Microbiological and Thin-Layer Chromatographic Identification of Benzylpenicillin and Ampicillin in Animal Body

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Abstract. Benzylpenicillin (PG-G) and ampicillin (AB-PC) were administered intramuscularly to pigs and calves. An attempt was made to identify these penicillins qualitatively in tissues and urine collected from the animals after slaughter. The kidney, liver and muscle were homogenized into fourfold dilutions. The diameter of an inhibition zone produced by each dilution was determined on *Micrococcus luteus* ATCC 9341 and *Bacillus subtilis* ATCC 6633 plates at pH 6.0 and 8.0. The urine was diluted up to 625-fold. Thin-layer chromatographic identification was performed for only tissue and urine samples characterized by the largest inhibition zone that was observed on *M. luteus* plate and inactivated by the addition of penicillinase. Next, a sample extracted with methanol was spotted on a silica gel plate, which was then developed in ethyl acetate-methanol-water (6:2:1). Rf values were determined by bioautography with *M. luteus* as the test organism. They were about 2 times as high in animals given PC-G as in those given AB-PC. They coincided closely with those of reference antibiotic solutions. Thus, it seems likely that PC-G and AB-PC residues may be identified in slaughtered animals by thin-layer chromatography with the spot volume of 10 and/or 30 μ l on the basis of inhibition zones produced on *M. luteus* plate. It was recommended that chromatography should be performed on extracts from the medulla rather than cortex of the kidney, which had been proved to contain penicillins.

The use of antibiotics for the treatment and prevention of infectious diseases in animals may have created a variety of problems involving a hazard to the consumer. Much attention has been paid to allergic reactions in sensitized individuals and the development of resistant pathogens in man and animals [6].

In some countries, a detection of antibiotic residues has been conducted qualitatively in slaughtered animals by official methods, such as *Micrococcus luteus* (*Sarcina lutea*) kidney test and general inhibitor test [12, 18, 21]. On the other hand, not only muscle but also at least kidney and liver have always been surveyed in slaughtered animals for antibiotic residues [3, 15,

20, 22]. Other surveys have been carried out on urine [7, 17, 19], into which certain antibiotics might have been excreted for a long period and at high concentrations. There is a possibility, however, that the antibacterial activities detected may have been originated from non-specific inhibitory substances, such as lysozyme and lactic acid, formed in a few tissues and urine from time to time [4, 14, 16]. Then, it is recommended to improve some procedures by which antibiotic residues can be identified in slaughtered animals in more detail.

In literature on antibiotic residues in slaughtered animals, the presence of penicillin was confirmed by using penicillinase [7, 17, 23]. However, in addition to benzyl-

Table 1. Test protocol

Animal No.	Species and sex	Approx. body weight in kg	Antibiotic administered	Dose in IU or mg	Time in hours until slaughter
2	calf (♂)	200	Benzyl-penicillin ¹⁾	1,500,000	2
4	calf (♂)	250		3,000,000	12
28	calf (♂)	250		3,000,000	24
6	pig (♂)	50		600,000	6
18	pig (♀)	50		900,000	6
1	calf (♂)	200	Ampicillin ²⁾	1,000	2
3	calf (♂)	300		1,500	12
5	pig (♂)	50		400	6
17	pig (♀)	50		600	6
11	calf (♂)	250	control		
12	pig (♀)	50			

¹⁾ Procaine penicillin in aqueous suspension (Meiji) for veterinary use, containing 300,000 IU of benzylpenicillin/ml.

²⁾ Vicillin sol-200 for veterinary use (Meiji), containing 200 mg of ampicillin/ml.

penicillin (PG-G) utilized since a long time ago, ampicillin (AB-PC) is now widely available for domestic animals. It is inactivated in the same manner as PG-G by the addition of penicillinase. The present paper deals with a procedure to identify PC-G and AB-PC contained in the kidney, liver, muscle and urine of animals given commercial products containing these antibiotics.

Materials and Methods

1. Animals

Six Holstein calves weighing from 200 to 300 kg and 5 Yorkshire pigs weighing approximately 50 kg were used. Of them, 3 calves (Nos. 2, 4 and 28) and 2 pigs (Nos. 6 and 18) were given a single intramuscular administration of an aqueous suspension of PC-G procaine. Two calves (Nos. 1 and 3) and 2 pigs (Nos. 5 and 17) were injected with AB-PC in oily suspension by the same route. After the antibiotics were administered, the animals were slaughtered at various intervals as scheduled in the test protocol (Table 1). The remaining 2 animals served as controls.

The kidney, liver, muscle and urine were collected from each animal and kept in frozen state until use.

2. Diameter of inhibition zones on *M. luteus* and *Bacillus subtilis*

By using antibiotic medium No. 1 (Difco) ad-

justed to pH 6.0 and 8.0 with hydrochloric acid or sodium hydroxide, agar plates were made to screen samples containing antibacterial activities. The plates were prepared in such manner as described below.

Ten ml of medium at each pH was poured in Petri dishes (10 cm in diameter) as the base layer. When solidified, 4 ml of the same medium previously inoculated with *M. luteus* ATCC 9341 or *B. subtilis* ATCC 6633 was overlaid as the seed layer. In the seed layer, *M. luteus* was added in a concentration of 0.5%, by using fresh cultures propagated in nutrient broth at 37°C for 18 hours. *B. subtilis* was added to give a bacterial count of 1.2×10^4 /ml, by using a spore suspension stored in a refrigerator.

A tissue sample (usually 5 g) was homogenized fourfold with distilled water and filled steel cups (8 mm in diameter) placed on *M. luteus* and *B. subtilis* plates at pH 6.0 and 8.0. Besides, a drop of penicillinase was added to one of the tissue homogenates, which filled steel cups placed on the *M. luteus* plate. After allowed to stand at room temperature for 1 hour, all the plates were incubated overnight at 37°C. The diameters of inhibition zones produced were measured and averaged. Urine samples were serially diluted with distilled water up to 625-fold with a dilution of 1:5. The diameters of inhibition zones produced on the plates were determined. By these procedures, it was possible to screen the tissue and urine samples containing more than 0.1 and 0.02 µg or IU/g of the antibiotic, respectively. Thin-layer chromatographic identification was carried out only on samples characterized by the largest inhibition zone

that was observed on the *M. luteus* plate and lost by the addition of penicillinase.

3. Thin-layer chromatographic identification

Tissues were homogenized threefold with methanol and centrifuged at 3000 rpm for 10 min [5]. The resultant supernatant was regarded as the solution to be chromatographed. Aliquots of 10 μ l of this solution were spotted to the origin of the silica gel plate (spot film for thin-layer chromatography (TLC), 20 $\times$ 20 cm, TOKYO Kasei), along with the same volume of the pure reference antibiotic solutions dissolved in methanol in concentrations of 0.5 IU/ml for PC-G and 0.2 μ g/ml for AB-PC. After allowed to dry, each plate was developed in ethyl acetate-methanol-water (6:2:1) until the solvent front reached approximately 15 cm from the starting line [9]. The plate was dried by the aid of a fan to remove the excess solvent and placed on the surface of the agar medium previously solidified in an aluminum plate (20.5 $\times$ 20.5 cm), on which was then poured 40 ml of antibiotic medium No. 1 (pH 6.0) inoculated with *M. luteus*. After overnight incubation at 37°C, Rf values were determined from the location of the area of inhibited bacterial growth on chromatograms. The chromatograms could be easily seen by adding 4% aqueous solution of 2,3,5-triphenyl-tetrazolium chloride.

When no area of inhibited bacterial growth was observed on the chromatograms by the method mentioned above, the solution was chromatographed once again with a spot volume of 30 μ l. In this case, the concentrations of the reference antibiotic solutions were 0.2 IU/ml for PC-G and 0.1 μ g/ml for AB-PC.

Urine samples were diluted with methanol to such concentrations as corresponding to the diameters of inhibition zones ranging from 25 to 30 mm which might be produced on *M. luteus* plated if diluted with distilled water and determined. A preliminary experiment indicated that the area of inhibited bacterial growth could easily be obtained and Rf values read by the use of urine samples diluted up to such concentrations as exhibiting antibacterial activities. Identification was carried out in the same manner as that conducted on tissue samples.

4. Qualitative assay of antibacterial activities in tissues and urine

Pieces about 1 cm square were cut from the tissue sample in frozen state and placed on *M. luteus* plated at pH 6.0 and 8.0. Paper discs were soaked into the urine before use. The antibacterial activities were detected qualitatively after incubation at 37°C for 18 hours. A sample was regarded as positive when it induced an inhibition zone

1 mm or more.

5. Antibiotic concentrations in renal tissues

An attempt was made to compare the antibiotic concentration between cortex and medulla of the kidney by a hole-plate method with a rectangular large plate (30 $\times$ 30 cm) modified from the method of Bennett et al. [2]. As the base layer was used 200 ml of No. 5 medium (pH 6.0) prepared in the same manner as prescribed in the Minimum Requirements for Antibiotics for Animal Use [10]. As the seed layer was used 100 ml of the same medium inoculated with *M. luteus*.

The cortex and medulla were separately homogenized fourfold with 0.1 M phosphate buffer (pH 6.0) in a glass mortar. The resulting supernatant was pipetted in holes 8 mm in diameter on the agar plate in 0.1 ml amounts. The plate was cut with a specially designed punch. The reference antibiotics used to prepare standard curves were dissolved in phosphate buffer. The threshold sensitivity of each antibiotic was 0.1 IU or μ g/g.

Results

1. Identification of penicillins in tissues

Table 2 shows the diameters of inhibition zones produced by tissue samples on *M. luteus* and *B. subtilis* plates, as well as Rf values obtained by TLC.

Regarding the test organisms, *M. luteus* was more inhibited than *B. subtilis*. Inhibition zones were a little larger on plates at pH 6.0 than on those at pH 8.0 for almost all the samples. In each animal, inhibition zones produced by the muscular tissue were apparently smaller than those by the other 2 tissues. No inhibition zone was observed on the *M. luteus* plate for any sample to which had been added penicillinase.

Examination of chromatograms spotted with 10 μ l revealed that Rf values were 0.41–0.44 and 0.19–0.20 in animals given PC-G and AB-PC, respectively, and that they coincided well with those of the reference antibiotic solutions. A similar result was obtained with a spot volume of 30 μ l.

In some samples (muscles of Nos. 2 and 18, livers of Nos. 18 and 3, and kidney of No. 17) their homogenates produced in-

Table 2. Diameter of inhibition zones on *M. luteus* and *B. subtilis* plates, and Rf values in chromatography on tissue samples

Animal No.	Tissue	Inhibition zone (in mm)				Rf	
		<i>M. luteus</i>		<i>B. subtilis</i>		Spot volume	
		pH 6.0	pH 8.0	pH 6.0	pH 8.0	10 μ l	30 μ l
2	Kidney	21.5	20.5	15.3	14.8	0.41	ND
	Liver	21.0	18.5	NZ	NZ	0.42	ND
	Muscle	17.8	17.5	NZ	NZ	no	0.34
4	Kidney	34.5	34.5	25.0	25.0	0.43	ND
	Liver	35.8	34.3	24.5	24.3	0.44	ND
	Muscle	9.5	9.5	NZ	NZ	no	no
28	Kidney	22.6	21.8	18.3	17.3	0.41	ND
	Liver	31.3	30.3	25.5	24.1	0.44	ND
	Muscle	NZ	NZ	NZ	NZ	ND	
6	Kidney	17.3	16.3	NZ	NZ	0.44	ND
	Liver	8.5	NZ	NZ	NZ	no	no
	Muscle	NZ	NZ	NZ	NZ	ND	
18	Kidney	22.4	20.5	18.3	15.1	0.44	ND
	Liver	18.5	17.5	NZ	NZ	no	0.32
	Muscle	13.3	12.3	NZ	NZ	no	0.35
1	Kidney	28.5	26.5	20.5	17.5	0.20	ND
	Liver	25.1	21.5	NZ	NZ	0.19	ND
	Muscle	20.8	17.3	NZ	NZ	0.18	ND
3	Kidney	18.8	16.3	NZ	NZ	0.20	ND
	Liver	15.6	13.2	NZ	NZ	no	0.14
	Muscle	NZ	NZ	NZ	NZ	ND	
5	Kidney	NZ	NZ	NZ	NZ	ND	
	Liver	NZ	NZ	NZ	NZ	ND	
	Muscle	NZ	NZ	NZ	NZ	ND	
17	Kidney	17.3	17.0	NZ	NZ	no	0.14
	Liver	12.5	13.0	NZ	NZ	no	no
	Muscle	NZ	NZ	NZ	NZ	ND	

NZ: No zone formed. ND: Not done. no: No area of inhibited bacterial growth on the chromatogram.

Rf values by reference antibiotic solutions spotted with 10 μ l were 0.43 and 0.21, and those spotted with 30 μ l 0.35 and 0.17 for PC-G and AB-PC, respectively.

inhibition zones less than 20 mm in width on the *M. luteus* plate, and there were no areas of inhibited bacterial growth on their chromatograms spotted with 10 μ l. Areas of inhibited bacterial growth became visible on their chromatograms when the spot volume was increased to 30 μ l. In other samples (muscle of No. 4 and livers of Nos. 6 and 17) their homogenates produced much smaller inhibition zones on the *M. luteus*

plate than those mentioned above, and no areas of inhibited bacterial growth were observed on their chromatograms even with a spot volume of 30 μ l.

The area of inhibited bacterial growth on the chromatogram was always round or oval in shape for all the samples, except the muscle of a calf (No. 1) given AB-PC, that exhibited tailing, as shown in Fig. 1.

2. Identification of penicillins in urine

Fig. 1. TLC pattern of inhibited bacterial growth on silica gel plate spotted with 10 μ l. 1, AB-PC standard; 2, PC-G standard; 3-5, Kidney, liver and muscle of calf No. 1; 6-8, Kidney, liver and muscle of calf No. 2.

Since the concentration of penicillin excreted in urine was very high, it gave rise to an inhibition zone much larger than that produced by a tissue sample. Some urine samples produced inhibition zones 30 mm or so in diameter, even if diluted 625 times with distilled water (Table 3).

PC-G and AB-PC excreted in a urine sample were easily identified by TLC with a spot volume of 10 μ l when the sample was diluted 20-500 times with methanol. No tailing or metabolites were observed in any urine sample.

3. Qualitative assay

In animals given PC-G, positive results were obtained from all the samples, including 2 muscle samples (Nos. 6 and 28) the homogenates of which failed to produce an inhibition zone for any test organism (Table 4). When animals were administered with AB-PC, the kidney was found to be positive in three, and the muscle in one of them. All the samples collected from control animals were negative for this assay.

4. Antibiotic concentration in kidney

Antibiotic concentrations were compared between the cortex and medulla of the kidney. The results obtained are shown in Table 5. They were several times as high in the medulla as in the cortex, regardless of the type of antibiotics administered.

Table 3. Diameter of inhibition zones on *M. luteus* and *B. subtilis* plates, and Rf values in chromatography on urine samples

Animal No.	Inhibition zone (in mm)*				Rf	
	<i>M. luteus</i>		<i>B. subtilis</i>		Spot volume of 10 μ l	
	pH 6.0	pH 8.0	pH 6.0	pH 8.0		
2	29.0	28.3	22.5	20.5	0.40	(500)
4	31.3	30.5	24.5	22.8	0.39	(500)
28	ND		ND		ND	
6	25.5	24.3	19.3	16.0	0.38	(200)
18	30.0	29.0	23.5	21.3	0.40	(200)
1	19.0	15.0	NZ	NZ	0.25	(200)
3	26.5	24.5	18.0	15.8	0.26	(200)
5	15.0	12.5	NZ	NZ	0.29	(50)
17	14.5	15.0	NZ	NZ	0.26	(20)

See the footnote of Table 2.

* Presented only inhibition zones produced by urine samples that were diluted 625-fold.

Rf values by reference antibiotic solutions were 0.39 and 0.27 for PC-G and AB-PC, respectively.

In parentheses is shown the dilution factory by which urine samples dissolved in methanol were chromatographed.

Table 4. Qualitative assay of antibacterial activities in tissues and urine, using *M. luteus* plates at pH 6.0 and 8.0

Animal No.	Kidney		Liver		Muscle		Urine	
	pH 6.0	pH 8.0	pH 6.0	pH 8.0	pH 6.0	pH 8.0	pH 6.0	pH 8.0
2	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+
28	+	+	+	+	+	+	ND	
6	+	+	+	+	+	+	+	+
18	+	+	+	+	+	+	+	+
1	+	+	+	+	+	+	+	+
3	+	+	+	+	—	—	+	+
5	—	—	+	+	—	—	+	+
17	+	+	+	+	—	—	+	+

ND: Not done.

Table 5. Antibiotic concentrations in cortex and medulla of kidney

Benzylpenicillin (IU/g)			Ampicillin (μ g/g)		
Animal No.	Cortex	Medulla	Animal No.	Cortex	Medulla
2	1.85	10.25	1	1.75	9.75
4	4.80	25.00	3	0.37	1.05
26	1.83	5.50	5	0	0
6	0.96	3.95	17	0.40	0.60
18	3.00	8.50			

Discussion

In their studies on the distribution of penicillins in tissues and body fluids, Rollins et al. [13], Mashimo et al. [9], and Tittiger et al. [17] used *M. luteus* as the most sensitive and reliable test organism. On the one hand, surveys have been done on antibiotic residues in slaughtered animals by the microbiological methods with agar plates at pH 6.0 and 8.0 inoculated with such highly sensitive test organisms as *M. luteus* and *B. subtilis* [1, 3, 7, 8, 12, 15, 17–19, 21, 22]. On the other hand, Murakawa et al. [11], Higuchi et al. [5], and Mashimo et al. [9] used TLC in their attempts to separate and determine penicillins in tissues and body fluids. In the present investigation to identify PC-G and AB-PC, TLC was applied to tissue and urine samples which had previously been

screened by using agar plates at different pH values inoculated with *M. luteus* and *B. subtilis*.

The presence of penicillins in tissue and urine samples was demonstrated by the formation of inhibition zones on agar plates, where these zone were larger for *M. luteus* than for *B. subtilis*, and a little larger at pH 6.0 than at pH 8.0 or of almost the same size at both pH values [8, 13, 15, 17, 19]. Some workers [7, 17, 23] employed penicillinase, an enzyme inactivating penicillin.

In this study, PC-G and AB-PC in tissue and urine samples screened with agar plates were shown to be identifiable by TLC with a spot volume of 10 and/or 30 μ l. From the results of the present study, it seems possible to identify these penicillins contained in tissue samples with a spot volume of 10 μ l when the homogenates of these samples produce inhibition zone 20 mm or

more in width on *M. luteus* plates. The antibiotics contained in the muscular or hepatic tissue from some animals (Nos. 4, 6, and 17) could not be identified even with a spot volume of 30 μ l, although the homogenate of such tissue produced inhibition zones on *M. luteus* plates.

No inhibition zones were produced on any plate by the homogenate of the muscular tissue from 2 animals (Nos. 6 and 28) which were positive for the qualitative assay. Thus, the residual antibiotics in meat are shown to be easily detectable by the qualitative assay, although their kind remains unknown. It is possible, however, to guess the kind of penicillin in meat if identification by TLC is successful in the kidney or liver of the same subject. This is because antibiotics are known to be distributed more densely in these excretory organs than in the muscle, and because a close correlation is maintained in concentration among the organs and tissues at various sites, except aminoglycoside antibiotics remaining in the kidney prolonged. In slaughtered animals, the residues of penicillins will be anticipated in meat by examining the kidney, liver, or urine occasionally in the same subject.

As listed in Table 5, penicillin concentrations were apparently higher in the medulla than in the cortex of the kidney. This result is in accord with the finding of Rollins et al. [13] who gave the product to cows by the intramammary route. From all these results it is concluded that the use of the medulla is preferred for the identification of penicillins in the kidney by TLC.

References

- [1] Beck, Gg., and Mantel, Th. (1972). Der biologische Hemmstofftest: Ein Weg zur Klärung des Rückstandsproblems in Fleisch. *Fleischwirtschaft* 52, 1605-1609.
- [2] Bennet, J. V., Brodie, J. L., Bonner, E. J., and Kirby, M. M. (1966). Simplified, accurate method for antibiotic assay of clinical specimens. *Appl. Microbiol.* 14, 170-177.
- [3] Bentler, W., and Zettl, K. (1973). Die Anwendbarkeit des "allgemeinen Hemmstoff-Testes" (AH-Test) bei der Untersuchung auf mikrobiologisch aktive Rückstände in Fleisch und Organen. *Dtsch. Tierärztl. Wochenschr.* 80, 35-38 & 62-63.
- [4] Heiner, H. H., van der Wail, G., and Brehner, H. (1976). Lysozym-Ursache von unspezifischen Reaktionen im mikrobiologischen Hemmstofftest. *Arch. Lebensm-hyg.* 27, 55-60.
- [5] Higuchi, S., Yakushiji, M., Eto, K., and Shigematsu, S. (1971). Differential assay and clinical application of ampicillin-cloxacillin. *Jpn. J. Antibiot.* 24, 1-7.
- [6] Huber, W. G. (1971). The impact of antibiotic drugs and their residues. In *advances in Veterinary Science and Comparative Medicine* 15, 101-132. Academic Press, New York & London.
- [7] Huber, W. G., Carlson, M. B., and Lepper, M. H. (1969). Penicillin and antimicrobial residues in domestic animals at slaughter. *J. Am. Vet. Med. Assoc.* 154, 1590-1595.
- [8] Langner, H. J., and Teufel, U. (1973). Der chemische und mikrobiologische Nachweis von Antibiotika. 4. Die qualitative mikrobiologische Bestimmung verschiedener Antibiotika im unteren Grenzbereich mit *Bac. subtilis* (ATCC 6633). *Fleischwirtschaft* 53, 1125-1128.
- [9] Mashimo, K., Fukaya, K., Kunii, O., and Suzuki, M. (1973). Studies on amoxycillin. *Chemotherapy (Japan)* 21, 1441-1445.
- [10] Ministry of Agriculture and Forestry (1970). Minimum Requirements for Antibiotics for Animal Use. Notification No. 1883 based on the Pharmaceutical Affairs Law, Tokyo, Japan, 1-20.
- [11] Murakawa, T., Wakai, Y., Nishida, M., Fujii, R., Konno, M., Okada, K., Goto, S., and Kuwahara, S. (1970). Chromatographic assay of mixed penicillins, ampicillin and cloxacillin in body fluids. *J. Antibiot.* 23, 250-251.
- [12] Nouws, J. F. M., van Schothorst, M., and Ziv, G. (1979). A critical evaluation of several microbiological test methods for residues of antimicrobial drugs in ruminants. *Arch. Lebensm-hyg.* 30, 4-8.
- [13] Rollins, L. D., Mercer, H. D., Carter, G. G., and Kramer, J. (1970). Absorption, distribution, and excretion of penicillin and dihydrostreptomycin in dairy cows following intramammary infusion. *J. Dairy Sci.* 53, 1407-

- 1414.
- [14] Schmidt, U., and Cremmling, K. (1975). Untersuchungen über unspezifische Reaktionen im Hemmstofftest beim Antibiotika-Nachweis in Schweinenieren. *Fleischwirtschaft* 55, 1736-1737.
 - [15] Takács, J., and Kovács, S. (1969). Demonstration of antibiotic residues in the meat of slaughtered animals. *Acta. Vet. Acad. Sci. Hung.* 19, 11-19.
 - [16] Terplan, G., Barthel, G., and Zaadhof, K. J. (1974). Zum Nachweis von Hemmstoffen im Harn. *Arch. Lebensm-hyg.* 25, 261-264.
 - [17] Tittiger, F., Kingscote, B., Meldrum, B., and Prior, M. (1975). Survey of antibiotic residues in Canadian slaughter animals. *Can. J. Comp. Med. Vet. Sci.* 39, 178-182.
 - [18] Vandenbrande, G., and van Hoof, J. (1975). A comparative study of some microbiological methods for the detection of inhibitory substances in slaughter animals. *Vlaams Diergeneesk. Tijdschr.* 44, 297-308.
 - [19] van Schothorst, M. and Peelen-Knol, G. (1970). Detection and identification of some antibiotics in slaughter animals. *Neth. J. Vet. Sci.* 3, 85-93.
 - [20] van Schothorst, M., Uijttenboogaart, Th. G., and van Leusden, M. (1973). Über die Leistungsfähigkeit des Nierentestes bei der Bestimmung von Antibiotikarückständen in Fleisch. *Fleischwirtschaft* 53, 703-705.
 - [21] van Schothorst, M., van Leusden, F. M., and Nouws, J. F. M. (1978). Antibiotic residues: Regulations, tolerances and detection in the European Economic Community. *J. Assoc. Off. Anal. Chem.* 61, 1209-1213.
 - [22] Wenzel, S. (1971). Allgemeiner Hemmstoffnachweis bei der Krankgeschlachteten Rindern und Schweinen und ein Vergleich mit den Hemmstoffnachweisen bei normal geschlachteten Tieren. *Arch. Lebensm-hyg.* 22, 200-205.
 - [23] Wuilleret, A. (1968). Contribution à la recherche de substances antibiotiques dans les viandes. *Schweiz. Arch. Tierheilkd.* 110, 523-531.

要 約

動物体内におけるベンジルペニシリンとアンピシリンの鑑別法: 吉村治郎・伊藤 治・米沢昭一 (農林水産省動物医薬品検査所)——ベンジルペニシリンまたはアンピシリンを筋肉内注射した牛および豚を用い、これらペニシリンの鑑別を、殺処分後に採取した腎、肝、筋および尿について行なった。まず、蒸留水による各組織の4倍乳剤を作成し、pH 6.0 と 8.0 に調整した寒天培地を用い、*Micrococcus luteus* ATCC 9341 および *Bacillus subtilis* ATCC 6633 に対する阻止径を、平板円筒法によって測定した。尿は、5倍希釈で625倍まで希釈し、同様にこれら試験菌に対する阻止径を測定した。*M. luteus* に対する阻止径が大きく、またその抗菌活性がペニシリンナーゼの添加により消失した検体だけを、薄層クロマトグラフィーによる鑑別に供した。次に、供試検体をメタノール処理し、3,000 rpm 10分間遠沈した上清 10 μ l を、シリカゲルプレートの原点にスポットした。酢酸エチル・メタノール・水 (6:2:1) で展開後、試験菌に *M. luteus* を用いた bioautography により Rf 値を求めた。この方法でクロマトグラム上に *M. luteus* の発育阻止ゾーンがみられなかった場合には、スポット量を 30 μ l として再試験を行なった。各検体とも、ベンジルペニシリンによる Rf 値はアンピシリンによるそれよりも約2倍高く、またそれぞれの常用標準品の値ともよく一致した。以上の成績から、*M. luteus* 寒天培地上の阻止径に応じて、スポット量を10ないし 30 μ l とする薄層クロマトグラフ法を利用すれば、屠畜におけるこれらペニシリンの残留の鑑別も可能と思われた。