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EXCRETION AND RE-EXCRETION OF A BROAD-SPECTRUM PENICILLIN IN BILE

BY

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A technique is described for estimating the biliary excretion, reabsorption and re-excretion of an antibacterial drug in rats. D(−)-6-(α-Amino-α-phenylacetamido)-penicillanic acid (BRL 1341) is rapidly excreted and re-excreted in the bile, in concentrations bactericidal to Salmonellae and other organisms which are usually refractory to antibacterial drugs in this situation. Recoveries of the drug accounted for 20 to 30% of oral or parenteral doses; much of the remainder is probably destroyed in the gut. Results from one patient indicate that D(−)-6-(α-amino-α-phenylacetamido)-penicillanic acid is excreted in high concentration also in human bile.

It is known that a few drugs, such as penicillin G and phenolphthalein, are rapidly excreted in bile. Reabsorption and re-excretion can obviously then occur, but there is very little quantitative data about this. In the case of antibacterial drugs, the amount of drug distributed in this way, and the relative concentrations thus obtained in the biliary tract and at different levels in the intestine, are obviously important in the therapy of alimentary infection.

We have recently drawn attention to the behaviour of methicillin in this respect (Harrison, White & Stewart, 1960). In the present paper we describe a technique designed to provide quantitative data about reabsorption and re-excretion, particularly with regard to D(−)-6-(α-amino-α-phenylacetamido)penicillanic acid (BRL 1341), another derivative of 6-aminopenicillanic acid which has wider antibacterial activity and may therefore be of use in the treatment of biliary and intestinal infections.

METHODS

White rats, bred in these laboratories, were selected from both sexes, weighing 250 to 300 g. They were anaesthetized with ether and incised 1.0 to 1.5 cm paramedially through the right side of the shaved abdominal wall. The loop of the duodenum and the bile duct were pulled through the incision. A soft polythene cannula (0.5 mm bore × 0.2 mm wall) was introduced through a lumbar-puncture needle into the abdomen and then inserted upwards into the bile duct. This cannula was secured by ligatures around the bile duct and at the point of egress on the abdominal skin. The wound was then closed in layers by cotton sutures. Rats thus treated served as donors of bile, samples being normally collected in 1-hr fractions for 4 to 7 hr, then overnight or longer. The rats were given 0.9% sodium chloride solution *ad lib.* to counteract loss of water and sodium, and light sedation with nembutal overnight. They were kept during this period in cages of soft wire which lightly restrained their movements. The flow of bile under these conditions was 0.5 to 1.2 ml. per hr.

Rats chosen as recipients were operated upon similarly and cannulated. The free end of the biliary cannula from a donor rat was then inserted through a needle-puncture into the duodenum of a recipient rat, immediately proximal to the ampulla of the bile duct. This cannula was fed into the duodenum for 1 to 2 cm and secured at the point of insertion by a purse-string suture through the muscular layer of the duodenal wall. The abdominal incision was then closed around the duodenal tube; donors and recipients were kept alongside each other in restraining cages.

Doses of drugs were then given, orally or parenterally, to the donor rats. Excretion was assessed by collecting and assaying the bile from a control group of cannulated donors; re-excretion was assessed by collecting and assaying the bile from recipient rats. Tail-blood was also collected at intervals from rats in both groups. Separate controls, kept in metabolism cages, were used for assays on urine and faeces, for it was found that laparotomy often caused a delay of 3 to 4 hr in the voiding of urine. D(−)-6-(α-Amino-α-phenylacetamido)-penicillanic acid was given orally via a stomach tube, or intramuscularly, in doses of 100 mg/kg to the donor rats.

The microbiological and chromatographic techniques employed in these studies have already been described (Stewart, 1960; Harrison, White & Stewart, 1960).

RESULTS

The results obtained in typical experiments are shown in Fig. 1. An oral dose of D(−)-6-(α-amino-α-phenylacetamido)penicillanic acid began to be excreted in the bile, in concentrations of 20 to 160 μg/ml., 1 hr after dosing. This concentration increased sharply during the next 2 hr, and peak values of 160 to 440 μg/ml. were obtained at 2.5 to 7.5 hr with considerable variation, in this respect, between individual rats. Plasma levels during this period showed peak values of 8.5 to 11.5 μg/ml., 1 to 3 hr after dosing, falling to 1.0 μg/ml. or less at 4 to 5 hr.

The re-excreted drug, assayed in the bile of the recipient rats, showed much lower peak values of 6 to 30 μg/ml. with a gradual rise spread over 3.5 to 15 hr after the time of administration of the drug to the donor rats. Plasma levels (0.3 to 4.3 μg/ml.) were also lower, though not proportionately. Assays in parallel showed

TABLE 1

EXCRETION (MG IN 24 HR) IN RATS WEIGHING 250 TO 300 G

Excretion of D(−)-6-α-amino-α-phenylacetamido)penicillanic acid in bile, urine and faeces of rats, and re-excretion in bile after 100 mg/kg orally

	Donor rats			Recipient rats.
Rat	Bile	Urine	Faeces	Bile
1	0·711	2·686	0·086	0·12
2	0·873	2·137	0·080	0·20
3	2·225	2·172	0·250	0·50
4	1·853			0·27
5	0·905			0·58
Mean	1·313	2·331	0·135	0·33
% dose	2·8–7·8	8–11	0·3–0·9	35–66

that the donor controls excreted on average about 5% of the dose in the bile in 24 hr (Table 1). The recipients re-excreted 20 to 60% of this quantity. During the same period about 10% of the dose was excreted in the urine and less than 1% in the faeces.

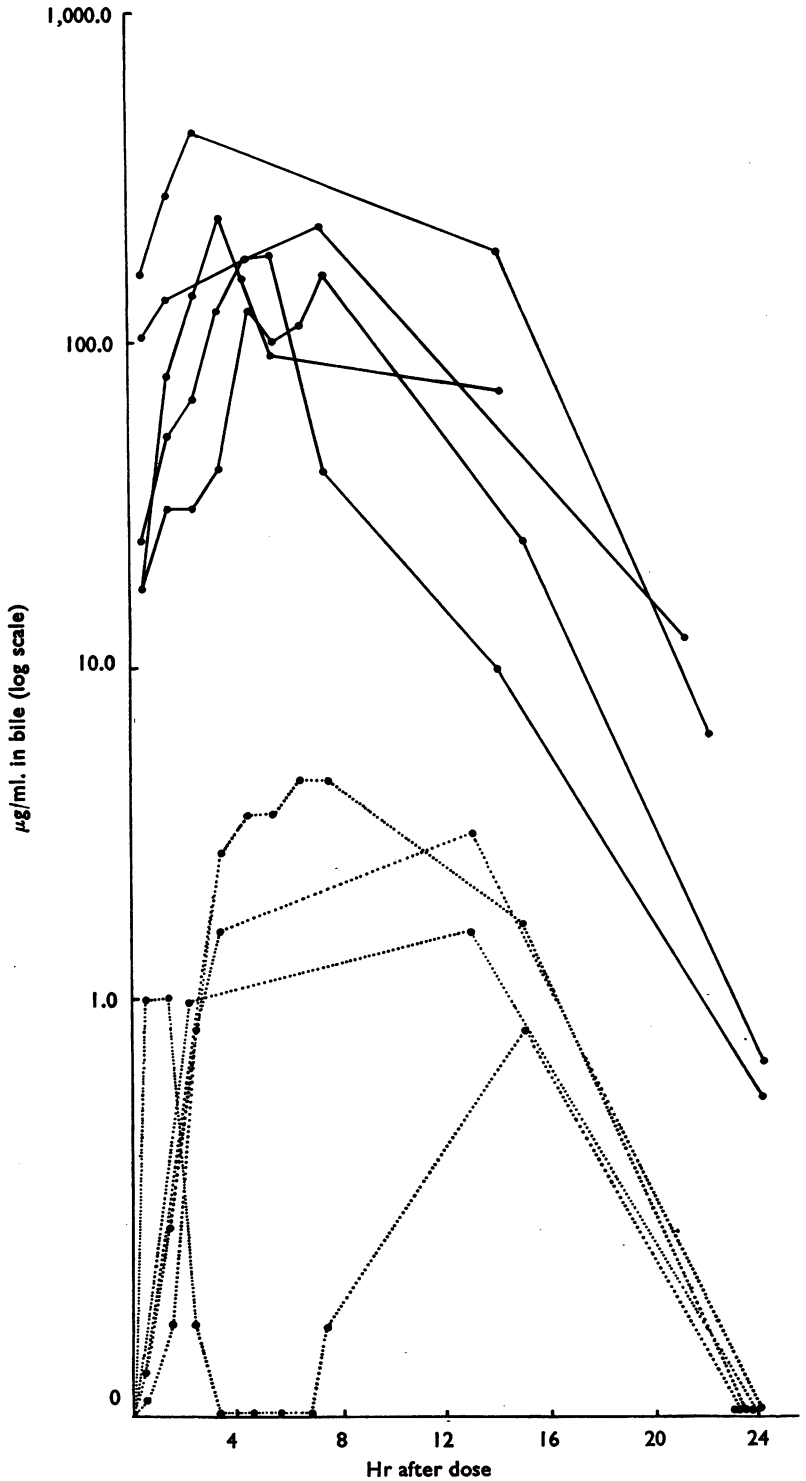

Fig. 1. Excretion and re-excretion of D(−)-6-(α-amino-α-phenylacetamido)penicillanic acid in bile of rats given 100 mg/kg. Donor rats ● —— ●; recipient rats ● · · · · ●.

To check the time factor in absorption from the upper part of the small intestine, the drug was injected directly into the duodenum in two rats. Plasma levels of 1.6 and 3.1 μg/ml. were detected in 15 min. In samples of bile collected during the first hour the concentrations were 114 and 160 μg/ml. Samples taken during the second hour gave values of 126 and 160 μg/ml., declining in succeeding hours. These results indicated that absorption proceeded immediately the drug entered the duodenum and that biliary excretion reached a maximum within 2 hr of this time.

This experiment gave an indication of the rate at which the drug entered the blood stream, but did not exclude absorption into the lymph. To investigate this, 2 rats had cannulae inserted directly into the thoracic duct. Lymph was collected for 3 hr after the standard oral dose and gave on assay only trace concentrations ($<$1.0 μg/ml.), that is, much lower than the corresponding plasma levels.

The experiments described above provided data from which the absorption and excretion of an oral dose could be calculated, but the actual rate of clearance through the liver could not be estimated accurately owing to the obvious variation in the rate of absorption from the gut. This point was investigated by injecting the drug intramuscularly and assaying samples of bile collected hourly. Under these conditions (Table 2) it was found that very high concentrations (718 to 1,575 μg/ml.)

TABLE 2

EXCRETION OF D(−)-6-(α-AMINO-α-PHENYLACETAMIDO)PENICILLANIC ACID (BRL 1341) IN BILE OF RATS AFTER INTRAMUSCULAR INJECTION (100 MG/KG)

	BRL 1341 in bile		
Hr after injection	Concentration (μg/ml.)	Excretion (μg)	% of dose excreted
1	1,225	980	3·4
	1,545	1,236	4·0
2	1,113	890	3·3
	1,575	2,200	7·3
3	718	790	2·6
	1,250	1,875	6·3
18	40	580	2·0
	20	263	1·0
Total			10·6–18·4

were excreted during the first 3 hr, accounting for a mean excretion of about 5% of the dose per hour. After 3 hr the rate of excretion fell rapidly, the cumulative clearance at the end of 18 hr being 11 to 18% of the dose.

In the evaluation of an antibacterial drug like D(−)-6-(α-amino-α-phenylacetamido)penicillanic acid the concentrations quoted in bile and other body fluids are of therapeutic significance only in so far as they equate to the bacteriostatic or bactericidal levels effective against organisms likely to be carried, or to cause infection, in the biliary tract and upper reaches of the small intestine. The relevant figures for a range of appropriate organisms are shown in Table 3.

Samples of bile and urine were examined chromatographically on Whatman no. 1 paper, by the technique previously described (Stewart, 1960), to see if the excreted drug was the original substance or a metabolite. The dried chromatograms were

TABLE 3

BACTERICIDAL CONCENTRATIONS OF D(−)-6-(α-AMINO-α-PHENYLACETAMIDO)-PENICILLANIC ACID

Organism	No. of strains	No. sensitive	Bactericidal concentration (μg/ml.)
Staph. aureus	282	216	0·1–5·0
Streptococcus			
Group A	57	57	0·05–0·1
Group D	24	22	1·0–5·0
Others	19	19	0·1–5·0
Bact. coli	102	48	1·0–5·0
Salmonella typhi	2	2	2·5
Salmonella typhi-murium	8	8	1·0–5·0
Salmonella, others	5	5	1·0–5·0

cut into strips and laid on nutrient agar already seeded with penicillin-sensitive *Sarcina lutea*. After overnight incubation at 37° C, the R_F values of the samples were calculated by measuring the distances of the solvent front and the zones of inhibition of the test organism from the origin. These measurements showed no difference in the R_F values of the excreted drug and of comparable amounts of pure drug added to bile and urine from control rats.

Since this paper was written, we have assayed D(−)-6-(α-amino-α-phenylacetamido)penicillanic acid in human bile, aspirated from the duodenum of a patient 5 hr after an oral dose, in capsules, of 50 mg/kg. Saline, 20 ml., was injected through the tube before aspiration so that the aspirated bile was diluted. On the assumption that the dilution factor was 1:10 (a minimal estimate) the assay value of the drug in the bile was 22.0 μg/ml. Plasma concentrations were 7.5 μg/ml. at 2 hr and 10 μg/ml. at 6 hr. These results suggest that absorption and biliary excretion proceed on similar lines in humans and rats.

DISCUSSION

These results show that D(−)-6-(α-amino-α-phenylacetamido)penicillanic acid is rapidly absorbed in the upper part of the small intestine in rats and is immediately excreted in active form in the bile. The liver is capable of clearing up to 7% of a large dose from the blood stream in 1 hr, producing a concentration in the bile which may be 40 times higher than the peak plasma concentration and 100 times higher than the concentration required to kill moderately sensitive organisms, such as *Salmonellae* and certain other coliforms; this concentration may actually be 1,000 times that of the level ordinarily effective against many pyogenic cocci.

The technique described enables an assessment to be made of the reabsorption and re-excretion of the drug originally cleared in the bile. Reabsorption produces plasma increments of about 1.0 μg/ml., occasionally more. Hepatic clearance of this amount of drug produces levels in the bile up to about 30 μg/ml., still sufficiently high to inhibit most pathogens. The first phase of absorption of the orally administered drug produces peak plasma levels, and peak excretion, between 1 and 3 hr; the second phase adds small increments to the plasma during this time and up to 6 hr.

In our rats the biliary flow as measured in the cannulated samples was 0.5 to 1.2 ml./hr, which gave an excretion in the post-absorptive stage of up to 0.5 mg of drug per hour. The original oral dose varied, according to body weight, from 25 to 30 mg, and the mean excretion of this, in 24 hr, was 1.3 mg, the greater part having re-entered the gut in the first 3 hr. Simultaneously, about 2.3 mg was excreted in the urine and a trace (0.13 mg) in the faeces. These figures suggest, therefore, that about 1/15th of the original oral dose is immediately excreted in the bile and presumably reabsorbed; during the next 20 hr about 1/5th of this is re-excreted in the bile. Urinary and faecal excretion during this period accounts for about 10% of the dose, so that, even when allowance is made for the fraction undergoing reabsorption and re-excretion, and for the inaccuracies of bioassay, a considerable proportion of the dose is not accounted for. This could mean either that there is retention in the body, or that the greater part of the dose is metabolized. The former possibility—retention—is unlikely, as blood and urine levels fall rapidly after 5 to 7 hr. Metabolism in the body is also unlikely on this scale, as the much higher blood levels produced by parenteral administration are cleared with greater rapidity via the liver and bile. There remains the possibility that the drug is partly destroyed in the gut, and this explanation is favoured by the apparent incompleteness of absorption and reabsorption. The comparative stability of the drug to *p*H and other changes (Rolinson & Stevens, 1961) makes it unlikely that it is destroyed by secretions; but it is known that D(−)-6-(α-amino-α-phenylacetamido)penicillanic acid is susceptible to the penicillin-destroying factors in coliform and other bacteria (Rolinson & Stevens, 1961; Stewart, Coles, Nixon & Holt, 1961) whose presence in the intestine may therefore account for considerable inactivation.

If D(−)-6-(α-amino-α-phenylacetamido)penicillanic acid behaves pharmacologically in man as in the rat, it should have interesting chemotherapeutic possibilities: in therapeutic doses it can attain and maintain in the biliary tract and upper intestine concentrations which are bactericidal to a wide range of organisms, including *Salmonellae* and other pathogens which are often located in these sites; the plasma levels attained by oral dosage indicate that a bactericidal concentration can probably reach many tissues; the urinary concentration is extremely high; and, in all these situations, effective concentrations of active drug are detectable for several hours after a single oral dose. The cycle of absorption-excretion-reabsorption *et seq.* in the gut, together with the possibility of some local destruction of the drug, suggests that it may not reach the ileum in a concentration inhibitory to pathogenic bacteria there. This may explain its failure to clear intestinal carriers of *S. typhi-murium* (Stewart *et al.*, 1961).

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