BENZYLPENICILLIN

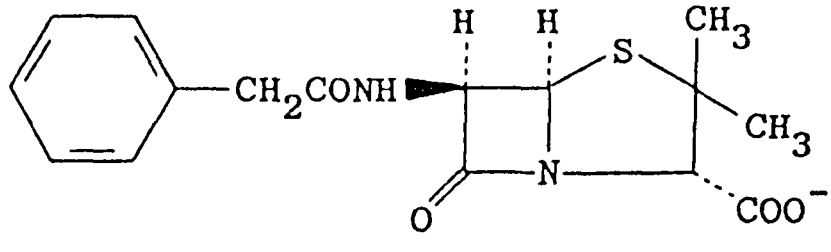
IDENTITY

Chemical name: 3,3,-Dimethyl-7-oxo-6[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid.

Synonyms: Free benzylpenicillin; Penicillin G; Penicillin II

Structural formula:

The basic structure of penicillins consists of thiazolidine ring connected to a B-lactam ring to which is attached a side chain. The penicillin nucleus itself is the chief structural requirement for biological activity, whereas the side chain varies in different penicillins and determines many of the antibacterial and pharmacological properties of the different penicillins.



Molecular formula: $C_{16}H_{18}N_2O_2S$ (Normally used as the sodium salt of the carboxylic acid).

Molecular weight: 334.38

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Physical Properties

Benzylpenicillin is an amorphous white powder and is unstable to moisture by breaking down to penicilloic acid.

Sodium benzylpenicillin (PEN-G) are white crystals. Very soluble in water, isotonic saline and glucose solutions. Soluble in primary alcohols and glycerol. Insoluble in acetone, ether, chloroform and fixed oils. 1 mg PEN-G is equivalent to 1670 I.Units penicillin. 1 I.U. is 0.6 ug PEN-G.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

Benzylpenicillin is one of the most widely used antibiotics in both animals and humans. It is active against gram-positive bacteria and is administered orally or by injection either intravenously (i.v), intramusculary (i.m.), intramammary or subcutaneously (s.c.). Benzylpenicillin is used for therapy in all farm animal species with a dose range of 3 - 25 mg per kg body weight. Major uses are for the control of mastitis in dairy cows and for treating infections of the urinary tract, gastro-intestinal system and the respiratory tract. Benzylpenicillin is also administered as a feed additive to pigs to control streptococcal meningitis and is included as an additive in the drinking water of poultry.

Benzylpenicillin has a short half life in the patient and repeated administration may be necessary to maintain the concentration of penicillin at the site of infection above the minimum inhibition concentration (MIC) for the bacteria. Many other penicillin derivatives with longer half-lives are also widely used. The procaine salt of benzylpenicillin is longer acting and the active ingredient in the patient is penicllin-G.

There are numerous formulations in which benzylpenicillin is combined with other antibiotics, e.g. combination with streptomycin for treatment of bovine mastitis. These are longer acting and have a broader spectrum of antibacterial activity. In the UK, for example, approximately 16 million intramammary antibiotic tubes or syringes are sold annually and there are over 50 different products containing benzylpenicillin. Thus the potential for residues appears considerable. In fact 99.9% of these treatments are administered without residues in milk for the consumer being detected. This is a reflection both on the accuracy of the withholding times and the management of milk production.

METABOLISM STUDIES

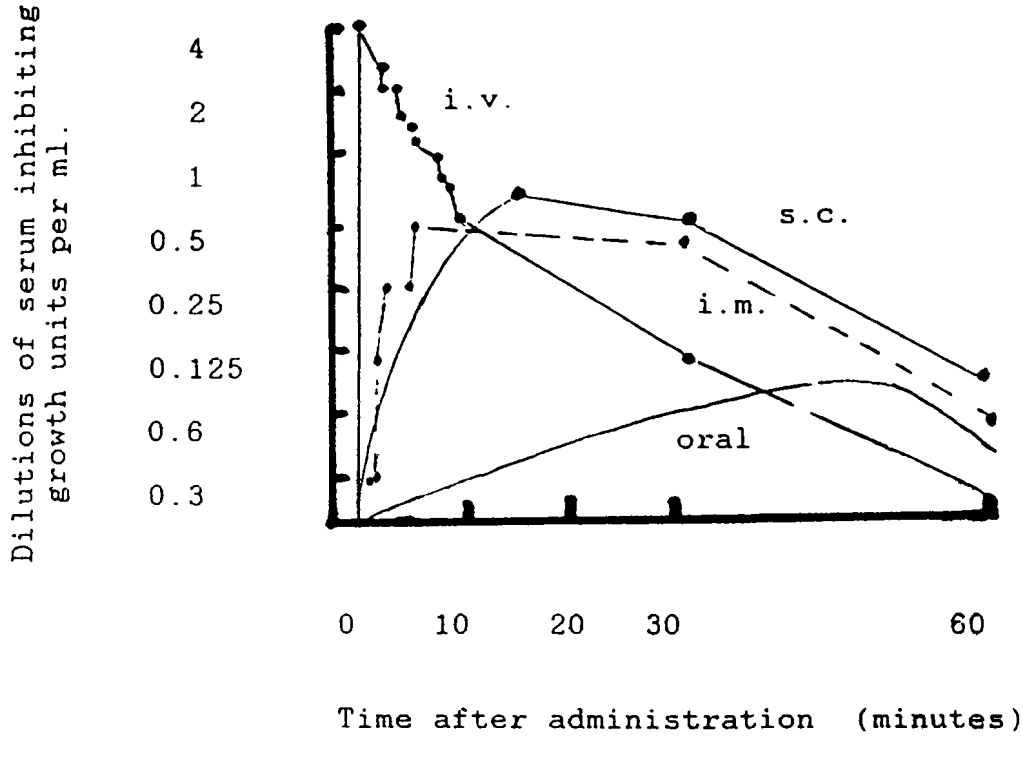
PHARMACOKINETICS

The rates of absorbtion, clearance and elimination are influenced by; - age of recipient - formulation - route of administration - derivatisation especially as procaine-benzylpenicillin. - disease status.

Absorption

A pattern typifying the absorption of benzylpenicillin following administration by several routes was established by the early work of Fleming (1944). The results as modified by Salter (1952) are shown in figure 1. Intramuscular and subcutaneous injections provide drug to the bloodstream more slowly and maintain concentrations longer than i.v. administration. There is a suggestion that there is no difference in the rates of absorption of benzylpenicillin from the i.m and s.c. sites. Benzylpenicillin is degraded in the digestive tract of many farm animals especially ruminants and only a fraction of the administered amount is absorbed. Other semi-synthetic penicillin derivatives which can resist enzyme and acidic degradation in the gut are used for oral therapy. Data based on Fleming (1944).

Figure 1. Absorption of benzylpenicillin



Absorption of benzylpenicillin from i.m or s.c sites can be slowed by the use of the relatively insoluble procaine salt. When equivalent doses of benzylpenicillin and procaine benzylpenicillin were injected parentally the peak concentration of benzylpenicillin in

blood occurred with the benzylpenicillin dose after ca. 2 hours and the drug had cleared the blood by 8 hours, whereas with the procaine benzylpenicillin the peak concentration of benzylpenicillin was ca. 5 hours after injection and the drug cleared the plasma about 24 hours after administration.

Ziv et al. (1973) administered benzylpenicillin i.v. at a dose of 10 mg/kg body weight to lactating cows. They collected serum and milk and measured the concentration of benzylpenicillin by the cylinder microbiological method. The resulting kinetic analysis showed that it took 2.5 hours for the benzylpenicillin to appear in the milk; the concentration range was 0.07 - 0.12 mg/L in milk and 0.65 - 1.20 mg/L in serum. The half life (t-B) was 0.7 $\pm$ 0.1 hours in serum.

Clearance Rates in Blood

Benzylpenicillin is rapidly cleared from the blood through the kidneys and excreted unchanged, almost entirely into the urine. The renal mechanisms involve both glomerular and proximal tubular secretion. Benzylpenicillin binds significantly (50 - 60%) to plasma proteins. Only the unbound benzylpenicillin molecules are available for filtration. However, protein binding does not interfere with tubular secretion, which is an active carrier-mediated process. In the early days of benzylpenicillin therapy, before long acting compounds were available, the agent probenicid or phenylbutazone, by competing for the transport mechanism for the excretion of benzylpenicillin would prolong the half life of benzylpenicillin (Kampmann et al, 1972).

Sodium benzylpenicillin was administered at a dose of 2.7 mg/kg body weight intravenously to calves aged 0.5, 5, 10 and 15 days of age. Serum was collected at various intervals over 2 hours after injection and analysed for benzylpenicillin by the cylinder plate method using micrococcus lutea as the test organism (Short et al, 1984). The mean total body clearance of benzylpenicillin increased after the day of birth but there were no significant differences in the half life. The half lives in minutes were: 29.7, 25.7, 27.6 and 23.5 at 0.5, 5, 10 and 15 days of age respectively. The study indicates that the half life in the adult cow is 25 min., a similar value.

McCracken et al. (1973) reported that in adult humans the the half life of benzylpenicillin in human serum is approximately 30 min. but is nearly 3 hours in infants aged 0 - 6 days and had decreased to 1.4 hours by 14 days of age.

Short (1982) reported a clear maturation pattern for the clearance and half life of benzylpenicillin in the first 15 days of life of the pig.

Distribution

Benzylpenicillin is readily absorbed into the blood stream where it is partially bound to blood proteins. Bogan (1983) lists the values for the extent of binding of benzylpenicillin to plasma proteins in different species as: Horse, 54%; Cow, 49%; Human, 65%.

During the first few hours after injection of benzylpenicillin the concentration of benzylpenicillin is higher in the blood than other tissues and milk (except liver and

kidney). Thereafter there is a more rapid clearance of the drug from the blood than the tissues and the concentrations in tissues and milk are higher.

An extensive pharmacokinetic study from France (Guillot, 1983) used 14C-benzylpenicillin in dairy cows. One animal weighing 450 kg was injected i.m. with 250 μCi 14C-benzylpenicillin at a dose of 6.7 mg/kg. The cow was slaughtered two hours after administration and the tissues and fluids analysed for residues of radioactivity. The ratios of the concentrations of activity in the plasma to that in tissues are shown in table I.

Table I. Tissue / Plasma ratios

Tissue	5 Muscles	Liver	Kidney	Bile	Adrenals	Heart
Ratio	0.14-0.28	5.12	11(a)16.7(b)	42.9	0.89	0.33

Plasma concentration was equivalent to 2.73 mg/L.
(a) is cortex, (b) is medulla.

It is important to maintain an effective concentration in the infected tissues and several studies have tried to determine the concentrations at the infected sites. Bacteria causing soft tissue infections are usually in the extracellular fluid although in the mammary gland they enter several cells. Penicillins are mainly distributed in the extracellular fluid and do not penetrate well into different cells (Bergman, 1984; Brown and Percival, 1978). The measurement of drugs in extracellular fluid is probably of greater clinical significance than is the measurement of whole tissue homogenates. A useful model has been the use of so-called "cages" which are basically hollow plastic golf balls with holes in which are placed at subcutaneous sites of the experimental animals and simulate infective sites. Fluid forms inside the cages and may be sampled by aspiration. However the model has been criticised because it does not represent the true concentrations in extravascular fluids (Franklin et al, 1984, Laber et al, 1980).

Luthman and Jacobsen (1986) using calves prepared with fluid cages, injected benzylpenicillin either i.v. with 10 mg/kg body weight or i.m. with 10 mg/kg body weight or with procaine penicillin at 30 mg/kg body weight. The concentrations of penicillin in cage fluid are shown in table II.

Table II. Mean concentrations of Penicillin (mg/L) in tissue cage fluid (TCF) and serum of calves (6 calves per group).

Time after injecting (hours)	Benzylpenicillin i.v. serum	Benzylpenicillin i.v. TCF	Benzylpenicillin i.m. serum	Benzylpenicillin i.m. TCF	Procaine-Pen-G i.m. serum	Procaine-Pen-G i.m. TCF
1	3.1	1.04	5.5	0.24	1.8	0.01
2	0.62	1.13	3.2	0.35	2.1	0.08
3	0.34	1.09	1.5	0.50	2.1	0.18
4	0.17	0.84	0.9	0.57	2.2	0.24
6		0.77	0.3	0.54	1.7	0.24
8		0.63	0.2	0.52	1.5	0.44
10		0.62		0.38	1.1	0.47
24		0.23			1.0	0.30

The i.v. route delivers a high concentration of drug to the cage very quickly, whereas it takes longer to reach a peak concentration by the i.m. route. The time to peak concentration of the penicillin from the procaine salt is at least four hours later and illustrates the slower absorption of the salt.

Bengtsson et.al (1989) injected potassium benzylpenicillin (6.3 mg/kg body weight) or procaine benzylpenicillin (30 mg/kg body weight) into groups of calves and measured the concentrations of benzylpenicillin in serum, synovial fluid and tissue cage fluid. Benzylpenicillin concentrations were initially lower in synovial fluid than in serum but from 2 hours after injection the potassium salt administration and 4 hours after the procaine salt administration, concurrent levels were very similar. After the potassium benzylpenicillin administration, peak serum levels of benzylpenicillin (4.7 $\pm$ 3.9 mg/L) occurred at 1 - 1.5 hours after injection and coincided with the lower peaks in synovial fluid (1.5 $\pm$ 0.6 mg/L). The elimination half-life in serum was 1.6 hours and in synovial fluid 2.3 hours.

Administration of procaine benzylpenicillin resulted in plateau shaped concentration-time curves with peak levels occurring 1.5 - 6 hours after injection in serum (1.6 $\pm$ 0.3 mg/L) and 1.5 - 4 hours post injection in synovial fluid (1.1 $\pm$ 0.2 mg/L). The elimination half-life in serum was 4.8 hours and in synovial fluid 3.5 hours. Franklin et al. (1986) injected lactating cows with benzylpenicillin in combination with dihydrostreptomycin and spriramycin by different routes, i.v., i.m. or intramammary. They measured the concentration of penicillin in plasma, mammary lymph and milk. After i.m. injection of 9.5 mg/kg body weight the concentration of penicillin peaked at 4.8 mg/L in plasma and 3.7 mg/L in lymph at 0.5 - 1 hour after injection. The values paralleled each other for about 7 hours after injection. The concentration rose in milk to peak about 0.3 mg/L at 5 - 6 hours after injection. Both the concentrations in milk and lymph were higher than that in blood at 8, 12 and 24 hours after injection. After the intramammary injection of 0.4 g procaine penicillin into both left side quarters, the peak concentrations of penicillin were 3.5 mg/L in lymph at 6 hours post injection and 0.07 mg/L in plasma at 4 hours after injection. There was almost no diffusion of the penicillin into the untreated quarters. Fever increases the distribution of benzylpenicillin. This is manifested by an increase in

the apparent volume of distribution (Baggot, 1983). The concentration of benzylpenicillin in mastitic milk was higher than that in normal milk (Franklin et al, 1984).

Elimination

Benzylpenicillin is rapidly cleared from the blood by the kidneys and is mostly excreted in the urine. Guillot (1983) injected i.m. at a dose of 6.7 mg/kg body weight a dairy cow weighing 450 kg with 14C-benzylpenicillin and measured the excreted radioactivity in the urine and faeces. Table III shows that 70.6% of the dose was recovered in the urine and only 6.6% in the faeces. Most of the excreted activity was recovered during the first 6 hours after injection and >98% of the radioactivity excreted over a 46 hours collection period was excreted in the first 22 hours. Data from Guillot, (1983)

Table III. Excretion of Radioactive residues into bovine urine and faeces after i.m. injection of 6.7 mg/kg body weight 14C-benzylpenicillin.

Time after injection (hours)	Cumulative % dose in	
	Urine	**Faeces**
6	62.8	1.9
22	70.2	5.3
30	70.4	6.0
46	70.6	6.6

Milk is not a major route of elimination of benzylpenicillin following i.v. or i.m. injection but it is a very important route of elimination following intramammary injection. Moretain and Boisseau (1984) followed the elimination of benzylpenicillin into milk after treating lactating cows with i.m. injections of commercial preparations of penicillins. In three preparations containing procaine benzylpenicillin one of which also contained benzylpenicillin, the highest concentrations of benzylpenicillin in milk were observed in the milking collected after the injection. No benzylpenicillin in milk was detected 3 - 5 days after the last injection. The recovery of benzylpenicillin in the milk was <0.03% initial dose.

In a follow up study Moretain and Boisseau (1989) investigated the elimination of benzylpenicillin into milk following intramammary injection. Lactating cows were injected with 3 different doses of procaine benzylpenicillin and the concentrations of penicillin measured in the milk. The dosage, the percentage of drug recovered in the milk and the time to reach baseline levels are shown in table IV.

Table IV. Benzylpenicillin after intramammary administration of procaine benzylpenicillin to lactating cows.

Dose	% Pen G recovered in milk	Number of first milking with no benzylpenicillin
2 admins 24 h apart 450,000 I.U./qtr	15.6 + 5.9	7 - 9
3 admins after consecutive milkings, 1,000,000 I.U./qtr	42.0 + 19.3	7 - 8
2 admins 24 h apart 100,000 I.U./qtr	70.8 + 30.4	4 - 7

Each product contained a second antibiotic.

METABOLISM STUDIES

No information was readily available on the metabolism of benzylpenicillin in farm animals.

Benzylpenicillin is susceptible to inactivation by penicillinases (B-lactamases) which split the B-lactam ring to form the biologically inactive penicilloic acid. This metabolic route becomes important when trying to treat organisms which possess the enzyme, an alternative drug might be needed.

The highly acidic (pH 2) gastric juices hydrolyse benzylpenicillin and reduce the amount of active drug absorbed from the gut. The gastric acidity of very young animals and humans is relatively low and more drug is absorbed by the very young.

The majority (>60%) of benzylpenicillin administered parentally to animals is excreted rapidly in the urine as the parent drug. One can assume that little metabolism of benzylpenicillin occurs and that any minor metabolites which are produced are not biologically active. A more definitve study in farm animals is needed in which residues are quantified by radiometry, bioassay and physicochemical methods and also the radiometric residues identified.

RESIDUE DEPLETION STUDIES

Milk

A survey (Booth and Harding 1986) in the UK showed that of 3484 violations of antibiotic residues in milk recorded in 1984-85 almost 80% were caused by intramammary antibiotics and about 5% by injections in sites other than the udder, most of the remaining failures were for unknown reasons. The majority of the treatments contained a penicillin and more than half the violations occurred following intramammary treatment

of lactating cows and about 27% failures following treatment of dry cows shortly before calving. The main reasons for the violations were, failure to observe the withholding period for the milk (16.5%), accidental transfer of the milk (16.7%), prolonged excretion of antibiotic (8.2%) and early calving (7.3%). In many countries there are more than 50 separate preparations containing a penicillin for the treatment of bovine mastitis and each formulation produces a different residue pattern in milk. Since all preparations cannot be covered some studies recently done in France are cited (Moretain and Boisseau 1984, 1989) as illustrations of the residues in milk.

The residues of benzylpenicillin in milk folowing the administration of i.m. and intramammary injections of benzylpenicillin and/or procaine benzylpenicillin are shown in table V. Above all they show that the persistence of the residues depends on the formulation of the commercial product. (Data from Moretain and Boisseau, 1984, 1989)

Table V. Residues (μg/L) of benzylpenicillin in milk

Milking	INTRAMUSCULAR				INTRAMAMMARY		
Number	A	B	C	D	E	F	G
-2	126 [168]	90 [150]	72 [108]	126 [216]	29100 [42000]	174600 [234000]	34800 [48000]
-1	21 [35]	22 [8]	41 [84]	40 [78]	1590 [3072]	109380 [192000]	420 [870]
1	114 [162]	120 [246]	90 [132]	120 [246]	51650 [76800]	173400 [264000]	20520 [31200]
2	23 [27]	34 [71]	38 [85]	29 [53]	3096 [8040]	5556 [11700]	184 [405]
3	7 [15]	16 [36]	20 [56]	11 [18]	329 [636]	331 [690]	15 [48]
4	2 [4]	5 [16]	8 [28]	13 [13]	48 [140]	26 [34]	1 [4]
5	ND	4 [11]	2 [10]	2 [8]	7 [23]	4 [7]	<1 [2]
6	ND	2 [7]	2 [4]	1 [5]	1 [5]	1 [2]	<1 [1]
7	ND	1 [5]	<1 [2]	<1 [3]	<1 [2]	<1 [<1]	ND
8	ND	<1[1]	ND	<1[3]	<1[1]	ND	ND
9	ND	ND	ND	ND	<1[1]	ND	ND
10	All residues are ND						

Each value is the mean of five animals with the highest value in brackets. ND is not detected.

In Table V, the following treatments and dosages were used for the intramuscular and intramammary administrations:

Treatments: using procaine benzylpenicillin (PBP) and/or benzylpenicillin (BP)

A = 40% BP, 60% PBP in aqueous solution.
B = PBP and dihydrostreptomycin in aqueous solution with polyvinylpyrrolidone.
C = B plus lecithin.
D = B plus carboxymethylcellulose.
E = PBP plus neomycin sulphate in fatty alcohols and petrolatum.
F = PBP plus dihydrostreptomycin, polysorbate-80 and colloidal silica in propylene glycolesters of saturated fatty acids.
G = PBP and glycerolmonostearate in peanut oil.

Dosage:

A,B,C,D = two injections i.m. of 6 mg/kg B.W. 24 hours apart.
E = two administrations of 270 mg/quarter 24 hours apart.
F = three administrations of 600 mg/quarter after three consecutive milkings.
G = two administrations of 60 mg/quarter 24 hours apart.

Meat

Definitive studies of residues of benzylpenicillin in meat are hard to find but there is evidence that benzylpenicillin therapy in meat producing animals leaves residues in meat even though there is a rapid absortion of the drug and fast clearance from the blood. Because procaine penicillin is absorbed more slowly the residues may persist longer.

Cattle

Guillot (1983) injected intramusculary a cow weighing 450 kg with 250 Ci 14C-labelled benzylpenicillin at a dose of 6.7 mg/kg B.W. The cow was sacrificed two hours after the injection and the radioactivity as benzylpenicillin equivalents was measured by combustion analysis in a number of tissues and fluids. The results are shown in table VI. Moats (1984) injected intramusculary a cow weighing 600 kg with benzylpenicillin at a dose of 6 mg/kg B.W. The cow was slaughtered two hours after the injection and the concentrations of benzylpenicillin in tissues and fluids measured by both bioassay and HPLC. The results are also shown in table VI. A disturbing feature of the results is the lack of agreement between the two types of assay. This is most probably the result of more efficient extraction of benzylpenicillin from cells using organic solvents. Moats found that about 90% of benzylpenicillin was recovered from tissues during the HPLC assay.

Table VI. Residues in mg/kg of benzylpenicillin in bovine tissues and fluids 2 hours after intramuscular administration.

Tissue	(Guillot) 14C-activity	(Moats) Bioassay	(Moats) HPLC
Plasma or serum	2.7	0.79	0.95
Bile	117	NM	NM
Liver	14	0.58	22
Kidney	30(a), 46(b)	0.55	6.1
Adrenals	2.4	NM	NM
Muscle	0.37-0.76(5)	0.03-0.05(4)	0.06-0.18(4)
Spleen	0.7	NM	NM
Tongue	0.8	NM	NM

NM is not measured; (a) is cortex; (b) is medulla; the numbers in parentheses are the number of different types of muscle examined.

McCracken et al. (1976) injected calves with 10 mg/kg B.W. benzylpenicillin and slaughtered the animals 24 hours later. Residues of benzylpenicillin were measured by bioassay and were >40 g/kg in muscle and kidney and >40 g/L in urine.

McCracken & O'Brien (1976) injected six calves twice each i.m. with 3 mg/kg B.W. benzylpenicillin 24 hours apart and sacrificed the animals 24 hours after the last injection. The residues were declared positive when they exceeded 40 g/kg, the lower limit of detection of the bioassay. All calves were positive in the following tissues; injection site (gluteus muscle), the gluteus muscle opposite to the injection site, pectoral muscle, diaphragm muscle, longissimus dorsi muscle, Masseter muscle, kidney, liver, heart, serum. Positive results were also observed for urine 5 of 5, psoas muscle 5 of 6 and oblique muscle 3 of 6.

Pigs

The only data submitted from sponsors on penicillin residues is a study using penicillin V added to the feed of pigs. Penicillin V is a semi-synthetic penicillin which differs from benzylpenicillin having a benzoyl (C_6H_5-O-CH_2-) side chain instead of the benzyl group. Nevertheless the residues pattern illustrates the type of situation which may arise when penicillins are used as in-feed additives. Forty pigs aged six weeks were fed feed for six weeks containing 2 kg penicillin V per ton feed. This dose is equivalent to about 10 mg/kg B.W. The pigs were then fed drug free diet. Pigs were sacrificed in groups of six at 0, 1, 5, 7 and 84 days after drug withdrawal. The residues in tissues were measured by a bioassay method with a lower limit of detection of 50 g/kg.

No residues were detected at any sampling point for muscle, liver,skin and fat. In the kidneys residues were detected in 3 of 6 pigs in the group sacrificed at day 0 of drug withdrawal. The values of the positives was 54, 60 and 62 g/kg which are only just above the limit of the assay. No residues were found in kidneys sampled at later time points.

METHODS OF RESIDUE ANALYSIS

The measurement of residues of penicillins is carried out using either bioassay or physicochemical methods. Microbiological methods measure the ability of the drug to inhibit the growth of selected bacteria. These methods are very sensitive for the penicillins and in many countries form the basis of screening meat, milk and other foods for the presence of residues of penicillins (and other antibiotics).Biochemical assay methods are based on using penicillins as substrates for the enzyme penicillinase. These methods are more specific than some of the microbiological methods and are also widely used.Physicochemical methods make use of chromatographic techniques of which HPLC has superceded TLC as the more suitable method. HPLC is very specific, precise and has lower limits of sensitivity of 50 g/kg in tissues and 10 g/L in milk.

MICROBIOLOGICAL ASSAY METHODS

Residues in milk

A list of the principle methods used world wide are shown in table VII. The inhibition of growth of selected strains of organisms is measured by a number of techniques. The 'Charm' test is a competive assay using 14C-benzylpenicillin and the receptor sites on the surface of a strain of B. stearothermophilis. Many of the methods in table VII are used throughout the milk industry and when combined with heavy financial penalties significantly reduce the number of violations due to residues of penicillin.

Table VII. Methods for detecting penicillins in milk.

Principle	Organism	Incubation Time (hours)	Incubation Temp (°)	LD g/L	Ref
(a) Morphological effect:					
Microscopy	Strep. cremoris	5	30	30	1
	Strep. thermophilis	1.5	37	9	2
(b) Growth inhibition:					
Cylinder plate	Micrococcus luteus	18	30	7.5	3
	B. stearotherm.	4.5	64	1.2	4
Disc plate	B. subtilis	3	37	30	5
	B. stearotherm.	5	55	1.5	6
Reversed phase disc	B. subtilis	6	37	30	7
(c) Reduction of redox indicator:					
	B. stearotherm.	0.7	62	1.2	8
	Strep. thermophilis	0.07	80	24	9
		2	37	24	9

(d) Inhibition of acid production:

pH indicator change	B. stearotherm.	2.5	66		3.6	10
	Strep. thermophilis	4	45		24	11
Lactate production	Yoghurt bacteria	0.75	37		7.5	12
	Strep. thermophilis	2.5	45		7.5	13
Radiolabelled antibiotic	B. stearotherm.	0.25	90	0.6	14	

LD is the lower limit of detection (in g/litre)

References: 1. Whitehead and Cox (1956); 2. Liska (1960); 3. FDA (1968); 4. Vilim et al. (1979); 5. Messer et al (1978); 6. Arret and Kirschbaum (1959); 7. Kosikowski and Ledford (1960); 8. Ingarashi et al (1961); 9. Neal and Calbert (1955); 10. van Os et al. (Delvotest) (1975); 11. Intervet and Oxoid kits; 12. Hamman et al (1980); 13. Wright and Trammer (1961); 14. 'Charm' test (US patent 1980).

Residues in animal tissues and fluids

The methods for blood (plasma and serum), urine and synovial fluids are essentially the same as those used for milk. The methods for milk have also been adapted to measure residues in tissues. The residues of benzylpenicillin in meat are presented to the test system with different levels of tissue preparation. In the Four Plate Test developed by Bogaerts and Wolf (1980) and used as a screening test for meat within the EEC, meat is placed in small pieces directly onto the surface of seeded agar. Other methods use exudate either drained from or soaked onto absorbent discs from fresh or frozen meat (van Schothorst and Peelen-Knol 1970, Billon 1976, Johnston et al 1980, Swab test, USDA/FSIS, 1989). Some methods use solvent extracts (Smither 1975, McCracken et al. 1976) and are the most sensitive because they involve a concentration step, but they are more time consuming. It is also probable that solvent extraction liberates more benzylpenicillin which is bound to cellular material. Bioassay methods are not generally specific but they are sensitive, e.g. the lower limit of sensitivity of the Four Plate Test for penicillins in muscle is 30 - 60 g/kg and for the Swab test the lower limit of detection is 12.5 g/kg in liver, kidney or muscle.

One approach to make the bioassay test more specific is first to separate the antibiotics by high voltage electrophoresis on large agar plates and then identify the regions which inhibit bacterial growth (Smither and Vaughan 1978). This works fairly well for penicillins.

PHYSICOCHEMICAL METHODS

Early workers used thin-layer chromatography (TLC) to identify the antibiotics but better results and quantitation are obtained with reversed-phase high performance liquid chromatography (HPLC). An outline of a selection of reports using UV detection in reversed-phase systems is shown in table VIII. The HPLC method of Tcyzkowska et al (1989) is further developed by linking the HPLC to a mass spectrometer and confirming the presence of the analyte.

Table VIII. HPLC Methods for benzylpenicillin.

Tissue	Solvent extraction	Column off line	Chrom on line	LD (g/kg)	Ref
meat	organic	no	no	50+	1
meat	aqueous	aluminum oxide	Sepak C-18	50	2
fluids	organic	no	no	500	3
milk	organic	no	no	10 UV	4
				50 UV & MS	4

LD is the lower limit of detection.

References: 1. Moats (1984); 2. Terada et al (1985); 3. van Gulpen et al (1986); 4. Tcyzkowska et al (1989).

APPRAISAL

Benzylpenicllin is widely used as a therapeutic drug in all food animal species. It is especially used in the treatment of bovine mastitis in the lactating cow. Thus the residues in milk are of concern.

Benzylpenicillin is distributed thoughout the body and enters the extracellular fluid of tissues. Following an intramuscular, intravenous or subcutaneous dose the drug is rapidly cleared via the kidneys and into the urine and when administered in this way to lactating cattle less than 0.03% dose is excreted in the milk. On the other hand most of the dose enters the milk following intramammary administration.

The persistence of residues in milk depends on the formulation and route of administration, but in a wide variety of examples residues did not persist beyond 5 days after the end of treatment.

There was very limited information on residues in meat and no tissue depletion study was available. Residues were easily detected in most tissues sampled within 24 hours following treatment. More information including a tissue depletion study is needed for residues in meat.

There are good, sensitive bioassay methods for measuring residues in milk and meat. These assays are not specific for benzylpenicillin and the presence of the drug would need confirmation by HPLC or mass spectrometry (MS). These latter methods can have lower limits of detection of 10-50 g per litre in milk and 50-100 g per kg in meat but there is a need to improve these methods.

The following points need consideration in setting an MRL:

- the drug is used in both meat and milk producing animals.
- bioassay methods are likely to the methods of choice in most member countries for the control of residues.
- the methods in milk are more sensitive than those for tissues.
- the methods for confirmation of benzylpenicillin are becoming more available but at the present time they are less sensitive than bioassays.

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