Effect of cooking and cold storage on biologically active antibiotic residues in meat

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SUMMARY

An investigation was undertaken to see if cooking or cold storage would destroy or decrease the level of biologically active antibiotic in tissues from animals given therapeutic doses of antibiotic on three occasions prior to slaughter. The effects of cooking and cold storage on the biological activity of the residues of ampicillin, chloramphenicol, oxytetracycline, streptomycin and sulphadimidine were varied; in some instances the effects were minimal, in others nil.

INTRODUCTION

The presence of drug residues – in particular antibiotics – in food, is a source of anxiety. In the U.K., the Swann Committee (1969) and in the United States, the Food and Drug Administration Task Force (Anon, 1972) considered the possible dangers to human health from the different applications of antibiotics in animal husbandry and veterinary medicine. A further working group convened in 1973 by the Regional Office for Europe of the World Health Organization, also considered the public health aspects and control of harmful residues in food for human and animal consumption. The Committees were concerned with the hypotheses that antibiotic residues in animal products might (*a*) produce harmful effects from direct toxicity or from allergic reactions in persons who had previously been sensitized to them, and (*b*) lead to the emergence of resistant strains of bacteria in animals and the passage of these via the food chain from animals to man.

The effect of heat on the stability of solutions of antibiotics *in vitro* has been studied (Pilet & Toma, 1969; Peric & Dakic, 1973). *In vivo* studies (Yonova, 1971; Sarkisov & Ezhov, 1972; Raseta, Peric & Dakic, 1975) described the effects of boiling on streptomycin, tetracycline and oxytetracycline residues in tissues from animals (mainly fowl) which had received therapeutic antibiotics before slaughter.

The work presented in this paper was undertaken to ascertain the effects of simulated domestic or catering establishment cooking (dry heat) and cold storage on the biologically active residues of five antibiotics used in general veterinary practices, which might be present in bovine tissues after parenteral administration.

MATERIALS AND METHODS

Antibiotics used

Five antibiotics, ampicillin (Penbritin, Beecham), chloramphenicol (Ertlen, Ciba-Geigy), oxytetracycline (Terramycin Q50, Pfizer), streptomycin (Streptomycin 33, Bayer, U.K.) and sulphadimidine (Sulphamezathine, Imperial Chemical Industries) were used in the injectable forms and administered at therapeutic levels once daily on three consecutive days. The levels used per kilogram body weight were 7 mg/kg (ampicillin), 4.5 mg/kg (chloramphenicol), 5 mg/kg (oxytetracycline), 2 mg/kg (streptomycin) and 14 mg/kg (sulphadimidine).

Test animals

Two bovine animals, aged 7–12 months (weight range 190–300 kg) were used per antibiotic, except in the cases of streptomycin, where only one test animal was used. Each animal was injected intramuscularly in the neck area and within two hours after the third injection, was slaughtered. Each carcass was skinned, eviscerated, split into two sides and left to hang at 4 °C for 5–7 days to simulate normal trade practice.

Immediately after slaughter, before the carcass sides were placed in the chill room, samples of the injection site, muscle, kidney (including medulla and cortex) and liver were tested for the presence of the antibiotic administered. At the end of the five to seven day hanging period and prior to the roasting and grilling of the prepared cuts, samples of the same tissues were again tested to make sure that antibiotic was detectable microbiologically. During the preliminary stages of the experiment it was intended to include streptomycin in the group of antibiotics under test but, while it was possible to detect it minimally in muscle immediately after slaughter, no biologically active residue was detectable in muscle after the hanging period. It was therefore decided to proceed with the cold storage procedures only for streptomycin, using kidney tissue where residues were detectable at higher levels.

Cooking practice

After the hanging period, one sirloin roast (average weight 1.5–2 kg) and three steaks (average 400–500 grams each) were cut from each carcass side. The roasts were rolled and secured with wooden skewers. Roasting commenced with the recommended cooker setting of 176 °C (see Table 1). However, this temperature proved inadequate for what was considered 'proper roasting' (judged by naked eye) and the thermostat was adjusted to 190 °C for all the subsequent roasts. The time allowed for roasting was approximately 20 min per 450 g plus 20 min extra as recommended by cookery books.

The steaks, 'rare', 'medium' and 'well-done' were placed on a grill pan and exposed (on either side) for 5, 10 and 15 min respectively at a distance of 7–8 cm from the grill element.

After each cooking session, samples of each roast were taken from (a) the outer surface to a depth of 1–2 cm (b) about mid depth and (c) at the centre. The samples of the grilled meat comprised the outer and inner tissue macerated together.

Temperature measurements

Thermocouples were inserted into the centre and subsurface of the roasts, and into the thickest part of each steak during cooking, and temperatures were monitored at these sites on a continuous flat bed recorder.

Storage practice

After the hanging period when the roasts and steaks were prepared from each carcass, additional individual samples (approximately 20 g each) of muscle tissue (gluteal; semitendinosus and semi-membranosus masses) were taken from the remainder of the hind quarters of the carcass, put in self sealing plastic bags and placed in refrigeration at -20°C . At the different time intervals indicated below, individual samples of these muscle tissues were retrieved, thawed at room temperature and assayed.

Extraction and Detection of the antibiotic residues

The methods of extraction and subsequent microbiological assay of chloramphenicol and oxytetracycline (McCracken, O'Brien & Campbell, 1976*a*, and for ampicillin (McCracken & O'Brien, 1976*b*) have been described previously.

The procedure for streptomycin and sulphadimidine extraction and assay were basically similar to those of the other antibiotics. However, the extractant for streptomycin was phosphate buffer (pH 7.9), the test spore suspension *Bacillus subtilis* (ATCC 6633: Difco) and the medium was Antibiotic Medium no 2 (Difco) adjusted to pH 7.9.

The tissues from the sulphadimidine injected animals were extracted with a mixture (98:2) of methanol and HCl (0.1 N). The determination of sulphadimidine was done by using the medium and test organism in the proposed EEC plate test method (Smither *et al.* 1980). The resuspending buffer for both streptomycin and sulphadimidine was phosphate buffer pH 7.9.

The minimum amounts of antibiotic detected in the tissues of the individual test animals (A and B) prior to cooking and cold storage were as follows:

	A ($\mu\text{g/g}$)	B ($\mu\text{g/g}$)
Ampicillin	0.6	0.8
Chloramphenicol	1.6	1.5
Oxytetracycline	0.5	0.6
Streptomycin	6.4	—
Sulphadimidine	8	8

RESULTS

The results of the microbiological assays of each antibiotic were recorded in millimetres as zones of inhibition. Each sample was assayed in duplicate and the results averaged. However, in order to make the results more meaningful and readily intelligible, the tables record the reduction in annular zone diameters after cooking or storage, as a percentage of the pre-cooking or pre-storage annular zone diameter. Tables 1–4 record changes in biological activity of the residual antibiotics after cooking. Tables 5–9 record changes in biological activities after cold storage.

Table 1. *Cooking effect on ampicillin residues in meat*

Test animal	Carcase side	Sample	Total cooking time (min)	Maximum temperature attained (°C)	Annular zone diameter reduction (%)
A	Right	Steak 'rare'	10	56	7.8
	Left		10	45	4.6
B	Right		10	44	2.7
	Left		10	35	2.9
A	Right	Steak 'medium'	20	71	30.4
	Left		20	90	33.6
B	Right		20	52	2.3
	Left		20	58	13.3
A	Right	Steak 'well done'	30	90	81.8
	Left		30	92	77.2
B	Right		30	78	25.3
	Left		30	87	34.8
A	Right*	Roast (centre)	180	62	27.8
	Left		210	73	77.6
B	Right		180	78	79.4
	Left		180	82	78.9
A	Right*	Roast (midway)	180	—	79.9
	Left		210	—	83.2
B	Right		180	—	87.3
	Left		180	—	100
A	Right*	Roast (outside)	180	96	100
	Left		210	93	100
B	Right		180	99	85.9
	Left		180	98	100

* Oven temperature setting (176 °C); see text. —, not recorded.

A typical example of the cooking temperatures recorded during the experiments is given in Fig. 1. This figure shows the temperature rise in a roast from the sulphadimidine injected animal B. It also records the range of temperature maxima of all 16 roasts used in the experiment. Figure 2 records similar data for the steaks from the same animal and the range of temperature maxima for the 48 steaks grilled in the experiment.

DISCUSSION

There is disagreement as to the effect of high temperatures and cold storage on the biological activity of antibiotic residues in animal tissues (Raseta *et al.* 1975). Yonova (1971) in his study showed that the inactivation of oxytetracycline in poultry meat and eggs depends on the amount present and duration of cooking and for the full inactivation of residual oxytetracycline in poultry meat it is necessary to cook for 60 min or more.

While percentage reduction of annular zone diameter is used to indicate reduction in biological activity it should be remembered that there is not a direct correlation between the two.

Table. 2. *Cooking effect on chloramphenicol residues in meat*

Test animal	Carcase side	Sample	Total cooking time (min)	Maximum temperature attained (°C)	Annular zone diameter reduction (%)
A	Right	Steak 'rare'	10	56	0
	Left		10	30	4.9
B	Right		10	52	0
	Left		10	46	7.1
A	Right	Steak 'medium'	20	58	50.0
	Left		20	70	46.7
B	Right		20	60	14.2
	Left		20	75	41.0
A	Right	Steak 'well done'	30	77	61.0
	Left		30	80	27.3
B	Right		30	82	35.7
	Left		30	82	50.0
A	Right	Roast (centre)	120	87	63.5
	Left		120	76	74.0
B	Right		120	51	37.4
	Left		120	59	42.8
A	Right	Roast (midway)	120	—	100
	Left		120	—	100
B	Right		120	—	46.4
	Left		120	—	42.8
A	Right	Roast (outside)	120	94	66.9
	Left		120	90	70.4
B	Right		120	87	55.2
	Left		120	101	75.0

—, not recorded.

The results of the present experiment (Tables 1–4) indicate that there was on average in all the 'rare' steaks less than 10 % reduction in the annular diameter size, the range being from no reduction (Table 2: chloramphenicol) to 15.8 % (Table 3: oxytetracycline). For ampicillin, chloramphenicol and oxytetracycline (Tables 1–3) the reduction in the 'medium' and 'well-done' steaks, and roasts varied considerably and appeared to depend on (a) the temperature attained during cooking, and (b) the duration of cooking. For example, no residue of ampicillin was detectable in the surface meat of the roasts from animal A when the maximum temperature was 93° or 96°, the total cooking times being 180 and 210 min respectively (Table 1). However, when the temperature recorded in the 'medium' steaks was in the 50's (52, 58 °C: animal B) there were only reductions of 2.3 and 13.3 % in zone diameters. Table 4 shows that sulphadimidine residues in any of the steaks or roasts were affected either minimally or not at all by either temperature rise or the duration of exposure.

As stated, no cooking experiments were carried out on the muscle tissue from the streptomycin injected animal. Previous experience of the authors with experimental animals had confirmed the difficulty of recovering biologically active streptomycin from muscle tissue.

Table 3. *Cooking effect on oxytetracycline residues in meat*

Test animal	Carcase side	Sample	Total cooking time (min)	Maximum temperature attained (°C)	Annular zone diameter reduction (%)
A	Right	Steak 'rare'	10	22	7.4
	Left		10	50	15.8
B	Right		10	48	10.4
	Left		10	44	4.3
A	Right	Steak 'medium'	20	58	15.1
	Left		20	62	18.0
B	Right		20	85	16.0
	Left		20	73	12.3
A	Right	Steak 'well done'	30	76	24.2
	Left		30	83	39.0
B	Right		30	85	27.7
	Left		30	73	22.5
A	Right	Roast (centre)	90	59	17.8
	Left		120	70	21.4
B	Right		120	65	32.0
	Left		120	63	23.1
A	Right	Roast (midway)	90	—	29.3
	Left		120	—	28.2
B	Right		120	—	76.3
	Left		120	—	23.1
A	Right	Roast (outside)	90	79	21.8
	Left		120	88	38.9
B	Right		120	94	74.0
	Left		120	94	58.2

—, not recorded.

Features of the experiment which require emphasis are:

(a) In general the very gradual rise, and temperatures attained (at the two depths recorded) during the cooking of a roast and the grilling of the 'rare' and 'medium' steaks (Tables 1–4).

(b) The great variation in temperature maxima recorded at similar sites in the different roasts and steaks (Figs 1 and 2); the maxima range being 82–98 °C (subsurface) and 51–84 °C (centre) for roasts, and 22–54 °C ('rare') and 55–98 °C ('medium') for the steaks.

(c) The slow rise in temperature in the depths of the roasts and steaks during the cooking process (Figs 1 and 2); and

(d) The attainment and retention of the 'cooking' temperatures for only brief periods (Figs 1 and 2).

When considering the influence of heat in the present context it should be borne in mind that while portions of meat are purchased by weight, considerable variation may occur in the shape and thickness of each purchase. The shape and thickness will have a great influence on heat penetration and hence affect the degradation of some antibiotic residues.

Table 4. *Cooking effect on sulphadimidine residues in meat*

Test animal	Carcase side	Sample	Total cooking time (min)	Maximum temperature attained (°C)	Annular zone diameter reduction (%)
A	Right	Steak 'rare'	10	32	0
	Left		10	32	1.5
B	Right		10	35	1.9
	Left		10	30	5.4
A	Right	Steak 'medium'	20	78	0
	Left		20	69	0
B	Right		20	81	0
	Left		20	80	7.6
A	Right	Steak 'well done'	30	81	0
	Left		30	84	0
B	Right		30	86	0
	Left		30	85	6.1
A	Right	Roast (centre)	120	48	0
	Left		120	48	0
B	Right		120	66	0
	Left		120	57	0
A	Right	Roast (midway)	120	—	0
	Left		120	—	0
B	Right		120	—	0.7
	Left		120	—	0
A	Right	Roast (outside)	120	82	0
	Left		120	88	0
B	Right		120	93	0
	Left		120	84	0

—, not recorded.

Table 5. *Cold storage effect on ampicillin residues in tissue*

Test animal	Sample	Storage temperature (°C)	% reduction in annular zone diameter after storage (weeks)					
			2	4	6	12	24	80*
A } B }	Muscle	4	{ 1.5 12.3	{ 29.5 58.1	{ 76.0 81.2	—	—	—
A } B }			{ 60.1 36.0	{ 100 85.4	{ 100 90.8	—	—	—
A } B }	Kidney	4	{ — 46.6	{ — 86.4	{ — 75.9	—	—	—
A } B }			{ 0 0	{ 0 0.2	{ — 0	{ 10.0 8.5	{ 0 19.8	{ 19.5 38.4
A } B }	Liver	-20	{ 38.3 7.1	{ 36.0 0	{ 27.8 9.2	{ 35.3 0.7	—	{ 93.9 53.7
A } B }			{ 13.2 0	{ 22.9 11.0	{ 7.6 0	{ 7.8 —	—	—

* Final test. —, not tested.

Table 6. *Cold storage effect on chloramphenicol residues in tissue*

Test animal	Sample	Storage temperature (°C)	% reduction in annular zone diameter after storage (weeks)					
			2	4	6	12	24	77*
A } B }	Muscle†	4	{ 85.4 100	100 —	— —	— —	— —	— —
A } B }	Muscle†	−20	{ 0 0	0 0	0 0	0 32.0	100 73.2	0 0

* Final test.

† Residues only recovered in muscle; see text.

—, not tested.

Table 7. *Cold storage effect on oxytetracycline residues in tissue*

Test animal	Sample	Storage temperature (°C)	% reduction in annular zone diameter after storage (weeks)						
			2	4	6	12	24	48	60*
A } B }	Muscle	4	{ 0 0	0 13.8	0 7.4	— —	— —	— —	— —
A } B }	Liver	4	{ 0 1.9	0 0	0 0	— —	— —	— —	— —
A } B }	Kidney	4	{ 0 0	0 0	0 0	— —	— —	— —	— —
A } B }	Muscle	−20	{ 0 0	0 10.3	0 3.2	0 6.3	0 0	0 0	0 1.0
A } B }	Liver	−20	{ 0 6.7	0 8.0	0 14.0	0 6.7	0 4.0	0 0	0 0
A } B }	Kidney	−20	{ 0 9.5	0 0	0 8.3	0 2.4	0 0	— —	— 0

* Final test. —, not tested.

Table 8. *Cold storage effect on streptomycin residues in tissue*

Test animal	Sample	Storage temperature (°C)	% reduction in annular zone diameter after storage (weeks)					
			2	4	6	12	24	66*
A	Kidney†	4	7.8	0	1.1	—	—	—
A	Kidney†	−20	5.6	0	1.1	1.1	17.9	0

* Final test.

† Residues only recovered in kidney; see text.

As the results in Tables 1–4 show, there was considerable variation in the reduction of the annular zone diameters of ampicillin, chloramphenicol, oxytetracycline and sulphadimidine under the conditions of the experiment. Such variation can in part be accounted for by the nature of the drugs themselves and their formulations, their pharmacodynamics and low temperatures attained during

Table 9. Cold storage effect on sulphadimidine residues in tissue

Test animal	Sample	Storage temperature (°C)	% reduction in annular zone diameter after storage (weeks)					
			2	4	6	12	24	50*
A } B }	Muscle	4	{ 0 4.3	— 0	0 0	— —	— —	— —
A } B }	Liver	4	{ 15.5 —	— 6.3	20.1 0	— —	— —	— —
A } B }	Kidney	4	{ 0 —	— 0	0 0	— —	— —	— —
A } B }	Muscle	−20	{ 0 2.8	— 2.6	0 0	0 0	3.1 12.8	0 1.3
A } B }	Liver	−20	{ 13.4 —	— 0.7	19.8 0	13.2 8.4	35.9 22.4	13.2 0.7
A } B }	Kidney	−20	{ 0 —	— 0	0 0	0 1.4	5.7 0	— —

* Final test. —, not tested.

Fig. 1. Temperature increases during cooking of roasts from sulphadimidine injected animal (B). Bars indicate the range of maxima temperatures of all 16 roasts in the whole experiment. ●—●, Roast (centre); ▲—▲, Roast (outside).

cooking. Schothorst (1969), Yonova (1971) and Peric & Dakic (1973) have shown that antibiotics can be refractory to degradation by heat in animal tissues unless the high temperature levels are maintained for considerable periods. As our own results show, such temperatures are not attained and maintained during normal cooking, and it seems logical to conclude, as did Raseta *et al.* (1975), that such heat treatment of animal tissues contaminated with antibiotics will not render them free of these substances.

Fig. 2. Temperature increase during cooking of steaks from sulphadimidine injected animal (A). Bars indicate the range of maxima temperatures of the 16 steaks used for each grilling regime. ○—○, Steak 'rare'; △—△, Steak 'medium'; □—□, Steak 'well done'.

During the cold storage part of the experiment it was not feasible to keep any tissues at 4 °C longer than six weeks as by then advanced autolysis had occurred. Therefore, the final testing of all tissues stored at this temperature was performed at 6 weeks.

Several features of the -20 °C storage should be noted. Firstly, the final test dates of the various antibiotic containing muscle tissues were different and are indicated in the respective Tables (5-9). Secondly, chloramphenicol residues were recoverable only in muscle and not in kidney or liver, and therefore these latter tissues were not included further in the study. Thirdly, streptomycin was recoverable only in the kidneys, and as a result, muscle and liver tissues were set aside. In all animals, only limited amounts of kidney tissue as compared with muscle or liver, was available therefore tests on this tissue were only carried out as indicated in the respective tables.

As can be seen from the Tables (5-9) the effects of cold storage at 4 or -20 °C on the residues of the different antibiotics was very variable. While storage at 4 °C may result in the reduction of ampicillin and chloramphenicol to low levels (Tables 5 and 6) minimal, if any, effects were observed in oxytetracycline, streptomycin and sulphadimidine residues at this temperature. Testing was not terminated until 6 weeks after slaughter even though animal tissues are not normally kept at this temperature for more than 7-10 days when 'off' taints and odours are perceived.

At -20 °C there was practically no reduction in the annular zone diameters of the residues of chloramphenicol, oxytetracycline and sulphadimidine (Tables 6, 7, 9) in muscle, and reductions of 19.5 and 38.4 % of the ampicillin residues (Table 5). The effect of cold storage on the residues in kidney and liver tissues was also slight except for ampicillin (Table 5) where storage over long periods did decrease the level detectable in the liver.

During the experiment it was found that, on occasion, there could be a reduction

in inhibition zone diameter and on subsequent testing of samples from the same tissue no reduction was detectable. Examples of this are tetracycline residues in muscle and liver of Animal B at -20°C . In extracts of muscle tissue reductions in zone diameter were detectable at weeks 4, 6 and 12 but subsequent testing at week 48 and 60 showed only slight inhibition.

What then are the likely hazards to the consumer of such meat or other animal product? Although hazards of direct toxicity (e.g. neomycin can produce serious nephrotoxicity or ototoxicity in people with pre-existing renal disease, Powell & Hooker, 1956), are potentially present in meat or meat products containing antibiotic residues, it appears the risk is small to the average consumer purchasing supplies from supermarkets where meat from animals is cut up and sold in small packs. Theoretically, there might be a greater risk for the family which bulk buys a whole or half side (for example beef) for deep freeze storage. Such a family could be exposed to a risk for a year or more. However, the idea of a direct toxic effect from ingesting meat or meat products containing antibiotic residues may be dismissed, for antibiotics in general have weak toxic effects on multicellular organisms as compared with unicellular organisms and it would require the consumption of several grams of antibiotic per kilogram body weight to produce a direct toxic effect (Pantaléon, 1966).

The eating of meat containing antibiotics as a source of allergic reactions remains an open question as it is nearly always an extremely difficult task to pinpoint any allergen in clinical situations.

Different estimates have been made of the propensity of people to allergy in general. One study of 1000 random hospital admissions in the United States showed an incidence of 14.9 % to various allergens (Algird, 1966). It has been estimated that the incidence of anaphylactic shock in patients treated with penicillin is somewhere between 0.1 and 0.3 % (Fernström, 1959; Brown, Price & Moore, 1964). It has been shown that sensitivity to streptomycin is common and it is estimated that 1–5 % of people handling it have developed allergies to it (Anon, 1953; Wilson, 1958). Neomycin, another antibiotic widely used in animal production, has cross sensitisation properties with streptomycin. Large quantities of streptomycin and dihydrostreptomycin are administered to food-producing animals (Huber, 1971). Streptomycin and dihydrostreptomycin are absorbed rapidly after parenteral administration but dihydrostreptomycin may be sequestered for long periods at injection sites. Residues, could therefore, be a source of risk to consumers of contaminated meat or meat products from these sites.

The possibility that meat containing antibiotics could induce drug resistance in the enteric bacterial flora of the consumer is cited as a potential hazard. Evidence to date would not support this. Knothe (1960, 1962) reports his experience over a 5 year period with human volunteers eating meat from animals receiving antibiotics in their diets. He states that no antibiotic resistance developed in the flora of the volunteers. The level of residue is another consideration and it is reckoned that in order to induce resistance it is necessary to consume between 3–5 mg/kg of food over a period of days. This level is not likely to exist in muscle tissue from animals either injected intramuscularly or subcutaneously or fed antibiotics in their diet since dietary antibiotics for growth promotion are usually included at levels 40 to 100 times less than therapeutic levels (Knothe, 1960, 1962).

The probability of multi-resistant organisms, for example, enteric *E. coli* passing their resistance factors to the consumers' flora would be very remote even though the *E. coli* of the slaughtered animal contaminated the meat during processing. R-factor transfer rarely occurs in the human gut except during chemotherapy (Anderson, 1975).

As the results of our experiment show, active antibiotic residues may be detected in animal tissue after roasting, grilling and prolonged cold storage. Present knowledge would appear to indicate that fears of direct toxicity to consumers are unfounded. The induction of antibiotic resistance in the microflora of the gut or the establishment of resistant organisms in consumers from contaminated meat are also very unlikely. However the possibility of allergic reactions occurring in a consumer of animal products containing antibiotic residues still exists. Present findings suggest that it would be unwise to rely on cooking or cold storage to minimise or destroy such residues. The only way to ensure no residues would appear to be the strict observance of the withdrawal period marked on the manufacturers data sheet for each drug administered to domestic animals.

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