Acta vet. scand. 1977, *18*, 15—22.

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RESIDUES OF SULFADIMIDINE/SULFANILAMIDE AND SULFAMETHOXYPYRIDAZINE IN SHEEP TISSUE*

By

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YNDESTAD, MAGNE and BJARNE UNDERDAL: *Residues of sulfadimidine/sulfanilamide and sulfamethoxypyridazine in sheep tissue.* Acta vet. scand. 1977, *18*, 15—22. — Following treatment of sheep with different sulfonamides, residues in kidney, liver, and muscle have been determined by microbiological and chemical methods. By the microbiological method residues could be detected in kidney until the third day after the combined treatment with sulfadimidine/sulfanilamide. Using the chemical method, residues of about 3.80 p.p.m. could be found that day in kidney, while the concentrations in liver and muscle were about 1.90 and 1.20 p.p.m., respectively. On the eighth day after the last treatment traces of the medicine could be found in kidney, liver and muscle by using the chemical method.

Residues of sulfamethoxypyridazine could be detected microbiologically in kidney the second day after the last administration of the drug. The concentration at that time in kidney, liver and muscle determined by chemical analyses was about 4.66, 2.45 and 1.23 p.p.m., respectively. Traces of sulfamethoxypyridazine in kidney, liver and muscle could also be detected on the eighth day after the last medication.

Considering altered metabolic rates of sick animals and variations in excretion rates between individuals of the same species, as well as variation in size of the doses applied, a 10 day withdrawal period for sulfonamides is proposed.

sulfonamides; residues; kidney; liver; muscle; sheep.

Sulfonamides are commonly used in the treatment of infectious diseases of farm animals in Norway. Acute mastitis in cows for instance is often treated by intravenous injection of sulfadimidine followed by sulfanilamide administered orally on the suc-

* This study was made possible by a grant from the Agricultural Research Council of Norway.

ceeding days. A variety of diseases in sheep and goats are treated, preferentially, with slowly excreted sulfonamides, such as sulfamethoxipyridazine.

Although this class of therapeutics are widely used, few references investigating residue levels of sulfonamides in carcasses can be found in the literature. *Righter et al.* (1971 a) found traces of sulfathiazole in muscles, liver, kidney and fat tissues of swine 7 days after oral administration. The same authors found less than 0.1 p.p.m. of sulfamerazine in liver, kidney and fat tissues of sheep 7 days after the last application. In muscles, traces of sulfamerazine could be detected up to 10 days after the last medication (*Righter et al.* 1972). When sulfamethazine was given to calves and chickens, less than 0.1 p.p.m. could be detected in the tissues of calves 8 days after treatment, while chicken tissues contained more than 0.1 p.p.m. 10 days after the last application (*Righter et al.* 1971 b).

The results for poultry (*Righter et al.* 1970) indicate that withdrawal periods of at least 7 days would be necessary to reduce the levels of sulfonamides in kidney and eggs to 0.1 p.p.m. or less.

After the administration of sulfadimidine to pigs, *Rasmussen et al.* (1973) could not, after 1 week, detect any residues in muscles except at the injection point. Traces of sulfadimidine could, however, be detected in the kidneys.

In a survey on calves, cattle and swine sampled from different slaughter-houses in W. Germany, *Jüntgen* (1973) found sulfonamide residues in kidneys in 12.6, 8.7 and 2.3 %, respectively, of the investigated carcasses. In 18 out of 120 kidneys brought to the laboratory in connection with emergency slaughtering, *Nielsen et al.* (1974) found traces of sulfonamides. Information on doses and time intervals from administration of the medicine to slaughtering was, unfortunately, incomplete.

Detection of sulfonamides can be done microbiologically or chemically. When using microbiological methods it is important to use growth media with the lowest possible concentrations of p-aminobenzoic acid and folinic acid, as these substances are antagonists to sulfonamides. At our laboratory, Müller-Hinton medium (*Müller & Hinton* 1941) has, therefore, been used for years, in the detection of growth-inhibiting substances in milk and tissues. Various microorganisms have been used in this test system. *Read et al.* (1971) investigated the sensitivity of dif-

ferent microorganisms to antibiotics and sulfonamides, and found Bacillus megaterium most suitable as test organism for the detection of sulfonamides in milk. Micrococcus luteus (Sarcina lutea) is usually used in Norway for the biological testing for growth inhibiting substances in carcasses.

Bratton & Marshall (1939) described a chemical method for the detection of sulfonamides utilizing a colorimetric determination. *Tishler et al.* (1968) modified the method which is, at present, most commonly used.

As traces of sulfonamides in meat and meat products may have allergenic effects on man, in addition to causing technological problems in the fermentation process in sausage production, it is of great interest to establish well-defined withdrawal periods for the most commonly used sulfonamides. In addition to the determination of excretion rates and, thus, withdrawal periods, the aim of the present investigation was to compare the sensitivity of the chemical and microbiological methods, using different test organisms.

MATERIALS AND METHODS

Animals and medication plan

The test animals, consisting of 14 adult sheep, were divided into 2 groups. One group was initially treated intravenously with 150 mg sulfadimidine/kg body weight.

On the same day, an oral dose of 100 mg sulfanilamide/kg was administered. This oral dose was repeated twice daily the following 3 days.

The other group was, initially, given 35 mg sulfamethoxypyridazine (Longamid®)/kg body weight orally, and a maintenance dose of 25 mg/kg body weight the succeeding 3 days. In both groups animals were slaughtered 2—8 days after terminated medication.

Microbiological determinations

Micrococcus luteus (Sarcina lutea) ATCC 9341 and Bacillus megaterium ATCC 9885 were used as test organisms. The microorganisms were inoculated into Müller-Hinton agar in numbers of approx. 6×10^5 of M. luteus and 1×10^5 of B. megaterium per ml agar. The agar was poured into dishes to a thickness of 4 mm.

Sulfonamides were identified by adding 0.1 mg p-aminobenzoic acid per ml of agar.

Samples, 1.5 cm×1.5×1.0 of kidney (cortex and medulla), liver and muscles were placed, aseptically, on the agar dishes containing the test organisms and agar dishes also containing p-aminobenzoic acid.

All the dishes were held at 4°C for 2 hrs. before the final incubation. Agar dishes containing M. luteus were incubated at 37°C for 18 hrs. while those containing B. megaterium were incubated at 37°C for 12 hrs. The inhibition zone was determined by measuring the clear zone between the edge of the tissue and the boundary of the bacterial growth. The results are given in mm.

Chemical determination

The concentrations of sulfonamides in different tissues were determined according to the method described by *Bratton & Marshall* (1939) as modified by *Tishler et al.* (1968).

The results have been corrected for nonspecific compounds using tissues from 3 non-medicated sheep as controls.

RESULTS

Tables 1 and 3 show inhibition zones from kidney, liver and muscle due to residues of sulfadimidine/sulfanilamide and sulfamethoxypyridazine. Similarly Tables 2 and 4 show sulfonamide levels in the different tissues when the sulfonamides used in the experiments were administered according to the plan outlined in the methods. The results for the combined treatment with sulfadimidine/sulfanilamide were calculated as sulfanilamide, even though the levels measured during the first days are from

Table 1. Inhibition zones (in mm) of Micrococcus luteus (M. l.) and Bacillus megaterium (B. m.) 2—8 days after treatment with sulfadimidine/sulfanilamide.

	2 days		3 days		4—8 days	
	M. l.	B. m.	M. l.	B. m.	M. l.	B. m.
Kidney	5	7	1.5	1.5	—	—
Liver	4	3	—	—	—	—
Muscles	3	3	—	—	—	—

Table 2. Trace levels of sulfadimidine/sulfanilamide, calculated as sulfanilamide, 2—8 days after treatment (in p.p.m.).

	2 days	3 days	4 days	5 days	6 days	7 days	8 days
Kidney	19880	3800	850	650	507	300	119
Liver	16500	1900	600	400	265	180	79
Muscles	16900	1200	261	175	109	75	50

Table 3. Inhibition zones (in mm) of Micrococcus luteus (M. l.) and Bacillus megaterium (B. m.) 2—8 days after treatment with sulfamethoxypyridazine.

	2 days		3—8 days	
	M. l.	B. m.	M. l.	B. m.
Kidney	2	2	—	—
Liver and muscles	—	—	—	—

Table 4. Trace levels of sulfamethoxypyridazine (in p.p.b.) 2—8 days after treatment.

	2 days	3 days	4 days	5 days	6 days	7 days	8 days
Kidney	4660	1100	380	250	198	150	115
Liver	2450	520	166	150	144	95	55
Muscles	1230	250	83	65	60	50	41

both sulfadimidine and sulfanilamide. For the other days the residues measured most probably consist of sulfanilamide alone.

Using the biological method, residues of sulfonamides could be detected in kidneys of sheep slaughtered until the 3rd day after the administration of sulfadimidine/sulfanilamide (Table 1). The inhibition zones were, however, so small (1.5 mm) that, under practical conditions, detection may be difficult and unreliable.

The chemical analyses showed that kidney, liver and muscle at that time contained about 3.80, 1.90 and 1.20 p.p.m. sulfonamides, respectively. On the 8th day after medication the concentration was about 0.12 p.p.m. in kidney, 0.08 p.p.m. in liver and 0.05 p.p.m. in muscles.

By the microbiological method residues of sulfamethoxypyridazine could be detected in kidneys of animals slaughtered 2 days after the last treatment.

The concentration at that time in kidney, liver and muscle determined by chemical analyses was about 4.66, 2.45 and 1.23 p.p.m., respectively. On the 8th day after medication the kidney contained about 0.12 p.p.m. sulfamethoxypyridazine, while in liver and muscle 0.06 and 0.04 p.p.m. could be detected.

DISCUSSION

After treatment of sheep for some days with a combination of sulfadimidine/sulfanilamide, traces of the therapeutics could, by microbiological methods, be detected in kidneys up to 3 days after the last treatment. The detection limit for sulfamethoxipyridazine was 2 days. However, using chemical methods traces could still be detected in both kidney, liver and muscles 8 days after the administration of the medicines in both experiments. On this basis it seems reasonable to propose withdrawal periods of 10 days for medicated animals about to be slaughtered.

The investigation shows that, at present, the microbiological methods available are not sensitive enough for the detection of sulfonamide residues in tissues of slaughtered animals. In spite of optimalizing the method with respect to the choice of microorganism, growth conditions, density of bacteria and agar thickness in the dishes, no inhibition zones were observed in the agar although the sulfonamide concentration was in the order of about 1.0 p.p.m. in kidney as determined by chemical methods. No significant difference in sensitivity was observed between M. luteus and B. megaterium.

In this study the sulfonamides were given to healthy sheep. In the case of sick animals, the metabolism of the sulfonamides may be altered, thus affecting the retention of the medicine in tissues. Furthermore, variation in excretion rates between individuals of the same species should also be taken into account. In addition, the practitioner may sometimes use higher doses for treatment than those used in the present investigation, resulting in higher levels in the various tissues. When fixing withdrawal periods, all these variables must be taken into account.

ACKNOWLEDGEMENTS

The authors are greatly indebted to Dr. S. Tollersrud at Kjeller animal research farm for providing animals. Special thanks are given to Mr. J. Andal for technical assistance.

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SAMMENDRAG

Restkonsentrasjoner av sulfadimidin/sulfanilamid og sulfamethoxypyridazin i nyre, lever og muskulatur fra sau.

Sau ble behandlet med forskjellige sulfonamider og avlivet på bestemte tidspunkter etter behandlingen. Restkonsentrasjoner i nyre, lever og muskulatur av de brukte medikamenter ble bestemt kjemisk

og mikrobiologisk. Ved kombinasjonsbehandlingen sulfadimidin/ sulfanilamid kunne rester av medikamentene påvises i nyrevev ved mikrobiologiske metoder i 3 døgn etter avsluttet behandling. Ved kjemiske analyser kunne spor av sulfonamider påvises i åtte døgn i nyre, lever og muskulatur.

Sulfamethoxypyridazin kunne påvises i 2 døgn fra nyrene ved mikrobiologiske metoder mens kjemiske analyser viste at rester av nevnte medikament forekom i nyre, lever og muskulatur åtte døgn etter avsluttet behandling.

På bakgrunn av de foretatte undersøkelser er det foreslått en tilbakeholdelsestid på 10 døgn for dyr som er behandlet med de brukte sulfonamider. En har da tatt i betraktning at syke dyr kan ha relativ langsom utskillelse av medikamenter. Det kan også være individuelle variasjoner i utskillelsen og dessuten brukes ofte større behandlingsdoser enn de som er anvendt i forsøket.

(Received November 29, 1976).

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