridostigmine is greater and the metabolism substantially less than that of neostigmine.

After an oral dose of neostigmine, 20% is found in the urine mainly as a metabolite and 50% in the faeces. The drug seems to be poorly absorbed though most of what is absorbed is rapidly metabolized in the liver (Roberts, Thomas & Wilson, 1966). This may explain the limited clinical usefulness of neostigmine when given by mouth. The present paper describes studies with orally administered labelled pyridostigmine, whose renal excretion after intramuscular injection is similar to that of neostigmine (Roberts, Thomas & Wilson, 1965a, b) but whose rate of metabolism is significantly slower (Birtley, Roberts, Thomas & Wilson, 1966).

Methods

Male rats weighing 150–160 g were allowed food and water *ad libitum* until the morning of each experiment. They were given two doses of warm tap water 5 ml/100 g body weight by stomach tube with an interval of 1 hr between doses. Thirty minutes after the second dose of water, ^{14}C-pyridostigmine iodide (500 μg) was administered in 0.5 ml. of water by stomach tube. Each rat was then placed under restraint in a Bollman cage (Bollman, 1948). Urine and faeces were collected separately by the method described by Brittain & Spencer (1963) 1, 2, 3, 5, and 24 hr

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after drug administration. After 24 hr the animal was killed and the intestine was ligated at the pyloric sphincter. The intestinal tract distal to the ligature was dissected and the intestinal contents were washed out with distilled water and mixed thoroughly with the collected faeces. The volume was recorded and after centrifugation the supernatant fluid was estimated for radioactivity.

In another series of similar experiments designed to study the concentration of pyridostigmine in the blood and liver, rats were killed by decapitation at 1, 2, 3 and 5 hr after administration of ^{14}C-pyridostigmine. Blood was collected in beakers containing 1 ml. of 4% trisodium citrate. The liver was dissected, drained on blotting paper and weighed. Extracts of blood and liver were prepared using trichloracetic acid as previously described by Roberts *et al.* (1965a). Each extract was mixed with 3 volumes of ethyl alcohol and set aside at 0° C until the supernatant was clear; it was then estimated for radioactivity.

Estimation of pyridostigmine and its metabolite

Urine and extracts of blood and liver were estimated for total radioactivity and for radioactive metabolite, using paper electrophoresis as described by Roberts *et al.* (1965b). The radioactivity separated into two distinct bands ; counts recorded between 10 and 13 cm from the origin are a measure of pyridostigmine and those recorded between 5 and 9 cm are a measure of metabolite (Birtley *et al.*, 1966). The concentration of total radioactivity and metabolite in the liver is expressed as μg of pyridostigmine/g wet weight.

Results

Excretion in urine and in intestinal contents and faeces

The mean radioactivity excreted in the urine of five individual rats, expressed as a percentage of the dose, is shown in Fig. 1. It will be seen that very little radioactivity is excreted during the first hour after drug administration and that the

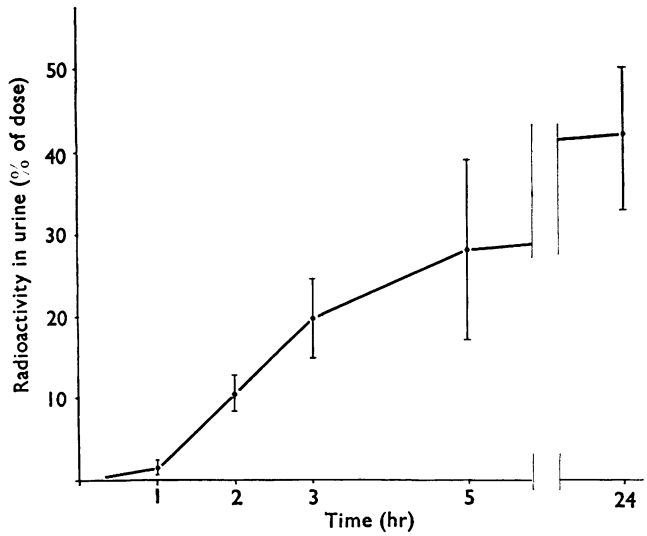

FIG. 1. Radioactivity excreted in the urine of the rat after oral administration of 500 μg of ^{14}C-pyridostigmine. Each point is the mean of five experiments. Standard deviations are represented by the vertical lines.

maximum rate of excretion occurs between 1 and 3 hr after administration. Throughout the 24 hr period of collection a total of 42±9.2% of the drug is excreted in the urine. The radioactivity present in the extracts of intestinal contents and faeces of three rats was estimated and the results, expressed as a mean percentage of the dose, gave a value of 38.4±2.6%. The combined results of these experiments show that about 80% of the dose was accounted for and that at least 42% of the dose was absorbed from the alimentary tract.

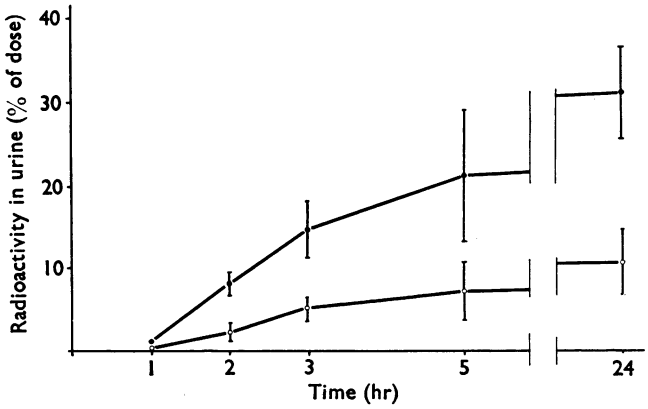

FIG. 2. Excretion of pyridostigmine (●) and metabolite (○) in the urine of the rat after oral administration of 500 μg of ^{14}C-pyridostigmine. Each point is the mean of five experiments. Standard deviations are represented by the vertical lines.

TABLE 1. *Pyridostigmine and metabolite in liver at different time intervals after oral administration of ^{14}C-pyridostigmine* (500 μg)

	Concentration calculated as pyridostigmine (μg/g wet weight)		
Time (hr)	Total	Pyridostigmine	Metabolite
1	1·10±0·47	0·91±0·42	0·19±0·04
2	2·34±1·71	2·04±1·58	0·30±0·13
3	1·10±0·74	0·84±0·60	0·26±0·16
4	0·31±0·19	0·23±0·14	0·08±0·05

Values are means and standard deviations of the results obtained from three rats at each time interval.

TABLE 2. *Concentration of radioactivity in blood at different time intervals after oral administration of ^{14}C-pyridostigmine* (500 μg)

	Time (hr)			
	1	2	3	5
	0·34	0·39	0·05	0·04
	0·15	0·17	0·08	0·06
	0·08	0·08	0·06	0·03
Mean	0·19	0·21	0·06	0·04

Individual results obtained from twelve rats are expressed as μg of pyridostigmine per ml. of blood.

Pyridostigmine and metabolite in urine

The results of the quantitative estimation of metabolite and unchanged drug in urine, collected at specified time intervals after oral administration of pyridostigmine, are shown in Fig. 2. At each of these periods the proportion of metabolite excreted is about one-quarter of the total radioactivity excreted; it is also evident that unchanged pyridostigmine is excreted throughout the 24 hr.

Liver and blood

The concentration of radioactivity in the liver of groups of three rats, collected 1, 2, 3 and 5 hr after an oral dose of 500 μg ^{14}C-pyridostigmine is shown in Table 1. The peak concentration of 2.34 μg/g occurs at 2 hr after drug administration; the concentration 3 hr after is the same as 1 hr after administration. The extracts of liver were also estimated for pyridostigmine and metabolite. It will be seen that at each time interval the proportion of metabolite did not exceed 25% of the total radioactivity in the liver.

Table 2 shows the concentration of radioactivity in the blood. As was found with liver extracts, the blood level reaches a peak in the first 2 hr but then abruptly falls.

Discussion

After oral administration of ^{14}C-pyridostigmine, at least 42% of the dose is absorbed and excreted in the urine. The presence in the faeces and intestinal contents of about 38% of the radioactivity suggests that this represents the proportion of the dose which is not absorbed. There is evidence, however, that after intramuscular injection about 3% of the radioactivity in intestinal contents is due to excretion into the intestine (Birtley *et al.*, 1966).

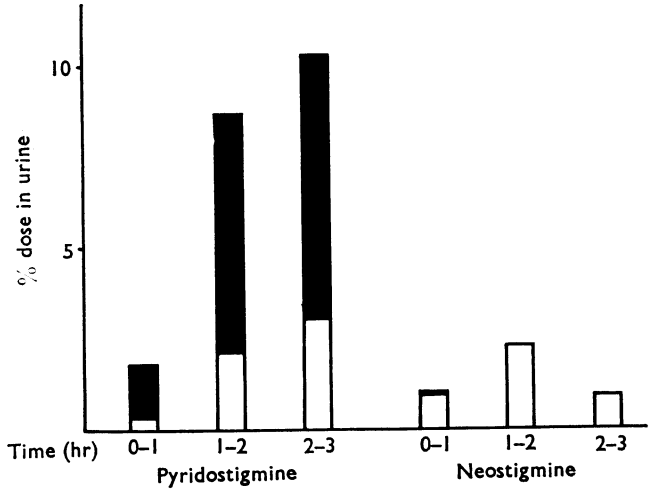

FIG. 3. Excretion of unchanged drug (■) and metabolite (□) in the urine of the rat after oral administration of ^{14}C-pyridostigmine (500 μg) and ^{14}C-neostigmine (250 μg).

It is reasonable to assume therefore that approximately 45% of an oral dose of pyridostigmine is absorbed. The results of the present work also show that the period of maximum absorption of the drug from the alimentary tract occurs within 3 hr after administration ; this is reflected in the amounts excreted in the urine and the peak concentrations of drug found in the blood and liver. The continuous slow excretion in the urine is probably due to further absorption from the intestine and not to retention of the drug in the liver, for the proportion of the dose excreted in the urine after 3 hr (20%) cannot be attributed to the amounts detected in the liver and blood 3 hr after administration because these represent only about 1% of the dose.

A characteristic feature of these results in common with those previously reported with oral neostigmine (Roberts *et al.*, 1966) is the wide variation in levels of radioactivity observed in urine, blood, liver and intestinal contents. This is a well recognized feature of quaternary nitrogen compounds and probably accounts for some of the variations in response of myasthenic patients during oral therapy with anticholinesterase drugs (Nowell, Scott & Wilson, 1962).

These results also provide evidence that pyridostigmine after oral administration is only slowly metabolized ; the amount of metabolite detected in the urine did not exceed 25% of the excreted radioactivity. This is also confirmed by the evidence from estimations of metabolite in the liver. A comparison of the excretion and metabolic patterns after oral pyridostigmine and neostigmine is shown in Fig. 3, where the data for the latter has been taken from the findings of Roberts *et al.* (1966). It will be seen that whereas 3 hr after administration of neostigmine only about 4% of the dose is excreted in the urine about 20% of the dose of pyridostigmine is excreted. Furthermore, the proportion of metabolite in the excreted product differs with the two drugs ; neostigmine is almost completely metabolized while a substantial proportion of pyridostigmine is excreted unchanged. We therefore conclude that in the rat the absorption from the gastrointestinal tract of pyridostigmine is greater and more prolonged than that of neostigmine and that pyridostigmine is more slowly metabolized than neostigmine. These results are in general agreement with those reported by Nowell *et al.* (1962) for patients with myasthenia gravis ; after oral administration of neostigmine little or no unchanged neostigmine was detected in the urine, whereas up to 16% of a dose of pyridostigmine was identified as unchanged drug.

It is tentatively assumed that in man also pyridostigmine is more adequately absorbed after oral administration and more slowly metabolized than neostigmine and that this affords a likely explanation for the general clinical impression that oral pyridostigmine is more prolonged in effect than oral neostigmine in the treatment of myasthenic patients.

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